Bull. Org. mond. Santé
Bull. Wld Hlth Org. } 1960, 23, 313-400

A Review of Recent Literature on Plague

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In his comprehensive monograph on plague, published by WHO in 1954, Dr Pollitzer pointed out that despite the marked drop in the incidence of this disease in recent years, he considered it impossible for various reasons to be complacent about the situation. Since this monograph appeared, plague has shown a truly spectacular decrease, but in case this is partly the outcome of a natural periodicity of the infection, the author still feels that the disease " should be given continued attention by those interested in global public health ". To this end he summarizes here the latest information on the subject, his review covering not only works published since 1954, but also some earlier literature (particularly from the USSR) which was not available to him at the time of preparation of his monograph.

BACTERIOLOGY

MORPHOLOGICAL CHARACTERISTICS

Capsule and envelope

Investigations on the problem whether the plague bacillus possesses a capsule or an " envelope " or both, which have led to discrepant results in the past, have been continued by Englesberg & Levy (1954a) and by Crocker and colleagues (1956). The subject has also received attention in a profound disquisition entitled *Bacterial Capsules and their Relation to the Cell Wall* by Tomcsik (1956).

The results obtained by Englesberg & Levy when cultivating an avirulent plague strain in a fluid casein-hydrolysate medium strongly supported the view that the organisms were surrounded by a readily soluble envelope, the substance of which consisted wholly or at least in considerable part of the antigenic fraction I (see Baker and associates, 1952). Dissolution of this layer left behind a small amount of material which microscopically appeared to be similar to a typical bacterial capsule, but possibly consisted of additional fraction I held somewhat tenaciously to the cell wall.

In partial agreement with these observations, Crocker and co-workers were able to demonstrate with the aid of the electron microscope the existence of a layer of structurally undifferentiated material of lower electron density between and among cells of *Pasteurella pestis* grown on collodion films over agar. However, they found no evidence that, in addition to this soluble envelope or slime layer, to which the non-somatic antigens of the organism were related, the plague bacillus also possessed a capsule.

As asserted by Tomcsik, there was no reason to assume that in the case of the plague bacillus " any capsular structure occurs corresponding to the separate morphological distribution of two different substances in capsulated *Bacillus megatherium* ".

However, Tomcsik drew attention to the possible existence of a microscopically invisible capsulation in the case of apparently non-capsulated bacteria, as had been postulated in the case of *P. pestis* by Burrows (1955) on account of an identical resistance to phagocytosis by a capsulated plague strain and by a virulent plague strain without visible capsule.

Motility

Observations confirming that *P. pseudotuberculosis*, though typically motile, in contrast to the invariably immotile plague bacillus, may show absence of motility, even if cultivated at a suitably low temperature (26°C or less), have been recorded by Knapp & Masshoff (1954) and by Knapp (1956).

The first-mentioned two workers noted absence of motility in three pseudotuberculosis strains of

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human origin, regardless of whether they had been cultivated at any temperature between 37°C and 18°C. According to Knapp (1956), re-examination of two of the strains in San Francisco not only confirmed their immotility but also showed absence of flagella.

In his 1956 study, Knapp drew attention to claims made by some previous observers that, in contrast to pseudotuberculosis growths isolated from animals,[1] those of human origin were immotile even if incubated at a suitably low temperature. Testing himself 14 strains of this nature, he found seven to be immotile and two weakly motile at incubation temperatures ranging from 18°C to 37°C. However, Knapp maintained with much reason that the immotility of pseudotuberculosis bacilli might be apparent rather than real, being due not to an absence but to a paucity of flagellated organisms, the presence of which could be revealed neither with the aid of hanging-drop or other routine methods of examination nor even through flagellar staining. He proved the validity of this postulation by showing that the presence of motility could be demonstrated in all his strains if—according to a still unpublished method by Bader—their initial cultivation in a broth flask was followed by massive inoculation of the semi-solid agar contained in an attached U-tube.

CULTURAL CHARACTERISTICS

Growth inhibition

Dealing with the partial inhibition exerted by nutrient broth on the growth of *P. pestis*, Girard (1956) maintained that it was not the lack of suitable nutritive material (as he had formerly assumed), but the presence of inhibitory substances in this culture medium, or in solid media prepared with it, which prevented the growth of the organism from small inocula, unless these untoward influences were counteracted by the addition of blood, serum, or bacterial lysates.

In support of this view Girard recorded a series of observations, according to which a medium prepared merely by addition of 20 parts of a suitable peptone and 5 parts of sodium chloride per 1000 parts of 2.5% nutrient agar proved of equal value to blood agar, permitting the growth of *P. pestis* from quite small inocula, even from a single bacterial cell, whereas at least 1000 organisms were required to start growth on agar media containing the same amount of peptone, but prepared in the usual manner with beef broth.

It is interesting that satisfactory results could be obtained with a slight delay through implantation of small inocula on media prepared with 150 parts of gelatin per 1000 parts of beef broth instead of with agar. Evidently, therefore, gelatin had to be classed among the substances capable of neutralizing the growth-inhibiting substances of nutrient broth.

Referring to the inability of *P. pestis* to grow from small inocula on agar media prepared in the usual manner, Englesberg (1957a) stated in general agreement with Girard that this inability " is not due to the failure of such a medium to provide the necessary growth requirements for this organism since the same medium without agar, vigorously aerated, does support growth of small inocula of *P. pestis* ".

Englesberg found also that ordinary agar could be rendered suitable for this purpose if it was autoclaved under acid conditions (pH 5.0 with HCl) rather than at pH 7.0, while autoclaving under alkaline conditions enhanced the inhibitory effect. In his experience the absence of growth from small inocula on media prepared in this unsuitable manner was a stable fundamental characteristic of all plague strains, attempts to isolate resistant mutants having proved invariably unsuccessful. As noted also by Girard, pseudotuberculosis bacilli on the contrary grew well on agar media prepared under alkaline conditions.

Further work by Englesberg & Ingraham (1957) confirmed the observation of earlier investigators that addition of 0.1% sodium sulfite was another means of rendering ordinary agar suitable for the growth of *P. pestis*. A dosage of 0.05% of the sulfite, on the other hand, was found to be inadequate fully to neutralize the inhibitory effect of agar on the plague bacilli.

Growth stimulation

In regard to the methods which have been recommended within recent years for promoting the growth of plague bacilli, especially that from small inocula, it has to be noted first that Girard & Gallut (1953), confirming observations by Sokhey

[1] In agreement with this claim, Klimova (1956), examining 41 strains isolated from commensal rats or mice or, in two instances, from grain supplies contaminated by these rodents, established with the aid of hanging-drop preparations and cultivation in U-tubes that at an incubation temperature of 16°-18°C 39 of the strains showed a marked and 2 a slight motility. If grown at 37°C, the organisms were invariably immotile.

(1952), found a lag phase to be absent if cultivations were made in the filtrates of broth cultures previously used to grow a plague strain. Such filtrates, obtained from broth cultures kept from 1 to 24 days, were capable of promoting the development of *P. pestis* from inocula which had been found too small to ensure growth in the plain media (meat-infusion or peptonized meat-broth) used to obtain the material for filtration.

In place of fresh blood which, as noted above, was amply used for stimulating the growth of *P. pestis*, Kivman and associates (1955a) recommended addition to the agar media in proportions of 0.5%-1% of a dried, lysed blood preparation manufactured thus:

Sterile defibrinated blood of rabbits, sheep or other species was lysed with distilled water added at a proportion of 1:1 to 1:4. One-ml quantities of the lysed blood were poured into ampoules of 5-ml capacity and frozen at −20°C to −30°C for 2½ hours and at −70°C for 30 minutes. Then the product was dried *in vacuo* at a residual pressure of 10^{-4} cm of mercury for 20-21 hours. During the first 12 hours of drying the temperature was slowly raised from −20°C to −30°C to zero; then the process was continued at room temperature. The ampoules were sealed while still *in vacuo*.

The solubility of the product could be markedly increased if, before drying, 1% glucose solution was added in a proportion of 1:3 to 1:4. The product was found to remain potent for at least 100 days at temperatures up to 42°C.

Of the various articles dealing with the growth stimulation of *P. pestis* through heterogeneous bacteria or their filtrates, it is convenient to refer first to a contribution by Kivman and colleagues (1955b). These workers reported good success with a medium prepared by the addition of culture filtrates of a spore-bearing saprophyte, *B. mesentericus fuscus* (best grown for 7-27 days at 28°C in sugar broth with a pH of 7.3), in a concentration of 5% to Martin agar, pH 7.3. Even if small inocula (100 organisms) were implanted on such reinforced media, characteristic colonies developed within 18 hours' incubation at 28°C. This good result was the more remarkable because Kivman and his co-workers experimented with the avirulent EV strain, which in the experience of the Soviet observers was more exacting in its nutritional requirements than glycerol-positive plague strains. It was noted in this connexion that the growth stimulator was active only in the case of R forms of plague and pseudo-tuberculosis bacilli and did not promote the growth of most other Gram-negative species. It remained efficacious if kept in the dry state.

In the course of an investigation on the growth of *P. pestis* on media of a known chemical composition, to which further reference will be made below, Gubarev and co-workers (1956) had occasion to study the development of the organism on agar media containing only one of the amino-acids tested by them. Almost invariably no or at most a weak growth resulted even if massive inocula were implanted on the agar plates. However, if the plates, kept at room temperature after incubation, became contaminated with air saprophytes (*Sarcina* or *Actinomyces* spp.), sometimes the plague bacilli were seen to grow well round the colonies of the contaminants. That the latter actually functioned as growth stimulants was confirmed through their artificial implantation on plates which had previously been inoculated with plague bacilli but had initially shown no growth of the latter.

Most satisfactory results were obtained by Karpuzidi & Makarovskaya (1956), when using media to which the sterilized lysates of sarcinae had been added in proportions of 0.1% to 10% for the cultivation of plague bacilli. As an interesting side issue of these investigations the two workers established that the M-concentration (Bail, 1929), i.e., the maximal number of organisms which can live simultaneously in a unit of fluid medium, was in the case of *P. pestis* 1600 million per ml. As was to be expected, the addition of the sarcina lysate did not exert an influence on the size of this figure.

Karpuzidi & Chochlova (1956) established that growth of the plague bacilli on media containing sarcina lysates did not alter the basic properties of the organisms. Though such media were not superior to the usual substrates as far as the prolonged preservation of the cultures was concerned, they not only accelerated the growth of the plague bacilli but also permitted their isolation from materials in which the organisms were contained in minimal numbers. These two authors therefore advocated the addition of the sarcina lysate in a proportion of 1% to the media used for practical laboratory work.

According to a further study, by Krichevskaya & Karpuzidi (1956), the sarcina lysates, after they had been freed from non-lysed microbial bodies, gave the chemical reactions for proteins. Comparative tests with the various protein (albumin) fractions obtained through ammonium sulfate precipitation showed that one of the fractions, which was readily

soluble in water and in phosphate buffer with a pH of 8.6 to 9.2, was alone responsible for the growth-stimulating action of the lysates. In order to obtain this active portion in a dry state, the following technique was adopted by the two workers:

After the lysate had been centrifuged at a speed of 3000 revolutions per minute (r.p.m.) for 10 minutes, the supernatant was put into a measuring cylinder and 45 g of sodium sulfate were added per 100 ml of fluid.

After the cylinder had been left standing for 1-2 hours, the protein, which had collected in the upper part of the vessel, was separated off by 10 minutes' centrifugation at 3000 r.p.m. The sediment was dissolved in water and the solution was centrifuged for 15 minutes. To the supernatant, which represented the active water-soluble portion of the protein, an equal part of acetone was added. The protein precipitate thus obtained was separated off by centrifugation for 5 minutes and was then washed 3-4 times with acetone, each new acetone addition being followed by 5 minutes' centrifugation. The finally obtained sediment was air-dried on filter-paper and this preparation was kept protected against moisture.

It was found that this dry product stimulated the growth of *P. pestis* even in a dilution of 1 : 1 000 000.

Nutritional requirements

Recent studies on the role played by amino-acids in the growth of *P. pestis* and on other nutritional requirements of this organism may be summarized thus:

For immunological studies, to which reference will be made in due course, Silverman and co-workers (1954) grew an avirulent plague strain in a synthetic medium containing 11 amino-acids,[1] salts of potassium, magnesium, manganese and iron, sodium gluconate and sodium bicarbonate, biotin, calcium pantothenate, thiamine and xylose. When the medium was used for cultivation of *P. pestis*, 5-ml quantities of a 25% solution of the last-mentioned sugar were added twice daily, besides the necessary quantities of either hydrochloric acid or sodium hydroxide to keep the reaction near neutrality.

In the course of studies on the mutation of *P. pestis*, Burrows & Bacon (1954a) cultivated their strains on the " minimal agar " (containing mineral salts and glucose) used by Bacon and co-workers (1950) for the cultivation of *Salmonella typhi*, supplementing it by the addition of haemin as well as of phenylalanine, cystine, methionine, glycine, valine and isoleucine. They explained that the last-mentioned three amino-acids, though not essential for the growth of plague bacilli at 28°C, were found to be very stimulatory and to improve the reliability of the medium.[2]

Using X-rays as the mutagenic agent and then incubating the plates for 4 days at 28°C, Burrows & Bacon were able to isolate small numbers of mutants with additional requirements for tryptophane, adenine, arginine and nicotinamide, respectively.

Reporting on the dissimilation of serine by *P. pestis*, Levine and colleagues (1954) referred to observations which showed that this amino-acid was not indispensable for the growth of the organism.

Working with the EV strain as well as with a virulent glycerol-acidifying strain, Gubarev and associates (1956) attempted to induce growth by adding various amino-acids singly to a basic medium which contained, besides a phosphate buffer (pH 7.3) and glucose, various mineral acids.

If large inocula were used, this basic medium in the fluid state could be made suitable for the cultivation of the experimental strains by adding singly the following amino-acids: phenylalanine; valine; methionine; tryptophane (very slight growth); cystine; glycine; cysteine; glutamic acid; norleucine and alanine. However, the strains did not become adapted to such media, so that after 3-4 passages through them no further growth of the organisms took place.

If the synthetic media containing one of the above-enumerated amino-acids were solidified through addition of 1.5% agar and 100 million inocula were implanted, almost invariably no or at most a slight growth of *P. pestis* resulted. However, an exception was formed by the quite satisfactory development of the virulent experimental strain and also of an avirulent glycerol-positive strain on cysteine-containing plates of the synthetic medium.

Trying to evolve a satisfactory substrate for the cultivation of plague bacilli by the addition of combinations of amino-acids, Gubarev and co-workers obtained success with only one medium, which contained, besides M/15 phosphate buffer

[1] *l*-Glutamic acid, *dl*-phenylalanine, *dl*-methionine, *dl*-valine, *dl*-leucine; *dl*-lysine hydrochloride, *l*-proline, *dl*-threonine, *l*-cysteine hydrochloride, glycine and *dl*-isoleucine.

[2] As added in the article, Burrows & Bacon found after conclusion of their studies that addition of sodium bisulfate at a final concentration of 0.02% greatly improved the growth of the organisms on the minimal agar.

(pH 7.3), 0.1% glucose and 1.5% agar, the following amino-acids and salts (amounts in mg%):

dl-Alanine	23.8
Asparagine	12.0
d-Cysteine	1.4
d-Glutamic acid	64.2
dl-Methionine	15.6
dl-Phenylalanine	6.6
d-Glycine	21.0
dl-Tryptophane	23.2
Tyrosine	11.0
dl-Valine	16.0
dl-Norleucine	10.0
Magnesium sulfate	4.0
Iron sulfate	2.0
Manganese sulfate	2.0
Sodium chloride	2.0

Note: Glucose, salts and cysteine were added under sterile conditions after the other ingredients had been autoclaved.

While evidently the EV strain did not, or at least not satisfactorily, grow in this medium, even if an inoculum of 100 million organisms was used, it proved suitable for the cultivation of the virulent experimental strain (minimal inoculum, 1 million organisms) and also 7 other virulent plague strains.

If the latter as well as the two principally used strains were inoculated into fluid media prepared according to the above-quoted formula, much better results were obtained. Even the EV strain grew if an inoculum of 100 000 organisms was used, while in the case of the 8 other strains usually an implantation of 2000 organisms sufficed.

The following plague workers besides Gubarev and his colleagues have recently used synthetic media prepared with amino-acids:

For studies on the appearance of pigmented mutants of *P. pestis* (see below), Jackson & Burrows (1956a) finally adopted a haemin-containing medium into which 6 amino-acids had been embodied. Of these, *dl*-methionine, *dl*-phenylalanine and *l*-cysteine appeared to be essential for the growth of the smooth avirulent Tjiwidej strain (Otten, 1936), while the other two plague strains mainly used by Jackson & Burrows proved to be methionine-independent. Glycine (like threonine which was originally used in its place), besides augmenting the growth of the organism, appeared to be essential for its cultivation at 37°C. In the case of some of the strains tested by these workers it was necessary to resort to an addition of arginine to the synthetic medium in order to obtain growth. As may be conveniently added, they found that omission of the ammonium salts from their medium resulted in very slow growth and reduced colony size. Another noteworthy point was that the use of galactose in place of glucose seemed preferable, because on media prepared with the former sugar (or with xylose) the plague colonies were of a more compact type than was the case when glucose, maltose or mannose served as the carbohydrate source. As added by Jackson & Burrows, these findings were in accord with observations which had shown that much more acid was produced when plague bacilli were grown in media containing glucose, fructose, maltose or mannose than when they were grown in media containing galactose or xylose.

For a large-scale study of the possibilities and limitations of cultivating *P. pestis* on synthetic media, Domaradsky & Ivanov (1957) used a basic fluid medium with the following formula (amounts in grams per litre):

l-Asparaginic acid	0.2
d-Glutamic acid	0.5
Glycocoll	0.5
dl-Alanine	0.2
dl-Valine	0.1
dl-Norleucine	0.1
dl-Serine	0.02
dl-Threonine	0.02
l-Cystine	0.15
dl-Methionine	0.05
dl-Leucine	0.2
dl-Arginine	0.1
dl-Tryptophane	0.2
l-Tyrosine	0.05
dl-Phenylalanine	0.1
dl-Proline	0.1
l-Histidine	0.2
Glucose	0.2

With the exception of the EV strain, all plague strains tested grew well in this medium. The minimal inoculum for virulent " continental " (i.e., glycerol-acidifying) strains was 20 organisms, while an inoculum of 200 organisms was required in the case of a virulent glycerol-negative (" oceanic ") strain.

Experimenting with media from which one or several of the amino-acids enumerated above had been excluded, Domaradsky & Ivanov reached the general conclusion that in order to ensure growth of the plague bacillus, the minimal requirements are the presence in the media of phenylalanine, methionine, cystine, serine and threonine.

However, as stated in the text of their article, these two workers found it possible to cultivate plague bacilli in the absence of methionine and

cystine, if at the same time alanine, glycocoll, valine and norleucine were also omitted. This seemed to furnish indirect proof that under certain conditions the plague bacillus had the property of synthesizing the sulfur-containing amino-acids (methionine and cystine) but that this synthesis was inhibited by the presence of one of the other above-mentioned amino-acids.

A further interesting study on the role played by the sulfur-containing amino-acids in the growth of *P. pestis* was made by Domaradsky (1957). Working with agar media containing 0.2 ml of radioactivated sodium sulfate ($Na_2S^{35}O_4$) per 50 ml, he found that, regardless of the length of incubation, the plague bacilli did not become radioactive. This confirmed the observation made in 1952 by Englesberg that *P. pestis* was incapable of assimilating sulfates.

If, on the contrary, radiocystine was added to the media, the plague bacilli became well marked with this substance. Obviously, therefore, the organisms assimilated cystine and embodied it into their protein complex. Moreover, as could be shown with the aid of paper chromatography, the plague bacillus was capable of using cystine for the synthesis of methionine—a transformation which appeared to be of an irreversible character.

As summarized in a review in the *Bulletin de l'Institut Pasteur*, Domaradsky & Semenuchkina (1957) had established in 1955 that glycocoll, though not indispensable for the growth of the plague bacillus, became oxidized by it with the formation of ammonia and carbon dioxide. Making further studies with glycocoll tagged with C^{14} isotopes, these two workers found the latter in the lipoid fraction, the nucleic acid and the protein fraction of organisms cultivated on the radioactivated media. They postulated that the products of glycocoll disintegration increased the content of the plague bacilli in specific polysaccharides and stimulated capsule formation.

Evaluating the importance of the assimilation of radioactivated carbon acetate by *P. pestis*, Domaradsky & Semenuchkina (1958) found this compound capable of synthesizing a variety of amino-acids, among which were glutamic acid and aspartic acid.

An interesting contribution to the solution of the problems at present under review was made by Englesberg & Ingraham (1957) who, using a subculture of an avirulent plague strain as "prototroph", tried to obtain *meiotrophic* mutants, i.e., mutants requiring fewer amino-acids for their growth. It was easy to isolate from the prototroph, which required cysteine, methionine, phenylalanine, valine and isoleucine for its cultivation, a methionine-independent mutant (me $^+$) on a thiosulfate-, valine-, isoleucine- and phenylalanine-supplemented agar. In studying the possibility of isolating mutants independent of the other amino-acids, unexpectedly complex conditions were met with, since the apparent requirements not only for both valine and isoleucine, but even for valine, leucine and methionine, could be lost simultaneously.

Working with a chemically defined medium, Higuchi & Carlin (1958) found phenylalanine, methionine and cysteine essential for the cultivation of *P. pestis*, while the 7 other amino-acids embodied (*l*-glutamic acid, *dl*-valine, *dl*-leucine, *dl*-lysine, *l*-proline, *dl*-threonine and glycine) were required for optimal growth at 27°C. For cultivation at 37°C isoleucine was essential, while addition of increased amounts of magnesium sulfate stimulated growth.

Other interesting findings made by Higuchi & Carlin were that:

(*a*) *dl*-serine exerted an inhibitory action on the growth of *P. pestis* which could be overcome by the presence of adequately large amounts of glycine;

(*b*) replacement of cysteine by sodium thiosulfate led to a prolongation of the lag phase of growth;

(*c*) ammonium acetate markedly stimulated the early growth of the plague bacillus;

(*d*) the use of *d*-xylose (in the place of glucose, which led to an accumulation of acidic products in the medium [1]) was satisfactory, especially if several 1% increments of this sugar were added in the course of cultivation;

(*e*) *d*-mannitol and *d*-galactose, though also ensuring an excellent growth of *P. pestis*, proved on the whole less satisfactory than xylose.

A further noteworthy observation was that there existed a difference in the length of the lag phase between virulent and avirulent plague strains grown at 37°C. Further studying this matter, Kupferberg & Higuchi (1958) found that the virulent strains grew well under these conditions if calcium was added to the synthetic medium, remaining fully pathogenic for intraperitoneally infected mice. As the two authors added, experiments were in progress to test the hypothesis that the presence of an adequate concentration of calcium in nutrient media was capable of preventing a loss of virulence caused

[1] The untoward influence exerted on the growth of *P. pestis* by glucose has also been dealt with in a recent short communication by Wessman and colleagues (1958).

by repeated subculturing in aerated liquid media at 37°C.

Casein hydrolysate media [1]

Besides Englesberg & Levy (1954a), to whose results attention has been drawn above, Gubarev and colleagues (1956) and Higuchi & Carlin (1957) have recently explored the possibilities of using casein hydrolysate media for the cultivation of *P. pestis*.

Describing the preparation of such media, the first-mentioned group of workers stated:

Mixtures of commercially available casein in amounts of 100-150 g and of tenfold quantities of 25% sulfuric acid were heated under reflux condensation in large flasks. After 24 hours the product was neutralized first with a saturated solution of barium oxide and then with either calcium or barium carbonate so as to remove the sulfuric acid. The end of hydrolysis was determined with the aid of the biuret reaction. After the sediment had been removed by filtration, the hydrolysate was treated with activated charcoal so as to remove its dark colour. The transparent and slightly yellow fluid thus obtained was used for cultivation either in concentrated form or in dilutions ranging from 10% to 50% with additions of the mineral salts used for the manufacture of synthetic media. If plates were needed, agar was added in a proportion of 1.75%.

Though media prepared with different lots of casein hydrolysate did not behave in a uniform manner, on an average an inoculum of 500 organisms sufficed to initiate growth. It was noted in this connexion that the EV strain grew less well than a virulent plague strain used in parallel tests.

After the latter strain had been subcultivated at intervals of 10 days for a period of 7 weeks on solid casein hydrolysate media, it showed a somewhat lessened virulence for white mice.

While addition of nicotinic acid or thiamine [2] to the casein hydrolysate media did not prove growth-stimulating, addition of *yeast autolysates* produced such an effect, The method of preparing the latter was as follows:

Bread yeast, broken into small pieces, was put in 1-kg quantities into flasks and the latter were kept for 2 days in a thermostat at a temperature of 45°C. Then 2 litres of tap-water were added per kg of yeast. After sterilization at 120°C for 30 minutes the flasks were kept standing until a sediment had formed; this sediment was then used for the preparation of solid or fluid media in concentrations ranging from 4% to 20%.

Yeast autolysates with a high amino-nitrogen content were found to promote a better growth of *P. pestis* than took place on the usual meat-peptone media. However, in comparison to the latter, growth on media prepared with yeast autolysates seemed to impair slightly the virulence of the organisms.

In order to obtain casein hydrolysate media for the cultivation of *P. pestis* with a high yield, Higuchi & Carlin resorted after hydrolysis for two hours in the autoclave to treatment of the hydrolysate with an anion-exchange resin to remove the sulfuric acid. Optimal results were obtained if the resin was used in amounts of about 2-3 g per gram of casein. The material finally obtained was incorporated into the test media at a concentration of 2.5% casein, and mineral substances and xylose were added. For cultivation, aeration was provided by placing 25-ml quantities of the fluid medium in 500-ml Erlenmeyer flasks and putting the latter into a reciprocating shaker operating at a rate of 100 cycles with a 3-inch (7.6 cm) stroke.

When making tests with media generally prepared according to the above method, but containing glucose instead of xylose, the two workers found that the former stimulated growth only at low concentrations (1-3 mg per ml), the use of higher concentrations inhibiting growth and leading to a very low terminal pH. Satisfactory results could be obtained by the often-repeated addition of 1-mg to 3-mg quantities of glucose per ml to the growing cultures, but, instead of resorting to this tedious procedure or to the use of a continuous-feeding device, it seemed desirable to search for alternative carbohydrates, among which xylose was finally chosen.

Though admitting that, as had been recently re-asserted by Girard (1955), generally speaking the optimal incubation temperature for *P. pestis* ranged from 26°C to 30°C and increased nutritional requirements were observed in the case of cultivation at 37°C, nevertheless Higuchi & Carlin pointed out with much reason that under certain circumstances growth of the organism at the latter temperature was essential and that, moreover, as the two authors put it, " the *in vivo* state of physiology of the

[1] It is interesting to note that recently Won & Lieu (1958) recommended for the sake of economy and easy availability the use of a fish hydrolysate medium in place of the casein hydrolysate media for the cultivation of *P. pestis*.

[2] As noted in the conclusions to their article, Gubarev and co-workers found that, besides these two bacterial vitamins, haemin and pantothenic acid also failed to stimulate the growth of *P. pestis*.

organism in a host animal probably is more clearly approached by culturing at 37°C rather than at 27°C ".

Attempting, therefore, the cultivation of *P. pestis* at a temperature of 37°C, the two workers found that good yields could be obtained by increasing the amount of magnesium sulfate used, substituting cysteine hydrochloride for sodium thiosulfate and reinforcing the media by addition of glycine, calcium pantothenate, thiamine hydrochloride and biotin.

Pigment formation

As established by Jackson & Burrows (1956a), many plague strains, when grown at 28°C on the above-described haemin-containing medium, formed dark-brown colonies, this pigmentation resulting from the absorption of haemin from the substrate.[1] After prolonged incubation, the pigmented colonies produced unpigmented secondary colonies, which in their turn yielded only non-pigmented growths on subculture.

Making a survey of 20 stock strains of *P. pestis* which had been kept for two years or more as dried cultures and had been subsequently freeze-dried, the two observers found that 16 of these growths consisted of mixtures of pigmented (P) and non-pigmented (NP) types, while two virulent strains yielded solely pigmented colonies and two avirulent strains (including the EV strain) only unpigmented colonies. On subculture the pigmented colonies gave predominantly the P type, with some non-pigmented mutants appearing as secondaries. In marked contrast to this, the subcultures from non-pigmented colonies invariably bred true.

Jackson & Burrows postulated that in nature the plague bacillus was probably of the P type, non-pigmented mutants accumulating during storage *in vitro*. Dealing with the relation of this phenomenon to the virulence of the organisms, they stated that:

" While loss of ability to pigment leads to reduction in virulence, possession of the ability obviously does not, by itself, confer high virulence. Ability to pigment appears to be one of a number of properties collectively determining high virulence of *P. pestis*. Further, it is evident that ability to pigment is not connected with ability to resist phagocytosis, which sharply differentiates virulent from the majority of protective avirulent strains . . ."

Further interesting observations in point by Jackson & Burrows (1956b) will be discussed later in this report, when attention is given to the problems of immunology.

Mutation

In addition to the above findings by Burrows & Bacon (1954a), Jackson & Burrows (1956a) and Englesberg & Ingraham (1957), observations on the mutation of *P. pestis* falling within the scope of the present disquisition have also been recorded by Elfimova & Chachina (1956) and by Anisimov (1958).[2]

The 11 virulent plague strains which Elfimova & Chachina used for their studies all acidified glycerol, while two also produced acidification of rhamnose within six days. Ten of the strains were daily subcultivated on freshly made agar slants containing plenty of condensation water, while the 11th was subcultured every 3-4 days. The incubation temperature was 37°C, the pH of the media apparently 6.9.

While the changes produced through these frequent passages in regard to the biochemical and immunological properties of the organisms will be discussed in due course, it deserves attention at the present juncture that:

(*a*) Deviations from the normal type of colonies, studied with the aid of subcultures on agar plates, were observed from the 94th generation onwards (no details given), while the single organisms tended to assume a coccus-like appearance after repeated subcultivation.

(*b*) From the 29th generation onwards disappearance of the capsules was noted, but the characteristic slimy growth shown by the capsulated forms continued to be apparent. Subcultivation on Martin agar to which 10% normal horse serum had been added led to a reappearance of the capsules only in some of the strains; and even these lost the faculty of capsule formation on serum agar upon further subcultivation, while retaining the slimy character of growth.

In order to study changes in the cytology of the plague bacillus, Anisimov made subcultures of the organism on agar plates which were incubated at 28°C and every hour cut out small squares of the agar with a sterile knife, mounted them on slides

[1] It is important to note that, according to Jackson & Burrows, the mechanism responsible for the absorption of haemin was not specific for this substance, since the plague strains showing the above-described brown colonies also absorbed basic dyes from liquid media more intensively than did non-pigment-forming strains.

[2] A further paper, by Ivanov and associates (1956), on the production of new forms in the vaccine strain of the plague bacillus under the influence of radioactive irradiation was not available to the present writer.

and, after adjustment of cover-glasses, examined them under the phase microscope. At the same time impression films were made from adjacent agar squares which, after drying and fixation, were subjected to staining methods appropriate to show the presence of nuclei.

To produce giant forms of *P. pestis*, cultivations were made on media containing lithium chloride, magnesium sulfate, glycocoll, penicillin or streptomycin.

Anisimov concluded from the observations made with the aid of these methods that:

" In contrast to some authors, who consider the nucleated giant forms as involution forms, we consider that the viability of these forms and the possibility of their further development by partition has been clearly demonstrated. The described type of multiplication gives reason to assume that the formation of giant forms is a peculiar adaptative process, directed towards preservation of the species, ensuring the rapid accumulation of a microbial mass of cells which are resistant to new conditions of life." [Trans.]

Dissociation

Akira Vake (1957) reported that he had been able to isolate from a culture of the EV strain, which had been stored for a long time at 4°C, a pure growth of smooth plague colonies. The organisms thus obtained continued to appear in the smooth form when, after preliminary mouse passage, they were seven times serially subcultivated on blood agar.

If the S strain was subjected to mouse passages, again R colonies could be cultivated besides colonies of the smooth type. The R type thus obtained proved to be stable when subjected to 8-9 serial subcultivations.

In an article on the colonial morphology and virulence of *P. pestis*, to which further reference will be made in the course of the present report, Eisler and colleagues (1958) stated that:

" Of 31 strains of *Pasteurella pestis* cultured upon thick blood agar base plates for 6 to 8 days, 19 revealed the presence of smooth and nonsmooth colonies. The remainder contained smooth colonies only. The two types were also derived from material aspirated directly from a human plague bubo.

" The smooth forms for the most part retained their characteristic morphology throughout subculture. The nonsmooth strains varied in homogeneity under such conditions. On the other hand pure cultures of both forms were maintained on agar plates inoculated with splenic material from mice infected with either smooth or nonsmooth strain Poona...

" Both morphological types of *P. pestis* have been isolated from parent strains collected from widely scattered places and hosts, indicating world wide distribution of strains composed of both smooth and nonsmooth variants."

Interesting as the observations of Eisler and his colleagues are, it seems questionable whether under natural conditions the plague strains are composed of both rough and smooth elements. Even the findings in a human plague bubo cannot be considered decisive in this respect in view of the untoward conditions to which the organisms temporarily or permanently held back in the regional lymph-nodes are apt to be subjected.

BIOCHEMICAL PROPERTIES

Metabolic processes

Working with the avirulent Tjiwidej strain, Santer & Ajl (1954) found that resting cells of *P. pestis* exhibited a high endogenous metabolism. However, using cells which had been rendered radioactive, the two observers could show that this endogenous process did not interfere with the oxidation of exogenous substrates. It could be further established that:

(*a*) Whole non-proliferating cells oxidized all members of the tricarboxylic acid cycle, the attacked fraction of the acids becoming converted into carbon dioxide and water.

(*b*) Acetate oxidation proceeded *via* the tricarboxylic acid cycle.

(*c*) The organisms contained isocitric dehydrogenase and aconitase which, when combined, converted citrate to α-ketoglutarate, this process proving to be reversible on addition of carbon dioxide.

As may be conveniently added, Santer & Ajl (1955a, 1955b), dealing in two subsequent papers especially with the processes of glucose fermentation by *P. pestis*, adduced evidence to show that resting cells of the organism fermented this sugar almost exclusively according to the Embden-Meyerhof series of reactions (i.e., according to the usual scheme of glycolysis), without showing the phenomenon of an operative hexose monophosphate shunt. Since, however, the component enzymes of the shunt pathway were found to be present, it appeared likely that a shunt took place during the period of the most active proliferation of the plague bacilli in order to provide pentose phosphates for synthetic reactions.

Englesberg, Gibor & Levy (1954), studying the problems of terminal respiration of *P. pestis*, noted the presence of a profound physiological difference between " aerobic " cells of the organism (i.e., those obtained through cultivation in an aerated casein-hydrolysate/mineral/glucose medium) and " anaerobic " cells produced through growth in flasks filled almost to the top with the same medium and sealed with a pyrogallol-NaOH plug, or by removing the oxygen from cultures grown in large Pyrex bottles by sparging the casein hydrolysate medium with oxygen-free nitrogen. Thus it was found that the aerobic cells rapidly effected an essentially complete oxidation of glucose, while the anaerobically grown organisms, though capable of a limited oxidation of glucose, caused an accumulation of large amounts of pyruvate and other organic end-products, which they could not oxidize further or oxidized at a very low rate.

The anaerobic cells could be converted to the physiological pattern of the aerobically grown organisms by aeration for several hours, but this conversion was blocked by ultraviolet irradiation.

Investigating the oxidative metabolism of *P. pestis* in a study to which further reference will be made below, Srikantan and colleagues (1957a) were unable to find any general correlation between the virulence and the oxidative metabolism of the strains. Older cultures showed a significantly lower oxygen consumption with glucose, ribose and serine.

Commenting on the detailed results of their investigations, Srikantan and co-workers stated that among the five amino-acids used for their tests, glycine and cysteine, which were the only ones to be deaminated appreciably by *P. pestis* (Sagar et al., 1956a), were also capable of being oxidatively metabolized by this organism. Threonine, structurally similar to serine, also showed a rapid rate of oxidation.

Of the three carbohydrates tested, glucose and galactose were more effectively metabolized than ribose, the oxidation of which was as a rule comparatively slow. Similar observations had been made already by Santer & Ajl (1955a).

Further interesting observations by Srikantan and his associates were that:

(*a*) Chlortetracycline, oxytetracycline and tetracycline considerably inhibited the oxidative metabolism of the plague bacilli in tests made with glucose and serine.

(*b*) Neomycin, streptomycin and chloramphenicol, while slightly activating the oxidation of glucose, exerted only a very minor inhibiting action on the oxidation of serine. Sulfathiazole and sulfadiazine were without any action.

Enzyme activity

Making comparative tests with aerobically and anaerobically grown plague bacilli derived from an avirulent strain, Englesberg, Levy & Gibor (1954) noted deficiencies in isocitric dehydrogenase, aconitase and fumarase in the case of the anaerobic growths, thus providing evidence that the tricarboxylic acid cycle did not operate under anaerobic conditions. Aeration in the presence of a casein-hydrolysate/glucose medium converted the anaerobic cells to the physiological pattern of the aerobically grown organisms.

Further investigations by Englesberg & Levy (1955) with the above-mentioned avirulent strain showed that the change from anaerobiosis to aerobiosis resulted also in the specific induced synthesis of phosphotransacetylase, the condensing enzyme, of malic dehydrogenase and of the enzymes involved in the oxidative decarboxylation of pyruvate and α-ketoglutarate. No difference was found between aerobically and anaerobically grown plague bacilli as far as the physiological pattern of glucose fermentation was concerned.

Slein (1955) recorded that he had been able to demonstrate in cell-free extracts of an avirulent plague strain, obtained after growth in the presence of *d*-xylose, an enzyme which catalysed the isomerization of *d*-xylose and *d*-xylulose. He also found that crude extracts of the organisms grown on *d*-xylose contained a xylulokinase, capable of phosphorylating xylulose in the presence of adenosine triphosphate.

A welcome addition to the rather scanty knowledge on the catalase activity of *P. pestis* was made by Zaplatina & Borodina (1956), who examined for this purpose 25 virulent and 6 avirulent strains as well as 6 pseudotuberculosis strains.

The method of examination used by the two workers consisted in the addition of hydrogen peroxide to suspensions of the bacteria under test and titration of the amount of this compound not decomposed by catalase activity with the aid of a decinormal solution of potassium permanganate, 1 ml of which corresponds to 1.7 mg of H_2O_2; hence the difference between the amounts of potassium permanganate used for the titration of the cultures and of control solutions of H_2O_2 multiplied by 1.7 gives the " catalase figure " in ml.

The mean values of the catalase figure were 2.23 in the case of the virulent plague strains, 3.27 in the

case of the avirulent strains, and 7.37 in that of the pseudotuberculosis strains. However, the catalase activity of each strain was found to vary under different extrinsic conditions.

Working with sets of 8 virulent and 8 avirulent plague strains and 20 amino-acids, Sagar and associates (1956a) found that:

(*a*) Washed cell suspensions of *P. pestis* could deaminate only serine, glycine, asparagine and cysteine to an appreciable extent.

(*b*) No general relationship existed between the virulence and the deamination processes of the organism.

The deaminase activity of plague bacilli grown in broth always exceeded that of the organisms which had been harvested from the corresponding agar cultures. This, the authors pointed out, was in agreement with previous observations, according to which *P. pestis*, if cultivated on solid media, was more exacting in its nutritional requirements, or in order to give good yields, had to be grown under reduced atmospheric pressure.

In contrast to these observations, Sagar and colleagues (1956b) found that plague bacilli grown on solid media had consistently more phosphatase than those cultivated in fluid media. Among the former media papain or tryptic casein digest agar proved most satisfactory for the production of the enzyme.

As pointed out in a subsequent study of the series at present under review (Saxena and co-workers, 1957), among the few amino-acids to be deaminated by *P. pestis*, cysteine alone had been considered by Rao (1939) essential for the growth of this organism. It thus appeared that the plague bacillus obtained the energy for growth in media containing amino-acids in some other way than through simple deamination. In particular, transamination was likely to serve as an energy source for the plague bacilli and it was for this reason that investigations were made to detect transaminase systems in them. Tests made with the aid of cell-free extracts of the organisms actually showed the presence of transaminases catalysing the conversion of α-keto-glutarate to glutamic acid with a number of amino-acids, among which phenylalanine alone was indispensable for the growth of *P. pestis*. As shown by comparative tests with sets of six strains each, there was no general correlation between virulence and transamination. Of the various chemicals tested, mercuric sulfate exerted the most inhibitory effect on the transaminase systems. Antibiotics and sulfonamides did not prove inhibitory.

Undertaking a systematic investigation of the dehydrogenations mediated by the plague bacillus, Srikantan and colleagues (1957b) found resting cells of the organism capable of dehydrogenating a large number of substrates in the presence of phenyltetrazonium chloride. Of the substances tested glucose showed the maximum activity, while glycerol showed no dehydrogenation. As the authors justly pointed out in the discussion of these findings, the last-mentioned observation was particularly noteworthy in view of the use of glycerol-containing media for the classification of *P. pestis*. Since the strains used for this investigation had all been obtained from the Haffkine Institute, Bombay, they were possibly all glycerol-negative, but this appears to have been definitely so in the case of only six of the strains (see list in the article by Sagar et al., 1956a). Certainly one must agree with Srikantan and his colleagues that this is a matter deserving further study.

Following up preliminary observations by Santer & Ajl (1955b), Srikantan and associates (1958) demonstrated aldolase activity in cell extracts of 8 virulent and 8 avirulent plague strains, but were unable to observe a correlation between this enzyme activity and the presence or absence of virulence. Some antibiotics were found to inhibit the action of this enzyme, but chlortetracycline as well as some other substances, particularly cysteine, exerted an activating influence.

Bacteriocin production

As recently described by Ben-Gurion & Hertman (1958), all but one of the 24 *P. pestis* strains examined by them were capable of producing a bacteriocin-like material, called " pesticin " by them, which inhibited the growth of *P. pseudotuberculosis*. The formation of this material could be induced by ultraviolet irradiation.

Action on carbohydrates

Observations on the reactions produced by plague and by pseudotuberculosis bacilli in media containing carbohydrates have been recorded recently by Devignat (1954) for 25 plague and 5 pseudotuberculosis strains, by Knapp & Masshoff (1954) for 3 pseudotuberculosis strains and by Klimova (1956) for 41 pseudotuberculosis strains. The findings of these workers have been embodied in Table 1, in which also the aggregate results of previous observations, as summarized by Pollitzer

TABLE 1

REACTIONS PRODUCED BY PLAGUE AND BY PSEUDOTUBERCULOSIS BACILLI IN MEDIA CONTAINING CARBOHYDRATES, ACCORDING TO DEVIGNAT (D.), POLLITZER (P.), KNAPP & MASSHOFF (K. & M.) AND KLIMOVA (KL.)

Carbohydrates tested	Plague bacilli according to:		Pseudotuberculosis bacilli according to:			
	D.	P.	D.	K. & M.	Kl.	P.
Adonitol	—	±	—	.	.	+ [a]
Amygdalin	—	—	—	—	.	±
Arabinose	+ [a]	+ [a]	+ [a]	+	+	+
Cellobiose	—	.	—	.	.	.
Dextrin	+	±	+	.	.	±
Dulcitol	—	±	—	—	—	— [a]
Erythritol	—	—	—	—	.	—
Galactose	+	±	+	+	+	+
Glucose	+	+	+	+	+	+
Glycerol	±	±	+	.	+	+
Glycogen	.	+ [a]	.	.	.	— [b]
Inositol	—	—	—	—	.	—
Inulin	+ [c]	— [a]	+ [c]	.	—	— [a]
Lactose	— [b]	— [b]	—	—	—	—
Laevulose	.	+	.	+	.	+
Maltose	+	±	+	+	+	+
Mannitol	+	+ [a]	+	+	+	+
Mannose	+	+	+	.	.	+
Melezitose	—	.	—	.	.	.
Melibiose	— [b]	—	+	.	.	+
Raffinose	— [a]	—	— [a]	—	±	±
Rhamnose	— [a, b]	— [b]	+	+	+	+
Saccharose	—	— [b]	—	—	—	±
Salicin	+	±	+	—	.	+ [a]
Sorbitol	— [a]	— [a]	—	—	.	±
Starch	+	±	+ [a]	.	.	±
Trehalose	+	±	+	.	+	+
Xylose	+	±	+	+	+	+

[a] Some exceptions noted.
[b] Late acidification in some instances.
[c] Positive only if phenol red was used in place of Andrade's indicator.

(1954), are shown. As will be noted, the latter, much more ample data do not invariably tally with the results of the above-mentioned recent observations, thus illustrating the deplorable fact that there still exist more or less marked discrepancies in the statements available in regard to the reactions produced by the pasteurellae in a considerable number of carbohydrate-containing media.

It has to be added that de Issaly & Issaly (1954), considering with much reason that tests with salicin or amygdalin were not fully reliable for a differentiation of the pasteurellae, recommended in their place arbutin-containing media for such diagnostic work. They recorded that in such media (*a*) acidification without gas formation was produced by all their 11 plague strains and by 10 of the 11 pseudotuberculosis strains tested, whereas (*b*) all 8 strains of *P. aviseptica* as well as 37 of the 40 pasteurellae cultures of mammalian origin examined gave negative results. Thus tests with arbutin-containing media were of considerable, but not of absolute, differential-diagnostic value.

The problem of the reactions produced by plague and pseudotuberculosis bacilli in rhamnose-containing media has continued to attract the attention of recent observers. An important study of this subject was made by Brygoo & Courdurier (1955b), who tested for this purpose 105 glycerol-negative strains isolated at Madagascar and kept in stock for periods ranging from 3 months to 29 years in a fluid medium containing 0.5% Bacto tryptose, 1% proteose peptone, 0.5% casamino-acid, 0.5% sodium chloride and 1% rhamnose, with phenol red as indicator. As shown by tests with this medium, the 105 plague strains of Brygoo & Courdurier fell into the following groups:

(1) 2 strains of human origin invariably acidified rhamnose media, one 2-3 days after inoculation (i.e., as rapidly as the pseudotuberculosis strains used as controls), the other after 6-10 days.[1]

(2) 51 strains, tested 2-4 times (35 only twice), gave consistently negative results.

(3) The remaining 52 strains gave irregularly positive results, acidifying the rhamnose media in the course of various tests at least once.

Since, moreover, it was possible to isolate from many of the strains rhamnose-acidifying variants, the two workers felt entitled to postulate that " the

[1] Another strain producing late rhamnose acidification (after 7 days) has been recently detected among about 50 strains examined during a 1956 plague outbreak in Indochina (Nguyen-Van-Ai, 1957).

plague bacillus, in an almost constant manner, is capable under certain conditions of using rhamnose or at least of giving rise to variants capable of utilizing this substance ".

In contrast to the statements of previous observers, Brygoo & Courdurier (1955b) stressed that bacteriophage action was not indispensable for the production of rhamnose-acidifying variants and that the latter could be derived from glycerol-acidifying plague strains.

The problem of the appearance of rhamnose-positive mutants in plague cultures was also studied by Englesberg (1955, 1957a) who, according to his 1957 publication, used a total of 8 avirulent and 8 virulent strains, no less than 14 of which were glycerol-negative, growing them on a modified Endo medium. If subcultivated on plates of this medium, the plague strains under test required 48-72 hours for growth, the initially appearing colonies manifesting no evidence of rhamnose fermentation. However, over a period of time varying from 6 to 20 days, dark-red colonies, corresponding to rhamnose-utilizing (R+) mutants, could be noted at an average of 1-2 per plate in the case of all the strains tested. Once formed, these mutants were found to breed true.[1]

Evaluating these findings, Englesberg stressed that mutation to rhamnose utilization was not associated with other changes indicating a conversion of the organisms in question into pseudotuberculosis bacilli and that on account of the early manifestation of rhamnose acidification in case of the latter, as contrasted with the tardy appearance of rhamnose-positive mutants of plague strains, the ability to acidify rhamnose remained " a reliable criterion for distinguishing *Pasteurella pestis* from *Pasteurella pseudotuberculosis* ".

As noted above (see page 320), Elfimova & Chachina (1956) reported that two of the 11 virulent strains used by them to study the mutability of *P. pestis* acidified rhamnose. These two workers further stated that (*a*) when subjected to serial subcultivation, three other strains acquired the property of rhamnose acidification, and (*b*) when grown in the medium of Bessonova (1% rhamnose in 0.5% peptone water, pH 7.2, with litmus as indicator), five of these strains produced an acid reaction after incubation for 4-9 days.

Subjecting the publications of Brygoo & Courdurier and of Englesberg to a critical review, Girard & Chevalier (1956) maintained, in agreement with the latter observer, that the practical value of rhamnose tests for a differentiation of plague and pseudotuberculosis bacilli remained uncontested. In support of this contention, Girard & Chevalier recorded that out of 77 plague strains of their collection, tested in the usual manner by cultivation in peptone water containing Andrade's indicator, none had shown evidence of rapid rhamnose acidification, 8 giving a positive result after 4-9 days, 9 after 10-20 days, and 5 between 20 and 30 days.

Girard & Chevalier inclined to the belief that a time interval between isolation and testing as well as repeated cultivation favoured the appearance of rhamnose-positive mutants, which, they maintained, had thus far never been detected in freshly isolated growths. It deserves great attention that recent findings by Kovaleva (1958) were not in accord with this contention.

Working in a district of Mongolia where an epizootic involving mainly *Microtus brandti* was present, Kovaleva was able to isolate 33 plague strains, only four of which, including the only human strain encountered, showed the typical behaviour of *P. pestis*. The other 29 strains, derived with few exceptions from *M. brandti*, showed, besides evidence of the presence of bacteriophage, an atypical colonial morphology and ability to acidify rhamnose [2] after incubation for 1-2 days at 28°C—properties which were evidently manifest as soon as the strains in question had been isolated.

Subsequent examinations showed that the atypical strains also resembled *P. pseudotuberculosis* by exhibiting a comparatively low virulence for guinea-pigs. However, Kovaleva emphasized, her atypical strains differed from the latter organism on account of their immotility, absence of urease activity and pathogenicity for white rats.

In view of these observations it would seem unwise to place sole trust in tests with rhamnose-containing media for a differentiation of plague and pseudotuberculosis bacilli. Results obtained with such media must be evaluated side by side with those obtained with the aid of other methods.

Studies on the physiological basis for rhamnose utilization made by Englesberg (1957b, 1957c) with his rhamnose-positive mutants showed that:

[1] However, as pointed out with good reason by Girard & Chevalier (1956), it would be well to subject the mutants to animal passage before coming to final conclusions regarding their irreversible character.

[2] As was afterwards found, the 29 strains failed to acidify arabinose. They, as well as the four typical strains, were glycerol-positive.

(*a*) Growth in the presence of rhamnose led to a gain on the part of the mutants of the potentiality for oxidizing rhamnose, but the oxidation of this compound by the R+ mutants was incomplete and small amounts of lactic aldehyde could be isolated.

(*b*) Two new enzymes were present in rhamnose-utilizing mutants of *P. pestis* grown in the presence of this carbohydrate, of which one, rhamnose isomerase, converted rhamnose into rhamnulose, while the other, rhamnulokinase, phosphorylated rhamnulose. Neither enzyme could be detected if the mutants were grown in the absence of rhamnose, or in the R-negative cells of the parent culture, regardless of whether the latter had been grown in the presence or the absence of rhamnose or rhamnulose.

Classification of plague strains

As summarized by Pollitzer (1954), in addition to the long-accepted distinction between glycerol-acidifying and glycerol-negative races of *P. pestis*, Devignat (1949, 1951), evaluating also the presence or absence of the ability of the strains to reduce nitrates to nitrites, or to produce nitrous acid in ordinary broth, had suggested a classification of the organisms into three varieties as follows:

Proposed sub-groups of P. pestis	*Tests with glycerol*	*Reduction of nitrates (Nitrous acid production)*
var. *orientalis*	−	+
var. *antiqua*	+	+
var. *mediaevalis*	+	−

The merits of these classifications have been assessed in a whole series of recent publications, among which attention has to be paid first to three important articles by Devignat (1953a, 1953b, 1954). Summarizing in the second of these publications the statements made in his first elaborate memoir, Devignat furnished a table showing the geographical distribution of the varieties of *P. pestis* (see Table 2).

TABLE 2

GEOGRAPHICAL DISTRIBUTION OF VARIETIES OF *P. PESTIS* *

Countries	Glycerol fermentation		Production of NO_2 ions (in countries italicized)	
	Proportion	Percentage	Proportion	Percentage
1st category: *Pestis orientalis*				
Arabia, Argentina, Brazil, Ceylon, *Egypt*, England, Formosa, France, Germany, Greece, *Hawaii*, *India*, Indochina, *Italy*, *Java*, *Madagascar*, *Morocco*, Palestine, Senegal, Sumatra, Thailand, *United States of America*	Negative		Positive	
	482/492	98.0	108/117	92.5
2nd category: *Pestis antiqua*				
Belgian Congo,[a] *Manchuria*, Mongolia, *Transbaikalia*, Turkestan, Uganda	Positive		Positive	
	175/176	99.0	107/109	98.0
3rd category: *Pestis mediaevalis*				
Iranian Kurdistan, *South-east Russia* [b]	Positive		Negative	
	240/240	100.0	213/215	99.0
4th category: Mixed	Negative		Positive	
	108/148	73.0	4/4	100.0
Japan, *Kenya*, Philippines, *Union of South Africa*	Positive		Positive	
	40/148	27.0	22/22	100.0

* After Devignat (1953b).

[a] As recorded by Jesierski et al. (1954), an avirulent plague strain showing the properties of the oriental variety had been isolated from the nasal mucus of a horse in an entirely plague-free area of the Belgian Congo. Subjecting this strain to a painstaking re-examination, Girard (1957b) found it in every respect identical with the EV strain, which was used for plague vaccination in the Belgian Congo.

[b] As noted by Özsan & Akyay (1954), a plague strain isolated during the 1947 outbreak at Akçakale near the Syrian border in Turkey, which was presumably traceable to a wild rodent focus extending from Iranian Kurdestan, was also of the mediaeval type.

Dealing with the fourth category of strains, Devignat pointed out that in the countries concerned glycerol-acidifying and glycerol-negative strains had never been found to be present simultaneously in a haphazard manner, but had been separately isolated either during different epidemics, as in Japan, or in different foci, as in South Africa and Kenya, where de Smidt (1928) had found only glycerol-negative strains at Nairobi, whereas, as confirmed by Heisch and co-workers (1953), the *antiqua* variety alone was met with during a plague outbreak of presumably wild rodent origin in the interior of the country.[1]

As pointed out with much reason by Devignat: "the *antiqua* variety, which is the most likely to be ancestral owing to its probable origin in Central Asia, also possesses the most complete enzyme equipment, since it ferments glycerol, reduces nitrates to nitrites and produces nitrous acid at the expense of certain proteins. Travelling through the world since biblical times to penetrate to the heart of Central Africa and even to reach South Africa, *Pasteurella pestis* var. *antiqua* encountered new hosts, different from its original hosts but favourable to the perpetuation of the normal genotype. However, during its century-old contact with the *Meriones* encountered near the Caspian Sea, the *antiqua* variety could have lost the enzyme or enzymes responsible for the reduction of nitrates and the production of nitrous acid, thus being transformed into a new genotype: *Pasteurella pestis* var. *mediaevalis*. On the other hand, through long adaptation to *Rattus rattus* . . . in the Far East, the *antiqua* variety might have lost the faculty of acidifying glycerol, thus giving rise to yet another genotype: *Pasteurella pestis* var. *orientalis*." [Trans.]

In his 1954 contribution, Devignat reported in a detailed manner on comparisons he had made, in technical co-operation with Boivin, of the biological and biochemical characteristics of the three varieties of *P. pestis* and of *P. pseudotuberculosis*. Though admitting that his results were not conclusive, Devignat drew attention to the tendency of his mediaeval strains to produce lung involvement in white mice and also in white rats, adding that, if confirmed, these observations would throw light on the epidemiology of the plague manifestations at the time of the Black Death (believed by him to have been caused by *P. pestis* var. *mediaevalis*) as well as those in South-east Russia and Kurdistan. However, considering that by far the most violent pneumonic outbreaks of modern times, taking place in Manchuria and North China, have been caused by the *antiqua* variety and that pneumonic plague manifestations are frequent in Madagascar, where organisms of the oriental type are the source of infection, one cannot share Devignat's belief in the significance of the experiences he gained with a few strains of the mediaeval type of *P. pestis* in laboratory animals. Moreover, in view of the evidence adduced by Pollitzer (1954) it is probable that the Black Death originated in the ancestral home of the infection in Central Asia.

Working with the technical assistance of Chevalier, Girard (1954a) experimented with two strains of *P. pestis* var. *mediaevalis* from Iranian Kurdistan, which he subjected to 50 direct passages through white rats by using the material from the bubo, spleen and blood of one animal for subcutaneous infection of the next. The biochemical properties of the two strains were checked with the aid of cultures made from the heart blood of the rats after every 10th passage. It could be established in this manner that the ability of the strains to acidify glycerol as well as their inability to produce nitrous acid in broth or peptone water remained unchanged. It thus appeared that the biochemical properties essential for the classification of *P. pestis* were of a stable character, not becoming modified either in the laboratory [2] or through passage from one host species to another in nature. Hence in the case of areas like Kenya, where two distinct varieties of *P. pestis* had been found, assumption of the existence of two independent reservoirs of the infection was indicated.

Again dealing with the problem at present under review, Girard (1954b) stressed that the classification of *P. pestis* into varieties, though of no clinical importance, was of great epidemiological interest. If in a given area the oriental (glycerol-negative) type was found either in rats or in wild rodents, or in both, it could be safely assumed that the rats had been the source of the infection, which was subsequently passed to the wild rodents, whereas the presence of one or the other of the glycerol-acidifying varieties indicated that the infection was traceable to a pre-existing wild rodent focus.

Supplementing the above observations, Girard (1954c) stated that the live avirulent EV plague vaccine had been found capable of protecting mice and guinea-pigs against infection with a highly

[1] A strain which Heisch and his colleagues had obtained from Tanganyika was also of the *antiqua* type.

[2] It deserves attention in this connexion that Elfimova & Chachina (1956) recorded one instance in which a serially subcultivated plague strain acquired the property of late glycerol acidification (after 22 days).

virulent *mediaevalis* strain. Since identical findings had been made previously in the case of the two other varieties of *P. pestis*, it thus appeared that the EV vaccine conferred protection against any type of plague infection.

Polemizing against Devignat, Tumansky (1957) upheld that

" the cause of the varieties of the plague bacillus must be looked for not in geographical factors and the history of the plague pandemics but in the physiological peculiarities of the different rodent species. Therefore we propose to designate the varieties of the plague bacillus according to the rodent species from which they were first isolated and in which they are observed in some localities up to the present time." [Trans.]

Accordingly, Tumansky proposed the following classification:

Varieties of P. pestis	*Glycerol acidification*	*Production of nitrous acid*	*Reduction of nitrates*
var. *ratti*	−	+	+
var. *marmotae*	+	+	+
var. *citelli*	+	−	−

In Tumansky's opinion it was difficult to decide with what rodent species the plague bacillus had first entered into a host relationship. However, taking into account that in hibernating species the organisms are apt to find more favourable conditions for persistence (such as high and stable levels of the rodent herds and a peculiar course of the infection during hibernation), one was entitled to assume that the plague bacillus first became entrenched in hibernating species and only afterwards also in non-hibernating rodents, changing at the time of this transition some of its original properties.

Since, as maintained by Tumansky, (*a*) phylogenetically the plague bacillus was preceded by the pseudotuberculosis bacillus, and (*b*) in some respects the var. *citelli* stood nearer to the latter organism than the other two varieties of *P. pestis*, there was reason to claim that the first-mentioned variety (var. *citelli*) was the oldest. However, he admitted that this was still an unsettled problem.

In Tumansky's opinion the study of the varieties of *P. pestis* is not merely of theoretical interest but of great practical importance " because it elucidates the knowledge of the focality of plague and the liquidation of the natural foci of the infection, and also permits the manufacture of more effective plague vaccines ". He maintained in the latter connexion that, according to observations in the Soviet Union, vaccines made from two varieties of the plague bacillus were more efficacious than those manufactured with only one type of the organism.

Hesitant though one must be to advocate changes in nomenclature, there can be no doubt that it would be more to the point to use the designations " var. *ratti* " and " var. *marmotae* " in place of the terms " var. *orientalis* " and " var. *antiqua* ". However, the same does not hold good of the proposal to designate the third variety of plague bacillus " var. *citelli* ".[1] To accept this name would be rather misleading because the still-continuing epizootics among the *Citellus* species of North America are caused by the var. *orientalis* (*ratti*). At the same time, however, one must state that Devignat's name of var. *mediaevalis* is also not at all felicitous. Since this variety of the plague bacillus is responsible for the infection of the wild rodents in Iranian Kurdistan, an area probably forming part of a much more extended focus of long standing, it might be justified to adopt the name " var. *merionis* " in place of var. *mediaevalis* or var. *citelli*. However, neither this name nor the terms " var. *marmotae* " and " var. *ratti* " should be used before the consent of the leading plague experts for these changes in nomenclature has been obtained.

VITAL RESISTANCE

Making laboratory studies on the action of methyl bromide on *P. pestis*, Gizatullina (1956) found that, if used in a proportion of 150 ml per cubic metre of air, this fumigant exerted a lethal action on the organisms in agar cultures as well as in sputum or blood within 24 hours. Since methyl bromide caused no harm to foodstuffs or utensils and seemed a little-dangerous poison, Gizatullina considered it suitable for the disinfection of plague-infected premises.[2]

Studies on the action of antibiotics on plague cultures have been made with a limited number of strains by Devignat (1954) and Nguyen-Van-Ai

[1] Devignat, in an unpublished working document (WHO/Plague/43) presented at the meeting of the WHO Expert Committee on Plague in September 1958, also objected to the use of this term (as well as to that of var. *marmotae*).

[2] However, if this advice is taken, one must keep in mind that, though not necessarily harmful in even relatively high concentrations, methyl bromide is apt to exert a delayed toxic action on man. Workers handling this chemical must, therefore, wear suitable masks, the more so because the sweetish odour of the gas is not pronounced (Pollitzer, 1954).

(1957), and on a larger scale by Brygoo & Courdurier (1955a).

Testing 24 strains, Devignat found the organisms markedly sensitive to chloramphenicol and moderately sensitive to chlortetracycline, oxytetracycline and streptomycin. Penicillin exerted a slight action on the *orientalis* and *antiqua* strains, but none on the var. *mediaevalis*.

Working with 94 stock strains and 7 recently isolated cultures from Madagascar and with 6 strains from other parts of the world, Brygoo & Courdurier found that the antibiotics most active *in vitro* were chloramphenicol, chlortetracycline, oxytetracycline, streptomycin and neomycin.

Analogous observations by Nguyen-Van-Ai (1957) with 14 plague cultures isolated in Indochina showed a sensitivity of all the strains to streptomycin and chloramphenicol as well as a variable, but generally limited, sensitivity to Framycetin, erythromycin, chlortetracycline, oxytetracycline and neomycin (to the last-mentioned antibiotic, however, two of the strains were quite sensitive).

The bacteriostatic action of dyes on the plague bacillus and some other organisms was studied by Brygoo & Daod Nathoo (1956). Among the 47 substances tested, promising results were obtained with dahlia violet, magenta fuchsin, hexamethylene violet and especially with methylene green.

According to recently published observations by Girard (1958a), certain streptococci, specially *Str. sanguis* and *Str. mitis*, have an inhibitory action on *P. pestis* and other pasteurellae. These experimental results appear to confirm the influence exerted by pyogenic bacteria on the progressive disappearance of the causative organisms from the buboes commonly seen in human plague.

The results of an interesting study on the changes undergone by plague bacilli subjected to aerosol dissemination have recently been published by Berendt (1958). It was found that this process of dispersing the organisms (*a*) significantly decreased their virulence for intrapleurally infected guinea-pigs; (*b*) increased their oxidative metabolic rate in various substrates; and (*c*) induced marked changes in the antigenic properties of the organisms which became manifest when suspensions of aerosolized and non-aerosolized (control) bacilli were subjected to agglutination tests with sera raised against the corresponding types. Moreover, it was found that the plague bacilli which had been aerosolized multiplied more rapidly and grew to greater dimensions than the control organisms.

Evaluating the significance of these findings, Berendt postulated that in *P. pestis* populations there were different types of organisms: " some which multiply rapidly, grow to a large size, possess little or no infectivity for guinea-pigs and are resistant to aerosolization, and others which multiply more slowly, do not attain the dimensions of the above-mentioned cells, are more virulent, and are affected to a marked degree by aerosolization ".

From the practical point of view, the most important conclusions reached by Heckly, Anderson & Rockenmacher (1958), when studying the influence of lyophilization on *P. pestis*, were that:

" Survival of *Pasteurella pestis* during lyophilization was increased comparably by precooling the cultures or by adding an equal volume of buffered lactose before snap-freezing. Shell-freezing with or without additives at $-78°C$ resulted in significantly lower survival. Survival was the highest at about pH 7.6 . . . The volume of culture lyophilized per bottle was . . . not critical ; about 50 per cent of the cells survived in 60 ml bottles containing between 0.5 and 10 ml culture . . .

" There was no appreciable loss in vitality in glass-sealed vials during 3 years' storage at 4°C where there was a log loss per year at room temperature. Viability in rubber stoppered bottles at room temperature decreased by approximately 3 log per year."

It was further found that addition of glucose, sucrose or lactose increased survival during lyophilization, but that after about a month's storage at room temperature the viability of the glucose-containing cultures decreased as rapidly as that of controls without additives. After 7 months' storage the cultures to which sucrose or lactose had been added showed a markedly higher bacterial content than the glucose-containing cultures.

As Heckly and colleagues finally stated:

" The virulence of lyophilized cultures which had been stored in rubber stoppered bottles for 9 months was approximately 10^4 organisms per LD_{50} immediately after reconstitution but less than 10^2 organisms per LD_{50} 24 to 32 hr after reconstitution with distilled water."

IMMUNOLOGY

VIRULENCE

General studies

As stated in an important study on the virulence of *P. pestis* by Englesberg and associates (1954), considerations of the nature of plague infection " have suggested that at least two factors contribute to the virulence of this bacterium: (*a*) the envelope substance (fraction I) produced by this organism, which has been shown to protect it from phagocytosis and probably blocks the action of antibodies on the cell, by virtue of the freely soluble nature of this substance; and (*b*) toxin production, which no doubt is responsible for death from this disease ".

In view of the occurrence of some avirulent plague strains producing both envelope and toxin, it seemed possible that the factor determining virulence was the quantity in which these substances were present. To test the validity of this assumption, comparisons were made of 6 virulent and 9 avirulent strains grown for 72 hours at 37°C on hormone agar. The saline washings of these growths were precipitated with acetone at −70°C. After the precipitates had been dried, the dry samples were weighed and extracted with saline, the cells were thrown out by centrifugation, and precipitin tests, using anti-Fraction-I serum, and intravenous mouse toxicity tests were performed on the supernatant fluid.

As shown by the proportions of envelope antigen in the various samples, a positive correlation existed between virulence and quantity of envelope produced, " thus suggesting that envelope production must reach a given level before it is effective in combating the host defenses ".

Though it was found that on the average the virulent strains produced more toxin, a high toxicity alone was obviously not sufficient to render an organism virulent. Thus the EV strain and another avirulent strain (14) were found to produce more toxin than three of the virulent strains. That the strain EV 76 was able to multiply in intravenously inoculated mice, producing necroses in the liver and spleen before being eliminated and causing death when given in a large doses, while strain 14 neither multiplied nor produced pathological signs, was probably due to the lower envelope production on the part of the latter strain. Similarly, the EV strain was probably comparatively avirulent, because it produced less envelope than the virulent strains. That the avirulent strain A 1122, though producing more envelope than the EV strain, failed to produce pathological signs in mice was probably due to the lower toxicity of the former.

However, inasmuch as a further avirulent strain and one of the virulent strains showed a close similarity with respect to the quantities of envelope substance and toxin produced, it appeared that high levels of envelope and toxin production, though essential for virulence, were presumably not the only factors involved. On the contrary, Englesberg and co-workers maintained:

> " Since virulence is a measure of the ability of a bacterium to grow and cause disease in a particular host, all aspects of energy metabolism and assimilation, for example, which may conceivably have no direct bearing on toxin and envelope production *per se*, may be involved here. The apparent correlation between virulence in *P. pestis* and catalase production should be noted."

Burrows and his associates, dealing with the problem at present under review in a series of papers appearing during the period 1954-58,[1] came to conclusions markedly different from those referred to above. Summarizing their earlier findings in their 1958 article, Burrows & Bacon stated that, in order to be fully virulent for both mice and guinea-pigs, a plague strain must (*a*) be toxigenic; (*b*) be capable of elaborating envelope antigen (Fl+) and producing also the V and W antigens (VW+) determining virulence according to Burrows & Bacon (1956b); and (*c*) form densely pigmented colonies on defined media containing haemin (P+).

> " The loss of ability to produce Fl (i.e., Fl−) ", the two authors continued, " still permits a strain to show full virulence for mice but reduces its virulence for guinea-pigs . . . Strains lacking only the ability to produce V and W antigens . . . are avirulent for both species . . . The loss of ability to produce pigmented colonies (Fl+ VW+ P−) results in reduced virulence for mice . . . and great loss of virulence for guinea-pigs . . . The virulence of such strains for mice but not for guinea-pigs can be fully restored by the simultaneous injection of minute, non-toxic amounts of iron salts (Jackson & Burrows, 1956b)."

[1] See Burrows (1955, 1956, 1957); Burrows & Bacon (1954a, 1954b, 1956a, 1956b, 1958); Jackson & Burrows (1956b).

For a further study of the properties of variants showing different combinations of the characters determining virulence, Burrows & Bacon (1958) relied upon a series of purine-dependent mutants derived from a fully virulent plague strain. As the two authors explained, such mutants, because they were incapable of growth in mice and guinea-pigs on account of the non-availability of their specific nutrients, were particularly suitable for the work contemplated.

Discussing their results, Burrows & Bacon stated that:

"Our assessment of the comparative virulence of genetically related strains leaves no doubt that full virulence for mice and guinea-pigs is intimately associated with the character VW+. Of the 3 virulence characters investigated here, namely F1+, VW+ and P+, loss only of the VW+ character, leaving F1+ and P+ intact, results in complete loss of virulence for both the above species. Loss of P+ alone renders a strain avirulent for the guinea-pig and reduces virulence for mice; loss of F1+ alone does not affect virulence for mice but reduces virulence for the guinea-pig. Strains dependent on an exogenous supply of purines for growth are avirulent for both mice and guinea-pigs, regardless of their complement of the above virulence determinants, as a result of nutritional limitation *in vivo*. There are indications, however, that such strains may not be so strictly limited in the rabbit."

Relation of virulence to phagocytosis

Studying the comparative behaviour of virulent and avirulent plague strains *in vivo*, Burrows & Bacon (1954b) found that both kinds of organisms, if harvested after 18 hours' growth on tryptic meat-agar, showed at first an equal sensitivity to the polymorphonuclear defence mechanism of the mouse. However, while after a short period *in vivo* the virulent plague bacilli became capable of resisting phagocytosis, the avirulent organisms failed to do so.

Again referring to the problem at present under discussion, Burrows & Bacon (1956a) adduced evidence that a virulent plague strain could be differentiated from the avirulent protective Tjiwidej strain by *in vitro* phagocytosis tests: the virulent organisms, when incubated after growth at 28°C for 3 hours in tryptic digest meat-broth at 37°C, changed to a condition in which they were highly resistant to phagocytosis, while the avirulent plague bacilli, subjected to the same treatment, retained their sensitivity to phagocytosis.

In his 1955 study Burrows had already drawn attention to the fact that virulent plague bacilli were phagocytosis-resistant regardless of whether they had a visible capsule or were apparently non-capsulated, whereas avirulent organisms, even if capsulated, were incapable of resisting phagocytosis. Thus, Burrows tentatively concluded, it appeared that:

"virulent organisms have the ability, under defined conditions *in vitro* and under the conditions occurring *in vivo*, to elaborate a soluble surface layer from few to many molecules thick which, on making contact with the polymorph surface, fails to evoke the stimulus necessary to initiate processes leading to their ingestion. Avirulent organisms, under the same conditions, produce a surface component in many respects identical with that of the virulent organism but sufficiently different to provide the necessary stimulus. This conclusion is not proven but . . . receives considerable support from the observation that normal mouse polymorphs treated with plague antiserum, which in all probability modifies their surface properties, have enhanced ability to ingest resistant virulent organisms."

Once more dealing with the same subject, Burrows & Bacon (1956b) considered it apparent that two types of resistance to phagocytosis existed in the case of *P. pestis*—one being associated with the presence of a visible capsule, the other occurring in the absence of such. Organisms of the first type could be rendered sensitive to phagocytosis by treatment with immune sera containing antibodies to fraction I, whereas plague bacilli in which resistance to phagocytosis was not associated with the presence of a visible capsule were not rendered sensitive to phagocytosis by the action of fraction-I antibody, and antisera capable of sensitizing these organisms retained this property after absorption with fraction I. While thus unconnected with the action of this fraction, the resistance to phagocytosis shown by non-capsulated plague bacilli appeared to be due to the presence of two other antigens, named V and W by Burrows & Bacon, to which further reference will be made in a later section of the present review. The two workers noted in this connexion that, in contrast to the strain Tjiwidej S, the avirulent vaccine strain EV 76 had the potentiality for V and W production and the ability to develop resistance to phagocytosis.

Experimental observations

Turning attention to recent studies on the virulence of *P. pestis* made with the aid of animal experiments, reference has to be made first to the observations

made by Brygoo (1956b) when determining the virulence of 30 strains isolated in Madagascar during the period 1954-56, either directly or with the aid of mouse inoculation, and used in the third subculture on agar or in broth to infect batches of 3-5 mice with varying numbers of viable organisms. In the case of more than 60% of the strains less than 100 organisms sufficed to produce a mortality of 100% in the test animals. As was to be expected, intraperitoneally infected mice succumbed in general to smaller infective doses than those infected subcutaneously.

Experiments by Fukui and associates (1957a) showed a different behaviour of virulent and avirulent plague bacilli when introduced into the lungs of guinea-pigs by aerosol inhalation or intratracheal injection. In the case of the avirulent organisms most of the bacilli were eliminated within six hours and thereafter the numbers continued to decrease, but at a slower rate. When virulent organisms grown at 26°C were introduced in the same manner, an identical rapid reduction in viable count was noted, but after six hours, multiplication of the bacilli commenced and continued increasingly until death of the animals. However, the initial reduction in viable count was not observed if (*a*) organisms grown *in vivo* (i.e., suspensions of the organs of infected animals or of the chorio-allantoic fluid of infected fertile hen's eggs) were administered intratracheally or (*b*) virulent organisms cultivated at 37°C in heart infusion agar supplemented with glucose and sodium sulfite were used. The early reduction in viable count was associated with a greatly increased rate of phagocytosis by lung macrophages, whereas the rate of phagocytosis was low if no initial reduction in viable count took place.

Studying, the fate of plague bacilli injected intracardially into guinea-pigs, Janssen and colleagues (1958) found that—regardless of whether the organisms were resistant or susceptible to phagocytosis—initially most of them were rapidly removed from the blood. However, the resistant plague bacilli began to increase in the blood and organs of the test animals about ten hours earlier than the susceptible organisms. It thus appeared that "following suitable incubation in vivo virulent phagocytosis-resistant organisms. It thus appeared that "following suitable incubation in vivo virulent *Past. pestis* acquire the ability to resist phagocytosis by circulating leucocytes and, in addition, the ability either to survive and multiply following ingestion by reticulo-endothelial cells or to reproduce at a rate which exceeds the capacity of the RES to destroy them".

In order to study the influence exerted on virulent plague bacilli in the course of their existence in the body of animals naturally resistant to the infection, Semenova (1958) experimented with dogs, under the aponeurosis or into the peritoneal cavity of which plague-contaminated pieces of paper inserted into filter-paper tubes were introduced. After 1-2 weeks the tubes were removed and cultures were made, which were again used for insertions into the test animals in the manner described above. One of the two plague strains used was thus subjected to 10 passages, the other to 6 passages. Summarizing the results of these experiments, Semenova stated that:

"in the body of dogs, a naturally plague-resistant species, a gradual dying of the organisms took place. This was shown by the appearance of scanty growth from the test objects and also by the appearance, after the first passage, of avirulent variants. Basically, notwithstanding the repeated passages and the prolonged existence in the body of the test animals, the organisms did not show changes in their cultural properties. However, existence in the body of the animals exerted a considerable influence on the virulence of the organisms: the virulence became considerably lowered and proved to be less stable if agar cultures were stored for a long time [than was the case in the original strains]." [Trans.]

Finally, mention has to be made at the present juncture of a curious observation by Parry (1955, 1956), according to which small white rats, if injected intraperitoneally with buffered dilutions of broth cultures of *P. pestis* grown with constant shaking at 28°C, were killed less quickly by doses of 10^6 than by doses of 10^4 organisms. In Parry's opinion this interference phenomenon was probably due to a non-specific stimulation of the defence mechanism of the experimental animals.

Relation of cultural conditions to virulence

Working with several virulent strains of *P. pestis*, Fukui and colleagues (1957b) found that growth of the organisms in aerated liquid cultures at 37°C consistently resulted in a marked loss of virulence. Such a loss did not occur when the strains were similarly incubated at 26°C or when the organisms were grown on solid media at either 26°C or 37°C. The authors attributed the loss of virulence to the selection of avirulent variants present in the inoculum or arising during growth.

Continuing these investigations, Ogg and co-workers (1958) obtained additional evidence that aeration of broth cultures at 37°C favoured the growth of avirulent mutants of *P. pestis*. The conditions favouring the growth of such mutants could be reduced or eliminated by the supplementation of the culture medium with filtrates of avirulent cultures of *P. pestis* or other bacteria (*Escherichia coli*, *P. pseudotuberculosis*, *Shigella sonnei*). It was also noted that no loss of virulence took place when the initial pH of the medium was adjusted to 7.8.

That reduced oxygen tension might play a role in reducing the virulence of plague bacilli grown in broth at 37°C was suggested by the observation that growths started from small inocula in a medium which contained a reducing agent (sodium thioglycolate) showed no reduction in virulence as long as they were not agitated, thus remaining in a static condition. Finally, no selection of avirulent mutants was noted if virulent plague bacilli were grown in Cellophane bags inserted into the peritoneal cavity of guinea-pigs. It is interesting that avirulent plague bacilli thus grown *in vivo* bred true even after 14-15 generations.

Eisler and co-workers (1958), dealing with the subject at present under review in a contribution to which reference has been made before, stated that, as far as it was permissible to judge from observations restricted to stock strains of *P. pestis*, their findings suggested the following:

" no virulent parent stock is entirely composed of smooth constituents; virulent parent strains may contain virulent or avirulent smooth forms, but generally possess virulent nonsmooth components; no avirulent parent is entirely composed of nonsmooth elements; many avirulent strains are completely smooth; no stock parent strain, virulent or avirulent, is free of smooth forms. The data further suggest that avirulence in a strain originally composed of virulent smooth and nonsmooth constituents may first have become an attribute of the majority of the smooth elements, secondarily of the nonsmooth as well as of the smooth, and that persistence of virulence is greater in the nonsmooth even though this form may be the minority population in the composite parent strain. It appears that the wild population of *P. pestis* is a complex of the smooth and nonsmooth both of which may be virulent."

Influence of hormone administration

Payne and co-workers (1955) established that after a single injection of cortisone mice challenged with the EV 76 strain or another ordinarily innocuous avirulent plague strain succumbed to a generalized infection.[1] That this result was due to an invasion of *P. pestis* and not to a latent infection of another kind was indicated by the high degree of bacteraemia as well as by the protection afforded to the test animals by specific immunization. In the doses tested, cortisone did not exert a significant influence on the susceptibility of laboratory mice to the action of plague toxin.

Confirming these findings, Girard (1957a) stated that the mice given a single dose of cortisone before intraperitoneal injection with the EV strain succumbed to a mixed infection, but that *P. pestis* could be isolated from the blood. Serial passage of the strain through cortisone-treated mice did not alter its behaviour in normal test animals.

According to Hayashida (1957), administration of adrenocorticotropic hormone (ACTH) for three days prior to challenge with the EV strain and for four days thereafter resulted in a significant depression of the resistance of white rats to the infection. Simultaneous administration of the pituitary somatotropic hormone (STH) not only counteracted this effect of ACTH but even increased the resistance of the animals in comparison with controls—an effect which could be produced also through administration of STH alone.

Blyakher (1958) confirmed that single administrations of cortisone markedly increased the sensitivity of laboratory mice to challenge with plague strains of low virulence and suggested that this experimental method could be used with advantage when dealing with suspect materials containing small numbers of the causative organisms or organisms of low virulence.

Action of X-rays

Investigations by Leshkovich (1958) showed that exposure of virulent plague strains to thrice-repeated intense irradiation with X-rays was apt to lead to a most marked loss of virulence so that 9 months after the commencement of irradiation 7 out of the 9 strains tested caused the death of guinea-pigs only in doses of 20 milliard organisms. Treated in an identical manner, the EV strain did not lose its immunogenicity.

Immunochemical investigations

Assessing the findings they had made when examining a virulent plague strain and the EV strain,

[1] As added by Payne et al., cortisone had no effect on the mortality rate among mice infected with the Tjiwidej strain or with a fourth avirulent plague strain (A 1122).

Akimenko & Aleshina (1956) postulated the existence of definite differences in the chemical composition of virulent and avirulent plague strains. Specially noteworthy were the differences in the phosphorus content of the organisms which seemed to indicate differences in the protein fractions of virulent and avirulent plague bacilli, particularly the nucleoprotein fractions.

PLAGUE TOXIN

Methods of production and principal properties

Of the various methods recently recommended for the production of *P. pestis* toxin, that of Englesberg & Levy (1954b) may conveniently be mentioned first. Using the casein-hydrolysate/mineral/glucose medium they had found suitable for the abundant growth of this organism (see Englesberg & Levy, 1954a), they found that plague toxin was released into the medium both during and after cell lysis, reaching a maximum by the seventh day. By precipitating the toxin with ammonium sulfate and then resorting to dialysis and lyophilization, a highly potent preparation could be obtained, the average yield being 0.85 g per litre of medium. It was found that the toxin yields of strain EV 76 were much higher than those of strain A 1122. As the two workers pointed out, this difference probably accounted for the divergent behaviour of the two strains *in vivo*, where multiplication and formation of necrotic areas in the spleen and liver of the test animals were observed only in the case of the EV strain.

Further studies with the toxin just described were made by Schär & Thal (1955), Schär & Meyer (1956) and Schär (1956).

Schär & Thal, comparing the properties of the plague toxin with those of *P. pseudotuberculosis* toxin, found that: (*a*) guinea-pigs and rabbits, while succumbing like mice and rats to the pseudotuberculosis toxin, were refractory to the plague toxin (to which the two last-mentioned species of laboratory animals were highly susceptible); and (*b*) the pseudotuberculosis toxin could be neutralized by its homologous serum *in vivo* and *in vitro* in multiple proportions, thus showing the properties of an exotoxin, whereas the plague toxin could be neutralized in the same manner only to a negligible degree. As was also noted, no cross-immunity existed between the two toxins.

Summarizing the results of an exhaustive study of the pathophysiological action of plague toxin in experimental animals, Schär & Meyer stated that:

"The toxin acts mainly on the peripheral vascular system and on the liver, causing haemoconcentration and shock. On local administration, oedema, often followed by tissue necrosis, is marked. There is no physiologic or pathologic evidence that the toxin acts selectively on the central nervous system and on the heart. The shock resulting after injection of a lethal dose of toxin is irreversible and cannot be markedly influenced by drugs or by administration of homologous plasma. The pathologic findings in mice and rats infected with virulent plague bacilli resemble closely those after injection of the toxin. In other animals no correlation exists between the symptoms and pathologic findings after infection and those after administration of toxin."

Schär, studying the specific neutralization of the plague toxin, found that: (*a*) the antibodies raised with virulent or avirulent plague bacilli or with purified toxin in experimental animals reacted quantitatively with the pure toxin in flocculation systems; (*b*) the toxin-antibody complex was unstable, dissociating *in vitro* when incubated with fresh normal sera at 37°C; (*c*) *in vivo* dissociation was extensive upon intravenous administration of the complex, less so upon subcutaneous or intraperitoneal administration; (*d*) owing to this dissociation no quantitative relation existed between the amount of toxin and that of antiserum necessary for neutralization.

Initiating a series of important studies, Ajl and colleagues (1955) stated that they had isolated from the Tjiwidej strain, by chemical procedures followed by continuous paper electrophoresis, a plague toxin in a state of considerable purity. This product, which contained 14% nitrogen, 1.9% sulfur and not more than 0.2% phosphorus, was found to be free from nucleoproteins, carbohydrates and capsular material, and behaved as a homogeneous protein in the ultracentrifuge and electrophoresis cell. When injected into rabbits, the purified toxin gave rise to a specific antitoxin.

In the second article of the series at present under review, Warren and associates (1955) stated that the purified plague toxin produced in rabbits a potent antitoxin which neutralized the toxins extracted from various virulent and avirulent *P. pestis* strains, and that *vice versa* heterologous antiplague sera reacted with the purified Tjiwidej toxin.

As reported in a note by Ajl and colleagues (1956), diphosphopyridine nucleotide (DPN) was

split by the purified plague toxin into at least two components, this reaction proceeding in an enzymatic manner. A close correlation was found to exist between the capability of the purified products to break down DPN *in vitro* and their toxicity for mice. It was also noted that these animals, if injected with a few LD_{50}'s of the plague toxin, developed a reduction of the DPN levels in their erythrocytes. Other bacterial toxins, e.g., that of *E. coli*, failed to produce this effect in moribund mice.

As stated in a further report on the action of the plague toxin on an enzymatic level, Ajl, Woebke & Rust (1958) found that the toxin inhibited the oxidation of several compounds where DPN acted as an intermediary between the substrates and oxygen, the inhibition being relieved on addition of an excess of DPN. The observation that inactivated (heated or formol-treated) toxin continued to inhibit the oxidation processes stood in contrast to the findings of Packer and co-workers (1958) that only unaltered plague toxin exerted an inhibitory action on the respiration of the mitochondria of animals susceptible to the action of the toxin. However, Ajl and his colleagues expressed the opinion that further research would yield a satisfactory explanation for this apparent discrepancy.

A detailed chemical analysis, by Bent and coworkers (1957), of the purified plague toxin under discussion led to the identification of relatively large amounts of sulfur and 19 other elements, among which sodium, calcium and magnesium were of quantitative significance, as well as to the detection of 18 amino-acids.

As stated in the last available report by Ajl and associates (1958) they had been able to obtain, with the aid of continuous-flow electrophoresis according to the method of Karler (1955), a plague toxin purified to such an extent that it exhibited only one band in gel precipitation tests. Nevertheless, it was probable that, as the authors put it, " not all of the molecular substances present in the highly purified ($0.7\ \mu g/LD_{50}$) toxin solution is actually involved in the toxicity of this protein molecule ".

Since, however, with the aid of the gel precipitation technique no distinction could be made between toxin and toxoid, possibly a portion of the finally purified product was inactivated toxin.

The outstanding value of continuous-flow electrochromatography for obtaining a purified plague toxin was also stressed by Spivack & Karler (1958). They established the interesting fact that the toxin isolated from the avirulent strain EV 76 behaved, in every way tested so far, in a manner similar to that of a highly virulent plague strain.

Interesting observations by Goodner and coworkers (1955) showed that labile products of high toxicity for mice could be obtained by the addition of bile salts (particularly of sodium desoxycholate) to suspensions of the strain EV 76 and that similar end-products also resulted from the addition of bile salts to filtrates of *P. pestis* autolysates. As was established by various comparative tests, including mouse experiments, the labile toxic products (called desoxycholate toxin by the authors) differed from the usual type of lysate toxins. It was also found that by immunization against the latter type of toxin one obtained sera with fair antitoxic titres against the homologous toxin, but low titres against the desoxycholate toxin. Sera produced with formolized desoxycholate toxin, on the other hand, showed excellent antitoxic levels against this toxin and good levels against the lysate toxin. It thus appeared " that all desoxycholate toxins contained some lysate toxin but that preparations of the latter may contain little of the highly labile toxin ".

Continuing studies on the plague toxin, Goodner (1955) came to the conclusion that " suppression of vegetative multiplication of an avirulent strain of *Pasteurella pestis* [EV 76], whether by temperature of incubation, adverse culture mediums, or gaseous environment, provided bacterial cells with enormously enhanced toxigenic properties ".

Goodner emphasized that, depending upon known and also probably upon unknown causes, his experimental strain exhibited wide differences in toxigenicity. Accordingly he questioned the validity of claims made in regard to a correlation between the virulence or avirulence and the toxigenicity of plague strains. In his opinion it " would seem hazardous . . . to attempt to relate toxin potential to virulence ".[1]

Supplementing these studies, Pannell (1955) found that besides sodium desoxycholate some other surface-active agents also enhanced the toxicity of filtrates prepared from the plague strain EV 76.

According to studies by Korobkova (1957b) two different kinds of plague toxin could be obtained by either growing the EV strain for 48 hours at 28°C on a specially prepared agar (Hottinger agar)

[1] It is interesting to compare this statement with the observation of Chen & Meyer (1955a) that apparently no correlation existed between the toxicity and the immunizing power of avirulent variants of virulent plague strains obtained through single colony picking.

containing 0.4% glycocoll [1] or treating 2-day-old EV cultures with glycocoll solution added at a rate of 1%-3%. Both these toxins had antigenic properties and produced antitoxins in the immunized animals. It was noted in this connexion that the sera of horses which had been immunized repeatedly with living organisms of the EV strain contained antitoxin against the toxin elaborated during the growth of the organisms and, to a lesser extent, also against the lysate toxin. The sera of rabbits immunized with heat-killed EV cultures (60°C for 1 hour) contained an antitoxin neutralizing the lysate toxin, but not the more potent toxin formed during the growth of the organisms. Analogously, the sera of rabbits immunized with the lysate toxin were potent in regard to this toxin but had only a slight antitoxic titre for the toxin developed during the growth of the bacilli.

In a note published in 1957, Keppie and his associates stated that they had obtained toxic substances from *P. pestis* by suspending the organisms, grown *in vivo* by the method devised by Smith and co-workers (1953), in water, treating the suspensions at 3°C for 1 hour with ultrasonic oscillations, then centrifuging and finally passing the product through a " Millipore " filter. Tests with the filtrate showed that, if given in sufficiently large doses, it was lethal for guinea-pigs as well as for mice, the resistance of the former animals to the toxic extract being about 250 times that of the latter. Stating that they had also succeeded in obtaining similar extracts *in vitro*, Keppie and his colleagues postulated that *P. pestis* contained a toxic complex which, owing to deficiencies in the extraction methods formerly used, had hitherto been extracted but in part. They added that their technique " has been the means of separating *P. pestis* grown *in vivo* into two constituents, namely, the highly toxic soluble material which appears to contain little protective antigen and a residue which readily immunizes guinea-pigs ".

Keppie dealt further with the immunogenic properties of this residue in a second communication, which appeared in 1958, to which due reference will be made when the problems of active immunity against plague are discussed.

[1] Korobkova noted that, as she had described in the 1957 report of the Saratov Anti-Plague Institute, *P. pestis*, when grown on glycocoll-containing agar, produces a considerable amount of mucus, which contained a particular polysaccharide almost absent when the organisms were grown on the usual media. Cultivation of the EV strain on glycocoll-agar markedly increased the toxicity as well as the sliminess of the organisms.

Observations on experimental animals

In addition to those already recorded, the following observations on the action of plague toxin on experimental animals deserve attention:

As alluded to above, Goodner and co-workers (1955) found that their two different toxin preparations exerted markedly different effects on laboratory mice, the animals inoculated with desoxycholate toxin succumbing within 24 hours, those which received lysate toxin dying within a period of days. As described by Goodner and his associates, there existed also significant differences in the symptoms and signs produced by the two toxic products.

According to Kratinov & Maximenko (1956), administration of toxic products of *P. pestis* to experimental animals (guinea-pigs as well as white mice and rats) led to a state of sensitization to histamine as was observed also in animals given single doses of living organisms of the EV strain and in guinea-pigs surviving experimental plague infection. Administration of anti-histaminic drugs temporarily decreased the sensitivity to histamine.

Observations on factors causing an increased susceptibility to the action of plague toxin have been recorded by:

(*a*) Silverman and co-workers (1954), who concluded that the fatality noted in mice inoculated with avirulent plague bacilli after they had been exposed to sub-lethal doses of X-rays was due to an increased susceptibility to the plague toxin; and

(*b*) Dzhaparidze & Kulikova (1958), who found that non-lethal doses of ferment inhibitors of the tricarboxylic acid cycle, e.g., barium monofluoracetate, increased the toxicity of plague toxin (whereas sodium citrate and sodium succinate decreased it).

ANTIGENIC STRUCTURE OF THE PLAGUE BACILLUS

Though, as will be discussed below, most workers recently studying the problem of the antigenic structure of *P. pestis* placed reliance on two recently introduced techniques, to which reference will be made shortly, it is necessary to deal first with observations by Englesberg & Levy (1954a), Thal (1956) and Spivack and associates (1958) made solely or mainly with the aid of conventional methods.

The first-mentioned two workers, studying the growth and antigen distribution of *P. pestis* in the casein-hydrolysate/mineral/glucose medium devised by them noted that: " About 82 per cent of the antigen that could be removed from the cells was released into the supernatant by the sixth day; only

an additional 4 per cent was removed by saline washings of the cells, and 14 per cent by a further acetone drying and saline extraction ".

Assaying the antigen content of the supernatant fluid with the aid of sera raised against the fraction I and living bacilli respectively, Englesberg & Levy found that: " Soluble antigen was produced in two distinct stages: the initial stage between 0 and 3 days of incubation, prior to cell lysis, and the secondary stage, which appears to depend on the lytic processes."

These two stages of antigen production appeared to be related to the production of at least two different antigens—first, fraction I and then, during the lytic stage, one or several more antigens, probably somatic in origin.

The main conclusion reached by Thal (1956) when studying the immunological relations between plague and pseudotuberculosis bacilli was that the two organisms possessed a common water-soluble R antigen, *P. pestis* consequently giving serological cross-reactions with sera raised against rough pseudotuberculosis bacilli, but not with OH or O sera. The existence of Vi antigen in *P. pestis* appeared to be likely.

Studying the response of the guinea-pig to *P. pestis* antigens, Spivack and associates (1958) found no evidence of the existence of a qualitatively distinct guinea-pig antigen and came, therefore, to the conclusion that: " The antigen in the plague bacillus that confers protection to the guinea-pig is the envelope antigen, Fraction I". It served as a corollary to this conclusion that the antibody found in the immune animals by serological tests was " primarily Fraction I antibody ".

Haemagglutination tests

As aptly stated by Landy & Trapani (1954), when dealing with the initial use of haemagglutination tests in plague work:

" Amies [1951], when he used a partly purified preparation of the capsular substance, obtained good results with a normal haemagglutination technique. Later, however, when he was able to use a pure antigen that was free from carbohydrate these results could not be repeated. The subsequent work of Chen [1952] provides ample evidence that a polysaccharide antigen is responsible for the sensitization of normal erythrocytes whereas the protein antigen (fraction 1 B . . .) is not adsorbed by normal erythrocytes. It is only after the treatment with tannic acid that the cells can be sensitized with the capsular protein." [1]

Besides being discussed by Landy & Trapani, the value of protein haemagglutination tests for plague work was established independently by (*a*) Néel & Baltazard (1954)—see also the studies by Néel & Taslimi, 1954; Néel et al., 1954; Néel & Girard, 1955—and (*b*) Chen & Meyer (1954). Applying the knowledge they had gained in the course of their studies, the last-mentioned observers arrived at the following working hypothesis in regard to the antigenic structure of *P. pestis*:

" The bacillus proper is considered to be surrounded by a protein envelope of varying thickness. The polysaccharide is considered to be either a molecular layer beneath the protein layer or a part of the cell body which is released when the cell is lysed by natural or artificial processes."

As Chen & Meyer added, the results of a serological analysis of a variety of plague strains, besides supporting this hypothesis, suggested that, while the polysaccharide content of virulent and avirulent organisms was approximately the same, virulent strains appeared to be characterized by the presence of a thick protein envelope. It thus seemed possible that " the amount of protein in the envelope is directly related to virulence ".

A study of the synthesis of the fraction I antigen in non-proliferating washed cell suspensions of *P. pestis* incubated at 37°C has been made by Fox & Higuchi (1958) with the aid of haemagglutination-inhibition tests. It was found that:

(*a*) " In the presence of phosphate buffer and adequate aeration only casein hydrolyzate was required for protein synthesis. Neither carbohydrates nor peptide fractions were stimulatory."

(*b*) Chloramphenicol completely inhibited antigen formation, while dinitrophenol and diazouracil proved partially inhibitory.

(*c*) " Sonically disrupted cells were capable of antigen synthesis only if the supernatant as well as the debris (separated by high-speed centrifugation) were recombined. The active constituent in the soluble fraction was

[1] It deserves attention that Silverman (1954) obtained apparently specific results when making haemagglutination tests with chemically separated fractions of *P. pestis* without resorting to tannic acid treatment of the erythrocytes. As pointed out by Girard when reviewing the article by Silverman in the 1955 volume of the *Bulletin de l'Institut Pasteur*, the fractions of the plague bacillus used by this worker presumably contained polysaccharides besides proteins. There can be no doubt that, as noted in a review by Girard, the " antigenic substance of *Pasteurella pestis* concerning hemagglutination " investigated by Fukumi and colleagues (1954) consisted mainly, if not solely, of polysaccharides.

nondialyzable and resistant to trypsin, ribonuclease and desoxyribonuclease. The site of antigen synthesis was associated with the debris; no fraction I antigen was detectable in solution."

Haemagglutination tests have also been used by Crumpton and associates (1958) to study the serological specificities of *P. pseudotuberculosis*. It was found that smooth *P. pseudotuberculosis* lipopolysaccharides considered to bear the specificity of the somatic antigens " failed to inhibit the agglutination by *P. pestis* antisera of erythrocytes sensitized with *P. pestis* lipopolysaccharide, but inhibition of this system was obtained with lipopolysaccharides of " Rough " *P. pseudotuberculosis* strains derived from various serological groups".

As also found by Davies (1958), these rough lipopolysaccharides were closely related to the rough cell products of *P. pestis* but were not identical in specificity.

Gel precipitation tests

Studying the specific precipitations of plague and pseudotuberculosis bacilli in gels against antivirulent *P. pestis* gamma-globulin, mainly by a modified Elek technique (see Elek & Levy, 1950), Chen & Meyer (1955b) were able to demonstrate two antigen-antibody reactions common to the two organisms and to identify reaction zones characteristic of the plague-specific fraction I and the toxin fraction II. Previous laboratory findings made in the case of the various plague strains used were thus confirmed.

Continuing these studies, Bhagavan, Chen & Meyer (1956) found that both virulent and avirulent plague bacilli contained seven antigens demonstrable by the technique of Oudin (1952). Of these, five (3 thermolabile and 2 thermostable antigens) were also present in pseudotuberculosis bacilli. Four of the seven plague antigens were found to be thermolabile and two thermostable; the seventh was a hapten of fraction I.

Summarizing the results of a study on the fractions of the soluble antigens of *P. pestis*, Bhagavan, Nimbkar & Rao (1957) stated that:

" 1. Using the gel diffusion-precipitin test, the supernatant of plague vaccine was found to contain 3 antigens in large amounts and at least 4 antigens in small amounts.

2. Crude powder of total antigens of *P. pestis* precipitated by saturation with ammonium-sulphate was first extracted by ethylene glycol (Fraction A) and then with diethylene glycol (fraction B) and the residue with saline (fraction C). These fractions represented fairly pure samples of the three major water soluble antigens.

3 Fractions A and B were highly antigenic and protective for mice, while fraction C was inactive. Fraction A did not precipitate with anti-*P. pseudotuberculosis* serum, while fractions B and C reacted strongly.

4. The virulence factor was not detected in the preparations A, B and C by the use of the gel diffusion-precipitin test."

As stated by Bhagavan and his associates in the discussion of these findings, their fraction A was apparently identical with the fraction 1A of Baker and colleagues (1947), while it was likely that their fraction B was identical with the fraction 1B of Baker et al.—a postulation difficult to reconcile with the observation that the fraction B strongly reacted with immune sera raised against pseudotuberculosis bacilli.

In contrast to the above findings, Ransom and associates (1955a) found a more variegated pattern when using the gel-precipitation technique of Oakley & Fulthorpe (1953) for an examination of five plague strains, in which more than ten different precipitation-producing components seemed to be present. According to their behaviour in these tests, the strains appeared to fall into three different groups, the first of which comprised the two virulent strains and one avirulent strain, the second and third one virulent strain each.[1]

Summarizing the results of a further study on the antigenic relationship between plague and pseudotuberculosis bacilli with a gel-precipitation technique similar to that used in the investigations just reviewed, Ransom (1956) stated that:

" It has been shown that high speed vibration of cells of *P. pestis* in the presence of 'ballottini' [glass pearls] is accompanied by disintegration of the bacillary forms with concurrent release of antigenic material. At least one antigen was present in the lysed material which could not be demonstrated when cells were not lysed ... *P. pestis* and *P. pseudotuberculosis* appear to share 2 free ' envelope ' antigens as well as a single somatic antigen. In addition, it has been shown that *P. pestis* possesses a minimum of 1 ' envelope ' and 3 somatic antigens which are not shared with *P. pseudotuberculosis*. No antigens which could be considered specific for *P. pseudotuberculosis* were revealed."

In contrast to the above-mentioned statements, Burrows (1956) and Burrows & Bacon (1956b) postulated the presence of special antigens determin-

[1] In a further article Ransom and his colleagues (1955b) recorded observations showing that immunologically inert proteins present in normal rabbit serum were of significance in diffusion-precipitin tests on account of their unspecific combining affinities for antigens.

ing virulence in *P. pestis*. Summarizing the findings they had made in this respect, Burrows & Bacon stated that:

" Strains of *Pasteurella pestis* possessing the ability to develop resistance to phagocytosis in the absence of visible capsulation, can produce two antigens (V and W) which other strains, unable to develop such resistance, cannot. All virulent strains examined can elaborate V and W antigens whereas the majority of avirulent strains cannot. Production of V and W antigens among avirulent strains is restricted to those capable of developing resistance to phagocytosis in the absence of visible capsulation. Anti-sera containing demonstrable amounts of V and W antibodies have high ability to render resistant-orga nisms sensitive to phagocytosis. Anti-sera appropriately absorbed can be used to demonstrate these two antigens by the agar diffusion precipitation technique. V or W, or both, antigens apparently determine the property by which the majority of virulent and avirulent strains can be differentiated *in vitro*, namely the ability of the former to resist phagocytosis in the absence of visible capsulation. For high virulence strains must be capable of producing not only fraction I (envelope or capsular) antigen but also antigen V and/or antigen W. All strains shown to produce antigen V can also produce antigen W. Production of antigen W in the absence of V has not been observed."

As has been noted earlier in the present review, Burrows & Bacon once more emphasized the importance of the V and W antigens in an article published in 1958.

As a result of an exhaustive antigenic analysis of *P. pestis* by diffusion of antigen and antibodies in agar, using a technique similar to that of Ouchterlony (1949), Crumpton & Davies (1956) found evidence for the presence of at least ten lines of precipitation and adduced evidence to show that most, if not all, of these lines represented different antigens, including the plague toxin and a specific polysaccharide, the chemical and immunological peculiarities of which have been dealt with in a separate article by Davies (1956). The various plague strains differed in the number of antigens demonstrable in them, so that, for instance, one avirulent strain, which proved neither protective nor toxic for mice, produced only five precipitation lines.

Of special importance was the fact that three of the antigens demonstrated by Crumpton & Davies, and numbered 3, 4 and 5 by them, were found to be dependent on temperatures approaching those of mammalian hosts, their production being maximal at 37°C and infinitesimal at 20°C. Since antigens 4 and 5 were present also in *P. pestis* possessing no envelope, antigen 3 was evidently the envelope substance. Regarding antigen 4 (which like antigen 5 was possibly involved in the host-parasite relationship), Crumpton & Davies suggested that its absence was " responsible for the rough form of the colony ". Further evidence for this postulation was adduced by Crumpton & Davies in a note published in 1957, wherein it was stated that:

" The addition of a purified preparation of antigen 4 to a suspension of rough organisms has a stabilizing effect such that they sediment no more rapidly than smooth ones. This effect could not be obtained with any of several other proteins, some of which had been isolated from *P. pestis* cells. It seems likely, therefore, that antigen 4 is related in some way to the smooth colonial form . . ."

As the two authors added, antigen 4 was produced by all virulent strains of *P. pseudotuberculosis*, but not by avirulent forms, regardless of whether they were rough or smooth.

NATURAL RESISTANCE TO PLAGUE

Apart from observations on the insusceptibility of a considerable number of species or, usually, races of rodents to plague, to which reference will be made later, the only major study on the natural resistance of animals to plague published during the period under review appears to be that of Hoessly (1954). He found that while chickens and pigeons, owing to both cellular and humoral mechanisms, were resistant to plague infection with moderate doses, this resistance could be overcome by the administration of large inocula.

ACTIVE IMMUNIZATION

Comparative studies

Before attention is paid to observations on live or other special types of plague vaccines, reference has to be made to some studies of a more general nature.

The possibility of preventing pneumonic plague by vaccination were explored by Ehrenkranz & Meyer (1955), who experimented for this purpose with monkeys immunized with: (*a*) live avirulent vaccine (strain EV 76); (*b*) formol-killed vaccine manufactured with virulent *P. pestis*; and (*c*) fraction I. The main conclusions reached by the two authors were that:

" a vaccine to be effective in modifying or preventing pneumonic plague in man must contain adequate fraction I, or in the case of the living avirulent vaccines, have

the ability to produce sufficient fraction I in vivo. In addition, immunizations must be spaced over properly long intervals and the initial immunity must be reinforced by subsequent booster injections."

Another interesting observation was that monkeys which recovered from bubonic plague (cutaneous infection) showed an impressive resistance to pneumonic plague infection. However, as far as can be gathered, this refractory state lasted for a few months only.

Somewhat similarly, Blawat (1955) found that 2-3 doses of killed anti-plague vaccines or 1 dose of the EV vaccine did not lead to an increase of the bacteriotropin titre in rabbits. To obtain this result, it was necessary to continue vaccination for a considerable time.

Live plague vaccines

Discussing the possibilities for increasing the efficacy of inoculation with live plague vaccines, Korobkova (1955b) concluded, in agreement with the statements of Ehrenkranz & Meyer, that:

" Through two administrations of live vaccine, increase of the doses, and lengthening of the intervals between inoculations one succeeds in producing in the experimental animals a long-lasting immunity of a high degree, which protects them against any form of plague." [Trans.]

However, Korobkova emphasized that cutaneous vaccinations with live vaccine " are more effective than subcutaneous administrations, facilitate the implementation of specific prophylaxis, do not cause marked reactions and reduce the number of contraindications to vaccination ".

Again dealing with the methods for improving the results of immunization with live plague vaccines, Korobkova (1957a) stated that:

(*a*) Repeated passage of the EV strain through the peritoneal cavity of guinea-pigs led to an increase and stabilization of the immunogenic properties of the strain due to a process of selection, through which the organisms adapted to a saprophytic existence on artificial media were killed off.

(*b*) In order to preserve the most immunogenic organisms one ought to avoid making cultures from the animals of the last passage but should preserve their spleen (the organ best suited for this purpose) in the freeze-dried state.[1]

Experiments on guinea-pigs, made after the material from these spleens had been kept for three years, showed that subcutaneous doses of 20 000 organisms sufficed to protect all 10 animals tested against challenge with 1000 MLD of a virulent plague culture which killed 7 out of the 10 animals vaccinated with the original EV strain and all the control animals.

Postulating that the EV strain did not confer sufficient protection against plague, especially against pneumonic infection,[2] Zaplatina (1956) tested the immunizing properties of five other plague strains which had lost their virulence through prolonged storage at room temperature with infrequent subcultivation. In contrast to the EV strain, these five strains acidified glycerol (one also acidified rhamnose) and did not reduce nitrates to nitrites; otherwise they showed properties corresponding to those of the EV strain except that they were atoxic for white mice. However, as shown by comparative tests, two of the experimental strains showed a higher immunogenicity than the EV strain and therefore seemed worthy of further investigation.

While, as just noted, Zaplatina's proposals for the introduction of new types of monovalent live plague vaccines have not been adopted so far for actual work, ample use is being made in the Soviet Union of divalent live plague vaccine, consisting of a glycerol-negative strain " 1 " and a glycerol-acidifying strain " 17 " and therefore designated " plague vaccine 1.17 ". Discussing the reasons for the adoption of this new product, Novikova and co-workers (1956) pointed out that the EV strain, though well tolerated and furnishing better protection than killed plague vaccines, was not fully satisfactory because:

(*a*) It had recently been found that some subcultures of the EV strain were not fully immunogenic so that multiples of the immunizing doses had to be given.

(*b*) It was desirable to use a vaccine manufactured not only with a glycerol-negative but also with a glycerol-acidifying strain of *P. pestis*—the more so because it had been shown that the continental (i.e., glycerol-positive) strains contained an additional antigen Pt, which was absent in the glycerol-negative races. It had been found also that the vaccinal strains possessed different antigens and that depending upon these variances some strains

[1] It deserves attention that Quan, Chen & Meyer (1956) had previously recommended lyophilization of suspensions from cultures of the EV 76 strain as a fully satisfactory means of preserving avirulent plague strains.

[2] This contention is not in accord with the results recorded by Korobkova (1956b), who reported that both cutaneous inoculation with live vaccine and subcutaneous administration of massive doses conferred protection against lung processes caused by intranasal or eye infection.

better immunized guinea-pigs, others grey rats, and a third group white mice.

Though it was possible to administer the new vaccine subcutaneously, intradermally or epidermally, Novikova and her colleagues recommended giving preference to the last-mentioned route, because vaccination by it produced much milder and mainly local reactions. The same advice was also given by Demina and associates (1956).

Kalacheva (1958), making an experimental study of combined vaccination against plague and tularaemia, established the superior value of cutaneous (epidermal) plague vaccine administrations as compared with that of subcutaneous inoculation. It is noteworthy that she used for cutaneous vaccination a dry vaccine manufactured from the EV strain, but preferred for subcutaneous administration the 1.17 vaccine, which proved more efficacious in her hands. Making analogous laboratory studies Korobkova and co-workers (1958) found that it was possible to combine immunization with killed cholera vibrios and live plague vaccine, while Vereninova and her colleagues (1958) demonstrated the possibility of simultaneous inoculation with live plague, tularaemia and brucellosis vaccines.

Important information on the immune reactions produced by administration of the EV strain was obtained by Payne, Smadel & Courdurier (1956) in the course of immunological studies on persons residing in a plague endemic area, the main results of which were as follows:

(*a*) Unvaccinated persons who recovered from plague, when examined by haemagglutination tests, usually continued to show for months or years appreciable antibody levels against the envelope antigen of *P. pestis*, but rarely showed evidence of significant amounts of antitoxin. By contrast, individuals who had been vaccinated before contracting plague usually had appreciable amounts of both types of antibodies for months or even years after recovery.

(*b*) It appeared, however, " that a single inoculation of living attenuated *P. pestis*, strain EV, elicits demonstrable antibody and antitoxin in relatively few persons ", but that subsequent administrations of the vaccine over a period of years " progressively enhance the production and maintenance of the antibodies by the individual and by the group ".

(*c*) The occurrence of positive serological reactions in a few individuals without a history of either plague or vaccination was suggestive of the occasional presence of unrecognized and possibly clinically inapparent *P. pestis* infections.

(*d*) Dealing with the results of skin tests, Payne and his colleagues stated that: " Purified murine toxin of *P. pestis* produces erythema and edema in the skin of normal persons but intradermally injected capsular antigen is relatively non-toxic . . . As little as 11μg of intradermally injected capsular protein, unlike a similar amount of toxin, elicited an appreciable specific antibody response in the majority of previously vaccinated, but not unvaccinated, persons . . .; no clear-cut relationship between skin reactivity and circulating antibody was forthcoming."

The following observations demonstrating or suggesting the development of an *allergic state* in connexion with the administration of live plague vaccine have been recently recorded:

Korobkova (1955a) established that intradermal administration of an antigen consisting of a heat-killed culture of the EV strain (exposure to 60°C for 1 hour) produced a marked reaction in immunized guinea-pigs, while the control animals failed to react, and postulated, therefore, that this reaction could be used to determine the appearance and also the degree of immunity produced through inoculation with live plague vaccine. An interesting observation made in this connexion was that intracutaneously or cutaneously vaccinated animals reacted earlier and more strongly than subcutaneously vaccinated animals. It is also noteworthy that positive skin reactions were obtained as well in human volunteers who had been vaccinated 5-11 months previously. Since positive results were obtained likewise in guinea-pigs which had been infected with a virulent plague culture 2-3 months before, Korobkova upheld that the skin tests were also useful for a retrospective diagnosis of the disease. It was noted in this connexion that the results of the tests were usually negative in animals actually ill with plague but that a positive result was prognostically favourable.

As noted already (see page 336) Kratinov & Maximenko (1956) found in the course of an experimental study that single doses of the live EV vaccine produced in white mice and guinea-pigs an increased sensitivity to histamine.

Very severe reactions following intracutaneous vaccination with live plague vaccine, observed in one instance each by Kozlov & Norov (1956) and by Medinski & Razumenko (1957), were ascribed by these authors to the presence of an allergic state in the individuals in question. The person observed by the former authors was stated to have been vaccinated against plague by the subcutaneous route 9 months previously, but no such statement

can be found in the short communication of Medinski & Razumenko.[1]

For the convenience of record it is added that Okamoto (1955), histologically examining the organs of guinea-pigs immunized with an antigen prepared according to the method of Baker et al. (1947) and then challenged with a virulent plague culture, found in the lungs of the animals signs which he considered to be of an allergic nature.

To complete the present disquisition on live plague vaccine and vaccination it has to be added that, in order to facilitate the testing of the EV strains to be used for vaccine manufacture, Brygoo & Courdurier (1956) recommended using white mice in the place of the guinea-pigs hitherto used. However, while apparently accepting this proposal, in general, Girard, when reviewing the article by Brygoo & Courdurier in the 1957 volume of the *Bulletin de l'Institut Pasteur*, considered it indispensable to make guinea-pig tests from time to time so as to ascertain the continued production of the splenic reactions characteristic of immunogenic EV strains.

Killed plague vaccines

Dealing with the problem of killed vaccines in their study on the immune response of the guinea-pig to the antigens of *P. pestis*, Spivack and his associates (1958) stated that:

" with preparations composed of killed plague bacilli, protection of the guinea pig against adequate challenge has not been effective in primary immunization unless these preparations were given in excessively large amounts or were incorporated in adjuvant. When emulsified in an oil adjuvant, standard antiplague vaccines, such as the formalin-killed one being used by the U.S. Armed Forces, became potent prophylactics in this animal."

Other types of vaccines

In agreement with previous findings (see Pollitzer, 1954), Thal (1955) found that an avirulent pseudotuberculosis strain which, used as a live vaccine, was capable of protecting guinea-pigs against *P. pseudotuberculosis* infection, also immunized the animals againt plague infection. In Thal's opinion these immunizing properties were vested in the R antigen, which was common to both bacterial species.

As already alluded to, Keppie and co-workers (1958) obtained a non-toxic complex immunizing guinea-pigs as well as mice from the residue left after treating suspensions of plague bacilli grown *in vivo* with ultrasonic waves.

For this purpose they resuspended the residue in water and again subjected the suspensions twice to treatment with ultrasonic waves. The material thus obtained dissolved almost completely when treated with ultrasonic waves in 0.05 M sodium bicarbonate or 0.02 M tris(hydroxymethyl)-aminomethane. The solution was used for immunity tests on guinea-pigs and mice at a concentration equivalent to 1×10^{10} organisms per ml.

As Keppie and his colleagues added:

" A solution of the well-washed, lipid-containing residue in 0.05 $NaHCO_3$ at pH 8.5 showed one precipitation line on Ouchterlony diffusion plates against plague antiserum; this line was common with that produced by the freely soluble fraction I isolated as described by Baker et al. [1952]. When serum was used, absorbed with an avirulent organism to detect virulence antigens as described by Burrows and Bacon [1956b], the solution of the residue also formed a line of precipitation. The relation of these lines to one another and to those virulence antigens previously described is under investigation. In this connexion it is relevant to mention that the solution of the residue in bicarbonate buffer prevented the phagocytosis of *Past. pestis* by guinea-pig polymorphs ; fraction I and the virulence antigens have already been associated with phagocytosis resistance."

Commenting upon these findings, Keppie and his colleagues postulated that the protective complex first obtained from *P. pestis*, grown *in vivo* and afterwards also *in vitro* from a virulent plague strain as well as from the Tjiwidej strain, might form the basis of an efficient plague vaccine. However, great as the value of this new type of vaccine might be, one cannot help feeling that the long and involved process of its manufacture would rather limit its practical usefulness.

Production of antitoxins

Studying the antibody pattern in the sera of pneumonic plague patients or convalescents, McCrumb and co-workers (1955) observed, besides a rise in the level of other antibodies, a rise in the antitoxin level. While the amounts of antitoxin demonstrable in these sera were small, they nevertheless showed that the toxin of *P. pestis* was evidently antigenic.

As noted before, Payne and associates (1955), working like McCrumb and his colleagues in Madagascar, made observations similar to those of the latter workers when examining the sera of various categories of persons residing in a plague-endemic area.

[1] According to Novikova and colleagues (1956), high fever and other signs of a severe reaction, confining the individuals in question to their beds for days, were met with in 1.5%-2% of the intradermally vaccinated.

Warren et al. (1955), studying the antitoxin levels in the sera of monkeys which had been vaccinated with various types of plague vaccines and had then resisted infection with virulent plague bacilli, found that antitoxin was produced to a variable degree in the animals immunized with vaccine containing *P. pestis* somatic protein, but was not produced in those which received preparations containing predominantly capsular antigen or preparations low in toxin content.

The sera of 25 normal human adults were mostly either negative or reacted at insignificant levels when tested for the presence of plague toxin by the haemagglutination method; only in three of these persons were slightly higher titres (maximum 1 : 40 in one instance) found. As shown by a limited number of tests on the sera of pneumonic plague patients, there was in 50% a significant rise of the antitoxin level by the third week of convalescence; thereafter, the level appeared to decrease and was insignificant 20 months after the illness. Thus the conclusion reached was that " antitoxin is low during convalescence from pneumonic plague treated with antibiotics ".

Experimental evidence that the toxin of *P. pestis* was antigenic and capable of leading to the production of antitoxin was also adduced by Schär (1956) and, as has been discussed earlier, by Korobkova (1957b).

PASSIVE IMMUNITY

Apart from (*a*) a study by Derteva (1958), in which she came to the conclusion that the effect produced by plague immune serum in the animal body was due to an activation of the phagocytic process, and (*b*) two papers (Seal, 1954; Dzhaparidze & Sidorova, 1956) dealing with methods of assessing the potency of the sera which will be discussed below, the authors recently considering the problem of passive immunization against plague have paid main attention to the advantage of using, instead of native sera, fractions of these, particularly the gamma-globulin fraction, for the prevention and treatment of plague.

Experimental evidence supporting the value of these fractions has been adduced by Ehrenkranz & Meyer (1955), Semenova and co-workers (1956), Khundanov and associates (1958), and Khundanov, Kolesnik & Pletnikova (1958), as well as by Meyer (1957), to whose statements attention will be drawn when dealing with the clinical aspects of plague. Particularly noteworthy among the experimental observations are those of Khundanov and co-workers, who recorded the results of tests in more than 1400 animals. They found that: (*a*) administration of the gamma-globulin fraction to white mice 24 hours before, or simultaneously with, infection with *P. pestis* saved 50%-73% of the animals, as against 5%-30% in the case of the beta-globulins, 53%-60% in the case of the pooled globulin fractions and 26%-51% when the native sera were given; and (*b*) therapeutically the gamma-globulin fraction also proved most effective, since it saved 75% of the treated white mice, while those succumbing to the infection survived on an average 3.6 days longer than the controls. The corresponding figures in the case of the pooled globulins were 73% and 5.3 days, and in the case of the native sera 63% and 2.4 days.

As alluded to above, proposals for improved methods to assess the efficacy of plague immune sera *in vitro* were made by Seal (1954), who recommended for this purpose the use of a qualitative precipitin nitrogen test and a quantitative agglutinin nitrogen test, and by Dzhaparidze & Sidorova (1956), who described a precipitation test using the specific polysaccharide of *P. pestis*, which gave positive results with the beta- and gamma-globulin fractions of the sera but not with their albumin fraction.

IMMUNOCHEMICAL STUDIES

As already alluded to (see page 339), Davies (1956) obtained from acetone-dried *P. pestis* cells, by means of phenol extraction after removal of proteins by saline extraction, a specific lipopolysaccharide containing glucose, glucosamine and an unidentified aldoheptose sugar. Immunologically this compound proved to be a hapten, which could be made antigenic by combination with the conjugated-protein component of the somatic antigen of *Shigella dysenteriae*. The material was non-protective and relatively non-toxic, but was strongly pyrogenic.

Working with a virulent plague strain, the EV strain and another vaccinal strain, as well as with a rough pseudotuberculosis strain, Zaplatina & Konnova (1956) obtained by various methods, including those of Boivin & Mesrobeanu (1933), Raistrick & Topley (1934) and White (1929), lipopolysaccharides. All of these gave a positive Molisch reaction but since sometimes this appeared without heating, whereas in other instances application of heat was necessary to make the reaction

manifest, it was likely that the various polysaccharides did not possess a uniform structure. Considering this problem, Zaplatina & Konnova were inclined to support the postulation of Korobkova and her colleagues (1951) regarding the presence of two different polysaccharides in *P. pestis*.

Immunologically the polysaccharides had the character of haptens. They reacted positively, though slowly, in precipitation tests with plague immune sera and apparently also when tested with immune sera raised against pseudotuberculosis bacilli. The polysaccharides were not toxic for white mice or guinea-pigs and—as far as tested—were not immunogenic for the latter species of laboratory animals.

BACTERIOPHAGE INVESTIGATIONS

Before dealing with the series of recent articles in which reference has been made to the use of bacteriophage for the purposes of the laboratory diagnosis or differential diagnosis of plague, attention has to be drawn to observations made by Girard (1957c) and by Macchiavello (1957a).

The former worker obtained with a phage which was highly potent for *P. pestis*, from a virulent plague strain, secondary colonies characterized not only by retarded growth and production of turbidity in peptone water, but also by a lessened virulence. While most of these colonies were lysogenic, some were free from the phage and lysosensitive. It thus appeared that a situation similar to the state of pseudolysogenicity defined by Jacob et al. (1953) had developed, in which a kind of equilibrium between the bacilli and the phage existed. It was impossible to separate the latter from the bacilli by numerous subcultures or by formol treatment. However, the phage could be destroyed and a growth resistant to all plague phages could be obtained with the aid of anti-phage serum or by suspending the mutant in citrated human or rabbit blood. All the characteristics of the original strain, including phage-sensitivity and high virulence, could be restored through repeated mouse passages. Girard raised the question whether the phenomena observed by him might also account for the cyclical occurrence of plague in nature. He noted in this connexion that the plague bacillus was sensitive to phages of the Enterobacteriaceae.[1]

Support for these postulations was furnished by the observations of Macchiavello (1957a) in Ecuador in that he found evidence of the presence of a bacteriophage in four plague strains of apparently lowered virulence, three of which had been isolated from patients with pestis minor.

Discussing the possibilities of using plague and pseudotuberculosis phages for a differentiation of *P. pestis* and *P. pseudotuberculosis*, Pilenko (1956) found, in contrast to the observations by Gunnison and co-workers (1951), that, regardless of whether incubation was carried out at 18°-20°C, at 28°C or at 37°C, undiluted plague phages exerted a lytic action not only on the five plague strains tested by him, but also on some of the 13 pseudotuberculosis strains used. Pilenko also obtained no clear-cut results when using his two plague phages in various dilutions for tests with plague and pseudotuberculosis strains, but was successful when making tests with diluted pseudotuberculosis phages. However, while recommending the use of the latter for tests at 18°-20°C and at 37°C, he admitted that " since this method is based on inconsiderable quantitative differences between plague and pseudotuberculosis bacilli, it can be applied only for orientative tests followed by a detailed study of their properties ".

Tumansky, comprehensively dealing with the use of bacteriophage for laboratory work in the 1958 edition of his manual on the microbiology of plague, stated that while this method of examination was of considerable value for the differentiation of plague and pseudotuberculosis bacilli, it was not infallible and had to be used therefore in combination with tests in rhamnose-containing media and other recommendable differential-diagnostic procedures —a view also expressed by previous experienced workers.

Dealing with the methods of cultivation of *P. pestis*, Tumansky pointed to the difficulty or even impossibility of growing the organisms from materials also containing plague bacteriophages. These phages might be present in old laboratory cultures, and there is also reason to assume that their presence might be responsible for negative results in the examination of wild rodents for plague. For cultivation from material suspected of containing bacteriophages, Mariena (1941) [2] advocated the use of plates on which 0.1 ml of anti-phage serum had been spread out not more than 15-20 minutes before

[1] It is important to add the statement made by Girard (1957b) that " there exists no specific plague phage and that other organisms among the Enterobacteriaceae—especially *Shigella*—can be lysed by the phages acting on *P. pestis* ".

[2] Mariena again dealt with this subject in a 1946 report, where she quoted also studies by Dolomanova (1941) and Bibikova (1946).

the media were used. Tumansky found it advisable to use for this work selective media (e.g., blood agar) in place of plain agar.

Tumansky also stated that the diagnosis of plague could be considerably accelerated if a comparison was made between cultures inoculated in the usual manner and cultures implanted with material to which at the moment of cultivation a potent specific bacteriophage had been added.

He recommended using for this purpose highly selective media (particularly agar containing 0.1%-0.2% blood and 1:100 000 gentian violet) and also a strictly specific bacteriophage. In his experience evidence of phage action (" negative colonies ") became visible to the naked eye after incubation at 28°C for 2½-5 hours or, if the plague bacilli were scanty, after 5-7 hours, or finally, if only single organisms were present in the substrates under test, after 10-12 hours.

As Tumansky added, a similar but more involved method of using a highly specific phage for the rapid diagnosis of plague has been devised by Domaradsky et al. (1957, 1958).

PATHOLOGY

OBSERVATIONS ON EXPERIMENTAL ANIMALS

Bubonic infection

The development of plague in monkeys (*Macaca rhesus*) infected subcutaneously with 0.5-ml doses of a virulent plague strain has been studied by Hoessly and his associates (1955). Most important among the findings made was that the constantly appearing circulatory failure was of a peripheral nature, the profound drop in the blood pressure not being accompanied by signs of cardiac failure. It was not possible, therefore, to ascribe the circulatory collapse and the death of the animals predominantly to the action of the plague toxin on the heart. Hence, as Hoessly and his colleagues stated:

" The failure of these animals to respond to peripheral circulatory failure with an increase in heart rate might be interpreted as compatible with a toxic effect on the central nervous system and suggests that the peripheral circulatory failure could be due to alteration in the function of the vasomotor center."

However, as Hoessly and his co-workers admitted, the absence of tachycardia in their animals " contrasts sharply with observations on cases of severe human plague in which the heart rate usually becomes very rapid and quite often irregular ".

It is interesting to note that observations made in the Soviet Union have led to analogous conclusions. Summarizing the results of studies such as those of Mokhin and others published in 1957, Shishkin (1957) stated:

" The observations of the pathophysiological laboratory of the [Rostov Anti-Plague] Institute, devoted to an investigation of the pathogenesis of plague . . . showed the incorrectness of the formerly held opinion that acute heart failure was the basic cause of death in plague. Physiological, electrocardiographic and biochemical investigations of various animals under the sway of plague infection showed only an insignificant functional disturbance of the heart muscle. The fundamental causes of circulatory failure in plague are a lowering of the tonus of the vessels and a series of haemodynamic displacements. Tests with tracers showed that in experimental plague considerable changes take place in the blood volume, the circulation time, and the intensity of the capillary circulation, which finally leads to a lack of oxygen in the organs and tissues of the body." [1] [Trans.]

The findings made by Schär & Meyer (1956) when studying the action of plague toxin on experimental animals deserve great attention in comparison with these postulations. As noted before, the conclusion reached by them was that the cell-free toxic fraction of *P. pestis* did not exert a selective action on the central nervous system or on the heart, but acted mainly on the peripheral vascular system and the liver, causing haemoconcentration and shock.

Far more important than an elucidation of these discrepancies is the question of how far the results of observations in experimental animals are applicable to the pathology of human plague. The contention of previous workers that the plague toxin exerts a direct deleterious action on the heart seems to be supported by the recent findings of Scheidegger (1957) made when performing an autopsy on a victim of bubonic infection. Coming late to hospital, this individual had been treated with heroic doses of antimicrobial drugs. As a result his organs

[1] As stated by Rall (1958b), an aberrant view was advocated by Kratinov (1955) and his school who claimed that death in plague was due to a paralysis of the respiratory centre.

had become free from plague bacilli, and death was obviously due to the action of plague toxin. The lesions thus produced included, besides marked damage to the liver and kidneys, a severe serous myocarditis.

Meyer (1957), comparing the evolution of bubonic plague infection in non-immunized and in immunized animals, drew attention to the marked lung involvement regularly found in partly resistant or immune animals, which was not yet fully accounted for. It was formerly claimed that, like the staphylococcus toxin, circulating plague toxin also reduced the resistance of the lung tissue. However, while mice and rats, susceptible to the plague toxin, rarely had secondary pneumonic lesions, these were frequent in the toxin-resistant guinea-pigs and squirrels. Moreover, the location of the pneumonic foci beneath the pleura suggested that they were initiated by bacterial emboli. Possibly the formation of the latter was promoted by the rapid mobilization of agglutinins.[1]

The severity of the bacteraemia produced in white mice through plague infection was well illustrated by Girard (1958b), who estimated that the spleen of the animals contained from 5 to 25 milliards of the causative organisms.

Pneumonic infection

Attempting to learn something about the " more obvious physiological abnormalities " that may occur in the course of pneumonic plague, Ehrenkranz & White (1954) obtained the following interesting results in monkeys:

(*a*) Hepatic function studies revealed that diminished function became manifest in almost all animals not later than the second day of fever, i.e., considerably earlier than in monkeys with bubonic infection.

(*b*) While in monkeys with bubonic infection cultures from liver biopsy specimens were virtually negative through the first five days of fever, in the intratracheally infected animals the liver was found to be regularly invaded by *P. pestis* during the first or second day of fever.

(*c*) On the other hand, it was found that hypotension, " save in the terminal stage of infection, and hemoconcentration were conspicuously absent throughout the course of pneumonic plague. These observations together with that of continuing eosinopenia imply that the adrenal cortex is able to maintain a hormonal response during the disease."

[1] As alluded to before, Okamoto (1955) postulated that the fibrinous thrombi he found when studying the lungs of immunized guinea-pigs were due to an inflammatory reaction of a possibly allergic nature.

Speck & Wolochow (1957), using a special apparatus devised by Wolochow and associates (1957) for a further study of respiratory plague infection in monkeys, established the interesting fact that the LD_{50} in their experiments proved to be about 20 000 organisms as against the 100 organisms found sufficient by Ehrenkranz & Meyer. Discussing the reasons for this difference, Speck & Wolochow pointed out that the method of intratracheal infection, resorted to by Ehrenkranz & Meyer, possessed three drawbacks—namely, (1) the necessity for anaesthesia, which was apt to disturb the pulmonary physiology; (2) the likelihood of lesions being caused by the intubation; and (3) the necessity for introducing the inoculum in a fluid medium, which might for some time supply the organisms with a pabulum unapproachable by the defence mechanisms of the host.

Apart from the discrepancy just discussed, the findings in the aerosol-infected monkeys were quite similar to those observed by Ehrenkranz & Meyer. While establishing that streptomycin treatment was successful even when the disease was well developed, Speck & Wolochow found prophylactic inoculations with killed vaccines, with live avirulent vaccine or with the purified fraction I to be " essentially ineffective ".

Studying the development of pneumonic plague following aerosol infection of (*a*) normal mice and (*b*) mice which had inhaled formol vapour 24 hours before exposure to *P. pestis*, Smith and colleagues (1957) established that formol inhalation thus modified the course of plague:

" Compared with controls, the decrease in lung titers during the first 16 hours after exposure was much less in treated mice; the subsequent rise in lung titer was more rapid; positive cultures were obtained from livers, spleens and heart blood earlier. The 50% lethal respiratory dose (LRE_{50}) was one-tenth that for controls."

Discussing the possible causes of these differences, Smith and his associates pointed out that paralysis of the ciliary apparatus and perhaps also interference with mucus production might account for the modified character of the infection in the formol-treated mice. Possibly, however, formol inhalation also interfered with other defence mechanisms.

Ransom & Krueger (1954), also experimenting with monkeys, established that survivors from a group of these animals exposed to *P. pestis* aerosols developed sub-acute or chronic lesions, four of them apparently being on the road to recovery when they were sacrificed 54 days after infection.

Since, in contrast to what was the case in the acute form of pneumonic plague, the causative organisms were " hard to find and difficult to cultivate " in the chronic form, the authors felt entitled to suggest " the possibility that the changes produced were toxic in nature, and that the number of viable organisms administered to the monkeys must have been too low to set up more than a transient infection ".

Recently, important observations have been made on the fate of experimental animals kept under conditions in which they could contract pneumonic plague infection through contact. Rall (1958b) referred in this connexion to the experiments of Smirnov (1956) and other Soviet workers, who had failed to produce primary pneumonic plague in sisels or marmots kept in close contact with animals suffering from secondary plague pneumonia.[1]

Still more noteworthy are experiences recorded by Meyer (1957) showing that monkeys, when kept with or near intratracheally infected animals of the same species (*Macaca mulatta*), may contract plague but, instead of developing the pneumonic or the so-called primary septicaemic type of the disease, actually had a primary tonsillar process with cervical buboes, followed by bacteraemia.

These findings are of particular interest in view of the results obtained by Druett and associates (1956) when inducing respiratory infection in guinea-pigs with aerosol particles of different size.

Particles no larger than 1 μ (containing single cells of *P. pestis*) initiated a bronchopneumonia which terminated in bacteraemia. The animals exposed to large (12 μ) aerosol particles, on the other hand, showed oedema of the neck tissues and gross enlargement of the deep cervical glands followed by a more rapidly evolving bacteraemia and quicker death, but not by the appearance of lung consolidation. Deaths in healthy control animals kept together with the infected guinea-pigs were four times more numerous if the latter had been exposed to single-organism aerosol clouds than if the large particles had been used for their infection. However, it was not possible to produce real epizootics by cross-respiratory infection, presumably because the healthy contacts developed affections characteristic of exposure to large aerosol particles without lung consolidation.

It is probable that these most remarkable findings offer an explanation for observations made during pneumonic plague outbreaks in China (see Pollitzer & Li, 1943, and Pollitzer, 1954), which suggested that patients developing what was designated " the non-pneumonic type of lung pest " were incapable of spreading respiratory infection, the epidemics thus tending to become self-limited.

[1] Analogous observations were recorded earlier by Korobkova (1939) in the case of guinea-pigs infected with *P. pestis* by inhalation and their contacts.

OBSERVATIONS ON HIBERNATION AND ALLIED PHENOMENA

Seasonal changes in the metabolic processes of rodents have been recently studied by Michailov (1956) and Kalabukhov (1958).

The former worker, judging from the seasonal changes in the oxygen consumption in the case of two species of Dipodinae, found that in both the intensity of the metabolism was most marked in early summer. In the case of one of the species (*Scirtopoda telum*) the metabolism then steadily decreased, to become minimal in late autumn. This was taken to indicate that the organism of the animals became prepared for hibernation in the course of summer and that thus the extrinsic factors inducing the animals to commence their winter sleep acted upon a body already conditioned for a state of torpor. The sequence of events was less regular in the other species (*Alactagulus acontion*), which did not belong to the category of typically hibernating rodents.

The conclusion reached by Kalabukhov (1958), through elaborate observations mainly on gerbils (*Gerbillus meridianus*), was that seasonal changes in the process of thermo-regulation played a most important role in conditioning variations in the susceptibility of wild rodents to a generalized infection with *P. pestis*.

Recording the results they had obtained when studying the problem of plague infection in hibernating sisels (susliks) (*Citellus fulvus*), Zhigilev & Otdelskaya (1956) stated that experiments of this kind had been made previously on a lesser scale by Shabaev (1946), whose findings indicated that these rodents were highly susceptible to plague during the active period of their life, but became less susceptible as the time of hibernation approached and were quite refractory to the infection while in the hibernating state.

The main conclusions of Zhigilev & Otdelskaya were:

" 1. Yellow susliks, infected before or during hibernation, can succumb to plague after different intervals of time (from 4 to 133 days after infection) [2] and it is

[2] The animal which succumbed to plague after it had awakened from hibernation on the 133rd day showed at autopsy typical appearances of a generalized infection with *P. pestis*.

possible to isolate plague bacilli from all their organs as well as from their blood.

2. Non-hibernating yellow susliks, regardless of their age, are very sensitive to plague infection in spring and early summer as well as later in the year.

3. Yellow susliks can pass through a plague attack without generalization of the process, such a course of the disease being possible with infection either in spring or later in the year." [Trans.]

It is of interest to add that, according to findings made by Makarovskaya and co-workers (1956), drug-induced sleep did not exert a beneficial influence on the course of plague in guinea-pigs.

PRACTICAL LABORATORY WORK

DIAGNOSIS

Even if it were possible, it would be redundant to enter here into an elaborate discussion of the methods suitable for the practical laboratory diagnosis of plague, because these have been dealt with quite recently in a text approved by an international group of experts and published in a journal available in any part of the world (see Baltazard et al., 1956). Moreover, a number of additional methods recommended for this purpose have already received attention in earlier parts of the present review (see, especially, the references to the articles by Girard, 1956; Girard & Chevalier, 1956; Girard & Gallut, 1953; de Issaly & Issaly, 1954; Karpuzidi & Makarovskaya, 1956; Kivman et al., 1955a, 1955b and Kovaleva, 1958). However, in order to do full justice to the important subject now under review, mention has to be made also of interesting contributions by Brygoo (1956a), Winter & Moody (1957), Baltazard & Bahmanyar [1] and Bahmanyar (unpublished data).

Brygoo advised adding for the purposes of animal experimentation 1000 units of penicillin to the materials to be tested, which were then used for the intraperitoneal infection of mice instead of the time-honoured, but far more tedious, method of infecting guinea-pigs by the percutaneous route. The efficacy of the new method was proved not only by some preliminary laboratory comparisons but also when actually examining the expectorations of plague patients during a pneumonic outbreak in Madagascar (see Brygoo & Gonon, 1958). Nevertheless, as pointed out by Girard in a review of Brygoo's original article in the *Bulletin de l'Institut Pasteur*, diagnostic difficulties might arise if specimens contaminated with Gram-negative organisms (against which penicillin is practically ineffective) have to be tested. Actually Brygoo (1956) recorded a failure with this method when testing a specimen contaminated with *Proteus*.

Describing the method used by them for the rapid identification of *P. pestis*, Winter & Moody stated:

"*Pasteurella pestis* antiglobulin labeled with fluoresceine isocyanate was used to stain individual cells of *P. pestis* in dried smears from cultures and infected animal tissues. Antiserum specific for *P. pestis* was produced in rabbits by injecting formalin-killed whole cells from a fully virulent strain. The globulin fraction obtained by ammonium sulfate precipitation was conjugated with fluoresceine isocyanate. Individual cells in smears of *P. pestis* exhibited a bright fluorescence after treatment for 15 to 30 minutes with the specific antibody."

As Winter & Moody added, their method was fully specific, even pseudotuberculosis bacilli failing to react. With the aid of the new procedure it was possible to detect plague bacilli in suspensions containing only 40 organisms per ml even in the presence of heavy contamination, and occasionally *P. pestis* could be demonstrated in animal tissues from which no positive culture could be obtained.

As will be gathered from the report of Baltazard & Bahmanyar on plague research in India, published together with the present review,[2] (*a*) remarkably good results could be obtained when use was made of percutaneously infected white rats for the examination of plague-suspect materials; and (*b*) splenectomy of possibly infected wild rodents was a satisfactory means of arriving at a diagnosis without having to sacrifice the animals.

In an unpublished report on plague work in Java, Bahmanyar noted that for the sake of economy one guinea-pig, percutaneously infected at four different points on its back, was used to test material from each lot of four plague-suspected rodents, the appearance of a local reaction at any of these sites

[1] See article on page 169 of this issue of the *Bulletin*.

[2] See page 169.

indicating a positive result. In the rare cases in which positive findings were recorded at more than one of the sites, repeated tests on individual guinea-pigs were made with the materials in question.

DIFFERENTIAL DIAGNOSIS

Before entering into a discussion of the methods considered useful for distinguishing plague and pseudotuberculosis bacilli, it is important to point out that the practical importance of this differential diagnosis has become greater than it was formerly assumed to be, owing to recent findings of *P. pseudotuberculosis* in commensal rats. Observations in this connexion on these and also on some other rodent species may be summarized thus:

As alluded to by Pilenko (1956), Tokarevich-and co-workers (1940) had drawn early attention to the not altogether rare occurrence of pseudotuberculosis in rats.

Further findings were recorded by Sergeeva & Somova (1956), who stated in a short note that they had been able to isolate in 1954 three strains showing the characteristics of *P. pseudotuberculosis* from field-mice and *Microtus arvalis*.

Examining during the period 1939-53 more than 166 000 rodents, Klimova (1956) was able to isolate from them directly or indirectly 39 pseudotuberculosis strains; 26 of these had been derived from grey rats, 12 from *Mus musculus* and one from a pool of 106 lice collected from 7 rats. Moreover, positive results were obtained through inoculation of white mice with normal saline washings of two specimens of barley, which obviously had been contaminated in some manner by animals suffering from pseudotuberculosis. In spite of the infrequency of her positive results, Klimova felt entitled to postulate that commensal rats and mice were the fundamental carriers of the pseudotuberculosis bacillus under natural conditions and were thus apt to pass on the infection to other animals. She did not deny, however, that domestic animals (chicken, turkeys, pigs and cows) might also serve as reservoirs of the infection.

Interesting studies on the incidence of pseudotuberculosis among the rodents trapped during 1953 and 1954 in Moscow have been made by Bulanova (1956), who was able to isolate a total of 79 cultures of *P. pseudotuberculosis*—25 from 26 734 grey rats, 26 from 22 667 commensal mice, 21 from 1485 common voles (*Microtus arvalis*), 6 from field-mice and one from a forest mouse. The infection appeared to be more frequent among the rodents trapped in the outlying precincts of the city than among those caught in the centre. It was noted in this connexion that the rodents most heavily involved, the voles, had been caught mainly in localities where establishments for the storage of vegetables were placed. There could thus be little doubt that the infection among the commensal species in the city was derived from the field rodents infesting the outskirts.

Referring to the investigations just mentioned, Yushchenko (1957) recorded that the field and forest mice involved besides commensal rodents and *Microtus arvalis* belonged to the genus *Apodemus* (*A. agrarius* and *A. sylvaticus*).

While one must fear that the preparation of two new media recommended by Devignat & Boivin (1953) for the differentiation of plague and pseudotuberculosis bacilli is too complicated to be practicable for routine laboratory work, two simple differential-diagnostic tests advocated by Thal & Chen (1955) deserve attention.

In the introduction to their note these two authors point out that none of the previously recommended differential-diagnostic tests were fully satisfactory. Motility tests were " complicated by the development of immotile variants of the ordinarily flagellated pseudotuberculosis organism "; as claimed by Thal (1954), " white rats, though typically not susceptible to pseudotuberculosis infection, may succumb to the toxin of this organism "; differentiation by serological methods, though possible, was time-consuming; the difficulty of maintaining suitable bacteriophage strains militated against the use of this method; tests with rhamnose-containing media, though generally reliable, were not absolutely so.[1] For these reasons, Chen & Thal: (*a*) again drew attention to the proposal of Fauconnier (1950) to use for the differential-diagnostic tests a medium containing urea, which in their hands proved fully reliable for the identification of 204 pseudotuberculosis and 50 plague strains; (*b*) recorded that striking differences could be noted if plague and pseudotuberculosis bacilli were cultivated on desoxycholate citrate agar.

However, while one cannot deny the validity of the claim of Thal & Chen that a combination of the two tests recommended by them ought to suffice for a differentiation of the two pasteurellae, equally good results may also be obtained by considering the cumulative evidence obtained with some of the previously available tests,[2] as advocated long ago by Schütze (1929) and recently by authors such as Girard (1953b), Pollitzer (1954) and Yushchenko (1957).

[1] Recent observations on rhamnose-containing media have been referred to in a previous section of the present review.

[2] Or up-to-date modifications thereof, e.g., motility tests using suitable cultivation methods.

SPECIAL TECHNIQUES

Tests in man

As established through a limited number of tests by Hoyer & Courdurier (1954), positive results could be obtained when using ether extracts of the pooled serous fluids obtained by puncture from the lungs, livers and buboes of plague-suspect dead bodies for precipitin tests with anti-plague rabbit globulin. It is noteworthy that these tests, which could be read after about 15 hours, proved positive in an instance where guinea-pig experiments with the same material had failed to establish the diagnosis of plague.

As has been noted above (see page 341), Korobkova (1955a) postulated that tests consisting in the intradermal administration of a heat-killed antigen might be of value for a retrospective diagnosis of plague.

Flea examination

To supplement the directions given for the " Organization and techniques for epidemiological surveys on wild-rodent plague " in Annex 6 to the report of the WHO Expert Committee on Plague (1959) reference has to be made to some procedures recently recommended for improving the results of flea collection and examination.

As can be gathered from papers published in 1949 by Akopian, Shiranovich & Fomichev and Shiranovich & Mironov, as well as from subsequent publications such as those of Darskaya (1955a), and Ioff (1957), within recent years much attention has been paid in the Soviet Union to the advantages of collecting fleas from wild rodent (particularly sisel) burrows by a special technique, designated by the authors the " band " method.

As stated by Akopian, this procedure, which had been suggested by Ioff, consisted in the use of strips of flannel about 1-1.5 m long, or even longer, and 2.5-5 cm wide. To facilitate insertion of the strips into the burrows, some ballast material was sewn in at one end of the strips while, to permit insertion of the strips into deep burrows, pieces of tape, 0.5-1 m long, were affixed at the other end. If mounted on pieces of flexible wire or rubber tubes, the strips could be utilized also for insertion into oblique (slanting) sisel burrows.

According to the observations of Akopian, the " band " method gave better results than the formerly used practice of raking off material from the inside of the burrows (a procedure also amply used in other parts of the world). The new method also proved satisfactory when dealing with burrows in areas in which anti-rodent campaigns had been undertaken and when making flea collections after the rodents had commenced to hibernate. As noted by Shiranovich & Fomichev, it was even frequently possible with the aid of the strips to collect fleas from burrows where the openings were covered by the webs of spiders.

Recording the results of a comparative evaluation of the various methods used for flea collection from sisel burrows, Shiranovich & Mironov (1949) stated that, in the case of vertical burrows, short-term exposure (i.e., 10-30 seconds) of the flannel strips proved most satisfactory and exposure for 5-7 minutes almost equally good, while longer exposures offered no additional advantages. In contrast to Akopian's experiences, Shiranovich & Mironov found that in the case of slanting burrows better results could be obtained by scraping out material with a special instrument [1] introduced into the holes than by the introduction of flannel strips mounted on wires or rubber tubes. The two workers further established that two insertions of flannel strips into vertical burrows, or two scrapings in the case of slanting burrows, sufficed to collect the overwhelming majority of the fleas present. If, however, the first insertion of flannel strips did not lead to success, it was of no use to repeat the operation. On the other hand, repeated scraping of material from slanting burrows was apt to yield results.

As described by Shiranovich & Fomichev, after the flannel strips had been left in the burrows for the required length of time, they were quickly withdrawn and put into a white enamel basin so as to facilitate the picking-up of the fleas with entomological forceps.

A simple, yet effective, modification of the method of collecting fleas from captured rodents described and illustrated by Baltazard & Eftekhari (1957) consisted in holding the animals over an adequately large white enamel basin filled with preferably ice-chilled water and then blowing in a " gentle, regular and prolonged " manner against the lie of the fur, paying special attention to the root of the tail, the perineum and the thighs as well as to the head and shoulders. The fleas, which became stunned by the cold, could easily be picked up from the surface of the water and, before being further used, lined up on chilled slides for identification under the low power of the microscope or a binocular loupe.

A method of speeding up the collection of fleas from rodent nests sent without previous handling to a central laboratory with the aid of an apparatus generating a current of hot air has been devised by Miles & Kinney (1957).

Of importance for research workers is the fact that: (*a*) Smith & Eddy (1954) and again Baltazard & Eftekhari (1957) have described methods suitable for rearing fleas apt to serve as plague vectors; and (*b*) Kartman (1954) and Wheeler and colleagues

[1] The use of a special scraper had been recommended previously by Davis (1953).

(1956) have designed contraptions for the artificial infection of such insects with *P. pestis*. As will be noted below, a method for tagging rodent fleas with cerium-144, devised by Quan and colleagues (1956), has proved useful in ecological studies.

As to the actual method of examining plague-suspect fleas, the recent study by Quan, Fintel & McManus (1958) on the efficiency of bacterial culture as compared to animal inoculation for detecting *P. pestis* in wild rodent fleas deserves great attention. Discussing the relative value of these two methods, the authors stated with great reason:

" Both methods are simple in procedure and equipment and both are highly sensitive in detecting the presence of plague bacilli in fleas . . . The culture method is rapid, it does not require animal facilities, and it allows the processing of large numbers of fleas individually. The animal inoculation of flea pools has few interfering factors, it has a higher degree of efficiency than the culturing method, and it allows the pooling of large numbers of fleas for few injections. Because *P. pestis* occurs in fleas in nature so rarely, the method of choice for surveillance remains that of animal inoculation. On the other hand, for critical study of epizootic plague among wild rodents and their fleas, individual culturing of fleas provides invaluable epizootiological data."

HOSTS OF INFECTION

RODENTS AND LAGOMORPHA

General considerations

Commencing the publication of a valuable series of articles on plague in South America, Macchiavello (1955), in order to characterize the different role played by the various species of rodents and Lagomorpha in the manifestations of plague, proposed the elaborate system of classification shown in Table 3.

TABLE 3
CLASSIFICATION OF TYPES OF RODENT PLAGUE ACCORDING TO MACCHIAVELLO (1955)

Category of rodent plague [a]	Examples of rodents or Lagomorpha involved
Pestis nautica	*R. rattus*
Pestis urbana	*R. norvegicus*, etc.
Pestis urbanoruralis et rodentium sylvestrium	*R. rattus* and *Sigmodon peruanus*
Pestis murina ruralis	*R. alexandrinus*
Pestis murina campestris progressiva	Spreading, for example, from *R. rattus* to peridomestic Cricetinae
Pestis campestris regressiva	Reverting, for example, from Cricetinae to *R. frugivorus*
Pestis sylvestris	Caviidae, Cricetinae, Leporidae
Pestis sylvatica	Sciuridae Gerbillinae, Leporidae

[a] Following the example of the reviewer of Macchiavello's article in *Trop. Dis. Bull.*, 1956, **53**, 584, the various kinds of plague are designated by Latin names instead of the original Spanish names.

Valuable as this classification is because it does justice to the various ecological situations in which plague among rodents and Lagomorpha may become manifest, it seemed best for the purposes of the present necessarily brief disquisition to deal first with what is commonly called wild-rodent plague and then with the manifestations of the disease in the commensal rodents. Nevertheless, it will be constantly kept in mind that this distinction is rather arbitrary because (*a*) quite often so-called wild rodent species or subspecies, assuming peridomestic habits, live near or even with man, while (*b*) commensal rodents may lead an independent existence well away from human settlements. This is true, for instance, of *R. hawaiiensis* (see Kartman & Lonergan, 1955b) and the present writer has been urged repeatedly to embody this species in his wild rodent list. Should this be done, however, the question would arise whether the same policy should be adopted in the case of the " coconut-tree rat ", which is known to be implicated in plague manifestations in New Caledonia and has recently been identified as *R. exulans* by Grenier & Rageau (1956). However, it has to be kept in mind that both these species belong to the *concolor* (or, as some call it, the *exulans*) group of *R. rattus* which, as Schwarz [1] has pointed out, " has developed a series of commensals of small size which are found, alongside the larger commensals, in the Malay Peninsula and islands, north as far as Burma, and east all over the islands of the Pacific as far as Hawaii ".

[1] See note on page 411

Plague in wild rodents

Recent observations on prevalence.[1] Information, hitherto not available, on the occurrence of wild-rodent plague in the Soviet Union and also in Mongolia and China has been furnished recently by Pastukhov[2] and, more elaborately, by Rall (1958a, 1958b). The former observer distinguished between four main foci of this type of the infection in the Soviet Union thus:

Area	*Main plague reservoir*
Pre-Caspian region	In the steppes: *Citellus pygmaeus*
	In the sandy stretches: *Meriones* (*meridianus* and possibly also *tamariscinus*)
Central Asiatic focus	In the desert lowlands: *Rhombomys opimus* and also *Meriones erythrourus*
	In the high mountain areas: *Marmota baibacina* and *M. caudata*
Transcaucasian area	Gerbils
Transbaikalia	*Marmota sibirica*

Note. According to the WHO Expert Committee on Plague (1959), " during the last two years (1957-1958) plague has been altogether absent from the Pre-Caspian area and from Transbaikalia. The situation has remained serious in the Central Asiatic foci, where more than 4000 plague strains were isolated in 1957 and 1958, and to a lesser degree also in the Transcaucasus area (isolation of about 600 strains)."

Pertinent information embodied in the book on the epizootiology of plague by Rall (1958b), as far as it lends itself to condensation, is summarized in Table 4.

Dealing with the situation in the Mongolian People's Republic, Rall stated that the fundamental plague carriers in the central part of the territory and in the eastern Tien Shan were marmots (*M. baibacina* and *M. sibirica*), while in the southern zone apparently independent roles were played by *Meriones meridianus*, *Rhombomys opimus*, the Mongolian gerbil (*Meriones unguiculatus*), *Citellus pallidicauda* and *Citellus dauricus*. Commensal rats, becoming secondarily infected from these field rodents, were capable of acting as important, though temporary, carriers of the infection. Species of small rodents, like field-mice, and also ochotonae could become involved in the marmot epizootics. Thus Kovaleva (1958), working in a locality of western Mongolia where plague was apparently enzootic among the marmots, found evidence of an acute epizootic among *Microtus brandti* and also established the presence of the infection in *Citellus undulatus* and the high-mountain field-mouse *Alticola worthingtoni semicanus*.

According to Rall, recent observations by Soviet workers in the Tungliao area of South-west Manchuria had shown that plague persisted in *Citellus dauricus* and also in the Dauria hamster, *Cricetulus barabensis*, as well as in *R. norvegicus* and *M. musculus*, the commensal species probably contracting the infection from the Dauria sisel, which was considered the primary reservoir.

Besides those in the above-mentioned countries, important new observations on the prevalence of wild-rodent plague have been recorded in the following areas:

Kenya. Reappraising the situation in Kenya, Heisch & co-workers (1953) came to the conclusion that *Arvicanthis* and *R. natalensis* (commonly called the multimammate mouse), which were rather resistant to infection with *P. pestis*, formed together with the moderately resistant swamp-rats (*Otomys angoniensis*) the true plague reservoir in that focus. Appearance of the infection in *R. rattus*, formerly considered the main culprit in Kenya, appeared thus to be of a secondary nature.[3]

South Africa. As reaffirmed by Davis (1953) in a valuable study on wild-rodent plague in South Africa: " The primary reservoir in the gerbils [*Tatera brantsi*] was the source of infection to the semi-domestic *Mastomys* [multimammate mouse] and the domestic *R. rattus* living in and around the farm buildings."

Iranian Kurdistan. According to the WHO Expert Committee on Plague (1959): " Interesting new advances have been reported from Iranian Kurdistan. It was formerly believed that only three species of *Meriones* were present in the focus and that all were resistant to plague. It is now known that *M. tristrami* is not a subspecies of *M. shawi*, but an independent species existing in the area under investigation. It was also demonstrated that *M. libycus erythrourus* included two species, one of

[1] A revised list of rodents other than the commensal rats and mice known to be naturally plague-infected, which has been checked by the WHO Expert Committee on Plague (1959) and by some additional members of the WHO Expert Advisory Panel on Plague, is appended to this review (see Annex 1, page 387).

[2] See note on page 401.

[3] Recent studies on potential plague hosts and vectors in Tanganyika by Halcrow (unpublished working document WHO/Plague/35) and observations on plague manifestations in Northern Rhodesia and adjacent areas by Davis, Fisher & Goldring (see note on page 405), though furnishing valuable material for further work, did not yield definite new information on rodent plague in the areas concerned. The same is true of an earlier contribution by Worsfold (1955).

TABLE 4

WILD RODENT FOCI IN THE SOVIET UNION ACCORDING TO RALL (1958b)

Location	Rodents mainly involved	Principal fleas	Remarks
Pamir/Tien Shan area	*Marmota baibacina, M. caudata*	*Oropsylla silantiewi (Rhadinopsylla ventricosa, Citellophilus lebedewi)*	Recently plague also found in *Microtus gregalis* in the Tien Shan
Transbaikalia	*Marmota sibirica, Citellus dauricus, Ochotona daurica*	*Oropsylla silantiewi, Citellophilus tesquorum*	*Ochotona daurica* proved rather resistant to plague
Central Asiatic areas	*Rhombomys opimus* (with *Citellus fulvus* and *Meriones libycus erythrourus* as supplementary carriers)	*Xenopsylla* species	*Rhombomys opimus* proved resistant experimentally, but actually there was seasonal variation in the intensity of the epizootics
Volga-Ural area	In sandy stretches: *Meriones meridianus* and also *M. tamariscinus*	*Xenopsylla conformis, Nosopsyllus laeviceps* and some others	*M. wagneri* sometimes become involved and then are apt to spread plague to man
	In steppes: *Citellus pygmaeus*, Dipodidae	*(Citellophilus tesquorum, Neopsylla setosa)*	Also involved: *Citellus fulvus, C. major* and, in sandy enclaves, gerbils
Western Pre-Caspian area	*Citellus pygmaeus*	*Neopsylla setosa, Citellophilus tesquorum, Ctenophthalmus breviatus, Ctenophthalmus pollex*	Gerbils possibly play an important role in part of the area. Mice may become secondarily involved
Transcaucasian area	*Meriones libycus*	*Xenopsylla conformis, Nosopsyllus laeviceps*	Other gerbil species probably also involved in southern border region

which was *M. libycus erythrourus* and the other *M. vinogradovi*. Of these species, *M. tristrami* and *M. vinogradovi* are extremely susceptible to plague. This makes it clear that in the plague focus of Kurdistan there are not only resistant wild rodents (*M. persicus* and *M. libycus*), but susceptible ones (*M. tristrami* and *M. vinogradovi*). "

Turkey, Syria and Iraq. While recent investigations in Turkey, Syria and Iraq failed to confirm the existence of wild-rodent plague, it is noteworthy that a human plague strain isolated in the first-mentioned country near the Syrian border had, according to the observations of Özsan & Akyay (1954) quoted above, the same biochemical characters as the Iranian and Transcaucasian strains. These findings tend to support the view that all these foci form part of a large enzootic area where commensal rats are absent.

Northern India. As will be gathered from an article by Baltazard & Bahmanyar,[1] evidence for a primary role of field rodents has also been adduced in the northern provinces of India where, among the common species involved, one (*Tatera indica*) was found to be markedly resistant to the infection, while *Millardia* and *Bandicota* are markedly susceptible.

Java. According to the WHO Expert Committee on Plague (1959): " Researches in Java, using techniques like those employed in Kurdistan and the northern provinces of India, have demonstrated a similar epidemiological pattern [to that in the last-mentioned foci]. The permanent reservoir is a rodent now identified as *R. exulans* which, like *Tatera*, is markedly resistant to plague . . . The distribution of this rat coincides with the areas of endemicity."

[1] See page 169

USA. According to the WHO Expert Committee on Plague (1959): "As a result of recent studies in the western part of the United States, emphasis has shifted from the larger wild rodents, such as the ground squirrels, to species such as *Microtus* and *Peromyscus*. On account of their small size and ecological inconspicuousness, insufficient attention has been paid to these species in the past. Recent investigations have confirmed that they are markedly resistant to plague infection."

Mexico. Owing undoubtedly to a spread of the infection from adjacent parts of the USA, the presence of plague was recently established in Mexico in the prairie-dog, *Cynomys mexicanus* (Varela & Vázquez, 1954).

South America. Dealing with plague in Peru, Macchiavello (1957b) maintained that the comparative rarity of plague in Cricetinae was probably only apparent, because—in contrast to the rather resistant *Sciurus stramineus*—these rodents succumbed to acute plague and were then immediately devoured by birds of prey.

Comparative importance of the species involved. As will be gathered from the information furnished above and from Annex 1, numerous species or subspecies of wild rodents as well as several Lagomorpha have been found to suffer from plague, almost invariably more than one species of these animals being found involved in one and the same area. While it is generally agreed that these different species or subspecies do not play a uniformly important role in harbouring and perpetuating the infection, the question of their relative importance has remained the subject of much debate.

In the past most authors were inclined to consider the rodent species among which widespread acute epizootics were observed as the main reservoir of the infection, and this view is still being advocated by some modern writers. However, following the lead of a few early observers, many modern plague workers [1] maintain on the contrary that rodents resistant to plague rather than those apt to fall prey to acute forms of the disease function as the true reservoir of the infection.

While there can be no doubt that rodent species which are less susceptible to infection with *P. pestis* are apt to play an important role in the perpetuation of plague, for various reasons the influence of this factor should not be overrated. Various mechanisms are at work which tend to limit the progress, and consequently favour the persistence of plague even in susceptible species. Thus it must be realized that often the wild rodents live in colonies more or less separate and distant from each other, so that plague among them may remain restricted to the " pockets " of infection which form such a characteristic feature of enzootic plague. Of equal importance is the fact that the plague susceptibility or resistance of a given rodent species is not uniform under all circumstances, but is subject to change for various reasons. Thus even susceptible species may show seasonal variations in the readiness with which they fall prey to the infection. In particular, experimental evidence has been adduced by several observers that hibernation, to which several of the wild rodent species apt to suffer from acute epizootics are subject, may be instrumental in carrying over the infection from one plague season to the next, because animals which had become infected immediately before or during their winter sleep may develop a peculiar latent type of plague which may flare up after they have awakened in spring. There can be no doubt that even among the susceptible species plague is prone to attack particularly the young animals, while the adults prove more refractory to the infection. Further, even among the plague-susceptible species there exist different races markedly distinct in the proneness with which they are apt to contract plague. Such differences may also exist in the case of the plague-resistant species, among which—as noted by Baltazard and co-workers (1953) in the case of the *Meriones* [2]—susceptible and refractory animals may even live side by side in the same colonial burrows, so that:

" before infection one always finds enough sensitive rodents to permit its recrudescence and enough resistant rodents to permit its continued existence; in this way there becomes established in these permanently inhabited burrows a real rotation between sensitive and resistant *Meriones* and the fleas, the latter seeming to play the principal role in the preservation of the infection". [Trans.]

One may conclude, therefore, that owing to the interplay of the various factors enumerated above, a kind of balance is apt to become established which renders possible the survival of the plague bacillus as well as of the rodent herds it has invaded. It is,

[1] Specially noteworthy observations have been recorded recently by Bacon & Drake (1958); Baltazard et al. (1953) and the article on page 169; Heisch et al. (1953); Holdenried & Quan (1956); Kartman et al. (1958a, 1958b); Meyer (1955, 1957); Olitzki (1955); Quan & Kartman (1956). Pertinent findings reported in the Soviet Union are summarized in Rall's book (1958b).

[2] According to Rall (1958b), analogous observations among gerbil populations have been made also in the Soviet Union.

however, important to note that the equilibrium which may thus become established in the " natural " plague foci is apt to be upset by man and his works. Indeed, Mironov, in a recently published article (1958), ascribed the disappearance of a focus of this kind in the steppes of South-east Europe to agricultural activities. However, like practically every other general rule appearing to be valid in regard to plague, this is not without exceptions, Frank (1953), for instance, noting that an improvement of low-lying soggy districts and moorlands created favourable conditions of life for field-mice.

Commensal rats

Distribution of species. In a note on the classification, origin and distribution of commensal rats, submitted to the WHO Expert Committee on Plague, Schwarz [1] maintained that both black and brown rats (*R. rattus* and *R. norvegicus*) were derived from wild forms of which *Rattus rattus* L. was typical. Both the *roquei-diardii-rattus* series and the *argiventer-norvegicus* series thus developing " now have an almost world-wide range, although the *rattus* (black rat) series is generally more southern, the *norvegicus* (brown rat) series more northern in distribution. In the tropics *norvegicus* is found sporadically, and only along the coast in cities, never far inland. For instance, it is found in Bombay but not in Delhi, in Capetown but not in Pretoria or Johannesburg, in Mombasa but not in Nairobi."

The *rattus* series of commensals included (*a*) indoor, domestic commensals with grey bellies, and (*b*) white-bellied outdoor peridomestic varieties.

" Rats of the black group, " Schwarz continued, " both grey-bellied and white-bellied, are the common rats on ships, where the impossibility of segregation has produced mixed populations. The Norway rat is uncommon on ships, but does become ship-borne occasionally, which explains its occurrence in harbours all over the world. The small Polynesian rat (*exulans*) has been spread by native boats into the Pacific."

In Schwarz's opinion, " the grey-bellied domestic commensals are the most important contacts for human plague. The white-bellied peridomestic commensals and the *norvegicus* series maintain the contact between infected domestic commensals and human plague on the one hand and the wild animal reservoirs—i.e., the sylvatic (animal) type of plague—on the other. "

Referring again recently to the much-debated question of the date of arrival of *R. norvegicus* in Europe, Loosjes (1956) postulated that this species had existed there much for longer than usually assumed. [2] In this connexion he pointed out with great reason that, having really distinct biotopes, the brown and the black rats can, and often do, co-exist in the same vicinity, and also that the comparative frequency of the two species in the areas of their co-existence is in a state of constant flux. While in the nineteenth century conditions became unfavourable for *R. rattus*, changes in building methods and other developments once more proved propitious for this species. Therefore its recent spread is not solely due to immigration (as is sometimes postulated) but also to the extension of locally existing populations.

However, true as this undoubtedly is, it would be wrong altogether to ignore the importance of an influx of *R. rattus*. Thus Przyborowski (1948) ascribed the great increase in the proportion of Norway rats, observed in Poland during and soon after the Second World War, to a diminished importation of *R. rattus*, owing to a restriction of shipping.

These observations illustrate that many factors, often those of a local nature, may thus come into play to determine the relative frequency of the two rat species in a given locality at a given time.

In an interesting article on the changing pattern of rodents and fleas in Calcutta and other cities, Seal [3] recorded that:

(*a*) In Calcutta, which in general always had a low proportion of *R. rattus*, a further decrease of this species as well as a reduction of *R. norvegicus* had been observed, with a corresponding increase of *Bandicota bengalensis* to nearly 80%.

(*b*) In Bombay the proportion of *R. rattus* had fallen from 79% to 22.9%, whereas *B. bengalensis* had increased from 1% to 40%.[4]

(*c*) While *R. rattus* and *R. norvegicus* had remained highly resistant to plague in Bombay, they were only partially resistant in Calcutta.

(*d*) *B. bengalensis* was fully susceptible to plague.

In spite of the increased frequency and high susceptibility of the last-mentioned species, the

[1] See page 411.

[2] This postulation does not seem to be in accord with the views of a great authority like Elton (1954), who spoke of the arrival of *R. norvegicus* in Great Britain not earlier than the early or middle part of the eighteenth century.

[3] See page 293.

[4] Allusion to an increasing prevalence of *Bandicota bengalensis* in Bombay had been made in 1956 by Deoras & Tonpi.

common rats were implicated in the plague manifestations in Calcutta. The density of *R. rattus*, though low in general, was high in the wards severely attacked by plague, and the seasonal peak in the prevalence of this rodent species coincided with the plague peak in April. Both *R. rattus* and *R. norvegicus* showed a higher *X. cheopis* index than *B. bengalensis*. It is not surprising, therefore, that 80% of the rats found to be plague-affected in Calcutta belonged to the two species of common rats. Seal's conclusion was that " *R. rattus* and among the fleas *X. cheopis* are the two important elements (reservoir plus vector) in urban human plague infection in India. If either of them can be kept down, the risk of human plague can be reduced to a minimum."

Influence of density of populations. Following up fundamental studies on the characteristics of rat populations published in 1951 and 1953, Davis [1] adduced experimental evidence which (*a*) made it clear that a high density of rodent populations through physiological mechanisms affected reproduction and mortality, and (*b*) also suggested that density of the rodents altered their resistance to some pathogens. Hence, Davis et al. postulated, " it is apparent that the density of rat populations may influence the course of plague. In nature plague, of course, occurs at high densities. However, it may be that the rats are less resistant at such densities and thus accentuate an epidemic or permit an epidemic even at a low population of fleas."

In an excellent study devoted in particular to commensal mice, Lisitzin (1949) stated that the main factors favouring or restricting the numbers and fertility of these rodents, which in South-east Russia not rarely lived away from the houses or even settlements, were the following:

(*a*) Meteorological factors, which acted upon the number and fertility of the mice both directly and indirectly by influencing the vegetation;

(*b*) The amount and quality of the food supply;

(*c*) The activity of natural enemies of the mice which, however, exerted a direct influence only on the population density, and not upon the fertility;

(*d*) The presence of ectoparasites and endoparasites, which, however, was the outcome of a high rodent density rather than a cause of low population density, thus playing no substantial role as a population regulator in normal years;

(*e*) Human activities, such as a proper system of agriculture, eradication of weeds, rapid and adequate harvesting, proper storage of food and fodder, sanitation of human houses and compounds and, finally, a system of rodent destruction, were of great importance in restricting the size of rodent populations.

Petrov (1956) confirmed that the presence and density of *M. musculus* and other small rodents on cultivated ground (fields, gardens, etc.) stood in close relation to the intensity of agricultural operations, lessened activity on the part of man favouring a most marked increase in the number of rodents.

That this rule is not, however, without exceptions, in the case of commensal as well as of wild rodents, is proved by the observations of Steiniger & Knothe (1951) in Schleswig-Holstein; these showed that greater dryness of the ground, brought about by cultivation in combination with climatic changes, created favourable conditions for the rats, some herds of which were thus enabled to live the whole year round in the open. Extermination of the natural enemies of the rodents, particularly the birds of prey (many of which were killed by devouring poison baits or poisoned rodents), as well as the absence or inadequacy of rodent campaigns, also created favourable conditions for the rats and other rodent species.

Movement range. Recent studies on commensal rats and also on the free-living *R. hawaiiensis* (see Kartman & Lonergan, 1955b), have confirmed that under normal conditions rats move within a very narrow orbit. Pertinent observations undertaken in an urban environment (Calcutta) by Bhattacharji & Seal (1954) [2] showed that: (*a*) rats removed from their habitat had a tendency to go back to their home colonies, even if they had to cover long distances for this purpose; [3] and (*b*) though there was considerable intermingling of neighbouring colonies, ordinarily the distance covered by the rats did not exceed 50 yards and was never more than 200 yards. In agreement with these observations it was found that " the larger percentage (61.4 to 72.0%) of confirmed human cases could be explained on the basis of rat deaths detected between 50-200 yards, and 22.2% beyond 200 yards; only 10.4% remained unexplained ". Therefore, whenever plague-affected rats were detected, it was necessary to apply intensive control measures within an area of 200 yards.

[1] See note by Davis, Fisher & Goldring on page 405.

[2] See also article by Seal on page 293.

[3] It is, however, significant that only 7 out of 1905 rodents thus released were recaptured near their homes.

Studying the population dynamics of sewer rats (*R. norvegicus*), Barnett & Bathard (1953) were inclined to ascribe the rapid restoration of the numbers of these animals after a poisoning campaign not only to increased breeding, but also to an infiltration of adult rats. It is, however, important to note that, notwithstanding this observation made under exceptional circumstances, generally speaking there is but a poor chance for alien rats to become established in a new, already rat-populated territory (Davis, 1953).

Interrelation between wild-rodent and rat plague

As confirmed by recent plague studies such as those of Baltazard & Bahmanyar,[1] Davis (1953), Heisch and co-workers (1953), Kartman & Lonergan (1955b), Kartman and colleagues (1958a, 1958b) and also by interesting observations on the occurrence of "non-synanthropic" species of rodents in Moscow by Pojarkov (1956), owing to much variation in the species involved and to differences in ecological conditions in general a contact between wild and commensal rodent species may be effected in so many different ways as to preclude the framing of any generally valid statement. Both commensal rodents, temporarily leaving the settlements, and wild rodents coming or living near these or even entering them, may act as a means of contact between the various rodent species and consequently as links in the transmission of plague, effected through infected parasites, particularly fleas, of the primarily affected species (*pestis murina campestris progressiva* and *pestis campestris regressiva*, according to the concepts of Macchiavello (1955) mentioned earlier).

OTHER HOSTS

Writing in 1954, Pollitzer, while giving credit to reports from the plague foci in South-east Russia which more or less definitely incriminated the camels as hosts of the infection, pointed out that these claims had met with considerable scepticism on the part of some of the West European reviewers. However, as shown by the evidence adduced in a report by Fedorov [2] which, being published *in extenso* together with the present review, requires no detailed discussion here, the possibility that camels may contract plague has been confirmed through ample further experiments. It is particularly noteworthy that, according to Fedorov:

"The mechanism by which camels are infected does not differ from that observed in other plague-susceptible animals or in man. Their infection occurs as a result of an attack by blood-sucking rodent ectoparasites, primarily fleas. In exceptional cases they may be infected through the bites of *O. tartakovskyi* ticks which have not long before sucked the blood of a plague-sick rodent."

Vaccination of camels with the living vaccine 1.17, given subcutaneously in a single dose of 30 000 million organisms, was found to induce an adequate immunity against plague in adult camels.

In an article of which the original was not available to the present writer, Piedrola Gil (1956) referred to a disease present in 1953 in a large part of the Spanish Sahara and causing a great mortality among camels. Though the signs of this affection were said to resemble those of bubonic plague, the fact that goats and gazelles as well as rodents were involved rather speaks against the presence of this disease.

INSECT VECTORS

FLEAS

Prevalence

To supplement the flea list appended to this review (see Annex 2, page 397), the following recently published reports on the prevalence of fleas actually or potentially involved in the transmission of plague deserve attention:

North Africa. In the 1952 report of the Institut Pasteur de l'Afrique Occidentale Française, information on which was not available to the present writer at the time of compilation of his book *Plague*, published in 1954, it was pointed out that in the Dakar and St. Louis districts *Pulex irritans* had replaced *Synosternus pallidus*. However, the latter flea was apparently still prevalent at Thiès (Senegal) and was abundant in the regions near the Niger.

Making a special study of the fleas on *Cricetomys gambianus*, Larivière & Abonnenc (1957) found that in Dakar this rodent harboured *Xenopsylla aequisetosa*, *Ctenocephalides felis felis* and *Echidnophaga gallinacea*.

Kenya. Heisch and co-workers (1953), reporting on the results of flea examinations made in the course of their plague investigations in Kenya, stated that in the active

[1] See article on page 169.

[2] See article on page 275.

focus of Rongai *X. cheopis*, which was predominant on wild as well as on commensal rodents, was evidently the chief transmitting agent. However, *P. pestis* was also isolated from *Dinopsyllus lypusus*. Heisch and his colleagues postulated therefore that, contrary to previously held beliefs, this flea " will prove to be an important vector of wild rodent plague in Kenya and perhaps elsewhere in East Africa; the flea is large and robust and may be able to withstand arid conditions and to harbour plague for long periods ". In the old, not active focus of Keruguya, *X. brasiliensis*, though predominating on *R. rattus*, was not found on the wild rodents. It was also significant that 19 flea-pools, derived from 1564 *X. brasiliensis*, proved negative for *P. pestis*. On the other hand, when revisiting the Rongai focus after about a year, Heisch and his associates were able to isolate plague bacilli from two batches of fleas (consisting almost exclusively of *X. cheopis* and *Dinopsyllus lypusus*) collected respectively from *Arvicanthis* and *R. natalensis*. Since during their second survey on two occasions they also obtained positive results with tissue pools from *R. natalensis* and *Otomys angoniensis* respectively, Heisch and co-workers felt entitled to conclude that wild rodents and not *R. rattus* were the primary plague reservoir in Kenya.

South Africa. The interesting conclusions reached by Davis (1953) regarding the important role played by *Xenopsylla philoxera* in the plague manifestations of South Africa will receive due attention in the section dealing with the perpetuation of plague in the fleas.

Israel. Examining a total of 1066 rats (773 *R. norvegicus* and 293 *R. r. alexandrinus*) in the port of Haifa, Gratz (1957) found *Xenopsylla cheopis* to be by far the most prevalent ectoparasite. The index of this flea on the 42 Norway rats examined in August 1954 was 32.4.

India. As stated in an article by Baltazard & Bahmanyar,[1] examination of numerous fleas collected from the field rodents and from *R. rattus* in Uttar Pradesh showed that:

(*a*) *X. cheopis* was completely absent from the field rodents.

(*b*) A special flea, *Xenopsylla hussaini*, was found on these animals in one of the districts.

(*c*) *Xenopsylla astia* and *Nosopsyllus punjabensis* (the " *Ceratophyllus fasciatus* " of the Indian Plague Commission) were common to field and commensal rodents and numerous on both.

The presence of natural plague infection was proved in *X. astia* and *Nosopsyllus punjabensis* and also in batches of *Xenopsylla* (presumably comprising *X. cheopis*) and of *Ctenocephalides felis felis* trapped in the houses of a plague-infected village. While, as will be discussed later, the vector capacity of *X. astia* could be retested, that of *X. hussaini* could not be determined.

[1] See page 169.

Judging from small samples, Deoras & Tonpi (1956) stated that *R. rattus* as well as *Bandicota bengalensis* in Bombay were infested by both *X. cheopis* and *X. astia*.

A study of the " domestic " fleas in Calcutta led Bhattacharji (1954) to the conclusion that, the prevalence of *R. rattus* and *X. cheopis* being low, the commensal rats and fleas were principally represented by " *Gunomys varius* " (i.e., *B. bengalensis*) and *X. astia*, respectively. The predominance of these species was probably the reason why plague failed to get a real foothold in the city when it reappeared after a lapse of 50 years.

The findings of Seal,[2] to which reference has been made above, were in accord with the opinion that *X. astia*, even though generally more prevalent in Calcutta than *X. cheopis*, played no role in the spread of plague in the city.

Soviet Union. Data on the flea species found naturally plague-infected in the Soviet Union will be found in Annex 2 (see page 397). Moreover, reference to the principal flea species involved according to Rall (1958b) in the individual plague foci of the Soviet Union has been made in the preceding section of the present study.

Some observers in the Soviet Union, for example, Boshko (1956) and Ioff (1957), have drawn attention to the transport of rodent fleas by dogs, while, as quoted by Rall (1958b), Ter-Vartanov and associates (1954, 1956) have dealt with the role played in this respect by birds.

USA.[3] Exhaustive studies on the occurrence and comparative importance of wild rodent fleas were made in the course of thorough ecological investigations in a plague-affected area in Central California by Miles and associates (1957), Murray (1957), Kartman and colleagues (1958a, 1958b) and Stark et al. (unpublished data, quoted by Kartman et al., 1958b). Summarizing these findings, Kartman and his colleagues (1958b) stated that:

" Of several field rodents, the vole *Microtus californicus* is thought to be the chief plague reservoir because of its high degree of resistance to *Pasteurella pestis* and the fact that it was found to be parasitized by the important vector species as well as by more than 90 per cent of all fleas of all species taken in the area. The fleas *Hystrichopsylla linsdalei* and *Malaraeus telchinum*, the two most prevalent species, were both found to have relatively high natural plague-infection rates during epizootic incidents. Of the two, *H. linsdalei* is an efficient vector by blocked individuals, and *M. telchinum* transmits primarily by mass mechanical means. The former is probably the primary plague vector in this

[2] See article on page 293.

[3] Recent observations on fleas in the USA that have no direct bearing on the problems of plague were undertaken by Pratt & Good (1954) and by Smith (1955). A study of the ectoparasites of Richardson's ground-squirrel in the southern part of Saskatchewan, Canada, was made by Burgess in 1955, but no evidence of the existence of plague was found. Five new fleas of the genus *Thrassis* have been described by Stark (1957).

region, whereas the latter may be a secondary vector of importance."

A study of the seasonal prevalence of the fleas of the antelope ground-squirrel (*Citellus leucurus leucurus*) in the Great Salt Lake Desert of Utah by Parker (1958) showed that: (*a*) the most abundant flea was *Thrassis bacchi gladiolis* which, though found the whole year round, was most frequent during the cool season; (*b*) *Hoplopsyllus anomalus*, next in frequency and like the last-mentioned flea having been found naturally plague-infected elsewhere in the USA, was more prevalent during the warm season; (*c*) *Rhadinopsylla heiseri* could be collected in moderate numbers only in winter; and (*d*) other fleas, including one *P. irritans*, occurred only in insignificant numbers.

Investigating in 1955 an epizootic which involved primarily mice (*Peromyscus* and *Reithrodontomys*), Holdenried & Morlan (see also Morlan, 1955) isolated plague bacilli from six species of fleas (*Hoplopsyllus anomalus*, *Monopsyllus wagneri*, *Orchopeas leucopus*, *O. sexdentatus neotomae*, *Peromyscopsylla hesperomys adelpha*, and *Stenistomera* (*Miochaeta*) *macrodactyla*), collected from: *Citellus variegatus*, *Neotoma albigula*, *Peromyscus leucopus*, *P. maniculatus*, *P. truei*, and *Reithrodontomys megalotis*. It is interesting to note that the disappearance of infected fleas was not correlated with a depletion of the rodent population, but coincided with the gradual reduction in the number of *P. truei* infested with *Monopsyllus wagneri*, as well as with the beginning of the summer high temperatures.

Mexico. In a preliminary note on a flea survey made in the area in which Varela & Vázquez (1954) had proved the existence of plague in *Cynomys mexicanus*, Barrera (1956) recorded that this rodent had been found to harbour a few *Opisocrostis* fleas, but mainly *Pulex irritans dugesii* Baker. It thus seemed that the conditions for the persistence of plague enzootics in Mexico and the possibilities for an occasional spread of the infection to man markedly differed from those in the adjacent parts of the USA.

South America.[1] In a recent study on plague in Huancabamba, Peru, Macchiavello (1958a) stated that in addition to occurring in the known vectors—*Polygenis litargus* and *Pleochaetis dolens quitanus*—natural plague had been found in *Cediopsylla spillmanni*, *Hoplopsyllus andensis*, *Hoplopsyllus manconis*, *Polygenis brachinus* and *Sphinctopsylla* (*Plocopsylla*) *mars*. As far as frequency was concerned, a preponderant role was played apparently by *Pl. dolens quitanus*. Though it had a great tendency to stay in the nests rather than on its hosts, this flea seemed responsible for the transmission of the infection to man in the settlements as well as in the fields.

[1] Complete annotated lists of the vectors as well as of the reservoirs involved in the plague outbreaks in the various South American countries will be found in a catalogue published by Macchiavello in 1954. Corresponding lists for the north-east of Brazil have been published in a note by de Freitas (1957).

Ecological observations

In view of the wide practice in the Soviet Union of destroying wild rodents with poison baits, it is not surprising to find that great attention continues to be paid to the fate of fleas in burrows no longer inhabited by the hosts.

Recording experimental observations in regard to the small sisels (susliks), Mironov and co-workers (1949b) stated that, though during the first 10 days after the burrows had become uninhabited, an extensive migration of the fleas took place, thereafter " the flea index, while continuing to fall for a long time, maintains itself at a very low level, evidently up to the time of the natural filling-up of the burrows ". The logical conclusion was that flea eradication had to be combined with rodent destruction—an opinion also expressed by Ioff (1957).

Further observations on susliks led Gonchar and his colleagues (1956) to the following conclusions:

" 1) The eradication of the small susliks through bait-poisoning led to an active migration of the ectoparasites from the nests, and this led to an increase in their frequency in the mouths of the burrows.

2) The increased frequency of the burrow ectoparasites in localities freed from susliks was maintained for a long period—up to 12 months. According to our observations this length of time is not final.

3) That the fleas which had migrated from the burrow nests could sometimes obtain partial nourishment from the blood of other animals facilitated to a certain extent their survival under the prevailing unfavourable conditions. The possibility that the fleas may survive in the habitations of the susliks until reoccupation by the latter cannot be excluded." [Trans.]

Accordingly Gonchar and his associates also insisted upon combined anti-rodent and anti-flea campaigns.

It is important to add that according to the experimental observations of Ivanov (1956), plugging of the suslik burrows with earth did not prevent an emigration of the fleas from the nests.

Observations similar to those of Gonchar and associates were recorded by Darskaya (1955b) in the case of *Xenopsylla gerbilli caspica*, a flea of *Rhombomys opimus*.

Interchange of fleas

Noteworthy recent observations that further testify to the frequency and the diverse character of the interchange of fleas between the various rodent species may be summarized as follows:

Kenya. In the course of their plague investigations in Kenya, Heisch and his associates (1953) noted the presence of *X. cheopis* on wild rodents as well as on *R. rattus*, from which, on the other hand, the wild-rodent flea *D. lypusus* could be collected.

South Africa. Davis (1953), studying the above-mentioned focus in South Africa, found evidence of a transition of the infection from *Tatera* to *R. natalensis* and *R. rattus* and also recorded an observation suggesting that *X. brasiliensis* might be instrumental in bringing back plague from the rats to the multimammate mouse and possibly also to the gerbils.

Soviet Union. Following up earlier studies by Mironov and co-workers (1949a) in the semi-deserts of the Pre-Caspian region, Shiranovich & Mironov (1956) obtained evidence of an exchange of fleas between different rodents, including the commensal mice, and concluded, therefore, that pathogenic organisms could be passed in different directions and could attack different groups of the animals at all seasons of the year. Proof of an interchange of fleas between commensal mice and field-mice (*Microtus arvalis*) was obtained by Demin & Demjashev (1956), while Belkina & Korchevskaya (1956), working with the steppe lemming (*Lagurus lagurus*), found its flea fauna to comprise, besides the species peculiar to it, species associated with other rodents, including mice and sisels (susliks).

India. As noted earlier, Baltazard & Bahmanyar [1] found that in the plague area of India studied by them: " Two [flea] species were common to the rats and to the field rodents and were as numerous on the one as on the other: *Xenopsylla astia* and *Nosopsyllus punjabensis* ". [Trans.]

Thailand. Dealing with a plague outbreak in Thailand, Elbel & Thaineua (1957) recorded that: " The field rodents, *Rattus rattus* subsp., *Bandicota indica* subsp., and *Bandicota* sp., whether trapped in the houses or in the fields near villages, were often infested with *X. cheopis* in small numbers, as was also *R. exulans* when trapped in the fields . . . *X. cheopis* has been found occasionally on the ground squirrel, *Menetes berdmorei* subsp., the tree shrew, *Tupaia* sp., and the forest rat, *Rattus rajah* subsp."

USA. Important observations on the exchange of fleas between different rodent hosts were made in the course of recent plague studies in Central California, to which reference has been made above. Miles and colleagues (1957), discussing the findings they had made in this respect, stated that their observations " would appear to minimize the chance for plague transfers between ground squirrels and other rodents. The frequency of flea interchange here indicated between smaller rodent species, and between them and commensal rats, suggests a potential role of primary importance for native field mice in plague epidemiology. Of particular concern in the areas studied was the flea interchange convincingly shown between *M. californicus* and *R. norvegicus*. It appears evident that *Microtus* was the principal source for *M. telchinum* found on *Rattus* since the occurrence of *M. telchinum* on *Rattus* was generally proportional to the occurrence of associated *Microtus*. This was particularly apparent where occasional rats strayed into a predominantly wild type habitat. Apparent transfers of rat fleas, *N. fasciatus*, were evidenced by their occurrence on atypical hosts following a decline of rat populations. *C. wymani* may be important because of its wide choice of hosts, and its relatively high rate of occurrence on rats. *A. multidentatus* and *Hystrichopsylla* sp. may be implicated in plague transfers among 3 small wild rodent species on which these fleas appeared with considerable indiscrimination."

Referring to the subject at present under review in their recent study, Kartman and co-workers (1958b) emphasized that further evidence for a flea exchange had been obtained by Hartwell and others (1958) by tagging fleas with cerium-144 according to the method of Quan and associates (1957) mentioned above. Commenting upon these observations, Kartman and his colleagues (1958b) stated: " These preliminary experiments are not conclusive, but they suggest that a wild-rodent flea, *Malaraeus telchinum*, will readily transfer between individuals of its normal host species, whereas transfer to domestic rats may take place only under unusual circumstances. Nevertheless, the transfer of wild-rodent fleas to rats from dead wild-rodent hosts is of special significance when applied to the problem of plague spread from sylvatic to domestic environments . . . Undoubtedly, other important wild-rodent flea vectors of plague will also transfer to rats."

Role of Pulex irritans

Reiterating claims which he had made in the past, Blanc (1956) postulated that human parasites, particularly *P. irritans*, played the main role in the spread of human plague. Evaluating this thesis, one must admit that in areas like Morocco where, as confirmed by Swellengrebel (1953), thick layers of clothing and lack of cleanliness tend to increase human infestation with ectoparasites, *P. irritans* is apt to take an important part in the transmission of plague, the high incidence of this species compensating for what it lacks in vector capacity.[2] At the same time, however, it is certain that in other plague areas, for example, China, India and also Madagascar, the role of this flea is negligible, the

[1] See article on page 169.

[2] It is interesting to add that, as recently discussed in an excellently documented article on plague in Venice in 1575-77 by Rodenwaldt (1953), *P. irritans* evidently played a most important role in the spread of the mediaeval plague epidemics in Western Europe. Rodenwaldt maintained that these outbreaks ceased when wooden houses were supplanted by stone ones.

transmission of the infection depending upon the rat fleas, particularly *X. cheopis*. Plague is a disease of so protean a character that it would be misleading to generalize the results of observations in one or a few areas, however suggestive they appear to be.

Vector efficiency

Observations made in regard to the plague-vector efficiency of individual flea species, usually in comparison with *X. cheopis*, may be summarized as shown in Table 5.

Using the apparatus of Kartman (1954) for the experimental feeding of fleas, Quan and his colleagues (1954) and again Kartman, Quan & McManus (1956) were successful in producing blockage in fleas (first with *X. cheopis*, then also with *X. vexabilis hawaiiensis*) with an avirulent plague strain. Cavanaugh and associates (1956), directly feeding numerous fleas (*Monopsyllus anisus* and *X. cheopis*) on white mice to which avirulent plague bacilli had been administered parenterally, could produce no mass infection in these ectoparasites, but could occasionally isolate *P. pestis* from their gastrointestinal tracts, in two cases from *X. cheopis* which showed evidence of blockage. Taking advantage of the simplified apparatus for the experimental feeding of fleas devised by Wheeler and co-workers (1956), Suyemoto and colleagues (1956) were able not only to produce blockage in *X. cheopis* with the avirulent strain used by Kartman and his associates, but also in some instances to transmit the organisms by means of fleas which had ingested them.

As summarized by Kartman and colleagues (1958b), in the studies of Kartman, Prince & Quan (1956) and Kartman & Prince (1956), in addition to the infection potential, the vector potential and the transmission potential hitherto used to determine the plague-vector efficiency of fleas, a further factor, the blocking-survival potential, was taken into account in deriving a vector index. This new potential was " based on a ratio of the mean day of death of fleas after blocking over the mean day of blocking after the infectious meal " and was thus the greater, the shorter the incubation period and the longer the survival after blocking.

In a further study, Kartman (1957) proposed a still more elaborate model for the determination of the plague-vector potential of fleas,

" in which components from field data are used in conjunction with the experimental vector index. Vector potential is reflected by an index relating three components in the following manner:

(experimental vector index) × (field infection index) × (field prevalence index) × 100."

TABLE 5

PLAGUE-VECTOR EFFICIENCY OF INDIVIDUAL FLEA SPECIES

Authors	Flea species tested	Observations
Kartman, Prince & Quan (1956)	*X. cheopis* and *X. vexabilis hawaiiensis*	*X. cheopis* was found to be about twice as efficient in plague transmission as the Hawaiian flea, bred in the laboratory according to a method described by Stark & Kartman (1957).
Kartman & Prince (1956)	*X. cheopis*, *Leptopsylla segnis* and 11 North American wild rodent fleas	" None of the species tested approached the high vector efficiency of *X. cheopis*. Of the wild rodent fleas used, *Thrassis bacchi johnsoni* and *Hystrichopsylla* sp. had the highest vector efficiencies."
Kartman et al. (1958c)	*X. cheopis* and *Hystrichopsylla linsdalei*	" The lower efficiency of *H. linsdalei* under the conditions of the experiment is partially ascribed to a lower blocking rate and a shorter survival period for the blocked individuals. Thus, the wild rodent flea had fewer opportunities to transmit *P. pestis* than the rat flea."
Macchiavello (1958b)	*Polygenis litargus*	The vector potential of *Polygenis litargus*, according to transmission experiments with *Sigmodon*, *Cavia* and *R. rattus*, was found to be variable, this flea seeming to favour the first-mentioned rodent, its natural host. Still, its resistance to untoward environmental conditions, its longevity and its ability to feed on different hosts should be taken into account.
Baltazard & Bahmanyar [a]	*X. astia*	" ...the experiments with *X. astia* on its field hosts, though showing that this flea was fully capable of preserving the plague bacillus and, if heavily infected, of transmitting it easily from rodent to rodent, nevertheless confirmed the results previously obtained with rats regarding the comparatively mediocre vector efficiency of this flea if it was lightly infected, that is to say, infected not at the time of an epizootic."

[a] See article on page 169.

Role of fleas in perpetuation of plague

That, besides transmitting the infection, rodent fleas play a decisively important role in the perpetuation of plague has been reaffirmed by the following observations:

Baltazard and co-workers (1953), referring to the carriage of plague-infected fleas by *Meriones* resistant to the infection, stated that:

" If one knows, as we will show in the experimental part of this work, that such *Meriones* can be authentic ' healthy carriers ' of infected fleas, preserve an intact receptivity and die in their turn from plague if the conditions change; that *Meriones* of less marked individual resistance, put in contact with the former, can succumb to plague contracted by their fleas; then the persistence of plague in the resistant species with permanent burrows appears easily explainable." [Trans.]

Observations on plague in the South African gerbils led Davis (1953) to the conclusion that their flea, *X. philoxera*, " is not only an efficient plague vector but also, it seems, more suitable than other gerbil fleas . . . to retain *P. pestis* for relatively long periods . . .[1] The combination of *T. brantsi* and *X. philoxera* evidently provides an ideal host-vector relationship for the survival of plague."

In agreement with these statements, Macchiavello (1957a, 1957b) laid stress upon the role of *Polygenis litargus* in the perpetuation of plague. He referred in this connexion to an observation showing that a lot of these fleas found in a tree trunk still proved infective for a subcutaneously injected guinea-pig after six months.

The important role of rodent fleas in the carry-over of plague has also been upheld in a general manner by Ioff (1957) and Mikulin (1957).

OTHER INSECTS

Ticks

As will be gathered from the article by Fedorov,[2] recent observations in the Soviet Union on the role played by ticks in the transmission of plague have led to the following results:

(*a*) Natural plague infection was found fairly frequently in " *Ixodes* and *Argas* ticks (*Ixodes crenulatus, Hyalomma asiaticum asiaticum, Haemophysalis numidiana turanica, Rhipicephalus schulzei, Rhipicephalus pumilio, Ornithodorus tartakovskyi* and *Ornithodorus alactagalis*) in all stages of metamorphosis ".

(*b*) Experimentally *P. pestis* could be transmitted by means of *Ixodes* ticks only through interrupted feeding —that is, the transference of the tick from a plague-infected to a healthy animal before it has finished its meal. Apparently, therefore, these ticks merely transmitted the infection through their contaminated mouth-parts (Afanasyeva & Mikulin, 1957).

(*c*) Similarly, *Argas* ticks transmitted plague only within the two days following a meal on a plague-infected animal (Burlachenko, 1958).

(*d*) Plague could be transmitted occasionally to camels through *O. tartakovskyi*, provided these ticks were used on the day on which they had had an infective meal or on the following day.

Ioff (1957), while maintaining in contrast to Fedorov that naturally plague-infected ticks were found comparatively rarely, pointed to the long persistence of the organisms in them.

Triatominae

Working with experimentally plague-infected Triatominae, Ames and associates (1954) found that:

(*a*) Plague bacilli remained infective in *Triatoma protracta* at 30°C for 3, but not for 5, days.

(*b*) Four adult *Triatoma phyllosoma* transmitted plague to a healthy mouse through interrupted feeding. Identical tests with five other *Triatoma* species gave negative results.

(*c*) " *Triatoma protracta* and *Mestor megistus* (Burmeister) infected with *Trypanosoma cruzi* Chagas and *Pasteurella pestis* (Lehman and Neumann) were fed to mice; 4 positive plague infections resulted one of which was pneumonic; 3 mice were negative. Thirteen other tests with plague-infected Triatominae fed to mice failed to transmit *P. pestis* through the gastro-intestinal tract, but produced 3 pneumonic cases ".

(*d*) Out of 17 specimens tested, *Triatoma platensis* alone was found to yield plague-positive faeces.

TREATMENT

LABORATORY OBSERVATIONS

Of the various laboratory studies on the treatment of plague made during the period under review, it seems suitable to refer first to the observations of Quan, McManus & Meyer (1955), because these authors compared the chemotherapeutic action of the sulfonamides, the first drugs used in the modern therapy of the disease, with that of the later-introduced antibiotics (or, as some authors now prefer

[1] As far as observed, up to four months.

[2] See page 275.

TABLE 6
TREATMENT OF PLAGUE-INFECTED GUINEA-PIGS WITH VARIOUS THERAPEUTIC SUBSTANCES *

Treatment given	Daily dose (mg)	Number of animals used	Number of animals that:		Length of survival (days)
			survived	died	
Biomycin	15	5	0	5	8.8
Biomycin + gamma-globulin	15	5	0	5	12.4
Bacteriomycin	15	5	4	1	15
Bacteriomycin + gamma-globulin	15	5	5	0	—
Gamma-globulin	0.2 ml	5	3	2	19
Streptomycin	15	5	5	0	—
Unconcentrated plague immune serum	0.2 ml	5	0	5	8.8
Controls	—	5	0	5	6

* After Semenova et al. (1957)

to call them, antimicrobial drugs). The main conclusions reached by Quan and his associates were that:

" *in vitro* chloramphenicol was about 20 times more potent than thiomycetin, 500 times more than sulfisoxazole, and 5,000 times more than sulfadiazine. Since the mechanism of action of the synthetic antibiotics differs from that of the sulfonamides, the two types of drugs are not comparable. This difference is illustrated by the fact that sulfadiazine proved to be the most effective among the chemicals for experimental plague prophylaxis in laboratory mice, whereas chloramphenicol was the best therapeutic agent. The *in vivo* order of efficacy was chloramphenicol, sulfadiazine, thiomycetin and sulfisoxazole " (which was probably the most rapidly excreted by the kidneys).

Important comparative studies on the value of different agents for the treatment of plague have also been made by McCrumb, Larson & Meyer (1953) and by Semenova and colleagues (1957). Working with monkeys, the first-mentioned observers obtained good results with early treatment with chloramphenicol, chlortetracycline or streptomycin, regardless of whether the animals suffered from bubonic or pneumonic plague. These therapeutic substances proved ineffective if treatment was commenced in the late stages of the disease, and the unfavourable outcome could not be averted by administration of cortisone or of hyperimmune plague serum. Indeed, administration of the latter in the form of concentrated rabbit globulin was apt to cause sudden death of the animals. A further interesting point was the emergence of streptomycin-fast mutants of *P. pestis* in two instances. In one of these the animal in question could be saved with the aid of chloramphenicol.

Semenova and her colleagues (1957) summarized the results they had obtained when treating plague-infected guinea-pigs with different therapeutic substances in the form shown in Table 6.

The conclusions reached by Semenova and her colleagues were that:

" 1. Out of the three experimentally tested antibiotics, two—bacteriomycin and streptomycin—proved efficacious. Bacteriomycin, like streptomycin, showed itself as a highly effective drug in experimental plague of guinea-pigs, whereas under these conditions no therapeutic action could be observed in the case of biomycin.[1]

2. The gamma-globulin fraction of anti-plague serum, proving to be more efficacious in comparison with the original anti-plague serum, is considerably less effective than the active antibiotics— streptomycin and bacteriomycin. Nevertheless, this circumstance does not yet permit one to exclude the possible usefulness of administering it in some cases in combination with antibiotics." [Trans.]

[1] Makarovskaya (1956c) maintained that biomycin retarded the multiplication of *P. pestis* in guinea-pigs but was too toxic for the animals to be therapeutically tested. She claimed, however, that the product was but little toxic for man. Unfavourable, or at least not fully satisfactory, results of laboratory observations were also recorded in the case of spiramycin by Brygoo (1955) and in the case of laevomycetin and synthomycin by Makarovskaya (1956b).

A large-scale study of the prophylactic and therapeutic action of streptomycin in guinea-pigs which had been infected with plague by various routes was undertaken by Makarovskaya (1956a). The drug was introduced at various intervals after infection and in different doses, either intramuscularly or intranasally, or by means of an aerosol apparatus.

If subcutaneous infection was followed by intramuscular administration of streptomycin, it was found that commencement of the treatment 4 to 5 days after infection proved insufficiently effective, even if doses up to 80 000 units were given. However, if treatment was started at intervals of from 6 hours to 3 days after infection, streptomycin doses of 20 000-80 000 units per day proved satisfactory. Still, continuation of this therapy for 7 days was necessary, even with daily doses of 40 000 units or more. It deserves attention in this connexion that in a number of instances the administration of insufficiently effective doses led to the appearance of streptomycin-fast strains.

Intranasally or intraconjunctivally infected guinea-pigs were given streptomycin by three different routes—intramuscularly, intranasally or through an aerosol apparatus. The first-mentioned method of treatment, used for a week in doses of 20 000-40 000 units daily for the treatment of intranasally infected guinea-pigs, gave markedly good results, even if started after generalization of the infection. Intranasal administration of such doses proved rather unsatisfactory, even if commenced soon after infection.

If aerosol treatment, consisting in the administration of 200 000 units during a period of half an hour, was commenced 30 minutes after intranasal infection, success was complete. There were some fatalities among the animals receiving such treatment 1-2 hours after infection, while it was impossible as a rule to save those whose treatment had been started 1-2 days after infection.

Aerosol treatment of conjunctivally infected guinea-pigs did not give satisfactory results, there even being a number of fatalities among the animals whose treatment had been started 30 minutes after infection. Intranasal administration of streptomycin to conjunctivally infected animals proved quite unsuccessful, but intramuscular treatment, if started at intervals of from 6 hours to 2 days after infection and continued for 7 days, gave fully satisfactory results.

Commenting upon these observations, Makarovskaya felt entitled to recommend aerosol administration of streptomycin for the prophylaxis of pneumonic plague in man.

A special study on the treatment of experimentally produced pneumonic plague was made by Netcheskaya (1956), who used for this purpose the technique for nasal infection of guinea-pigs also adopted by Makarovskaya (introduction of *P. pestis* suspensions through a tuberculin syringe into the nostrils of animals kept under light ether narcosis). To treat her experimental animals, Netcheskaya first resorted to the intramuscular administration of daily streptomycin doses of 130 000 units. If this treatment was commenced 12 to 20 hours after infection and continued for 4½ days, 40% to 60% of the guinea-pigs could be saved, while those which eventually succumbed survived twice as long as the controls. However, as shown in Table 7, far better results were obtained by combining intranasal and intramuscular administrations of streptomycin (daily dosages of 285 000 units for 7 days). [1]

TABLE 7

STREPTOMYCIN TREATMENT OF INTRANASALLY INFECTED GUINEA-PIGS *

Method of administration	Number of animals used	Number of animals that:		Average length of survival of dying animals (days)
		survived	died	
Intramuscular and intranasal	10	9	1 [a]	17
Intramuscular only	10	7	3	14.5
Intranasal only	10	5	5	12.5
Controls	10	0	10	3.9

* After Netcheskaya (1956)

[a] Did not succumb to plague.

It deserves attention that, however effective the above methods of streptomycin administration were, they did not engender a state of immunity in the cured animals, which invariably succumbed to reinfection with *P. pestis* by the subcutaneous route.

As noted earlier (see page 346), Speck & Wolochow (1957) found streptomycin effective for the treatment of monkeys in which pneumonic plague had been produced by means of aerosol spraying. Previous administration of plague vaccine to the test animals failed to exert an influence on these therapeutic results.

Comparing in subcutaneously infected guinea-pigs the results of treatment with streptomycin with

[1] The value of this combined method of streptomycin administration has also been shown by observations on intranasally infected guinea-pigs whose treatment was started after bacteraemia had developed. The method likewise proved fully effective in the case of guinea-pigs in which a pneumonic process had been produced by conjunctival or intrapulmonary infection with *P. pestis*.

those of administration of the gamma-globulin fraction of plague immune serum, Abramova and colleagues (1956) established that while the former therapeutic method engendered no immunity in the test animals, gamma-globulin administration in 1-ml doses once daily for 10 days rendered them resistant to challenge with 250 MLD of *P. pestis* after two months. On account of these findings, Abramova and her colleagues suggested a combination of streptomycin treatment and administration of concentrated plague immune serum. They referred in this connexion to the earlier recommendation of Zhukov-Verezhnikov and co-workers (1949) to use for the treatment of plague a combination of sulfonamides, antibiotics and plague immune serum.

HUMAN PLAGUE

In view of the outstanding value of the antimicrobial drugs (antibiotics) for the treatment of all forms of plague, it is not surprising that modern workers prefer to use these therapeutic substances in place of the sulfonamides or to combine treatment with both kinds of drugs. No doubt, however, fully satisfactory results in the treatment of bubonic plague can be obtained with sulfa-drugs, Macchiavello (1958a) recently recording 40 successes in a series of 42 patients treated with 4-6 g of sulfadiazine or sulfamerazine daily for 1-3 days. The incontestably great value of sulfonamides (sulfathiazole) for the abortive treatment of contacts of pneumonic plague patients has been confirmed once more through the splendid results obtained by Brygoo & Gonon in the 1957 Madagascar epidemic.

Excellent results in the treatment of pneumonic plague with streptomycin and/or other antibiotics were recorded by the last-mentioned workers and, previously, in a somewhat larger series by McCrumb and associates (1953). It is, however, significant that the full adequacy of this method of therapy and the dangers which might be inherent in it are still under discussion. Thus the advisability of combining the antimicrobial treatment of plague with serum administration, which has been urged in the past, continues to be advocated, for instance, by Girard (1953, 1955), Nguyen-Van-Ai (1957), Meyer (1957) and Seal.[1]

Meyer (1957), discussing this problem and what one might call the philosophy of antiplague treatment in general in his masterly study, pointed in particular to two plague victims, one in Madagascar, the other in California,[2] whose deaths, taking place in spite of the administration of antimicrobial drugs, were presumably due to a liberation of plague toxin.

"Efforts to understand this intoxication", Meyer continued, "and its treatment have been only partly rewarding. Potent antiserums containing antibodies against both infection and toxin have ameliorated this damage in mice, but not in monkeys. In more recent preliminary studies on mice with the *P. pestis* strain isolated from the California patient, streptomycin was indeed highly bactericidal; in fact this strain was more rapidly lyzed by a combination of streptomycin and penicillin than was the control strain. When treatment with doses comparable to those used on the patient were begun late in the infection, animals died even though their tissues were completely free of *P. pestis*. That the deaths were probably attributable to the toxin was indicated by the observation that the effectiveness of the antimicrobial drugs was increased from 15 to 50 percent when one dose of purely antitoxic serum was administered."

That concentrated plague immune serum with adequate antitoxic properties should be available to treat patients suffering from, or in danger of, the action of liberated *P. pestis* toxin, is no doubt important. However, it is more important still to guard the patients against this danger by avoiding indiscriminate methods of treatment, in which, as Meyer eloquently puts it, "great quantities of every drug against every infection without proper guidance" are employed. On the contrary, treatment must be "qualitative", the therapeutic indications and the dosages of the drugs chosen being carefully determined for each sufferer individually.

ECOLOGY AND EPIDEMIOLOGY

It is in agreement with modern trends that a major, if not the overwhelming, part of the publications to be considered in this section deal with problems of the ecology of plague rather than with the epidemiology of the disease in the strict sense. Apart from already quoted contributions, such as those of Meyer (1957), Murray (1957), and Kartman et al. (1958a, 1958b), this new approach to the epidemiology of plague *sensu lato* is shown in articles by

[1] Unpublished working documents WHO/Plague/39 and WHO/Plague/40.

[2] The observations made on this patient and with the strain isolated from him are also discussed by Kartman and co-workers (1958b).

Fuller (1954) and Heisch (1956). According to the former of these authors:

" In studying the ecology of an arthropod-borne disease, one is concerned with the ecological niche occupied by each of the living things involved, and with the effects of environmental factors upon each, as well as their relationship with the communities of which each organism is a member. In other words, the epidemiology of an arthropod-borne disease is a problem of the syn-ecology and autecology of pathogen, vector and host."

Similarly Heisch (1956), adopting the " holistic " philosophy of Smuts (1936), declared that:

" In nature, every organism, whether plant or animal, is a whole, and the modern science of ecology attempts to show how these various wholes react on each other, becoming linked, or rather combined, into new wholes, which are continually modified by changes in the environment."

Though these principles of ecology are valid for all plague-affected localities and situations, the profound differences in the hosts, vectors and environmental conditions in different areas and even the changes in the situation apt to take place in one and the same locality cause the almost infinite variety of manifestations of the disease already alluded to earlier in this review. Still, difficult or even impossible as it generally is to demarcate the various types of plague, between most of which transitions exist, a clear distinction can be made between the more or less widely spread epidemic manifestations of the disease, and resulting endemics due to the more or less prolonged but ultimately ceasing presence of rat epizootics, and the persistence of the infection in a comparatively limited number of wild rodent foci in which, though occasional or even periodical epizootic outbursts may take place, usually a smouldering enzootic type of plague prevails.

The fundamental importance of these " natural " foci of plague, defined by Pavlovski (1946) as foci in which the disease had arisen and could exist indefinitely in the complete absence of man, has been recently reasserted by this observer and others in the Soviet Union (see Pavlovski, 1955, 1957; Pavlovski et al., 1955).

Evaluating the generally accepted concepts of Pavlovski and his school, one must note that the term " foci " as used by him might prove somewhat misleading in so far as it might convey the idea that the localities in question are limited in extent, whereas actually the areas where " natural " plague persists are as a rule of considerable size and are not rarely enormous. At the same time, as already alluded to, it is necessary to realize that within these areas the rodent hosts often show a patchy rather than a uniform distribution, localities suitable for them being separated not only by barriers such as mountains but also by zones unfavourable for their existence. Conditions are thus propitious for the formation of plague foci in the strict sense or, rather, pockets of the infection.

Though occasional stationary phases may be met with, as a rule a state of flux is characteristic not only of the natural plague foci but of all localities affected by the disease.

From what has been stated earlier in this review, it will be gathered that the following are the most important of the various factors accounting for these constant changes in the plague situation:

Rodents with markedly different susceptibilities to plague are apt to co-exist in the affected localities so that simultaneously there may be fuel for slowly progressing enzootics as well as for rapidly and widely spreading epizootics. The latter are apt to decimate the susceptible herds to such a degree that the infection becomes quiescent, or even absent, among them until the population density of the herds has been restored to a level creating conditions suitable for a new epizootic wave. In addition to this long-term periodicity, the incidence of plague in wild rodents is also governed by seasonal influences, such as the appearance and dispersal of the young susceptible animals in spring and early summer, the comparative insusceptibility of the older animals which becomes manifest in late summer or autumn, and in the case of several of the species concerned also by aestivation or hibernation.

Activities on the part of man, particularly agricultural operations, may exert a profound influence on plague in rodents. As noted earlier (see page 355) this may even be true in the case of natural plague foci, though, as justly pointed out by Meyer (1955), the ultimate disappearance of the infection due to occupation of the localities in question by man may be preceded by " an initial period of joint tenancy by people and wild and commensal rodents—a condition theoretically ideal for the propagation of plague ". More frequently changes in the plague situation among commensal and peridomestic rodents may be brought about by the successive phases of agricultural work: Movements of the commensal species to the planted fields are apt to reduce the population density of the animals in the settlements, but may at the same time bring these rodents into contact with infected peridomestic species. As a result the plague situation in the settlements may become exacerbated when, after the crops have been harvested, the commensal

species and not rarely also peridomestic rodents invade compounds and houses.

Periodical changes in the plague situation will also be brought about by seasonal changes in the frequency of efficient vector fleas and/or the presence of climatic conditions promoting a high incidence of actually infective fleas. If many susceptible rodents are present under such circumstances, widespread epizootics are bound to occur and the resulting disproportion between a low number of rodents and a high number of fleas, which impels the latter to search for substitute hosts, may create a most dangerous situation for man.

Though no doubt the risk of contracting zootic plague [1] is greatest under these circumstances, the chance or, one must often say, off-chance of infection exists also under other conditions. An increased incidence of the disease may become manifest in certain occupational groups—for instance, as was observed in the past, among dock workers handling cargo from infected ports or, as recently noted by Brygoo & Creff (1955) in Madagascar, among labourers using temporary shelters in rice-fields. As admitted by Pavlovski (1946), the infection may also be contracted by persons entering the natural plague foci or otherwise coming in contact with the reservoirs or vectors involved in their maintenance, for example, when skinning the carcasses of diseased animals or handling their furs.

Dealing with the problem of demic plague, Pollitzer (1954) drew attention to the occurrence of " purely " pneumonic epidemics, due to the arrival of patients with secondary or primary lung involvement in localities where the rodent populations were originally free from plague and where they remained entirely so even when the disease became rampant in man. The epidemic recently observed by Brygoo & Gonon (1958) in north-east Madagascar serves as a classic example of this type of the infection. It is consoling to note that all six patients with pneumonic plague met with after this outbreak had been notified with great delay, could be cured and that, more important still, the spread of the infection could be cut short by administration of sulfathiazole to the numerous contacts.

PREVENTION AND CONTROL

INTRODUCTORY REMARKS

It is obvious that, in order to be effective, plague prevention and control programmes must be implemented according to comprehensive plans which, taking account of the situations to be dealt with, lay a varying degree of emphasis on the three main branches of the work—namely, (*a*) action against the rodents; (*b*) anti-flea measures; and (*c*) application of methods directly to forestall or to combat manifestations of human plague—or, if this is warranted, prescribe steps only against the rodents and their fleas or even only against one of these enemies of mankind. Nevertheless, for the sake of clearness it is necessary to commence a discussion of this work by considering anti-rodent and anti-flea measures separately, then to assess to what extent they may be used independently or must be applied jointly, and finally to deal with the steps necessary in regard to human plague.

[1] The terms " zootic plague " (i.e., the usual insect-borne type of the disease) and " demic plague " (i.e., the forms of plague spreading directly from man to man) were proposed by Megaw in a review in *Trop. Dis. Bull.*, 1952, **49**, 858.

ANTI-RODENT MEASURES

Commensal rodents

As stated by Elton (1954) in the introductory chapter of the profound studies on the control of rats and mice edited by Chitty & Southern:

" Although poison baiting had seemed, and eventually proved, to be the most decisive way of tackling rat infestations on a country-wide scale, there were many other ways of killing rats for which someone or other would be making confident claims, or which might be good in the hands of particularly skilled operators, or which might indeed be the only way of clearing the residue of some particularly refractory population of rats."

It is in accordance with this statement that recommendations to use, apart from the fundamentally important procedures of environmental sanitation and house improvement, methods other than poisoning for rat control are few and far between in the modern literature. Indeed, it only deserves attention that, as described by Hentsch (1955), ditches 45 cm deep and 40 cm wide proved in Java to be an adequate means of keeping plague-sick rats away from the houses.

As can be gathered from recent publications,[1] apart from the anticoagulants, to be dealt with separately, attention is still being paid to the following rat poisons:

ANTU. According to recently published recommendations for rat poisons (WHO Expert Committee on Insecticides, 1958), " ANTU still holds a definite place as a quick-acting poison for the Norway rat. Its use to reduce large populations rapidly may be followed by the use of anticoagulant rodenticides to achieve complete and lasting control. ANTU should not be used against the same populations more often than about once a year . . . Its safety record is good . . ."

It has to be added that Bentley and co-workers (1955), comparing the toxicity of ANTU preparations of different particle size for albino rats, came to the conclusion that the use of fine parts for the manufacture of poison baits was to be avoided since particles of 100-110 μ proved most efficacious. Steiniger (1953), though admitting that ANTU was innocuous for man, considered it dangerous for domestic animals and, therefore, advocated its application in the form of powder (with a particle size of 10-20 μ) to be used only for the insufflation of the rat burrows. However, according to the specifications of the California State Department of Health (*Calif. Hlth*, 1955), it could be used as a tracking poison for dusting rat runways and burrow entrances. For bait preparation it was advisable to mix the poison with equal parts of tartar emetic.

Arsenious oxide. According to the work edited by Chitty & Southern, arsenious oxide is still extensively used in Great Britain without proving more dangerous than other poisons of comparable toxicity. Baiting with this poison, preceded by DDT application and followed by trapping, was also resorted to by Schulz & Crutkhow (1956) to free a plague-threatened town in Thailand from *R. rattus.*

Red squill. The opinion usually held that red squill may be considered the safest of all rat poisons is not universally shared. Thus Freeman and associates, in their contribution to the book edited by Chitty & Southern, regarded the belief in its harmlessness for man and domestic animals as a fallacy, though admitting that: " It is perhaps safer in use than other poisons because it is most unwillingly eaten, and because it gives rise to vomiting; but if these safeguards fail it is toxic."

Sodium fluoracetate. Though according to a statement in the journal *California's Health* the manufacture of sodium monofluoracetate has been given up, the possibilities of its distribution are still mentioned by the WHO Expert Committee on Insecticides (1958) and recent reports on the actual use of this most dangerous but equally potent rat poison have been made by Elbel & Thaineua (1957) and by Bentley et al. (1958), the latter report dealing with its use in sewers only. Promising laboratory results with other fluorine-containing compounds have been recorded by Falkenstein & Ershova (1957).[2]

Zinc phosphide. Zinc phosphide which, according to Freeman and associates (1954), enjoyed increasing popularity in Great Britain, was also recommended as a substitute for sodium fluoracetate in the article in *California's Health* on account of its unattractive colour and phosphorous odour which, while not deterring the rats, were repulsive to most domestic animals. The admixture of 3 parts of tartar emetic to 8 parts of zinc phosphide was recommended.

As has become well established, in contrast to the acutely acting poisons just mentioned, the various brands of anticoagulants, because they kill the rodents gradually, cause no bait-shyness and may, therefore, be used continuously until the animals have been exterminated. Though usually distributed in bait form, they may also be used in fluid form (*Calif. Hlth*, 1955) or, as Steiniger (1953) recommends for the sake of particularly great safety for the domestic animals, in a concentration of 0.5%-1% as tracking powder applied to ratruns and burrow entrances. An example of the outstanding success which may be obtained by the persistent use of anticoagulants for rat control has been given by Steiniger (1956). Elbel & Thaineua (1957), using Warfarin mixed with dry rice bran and peanut oil in bait form for rat control in Thailand, found that by this method the number of rodents could be definitely reduced.

It is noteworthy that, besides Warfarin and similar preparations, recently Pival (2-pivalyl-1,3-indandione) and other indandione compounds have been found effective as rodenticides.

Wild rodents

The truly heroic and remarkably successful efforts made in the Soviet Union to fight wild rodents have been described in the writings of numerous obser-

[1] See besides the work edited by Chitty & Southern (particularly the contribution by Freeman and associates): Bentley et al. (1955, 1958); *Calif. Hlth*, 1955; Elbel & Thaineua (1957); Falkenstein & Ershova (1957); Giannico (1958); Guillaume & Hellequin (1954); Melis (1955); Nedelin (1957); Saunders et al. (1955); Schulz & Crutkhow (1956); Steiniger (1953, 1956); Telle (1954); Vashkov et al. (1957); Wilk (1957); WHO Expert Committee on Insecticides (1958).

[2] The above-quoted article in *California's Health* mentions " Castrix " (2-chloro-4-dimethylamino-6-methylpyrimidine) as a possible substitute for sodium fluoracetate because, though it was equally dangerous, up to doses of 10 LD_{50} its effects could be counteracted by sodium pentobarbital.

TABLE 8

EXAMPLES OF METHODS OF RODENT EXTERMINATION RECENTLY USED IN THE SOVIET UNION

Authors	Rodent species dealt with	Method
Demjashev et al. (1956)	Steppe lemming (*Lagurus lagurus*)	Poisoning with baits prepared from fresh wormwood (*Artemisia*) with zinc phosphide, sodium arsenite or barium fluoracetate.
Edjikina (1956)	Water field-mouse (? *Arvicola terrestris*)	10 % zinc phosphide baits made with pieces of carrot.
Konnova (1946)	*Meriones meridianus*	Gassing with an HCN compound (" Cyanplav ").
Konnova (1949)	*Citellus pygmaeus*	Maize kernels impregnated with strychnine nitrate or thallium sulfate distributed by aeroplane.
Kreizberg & Sharetz (1956)	*Citellus fulvus* Gerbillinae *Ellobius talpinus* Marmots	Treatment of the burrows with methyl bromide so as to kill the rodent fleas as we as the rodents.
Latishev Siborkin (1940)	*Rhombomys opimus*	(*a*) Gassing with " Cyanplav "; (*b*) poisoning with wheat soaked in sodium arsenite solution.
Lisitzin et al. (1956a, 1956b)	*Citellus pygmaeus*	Used mainly oat baits impregnated with 15 %-20 % zinc phosphide and 5 %-6 % vegetable oil, but partly also oats soaked in 0.5 % fluoracetimide solution. Distribution by aeroplane proved most effective.
Lisitzin & Konnova (1956)	Mouse species	Recommended chloropicrin (90 g per cubic metre) for the destruction of mice in stacks of unthreshed corn.
Naglov et al. (1956	Gerbillinae	Grain baits impregnated with zinc phosphide distributed by aeroplane.
Rezinko (1949)	*Citellus pygmaeus*	Made tests with maize-base baits, impregnated with strychnine, thallium sulfate or zinc phosphide and distributed by aeroplane, which gave promising results.
Valejkov et al. (1956)	*Microtus socialis*	Found vegetable baits (carrots or beetroots) impregnated with 2 %-4 % zinc phosphide and 2 % vegetable oil best.
Zinin & Kartushin (1949)	*Citellus pygmaeus*	Obtained satisfactory results when testing a new compound containing 35 % carbon bisulfide and 65% carbon tetrachloride for treating sisel burrows.

vers [1] and important methods for rodent extermination used by some of these authors are summarized in Table 8. These anti-rodent measures have also been dealt with recently in summaries by Pastukhov [2] and Fenyuk [3] which, because they are published together with the present review, need only brief analysis here.

As will be gathered from the note by Pastukhov,[2] fundamental features of the plague eradication work in the Soviet Union are: (*a*) centralization of all services under the Ministry of Health—an arrangement that permits all necessary measures to be taken regardless of administrative boundaries with the combined efforts and equipment of the various regional anti-plague organizations and (*b*) systematic intelligence work, comprising examination of the rodents and/or their fleas on a truly enormous

[1] See, for example, Borodina (1956); Demjashev et al. (1956); Edjikina (1956); Ivanov et al. (1956); Jakovlev et al. (1956); Konnova (1946, 1949); Kreizberg & Sharetz (1956); Latishev & Siborkin (1940); Lisitzin et al. (1956a, 1956b); Lisitzin & Konnova (1956); Mamontov (1937); Naglov et al. (1956); Rezinko (1949); Stupnitski & Shishkin (1946); Valejkov et al. (1956); Vansulin et al. (1956); Zinin (1949); Zinin & Kartushin (1949).

[2] See page 401.

[3] See page 263.

scale, and a systematic watch over the health of the population.

Though, if indicated, attention is paid to the simultaneous extermination of the rodents and their ectoparasites (carried out in the instance mentioned by Pastukhov by the combined use of " black " cyanide and hexachlorocyclohexane), in general attention is being concentrated on rodent eradication, carried out mainly by poison-bait distribution through the large-scale use of aeroplanes. As described by the author, the aim of the campaigns is either to liquidate plague, or to forestall the development of epizootics, or to suppress epizootics and form " protective zones " to lessen the danger of plague infection for man. At the same time large-scale and systematic use is being made of deratization and desinsectization in the centres of population.

Similarly Fenyuk [1] distinguished between (*a*) complete eradication of natural plague foci, (*b*) " current " prophylaxis (i.e., anti-rodent campaigns in places where an epizootic had appeared so as to prevent or lessen the danger of human infection), and (*c*) long-term prophylaxis " aimed at preventing in a particular locality for some time the possibility of an intensive epizootic developing or penetrating from the outside ".

As stressed by Fenyuk, in the case of " current " prophylaxis it is " extremely important to combine destruction of the rodents with disinsectization of their burrows ". However, he continued, in the areas

" where enzootic plague is being eradicated on the so-called " complete-clearing " principle it is necessary only to exterminate the rodents and not to take anti-flea measures. In this method of work rodent extermination is carried out over large areas and to a certain extent independently of epizootics. The rodent population is kept at a low level for a number of years running. In this way, the fleas, deprived of their hosts, gradually die out and this makes it possible to dispense with special efforts to destroy them. "

As shown by Fenyuk's account, recently an important change was made in the methods of exterminating the sisels (*Citellus pygmaeus*) in the enormous North-west Pre-Caspian area: whereas from 1933 to 1941 reliance was placed upon gassing, first with chloropicrin, then also with " black " cyanide, in the period from 1947 to 1956 [2] emphasis was laid upon baiting, mainly using oats impregnated with 10%-20% zinc phosphide. While justly appreciating the excellent results of the second campaign, Fenyuk admitted that:

" conditions still exist in the North-West Caspian area for the re-establishment of natural plague foci if epizootics spread from its south-eastern portion into the territory freed from plague. In view of this, the task of complete elimination of enzootic plague as quickly as possible in that part of the North-West Caspian focus where it is still perhaps in existence has become extremely important in order to exclude the possibility of its further spread."

However, he pointed out with great reason that large-scale agricultural operations in the areas freed from plague " have essentially destroyed the factors favourable to natural plague foci ".

Briefly referring also to the Transbaikalian focus, Fenyuk stated that large-scale campaigns against the marmots, undertaken there from 1939 to 1955 with chloropicrin and " black " cyanide, had led to a disappearance of the infection, no trace of which could be detected since 1946. Commenting upon this result, he stated that:

" At the moment, the occurrence of plague among rodents in Transbaikalia is, it would seem, likely only when the frequency of the marmots there has been re-established and if epizootics spread from the Central Asian (Mongolian) natural plague focus, of which the Transbaikalian focus was a small projection. To prevent this, further control of the number of marmots is essential, even if only on a broad strip along the boundaries of Transbaikalia and the Mongolian People's Republic."

In a final evaluation of the results obtained in the Soviet Union Fenyuk admitted that the amount of labour and money necessary for the campaigns had been so great as to render the methods used in the Pre-Caspian area and in Transbaikalia impracticable for the extensive natural foci of plague in the sparsely populated, high-mountain, desert or semi-desert districts of Asia, Africa and America. Nevertheless he felt entitled to conclude his well-balanced article by stating that " as present natural plague foci are affected by improvements in civilization and farming, and as methods for enzootic plague control are improved, the elimination of enzootics will become more and more a practicable proposition ".

ANTI-FLEA MEASURES

DDT

While the value of DDT for the prevention and control of plague has been confirmed by most

[1] See page 263.

[2] As noted by Fenyuk, from 1942 to 1946 the scale of extermination work in the area had been greatly reduced.

recent workers,[1] stress has been laid by several of them upon using different methods of applying this insecticide so as to deal adequately with different sites or situations requiring disinsectization. It has first to be mentioned in this connexion that Gramiccia & El Din Sultan (1953) found it essential, when trying to combat the *P. irritans* abounding in the houses of Lebanon, to rely upon application of DDT in spray form, because distribution of 10% dust, prepared by diluting a wettable DDT powder with talc, proved completely ineffective.[2] It would seem, however, that 5% DDT dust, prepared in a similar manner with rice talc, was applied by Schulz & Crutkhow (1956) with success for the purpose of plague prevention in Thailand both by patch-dusting and by blowing into inaccessible places.

The above-mentioned method of patch-dusting, already utilized before the period at present under review (see Pollitzer, 1954), has been most emphatically recommended for plague control work by Baltazard & Bahmanyar [3] in their report on plague research in India. The relevant statements made by these observers deserve close attention on the part of all plague workers operating under comparable conditions.

As stated in a recent article, Vasilenko and colleagues (1958), to combat the rat fleas under urban conditions, relied mainly upon the introduction into the rodents' burrows of cotton plugs into which teaspoonfuls of DDT (or benzene hexachloride) had been inserted.

The use of DDT for dusting the burrows of wild rodents has been found effective by Davies (1951) in South Africa, Heisch and co-workers (1953) in Kenya and Ivanov (1949) in the Soviet Union. However, Ryckman and associates (1953, 1954), attempting to control wild rodent fleas in California with different insecticides, obtained better results with dieldrin, aldrin and heptachlor than with 5% DDT. It has to be added that Miles & Wilcomb (1953), judging from tests in which DDT was evenly spread over experimental plots harbouring *Neotoma micropus* infested mainly by *Orchopeas sexdentatus* and *E. gallinacea*, concluded that distribution of the insecticide by aeroplane might be capable of bringing about a temporary reduction of the fleas on wild rodents. The two workers admitted, however, that *E. gallinacea* had not been significantly affected and that the effect on the other fleas was lost within two weeks.

An interesting apparatus for the control of wild rodent fleas devised by Kartman & Lonergan (1955a), consisted, as described by Kartman and colleagues (1958b), " of a covered bait station with DDT-filled sacks hung at opposite entrances, so that the rodent would receive a shower of DDT on entering and leaving the box ".

Though excellent results were obtained with this apparatus, Kartman and associates used for their work in California a simplified insecticide bait box (Kartman [4]) which was found to control " fleas on the heavily parasitized *Microtus californicus* as well as on other species of meadow mice, both on the hosts and their nests ".

As summarized by Fedorov (1957):

" In principle, it is possible to exterminate rodents and their ectoparasites by using complex food-baits containing both rodenticides and insecticides. Simultaneous extermination of rodents and their ectoparasites may become particularly important if deratization is prompted by epidemic evidence." [Trans.] [5]

Though within recent years reports on the development of DDT-resistance in fleas, particularly in *P. irritans* and in *Ctenocephalides canis* and *C. felis*, have been made by several authors,[6] it is reassuring to gather from a recent statement by Brown [7] that " to date there is no valid evidence of resistance having developed in *Xenopsylla* ".[8] It serves as a corollary to this contention that, according to reports by Mercier & Razafindrakoto (1953) and by Courdurier (1954), the systematic application of DDT (or, to a lesser extent, BHC) in the houses of Tananarive (Madagascar) for three years was no

[1] In addition to the authors quoted in the text, the following, for instance, have recorded good results with DDT: Patel & Rodda (1952); Mercier & Razafindrakoto (1953); Courdurier (1954); and Mohr & Smith (1957), who used it for the control of flea-borne typhus.

[2] It also deserves notice that Chen and co-workers (1956), comparing in Taiwan the value of DDT residual spraying with that of general house-cleaning, found only the former method effective against fleas as well as bed-bugs and cockroaches.

[3] See page 169.

[4] Unpublished working document WHO/Plague/45.

[5] Some details of the methods used by Fedorov and the results obtained will be found in the review of his article in *Trop. Dis. Bull.*, 1957, **54**, 680.

[6] See, for instance, in addition to the statements quoted by Pollitzer (1954): Brown (1958); Busvine (1957); Floch (1954); Hess (1952); Quarterman & Schoof (1958); Sen (1958); and Shawarby (1953).

[7] See note on page 410.

[8] The discovery of resistance in *X. cheopis* reported by Patel et al. in the article on page 301 was not known to the author at the time of writing.—ED.

doubt responsible for a permanently very low *X. cheopis* index and the absence of plague.

To complete the present record it has to be noted that, according to Hentsch (1955), DDT-killed plague-infected fleas, if protected against exsiccation, remained infective for cutaneously inoculated guinea-pigs for periods up to 15 days. In the opinion of this writer such fleas might be dangerous for people walking about barefoot and for children at play.

Benzene hexachloride

Making comparative tests with DDT and the gamma-isomer of benzene hexachloride in the laboratory with *X. cheopis* and in the field with *P. irritans*, Shawarby (1953) found that the latter insecticide gave better and longer-lasting results. He considered it possible that the slight resistance to DDT which had developed by selection in the case of the laboratory tests with *X. cheopis* might have been a factor contributing to the greater efficacy of BHC.

As stated by Heisch and co-workers (1953), when human plague became manifest in 1952 in the huts of labourers near Rongai in Kenya, these premises

> " were vigorously treated with a gammexane powder (3 per cent. gamma isomer) applied with dust blowers to the walls, floors, and roofs, some of the powder being forced down *Rattus* burrows, the entrances of which were outside the huts where the walls joined the ground. After treatment, white rats freed of ectoparasites and held in wire cages were placed in the huts and examined at intervals for fleas . . . Twelve white rats placed in treated huts and examined daily remained uninfested for a month, whereas rats placed in untreated dwellings collected 25 fleas which included *Xenopsylla cheopis* and *X. brasiliensis*."

However, as noted earlier, these workers also obtained fully satisfactory results when using in place of BHC 10% DDT powder sprinkled on the floors and down *Rattus* burrows by means of perforated cigarette tins.

As summarized in volume 43 of the *Review of Applied Entomology*, Tagil'tsev (1954), working in Kirgizia, could eliminate fleas and other insects by blowing a dust containing 0.5% technical BHC in loess into the burrows of *Meriones erythrourus*. The method was evidently also useful for dealing with the burrows of commensal rodents, in which it killed ticks.

Organo-phosphorus compounds

Organo-phosphorus compounds, found by Fluno (1955) to be efficacious against *Ctenocephalides felis*, were recommended for campaigns against this and the dog flea by the WHO Expert Committee on Insecticides (1958).

Ship fumigation

Only passing reference need be made to a recently published study on the insecticidal effect of cyanic acid compounds used for the fumigation of sea-going vessels by Yazikov and associates (1958).[1]

Biological control

In a note submitted to the WHO Expert Committee on Plague in 1958 (unpublished working document WHO/Plague/36), Garnham referred to the possibility of using protozoa, especially microsporidia, for the biological control of fleas. It is an interesting subject for speculation whether such parasitic infestations of fleas might exert an influence under natural conditions and might thus account for a drop in the incidence of fleas in the absence of insecticidal campaigns, as observed for instance in the case of *X. cheopis* in Puerto Rico by Fox (1956).

Flea repellents

Diethyltoluamide, found by Gilbert and associates (1957) to be more effective against *X. cheopis* than other flea-repellents, was recommended in the above-mentioned report of the WHO Expert Committee on Insecticides (1958) as " a superior flea repellent, particularly when used to impregnate socks and outer garments as described in the section on chigger repellents . . . Clothing impregnated with diethyltoluamide repels fleas for more than one week. Temporary protection can be obtained by smearing the repellent on the socks and trouser legs."

The report added that: " Undecylenic (or undecanoic) acid, propylacetanilide and benzyl benzoate are also good flea repellents; clothing treated with them remains effective through several days of ordinary wear."

MEASURES AGAINST HUMAN PLAGUE

In regard to the active immunization of man against plague it has been stated already that (*a*) according to the serological studies of Payne, Smadel & Courdurier (1946, p. 49), in the case of live plague vaccine, administration of booster doses

[1] Two earlier articles on the use of this procedure were published by Yazikov & Raigorodskaya in 1957. However, to judge from an editorial appearing in 1955 in the German journal *Desinfektion und Gesundheitswesen*, opinions regarding the superior value of this method as compared with those formerly used for ship fumigation are not unanimous.

was essential for the production and maintenance of a solid immunity, while (*b*) as suggested by the guinea-pig experiments of Spivack and associates (1958, p. 51), it appeared to be indispensable for the production of a solid immunity to administer killed plague vaccines in an oil adjuvant.[1] Brief mention has been made also of the observations made by Speck & Wolochow (1957) regarding the impossibility of conferring on monkeys an active immunity against respiratory infection with *P. pestis*. The two workers made the following statement in this connexion:

"Monkeys were vaccinated with a living avirulent vaccine, a killed virulent vaccine, and the purified envelope substance, fraction I. These were given in a series of three weekly injections and the animals were challenged with an aerosol 5 to 12 weeks later. The vaccination procedures were found to be essentially ineffective even in animals receiving two consecutive series. The disease in vaccinated animals was found to be no more susceptible to streptomycin therapy than the disease in the unvaccinated animal."

Under these circumstances it is fortunate that administration of sulfonamides to the contacts of pneumonic plague patients offers a practically infallible means of protecting them against manifest attacks of the disease. Indeed, one may say that, in contrast to the tedious, if not difficult, procedures involved in the prevention and control of rodent-caused and flea-borne plague, control of the pneumonic form is comparatively simple, provided that sulfonamide treatment is started as soon as the presence of the disease has been ascertained.

CONCLUDING REMARKS

Discussing in 1954 the history and distribution of plague, Pollitzer pointed out that "in many of the countries dealt with the incidence of plague has markedly decreased within recent years and in some the disease has ceased to be manifest for the present", but nevertheless considered it impossible for various reasons to be complacent about this situation.

Since the time the above statement was made, plague has shown a truly spectacular progressive decrease in all hitherto affected areas,[2] and the question therefore arises whether it may now be maintained that the disease has ceased to be of concern for the world. Trying to frame an answer to this question, the present reviewer for one considers such an attitude to be over-optimistic. It is true that the information given in the foregoing pages has shown that there has been most satisfactory progress in the prevention and control of the infection, outstanding work in the Soviet Union having even demonstrated that it is possible successfully to deal with the situation in natural plague foci. Stress has been laid also upon the fact that, besides specific efforts, agricultural activities play a most important role in curbing the persistence of wild-rodent plague, thus forming a counterpart to the influence of environmental improvements in the settlements, which are apt to prove of greater, particularly more permanent, value in the fight against the commensal rodents than the usual type of direct anti-rat campaigns.

Nevertheless, it would be wrong to place too much hope on the combined success of the specific and unspecific measures taken against plague. The excellent statement of Fenyuk quoted above renders it clear that the success obtained in two natural plague foci of the Soviet Union required efforts of such a magnitude as to exclude the implementation of equally radical measures in other parts of the world, and even of the Soviet Union, where continued use has therefore to be made of palliative methods. Moreover, as can be gathered from the report of Fenyuk, even in the drastically treated areas constant vigilance is necessary to guard against a recrudescence of plague, which indeed has been seen to reappear within the territory brought under control. While there is much hope that this impasse in the Pre-Caspian area will be successfully dealt with, Fenyuk appears to be less sanguine in regard to the focus of marmot plague in Transbaikalia, in view of its contiguity with the still-active extended focus in Mongolia, designated as "potentially most dangerous" by one of the participants at the 1958 meeting of the WHO Expert Committee on Plague.

Necessary though it has been to illustrate the possibility of a recrudescence of plague with the above statements, there seems no reason to fear

[1] The same recommendation was made also by Seal (unpublished working documents WHO/Plague/39 and WHO/Plague/40) who, however, advocated relying upon immunization with the specific protein of *P. pestis*.

[2] See *Wkly epidem. Rec.*, 1958, **33**, 56; 1959, **34**, 36.

the reappearance of severe and widespread outbursts like those known to have originated in Mongolia and Transbaikalia; for the presence of human plague is apt to go unnoticed only as long as it remains restricted to sparsely populated or inaccessible areas, and once it reaches populous localities it can be quickly subdued.

A final point to be given most serious consideration when dealing with the present global plague situation and its prognosis has been expressed by Pollitzer (1954) thus:

"As its history teaches, plague has often shown a decline even if left alone but was apt to flare up again in due course. Hence, while appreciating the strenuous efforts now often made to combat the scourge, one should be careful not to ascribe to the measures implemented what might really be the outcome of a periodicity of the infection."

For this as well as for the reasons stated above it would seem, therefore, that the present manifestations of plague, insignificant though they appear at first glance, should not be considered local events of little importance (an attitude indeed adopted during the preliminary stages of the present pandemic), but should be given continued attention by those interested in global public health.

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Annex 1

RODENTS AND LAGOMORPHA OTHER THAN THE COSMOPOLITAN SPECIES OF RATS (*Rattus norvegicus* and *Rattus rattus* subspp.) AND OF COMMENSAL MICE (*Mus musculus* subspp.) PROVED NATURALLY PLAGUE-INFECTED OR STRONGLY INCRIMINATED THROUGH POSITIVE FINDINGS IN THEIR ECTOPARASITES *

RODENTIA

Family and subfamily	*Species*	*Locality*
BATHYERGIDAE	*Cryptomys* sp. White-toothed mole-rat	Angola
CAVIIDAE [1] Caviinae	*Cavia aperea* Restless cavy	Brazil
	Cavia pamparum Pampas cavy or Lund's guinea-pig	Argentina
	Cavia tschudii atahualpae Peruvian cavy [2]	Peru
	Caviella australis australis	Argentina
	Caviella australis joannia	Argentina
	Galea musteloides leucoblephara	Argentina
	Galea musteloides littoralis	Argentina
	Galea spixii " Preá "	Brazil [3]
	Kerodon rupestris Brazilian rock-cavy	Brazil
CHINCHILLIDAE	*Lagostomus maximus immollis* " Vizcacha " or Peruvian hare	Argentina
DIPODIDAE Dipodinae	*Allactaga elater* Small five-toed jerboa	South-east Russia ; Transcaucasia ; Central Asia
	Allactaga elater indica	Iranian Kurdistan
	Allactaga major (*A. jaculus* auctt.)	

* Reproduced, with slight modifications, from *Wld Hlth Org. techn. Rep. Ser.*, **165**, 16.

[1] The common guinea-pig, *Cavia porcellus* (*Cavia cobaya* auctt.), though repeatedly found plague-infected, has not been included in this list since it is a domestic rather than a wild rodent.

[2] Some other subspecies of *Cavia tschudii* have also been found infected in Peru.

[3] An unidentified species of *Galea* was also found naturally infected with plague in Bolivia.

Family and subfamily	*Species*	*Locality*
DIPODIDAE Dipodinae *(continued)*	*Allactaga sibirica saltator*	South east-Russia
	Allactaga sibirica sibirica Mongolian five-toed jerboa (*A. saliens* auctt.)	Transbaikalia
	Dipus sagitta Northern three-toed jerboa	South-east Russia
	Eremodipus lichtensteini	Central Asia
	Pygeretmus platyurus Greater flat-tailed jerboa	Transcaspia
	Scirtopoda telum Thick-tailed three-toed jerboa	South-east Russia
ECHIMYIDAE Echimyinae	*Cercomys cunicularius laurentius*	Brazil
	Cercomys inermis	Brazil
GEOMYIDAE	*Thomomys bottae* Western pocket-gopher	California, USA ; Colorado, USA (?)
	Thomomys fossor (*Thomomys talpoides fossor* auctt.) Mountain pocket-gopher	Fleas only : Colorado, USA
HETEROMYIDAE Dipodomyinae	*Dipodomys* sp.	Texas, USA
	Dipodomys ordi ordi Ord's kangaroo-rat	Washington, USA
	Perognathus parvus Pocket-mouse	Fleas only : Washington, USA
Heteromyinae	*Heteromys anomalus anomalus*	Venezuela
MURIDAE Cricetinae	*Akodon dolores*	Argentina
	Akodon mollis mollis	Peru-Ecuador border region
	Akodon mollis orophilus Mountain field-mouse	Huancabamba, Peru
	Cricetulus barabensis	Transbaikalia; Manchuria
	Cricetulus eversmanni	Mongolia
	Cricetulus migratorius Migratory (grey) hamster	South-east Russia
	Cricetus cricetus Common hamster	South-east Russia
	Eligmodontia hirtipes jucunda	Argentina
	Eligmodontia moreni	Argentina
	Graomys griseoflavus centralis	Argentina
	Graomys griseoflavus griseoflavus	Argentina

Family and subfamily	*Species*	*Locality*
MURIDAE Cricetinae *(continued)*	*Hesperomys bimaculatus*	Argentina
	Hesperomys fecundus	Bolivia
	Hesperomys laucha	Argentina
	Hesperomys murillus cordovensis	Argentina
	Hesperomys venustus	Argentina ; Bolivia
	Holochilus balnearum	Argentina
	Holochilus sciureus Sugar-cane rat	Brazil
	Mystromys albicaudatus White-tailed rat	South Africa
	Neotoma albigula albigula White-throated wood-rat	Arizona, USA ; New Mexico, USA
	Neotoma cinerea occidentalis Western bushy-tailed wood-rat	California, USA
	Neotoma desertorum Desert wood-rat	Nevada, USA ; Utah, USA
	Neotoma fuscipes Dusky-footed wood-rat	California, USA ; fleas only : Oregon, USA
	Neotoma fuscipes mohavensis Mohave desert wood-rat	Nevada, USA
	Neotoma intermedia intermedia (*N. lepida intermedia* auctt.) Intermediate (Rhoads') wood-rat	California, USA
	Neotoma lepida lepida	Utah, USA (?)
	Neotoma micropus Pack-rat	Texas, USA [1]
	Onychomys sp.	Fleas only : Texas, USA
	Onychomys leucogaster White-bellied grasshopper mouse	Fleas only : New Mexico and other western areas of the USA
	Onychomys torridus	Fleas only : New Mexico, USA
	Oryzomys andinus	Peru
	Oryzomys arenalis	Peru
	Oryzomys flavescens subsp.	Argentina ; Bolivia
	Oryzomys laticeps intermedius	Brazil
	Oryzomys laticeps nitidus	Fleas only : Ecuador
	Oryzomys palustris (*Hesperomys palustris* auctt.)	New Orleans, USA; Louisiana, USA
	Oryzomys phaeopus olivinus	Ecuador
	Oryzomys pyrrhorinus	Brazil

[1] Plague in *Neotoma* sp. was recorded in Oklahoma as well as in other western parts of the USA.

Family and subfamily	*Species*	*Locality*
MURIDAE Cricetinae *(continued)*	*Oryzomys stolzmanni stolzmanni* (*O. longicaudatus stolzmanni* auctt.)	Huancabamba, Peru
	Oryzomys xanthaeolus xanthaeolus	Ecuador ; Peru [1]
	Oxymycterus paramensis	Bolivia
	Peromyscus boylii Brush-mouse	Fleas only : Arizona, USA
	Peromyscus leucopus White-footed mouse	Fleas only : New Mexico, USA
	Peromyscus maniculatus Deer-mouse	New Mexico, USA ; fleas only : California, USA ; Washington, USA
	Peromyscus truei gilberti Gilbert's white-footed mouse	California, USA
	Peromyscus truei truei True's white-footed mouse	California, USA ; New Mexico, USA [2]
	Phyllotis amicus amicus	Peru
	Phyllotis amicus maritimus	Fleas only : Peru
	Phyllotis darwini vaccarum	Argentina
	Phyllotis fruticicolus	Ecuador
	Phyllotis wolffsohni	Bolivia
	Reithrodontomys megalotis Harvest mouse	Fleas only : California, USA ; Kansas, USA ; New Mexico, USA
	Rhipidomys equatoris	Peru
	Rhipidomys leucodactylus	Bolivia
	Sigmodon hirsutus	Venezuela
	Sigmodon hispidus Cotton-rat	Fleas and lice only : New Mexico, USA
	Sigmodon peruanus	Peru
Dendromyinae	*Dendromus haymani*	Belgian Congo
	Dendromus mesomelas kivu	Belgian Congo
	Malacothrix typicus Mouse-gerbil	South Africa
	Steatomys pratensis Fat mouse	South Africa
Gerbillinae	*Desmodillus auricularis* Namaqua gerbil	South Africa
	Gerbillus paeba	South Africa
	Meriones libycus erythrourus	Iranian Kurdistan ; Transcaucasia

[1] An unidentified subspecies of *Oryzomys xanthaeolus* (? *O. x. baroni*) was also found infected.

[2] The presence of plague in *Peromyscus* sp. has also been repeatedly recorded in the western parts of the USA.

Family and subfamily	*Species*	*Locality*
MURIDAE Gerbillinae *(continued)*	*Meriones meridianus* (*Pallasiomys meridianus* auctt.) Midday gerbil	South-east Russia ; Transcaspia ; Turkestan
	Meriones persicus persicus	Iranian Kurdistan
	Meriones tamariscinus Tamarisk gerbil	South-east Russia
	Meriones tristrami	Iranian Kurdistan
	Meriones unguiculatus	Mongolia
	Meriones vinogradovi	Iranian Kurdistan
	Rhombomys opimus Great gerbil	South-east Russia ; Central Asia
	Tatera brantsi (*T. lobengulae* auctt.)	South Africa
	Tatera indica India gerbil or antelope-rat	India
	Tatera schinzi Schinz's gerbil	South Africa
	Tatera valida beniensis	Belgian Congo
Microtinae	*Alticola worthingtoni semicanus* (*A. semicanus* auctt.)	Mongolia
	Arvicola terrestris	South-east Russia
	Ellobius lutescens	Iranian Kurdistan
	Ellobius talpinus Northern mole-vole	South-east Russia
	Lagurus curtatus Sage-brush vole	Fleas only : Washington, USA
	Lagurus lagurus Steppe lemming	South-east Russia
	Microtus arvalis Common vole	South-east Russia
	Microtus (Lasiopodomys) brandti Brandt's vole	Transbaikalia; Mongolia
	Microtus californicus Californian meadow-mouse	California, USA [1]
	Microtus (Stenocranius) gregalis raddei Narrow-skulled vole	Transbaikalia
	Microtus montanus Mountain vole	Oregon, USA ; fleas only : Washington, USA
	Microtus nanus	Fleas only : Washington, USA
	Microtus socialis Social vole	South-east Russia
	Microtus townsendi townsendi	Tacoma, Washington, USA

[1] Plague in *Microtus* sp. has also been recorded on several occasions in the western part of the USA.

Family and subfamily	*Species*	*Locality*
MURIDAE *(continued)* Murinae	*Acomys cahirinus* Cairo spiny-mouse	Egypt
	Aethomys kaiseri medicatus	Belgian Congo
	Apodemus agrarius	China
	Apodemus sylvaticus Common field-mouse	Transcaucasia
	Arvicanthis niloticus subsp.	Kenya
	Arvicanthis niloticus niloticus Nile-rat	Egypt
	Arvicanthis niloticus nubilans Unstriped African grass-rat	East Africa
	Arvicanthis niloticus rossi	Belgian Congo
	Arvicanthis niloticus rufinus	Senegal
	Bandicota bengalensis (*Gunomys bengalensis* auctt.) Lesser bandicoot-rat	Burma ; India
	Bandicota bengalensis kok (*Gunomys kok* auctt.)	India
	Bandicota gracilis	Ceylon
	Bandicota indica Large bandicoot-rat	India
	Bandicota indica (*Bandicota malabarica* auctt.)	Ceylon
	Cricetomys gambianus Giant rat	Belgian Congo ; Ghana ; Senegal
	Dasymys incomtus bentleyae [1] Swamp-rat	Belgian Congo
	Grammomys dolichurus	East Africa
	Grammomys dryas	Belgian Congo
	Lemniscomys griselda [2]	Senegal
	Lemniscomys striatus massaicus	East Africa
	Lemniscomys striatus striatus Spot-striped grass-mouse	Belgian Congo
	Lophuromys aquilus (*L. aquilus rita* auctt.)	Belgian Congo
	Millardia meltada Soft-furred field-rat or metad	India
	Mus booduga (*Leggada booduga* auctt.) Little Indian field-mouse	India

[1] It has been stated that " *D. i. nudipes* [*Dasymys incomtus nudipes*] is found in the enzootic plague area in Barotseland, N. Rhodesia, where it is subject to secondary infection from gerbils and *Mastomys* ". [Davis, D.H.S. (1950) In: Union of South Africa, Department of Health, Plague Research Laboratory, *Sylvatic plague in South Africa : reservoirs and vectors*, Johannesburg (Special Report No. 1/50)].

[2] This rodent was originally described as *Pelomys campanae* [Garnham, P.C.C. (1949) *Bull. Wld Hlth· Org.*, **2**, 271 but was later corrected to *Lemniscomys griselda* (see *Bull. Wld Hlth Org.*, 1951, **3**, 697).

Family and subfamily	*Species*	*Locality*
MURIDAE Murinae *(continued)*	*Mus deserti* (*Leggada deserti* auctt.) Dwarf-mouse	South Africa
	Mus musculoides emesi (*Leggada emesi* auctt.) Pygmy-mouse	Belgian Congo
	Mus triton fors (*Leggada triton fors* auctt.)	Belgian Congo
	Mylomys cuninghamei alberti (*M. dybovskii alberti* auctt.)	Belgian Congo
	Pelomys fallax iridescens [1]	East Africa
	Rattus natalensis (*Mastomys* or *Rattus coucha* auctt.) Multimammate mouse	South Africa ; Belgian Congo ; Kenya
	Rhabdomys pumilio Four-striped grass-mouse	South Africa ; Kenya
Otomyinae	*Otomys* sp.	East Africa
	Otomys angoniensis Swamp-rat	Kenya
	Otomys irroratus South African water-rat	South Africa
	Otomys tropicalis elgonis	Belgian Congo
	Otomys unisulcatus (*Myotomys unisulcatus* auctt.)	South Africa
	Parotomys brantsi luteolus Eastern Karroo rat or Brants' otomys	South Africa
PEDETIDAE	*Pedetes capensis* South African spring-hare	South Africa
SCIURIDAE	*Citellus armatus* Uinta ground-squirrel	Western part of the USA (Idaho, Nevada, Montana, Wyoming, Utah, Washington)
	Citellus beecheyi beecheyi California ground-squirrel	California, USA
	Citellus beecheyi douglasi Douglas ground-squirrel	California, USA ; Oregon, USA
	Citellus beecheyi fisheri Fisher's ground-squirrel	California, USA
	Citellus beecheyi nudipes	Fleas only : California, USA
	Citellus beldingi beldingi Belding's ground-squirrel	California, USA

[1] Davis (1950) noted that " in Barotseland [Northern Rhodesia] *P. f. frater* [*Pelomys fallax frater*] is associated with *Otomys* and *Dasymys* and has a similar flea fauna. It may act as a transient reservoir with these species." [Davis, D.H.S. (1950) In: Union of South Africa, Department of Health, Plague Research Laboratory, *Sylvatic plague in South Africa : reservoirs and vectors*, Johannesburg (Special Report No. 1/50)].

Family and subfamily	*Species*	*Locality*
SCIURIDAE *(continued)*	*Citellus beldingi oregonus* Oregon ground-squirrel	California, USA; Nevada, USA ; Oregon, USA
	Citellus columbianus columbianus Columbian ground-squirrel	Washington, USA
	Citellus columbianus ruficaudus Blue Mountain ground-squirrel	Oregon, USA
	Citellus dauricus dauricus Dauria sisel	Transbaikalia
	Citellus erythrogenys pallidicauda	Mongolia
	Citellus fulvus Large-toothed suslik	South-east Russia
	Citellus idahoensis Idaho ground-squirrel	Fleas only : Idaho, USA
	Citellus lateralis chrysodeirus Golden-mantled ground-squirrel	California, USA
	Citellus lateralis lateralis (*Callospermophilus lateralis* auctt.)	Fleas only : Wyoming, USA [1]
	Citellus leucurus leucurus (*Ammospermophilus leucurus* auctt.) Antelope ground-squirrel	Fleas only : Arizona, USA ; California, USA
	Citellus major Red-cheeked suslik	Western Kazakhstan
	Citellus mexicanus	Fleas only : New Mexico, USA
	Citellus pygmaeus Little suslik	South-east Russia
	Citellus richardsoni elegans Wyoming ground-squirrel	Wyoming, USA
	Citellus richardsoni nevadensis Nevada ground-squirrel	Nevada, USA
	Citellus richardsoni richardsoni Richardson's ground-squirrel	Alberta and Saskatchewan, Canada ; Montana, USA
	Citellus spilosoma major Spotted ground-squirrel	Fleas only : New Mexico, USA
	Citellus townsendi mollis Piute ground-squirrel	Fleas only : Idaho, USA
	Citellus tridecemlineatus 13-striped ground-squirrel	Fleas only : New Mexico, USA; Texas, USA
	Citellus undulatus (*C. rufescens*)	Mongolia
	Citellus variegatus grammurus Say's rock-squirrel	Utah, USA ; fleas only : Arizona, USA; Colorado, USA ; New Mexico, USA

[1] Plague was also confirmed in fleas from *Callospermophilus* sp. in California and in fleas and ticks from *Citellus lateralis* in Colorado.

Family and subfamily	*Species*	*Locality*
SCIURIDAE *(continued)*	*Citellus variegatus utah* Utah rock-squirrel	Utah, USA
	Citellus washingtoni loringi Loring's ground-squirrel	Washington, USA
	Citellus washingtoni washingtoni Washington ground-squirrel	Washington, USA
	Cynomys sp.	Fleas only : Colorado, USA ; Texas, USA
	Cynomys gunnisoni gunnisoni Gunnison's prairie-dog	New Mexico, USA
	Cynomys gunnisoni zuniensis Zuni prairie-dog	Arizona, USA ; New Mexico, USA
	Cynomys leucurus White-tailed prairie-dog	Fleas and lice only : Wyoming, USA
	Cynomys ludovicianus Black-tailed prairie-dog	USA : Colorado, Kansas, Montana, New Mexico, Texas, Wyoming
	Cynomys mexicanus	North Mexico
	Cynomys parvidens Utah prairie-dog	Utah, USA
	Funambulus sp. (? *F. pennanti*)	South India
	Funambulus palmarum Indian palm-squirrel	Ceylon ; India
	Glaucomys sabrinus lascivus Sierra Nevada flying-squirrel	California, USA
	Marmota baibacina centralis (*Arctomys centralis* auctt.)	Russian Turkestan
	Marmota caudata Long-tailed marmot	Central Asia
	Marmota flaviventris subsp. Yellow-bellied marmot	Colorado and Oregon, USA ; Fleas : British Columbia, Canada ; New Mexico, USA
	Marmota flaviventris avara	Fleas only : Oregon, USA
	Marmota flaviventris engelhardti Engelhardt's marmot	Montana, USA ; Utah, USA ; Wyoming, USA
	Marmota flaviventris nosophora Golden-mantled marmot	Montana, USA
	Marmota sibirica Siberian marmot or tarabagan	Manchuria ; Mongolia ; Transbaikalia
	Sciurus stramineus nebouxi Neboux's squirrel	Ecuador; Peru

Family and subfamily	*Species*	*Locality*
SCIURIDAE *(continued)*	*Spermophilopsis leptodactylus* Long-clawed ground-squirrel	Central Asia
	Tamias minimus (*Eutamias minimus* auctt.) Least chipmunk	Fleas only : Washington, USA
	Tamias quadrivittatus frater (*Eutamias speciosus frater* auctt.) Tahoe chipmunk	California, USA ; Nevada, USA
	Tamiasciurus douglasi albolimbatus Sierra Nevada chickaree	California, USA
	Xerus erythropus Central African side-striped squirrel	Senegal
	Xerus inauris (*Geosciurus capensis* auctt.) Bristly ground-squirrel	South Africa

LAGOMORPHA

Family and subfamily	*Species*	*Locality*
LEPORIDAE	*Lepus californicus* Black-tailed jack rabbit	California, USA
	Lepus capensis Cape hare	South Africa
	Lepus europaeus European hare	England ; Argentina
	Lepus saxatilis Scrub hare	South Africa
	Lepus tolai	Central Asia
	Oryctolagus cuniculus [1] Rabbit	England
	Sylvilagus sp. Cotton-tail rabbit	Bolivia ; Ecuador ; Peru
	Sylvilagus andinus	Huancabamba, Peru
	Sylvilagus audoboni Desert cotton-tail rabbit	New Mexico, USA
	Sylvilagus bachmani California brush-rabbit	Fleas only (?) : California, USA
	Sylvilagus brasiliensis	Brazil
	Sylvilagus brasiliensis gibsoni	Argentina ; Bolivia
	Sylvilagus caudatus " Tapeti "	Huancabamba, Peru
	Sylvilagus nuttalli nuttalli Washington cotton-tail rabbit	California, USA
OCHOTONIDAE	*Ochotona daurica*	Transbaikalia
	Ochotona pricei	Mongolia

[1] Instances of secondary plague manifestations among domesticated rabbits, due to the presence of the infection among rats, have been recorded by several observers.

Annex 2

FLEAS OF RODENTS OTHER THAN THE COSMOPOLITAN SPECIES OF RATS (*R. rattus* and *R. norvegicus* subspp.) AND OF COMMENSAL MICE (*M. musculus* subspp.) FOUND TO BE NATURALLY INFECTED WITH *P. pestis* *

Species	*Locality*	*Usual hosts*
Adoratopsylla (Tritopsylla) intermedia copha	Ecuador	*Didelphis*
Amphipsylla primaris mitis	Mongolia	—
Anomiopsyllus sp.	New Mexico, USA	*Neotoma*
Anomiopsyllus hiemalis	Texas, USA	*Neotoma*
Atyphloceras sp.	Western part of USA	*Lagurus, Peromyscus*
Atyphloceras multidentatus	Western part of USA	*Peromyscus*
Catallagia decipiens	Washington, USA	*Lagurus, Peromyscus*
Cediopsylla spillmanni	Huancabamba, Peru	*Sylvilagus*
Chiastopsylla rossi	South Africa	*Tatera, Otomys* and other wild rodents
Citellophilus lebedewi	Central Asia	*Marmota*
Citellophilus tesquorum subspp.	South-east Russia; Transbaikalia	*Citellus*
Coptopsylla bairamaliensis	Central Asia	*Rhombomys*
Coptopsylla lamellifer ardua (*Coptopsylla lamellifer fallax* auctt.)	Central Asia	*Meriones, Rhombomys*
Coptopsylla lamellifer dubinini	Central Asia	*Meriones, Pallasiomys, Rhombomys*
Coptopsylla lamellifer rostrata	Transcaspia	*Meriones, Rhombomys*
Ctenophthalmus breviatus	South-east Russia	*Citellus, Microtus*
Ctenophthalmus cabirus	Belgian Congo	*Arvicanthis* and other rodents
Ctenophthalmus dolichus	Central Asia	*Meriones, Pallasiomys, Rhombomys*
Ctenophthalmus phyris	Belgian Congo	*Arvicanthis, Lemniscomys, Otomys*
Ctenophthalmus pollex	South-east Russia	*Arvicola, Citellus*
Ctenophthalmus secundus	Transcaucasia	*Microtus*

* Reproduced from *Wld Hlth Org. techn. Rep. Ser.*, **165**, 29.

Species	*Locality*	*Usual hosts*
Delostichus (Parapsyllus) talis	Argentina	*Cavia*
Diamanus montanus	Western part of USA	*Citellus*
Dinopsyllus ellobius (*D. lypusus* auctt. nec J. & R.)	South Africa	*Rhabdomys*, *Tatera* and other rodents
Dinopsyllus lypusus	East Africa; Belgian Congo	*Arvicanthis* and other wild rodents
Echidnophaga oschanini	Transcaspia; Central Asia; Mongolia	*Rhombomys*
Foxella ignota	Colorado, USA	*Thomomys*
Frontopsylla semura	South-east Russia	*Citellus*
Hectopsylla eskeyi	Peru	*Cavia* and rats
Hectopsylla suarezi	Ecuador	*Cavia* and rats
Hoplopsyllus andensis	Huancabamba, Peru	*Sylvilagus*
Hoplopsyllus anomalus	Western part of USA	*Citellus*
Hoplopsyllus glacialis affinis (*H. affinis* auctt.)	New Mexico, USA	*Sylvilagus*
Hoplopsyllus manconis (*H. exoticus* auctt.)	Huancabamba, Peru	*Sylvilagus*
Hystrichopsylla linsdalei	California, USA	*Microtus*
Listropsylla dorippae	South Africa	*Tatera*
Malaraeus telchinum	Western part of USA	*Microtus*, *Peromyscus*
Megabothris clantoni clantoni	Washington, USA	*Lagurus*, *Peromyscus*
Meringis shannoni	Washington, USA	*Lagurus* and other wild rodents
Mesopsylla apscheronica	Central Asia	*Allactaga*
Mesopsylla eucta tuschkan	Central Asia	*Alactagulus*, *Allactaga*, *Scirtopoda*
Monopsyllus eumolpi	Western part of USA	*Tamias*
Monopsyllus exilis	Texas, USA	*Onychomys*
Monopsyllus wagneri	Western part of USA	*Lagurus*, *Peromyscus* and other wild rodents
Neopsylla mana	Mongolia	—
Neopsylla setosa	South-east Russia	*Citellus* and other wild rodents
Neotyphloceras rosenbergi	Ecuador	*Didelphis* and wild rodents

Species	*Locality*	*Usual hosts*
Nosopsyllus sp.	Iranian Kurdistan	*Meriones*
Nosopsyllus (Gerbillophilus) aralis	Central Asia	*Meriones*
Nosopsyllus consimilis	South-east Russia	Mice
Nosopsyllus laeviceps	South-east Russia	*Lagurus*
Nosopsyllus mokrzeckyi	South-east Russia	Mice
Nosopsyllus (Gerbillophilus) tersus	Central Asia	*Rhombomys*
Nosopsyllus (Gerbillophilus) turkmenicus	Central Asia	*Meriones, Pallasiomys, Rhombomys*
Odontopsyllus sp.	Huancabamba, Peru	*Sylvilagus*
Ophthalmopsylla volgensis	South-east Russia; Central Asia	*Alactagulus, Allactaga, Dipus*
Opisocrostis hirsutus	Western part of USA	*Cynomys*
Opisodasys keeni nesiotus	California, USA	*Microtus, Peromyscus, Reithrodontomys*
Orchopeas leucopus	New Mexico, USA	*Peromyscus*
Orchopeas neotomae	New Mexico, USA	*Neotoma*
Orchopeas sexdentatus	Western part of USA	*Neotoma, Peromyscus*
Oropsylla ilovaiskii	South-east Russia	*Citellus*
Oropsylla silantiewi	Manchuria; Mongolia; Transbaikalia	*Marmota*
Paradoxopsyllus dashidorzhii	Mongolia	—
Paradoxopsyllus teretifrons	Central Asia	*Meriones, Pallasiomys, Rhombomys*
Peromyscopsylla hesperomys adelpha	New Mexico, USA	*Peromyscus*
Pleochaetis dolens quitanus	Huancabamba, Peru	*Cavia, Oryzomys, Sylvilagus*
Pleochaetis equatoris	Huancabamba, Peru	*Akodon, Oryzomys, Sylvilagus*
Plocopsylla hector	Ecuador	*Thomasomys* and other wild rodents
Polygenis sp.	Ecuador Raquia, Peru Venezuela	*Akodon* *Oryzomys* *Heteromys, Sigmodon*, rats
Polygenis brachinus	Huancabamba, Peru	*Akodon, Oryzomys*
Polygenis litargus	Ecuador-Peru border region Huancabamba, Peru	*Oryzomys, Sciurus* *Akodon, Oryzomys*

Species	*Locality*	*Usual hosts*
Polygenis platensis cisandinus	Argentina	*Cavia* and other rodents
Rhadinopsylla cedestis	Transcaucasia	*Meriones*, *Pallasiomys*, *Rhombomys*
Rhadinopsylla ukrainica	South-east Russia	*Microtus*
Rhadinopsylla ventricosa	Central Asia	*Marmota*
Sphinctopsylla mars	Huancabamba, Peru	*Hesperomys*
Stenistomera (Miochaeta) macrodactyla	New Mexico, USA	*Peromyscus*
Stenoponia conspecta	Central Asia	*Rhombomys*
Stenoponia insperata	Iranian Kurdistan; Transcaucasia	*Meriones*
Stenoponia vlasovi	Central Asia	*Meriones*, *Pallasiomys*
Thrassis bacchi bacchi (=*Thr. gladiolis*)	Western part of USA	*Citellus*
Thrassis bacchi johnsoni	Western part of USA	*Lagurus*, *Peromyscus*
Thrassis fotus	Western part of USA	*Onychomys*
Thrassis stanfordi	Western part of USA	*Marmota*
Tiamastus cavicola (*Rhopalopsyllus cavicola* auctt.)	Ecuador; Peru	*Cavia*
Xenopsylla buxtoni	Iranian Kurdistan	*Meriones*
Xenopsylla conformis	Central Asia; Transcaspia	*Meriones*
Xenospylla eridos (*X.pasiphae*)	South Africa	*Otomys* and other wild rodents
Xenospylla gerbilli caspica	Central Asia; Transcaspia	*Meriones*, *Rhombomys*
Xenopsylla gerbilli minax	Central Asia; Transcaspia	*Meriones*, *Rhombomys*
Xenopsylla hirtipes	Central Asia; Transcaspia	*Allactaga*, *Meriones*, *Rhombomys*
Xenopsylla nuttalli	Transcaspia	*Rhombomys*
Xenopsylla philoxera (*Xenopsylla eridos* auctt.)	South Africa	*Tatera* and other wild rodents
Xenopsylla phyllomae	South Africa	*Desmodillus* and other wild rodents
Xenopsylla piriei	South Africa	*Desmodillus*
Xenopsylla skrjabini	Central Asia; Transcaspia	*Rhombomys*
Xenopsylla versuta	South-west Africa	*Rhabdomys*
Xiphiopsylla lippa	Belgian Congo	*Lophuromys* and other wild rodents