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THE USE OF MICRO-ORGANISMS FOR THERAPEUTIC PURPOSES*

BY

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There can be no one in the whole history of surgery who more deserves commemoration than Lister, and to be asked to deliver a memorial lecture is at once a great honour and a somewhat heavy responsibility. I am not a surgeon, and it did not seem appropriate for me to lecture on an exclusively surgical subject, but as Lister in his own person combined great capacities as an experimentalist with an ability to make epoch-making innovations in surgery, I thought that to-day it might be permissible to treat of some of the scientific background which is becoming more and more important in the practice of surgery.

It may not be realized by all that Lister made some most important contributions to bacteriology—a subject which was to him of the greatest significance when he had grasped the bearing of Pasteur's observations on his own problem of "putrefaction." Lister's aim was essentially what is now called aseptic rather than antiseptic, his object being to exclude all organisms at operation, including those of the air, by means of chemical antiseptics. It is true that he used antiseptics in the treatment of established infections, but his main aim was the exclusion of pathogenic bacteria, which he at first conceived to be those floating in the air. I can imagine that Lister would be delighted to know that in the course of time some of these same organisms have been made to yield substances with almost ideal properties for the treatment and prevention of sepsis. It is a widespread idea that our knowledge of the existence of such substances is of recent origin, so I have thought it might be of interest to trace the development of this subject, partly to give a background against which to measure modern achievements and partly to help to orientate further work from both the biological and the more strictly surgical points of view.

Early Conception of Antibiosis

The antiseptic substances which we now know to be produced by many micro-organisms are beginning to be known as antibiotics. The word "antibiosis" was first used by Vuillemin in 1889. He wrote:

"The lion that springs on its prey and the serpent that poisons the wound before devouring its victim are not considered to be parasites. There is nothing equivocal about it—one creature destroys the life of another in order to sustain its own, the first being entirely active and the second entirely passive; one is in unrestricted opposition to the life of the other. The condition is so simple that it has never been named, but instead of being examined in isolation it can be viewed as a factor in more complex phenomena. For simplicity we shall refer to it as *antibiosis*; the active participant will be the *antibiote*."

Marshall Ward blessed the word in 1899, and it has been frequently used since. The use of the word antibiotic for the actual chemical substances involved in bacterial antagonism was recently introduced by Waksman.

Just as Pasteur's conception of bacteria as the cause of disease was the starting-point of much of Lister's work so Pasteur, with his colleague Joubert, was the first to describe in 1887 the existence of this antibiosis affecting a disease-producing micro-organism. They were working on anthrax, and wrote:

"Neutral or slightly alkaline urine is an excellent medium for the bacteria. If the urine is sterile and the culture pure the bacteria multiply so fast that in the course of a few hours their filaments fill the fluid with a downy felt. But if when the urine is inoculated with these bacteria an aerobic organism, for example one of the 'common bacteria,' is sown at the same time, the anthrax bacterium makes little or no growth and sooner or later dies out altogether. It is a remarkable thing that the same phenomenon is seen in the body even of those animals most susceptible to anthrax, leading to the astonishing result that anthrax bacteria can be introduced in profusion into an animal, which yet does not develop the disease; it is only necessary to add some 'common bacteria' at the same time to the liquid containing the suspension of anthrax bacteria. These facts perhaps justify the highest hopes for therapeutics."

Pasteur himself seems to have done no more work on this phenomenon, but in 1885 several papers of considerable interest appeared. The most important of these was by Babès, who demonstrated on solid as well as in liquid media that one organism can elaborate a substance which will stop the growth of another. He wrote that he had "studied experimentally the way in which bacteria of a known species produce chemical substances or modify the culture medium in such a way as to harm bacteria of other species. If the study of the mutual antagonisms of bacteria were sufficiently far advanced a disease caused by one bacterium could probably be treated by another." And later in his paper he said: "A further and wider study of this reciprocal action of bacteria may lead to new ideas in therapeutics." In this year, 1885, also appeared a paper by Catani, who opened with the words: "The known fact that certain bacteria can destroy other, even pathogenic, microbes if they come in contact with them in any way gave me the idea of exploring this procedure for the treatment of various infectious diseases." He took an organism which was called *Bacterium termo* (it appears to have been a mixture of species) and showed its lack of pathogenicity for animals. He then treated a patient suffering from severe tuberculosis by insufflating

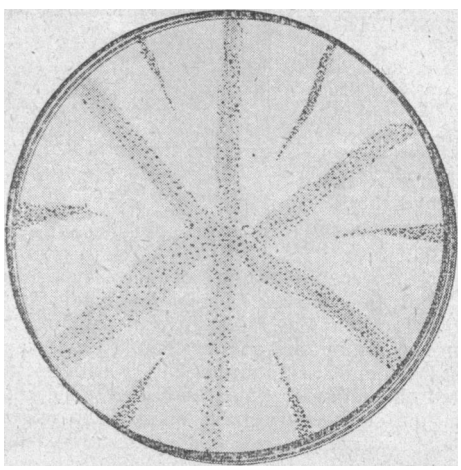

FIG. 1 (from Frost, 1904).—Drawing showing Garré's method of studying antagonism by means of alternate streaks. The large streaks of *Ps. fluorescens* were allowed to grow for 24 hours and then streaks of *Bact. typhosum* were made. These grew only where they were furthest from the streaks of *Ps. fluorescens*.

* The Lister Memorial Lecture, delivered at the Royal College of Surgeons of England, Oct. 11, 1945.

directly into the lungs by means of an atomizer a culture mixed with gelatin. The tubercle bacilli disappeared from the sputum and *Bact. termo* appeared in their place, while at the same time the patient's condition improved. He suggested that this method of bacteriotherapy might be applicable to infected surfaces. This, then, was the first example of the idea of replacing one organism by another less harmful—an idea which, we shall see, frequently recurs in succeeding years.

In 1887 an important bacteriological contribution was made by the Swiss, Garré. He introduced methods for the examination of bacterial antagonism which differ little from those in use at the present day. Fig. 1 is an illustration of one of Garré's methods which was given in a paper by Frost nearly 20 years later. In this the streaks of the antagonist and the organism to be antagonized are arranged alternately, radiating from the centre, but sometimes the streaks were put down parallel to one another at increasing distances. Garré wrote:

"I inoculated on the untouched cooled [gelatin] plate alternate parallel strokes of *B. fluorescens* and *Staph. pyogenes*. This was carried out so that the distance between the inoculated strokes increased from 3 to 15 mm. *B. fluorescens* grew more quickly. Its products of secretion diffused into the surroundings and were completely inhibitory for the near-by staphylococcal inoculation. . . . It is thus not a question of overgrowth or crowding out of one by another quicker-growing species, as in a garden where luxuriantly growing weeds kill the delicate plants. It is also not due to the utilization of the available foodstuff by the more quickly growing organism, but it is a question of antagonism caused by the secretion of a specific, easily diffusible substance which is inhibitory to the growth of one species but completely ineffective against the species producing it."

He also had the idea of using this phenomenon in therapeutics.

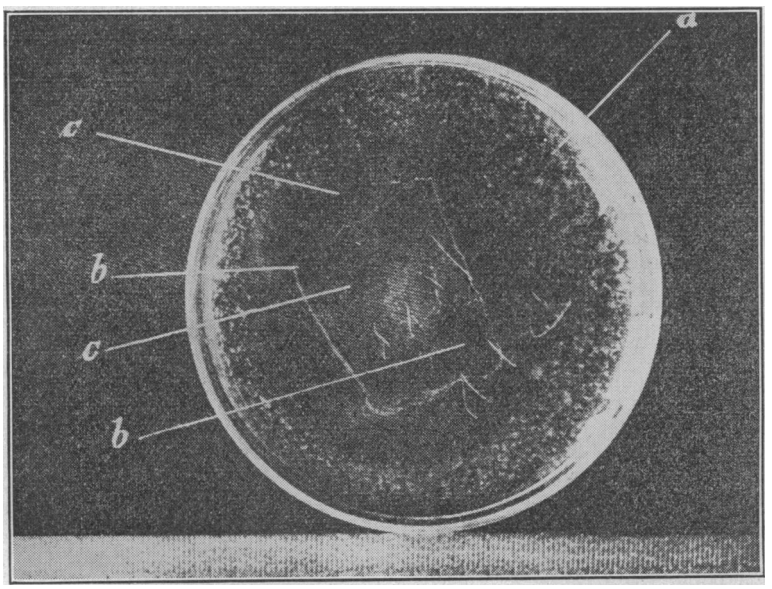

FIG. 2 (from Doehle's thesis, 1889).—A plate was poured with gelatin containing anthrax bacilli. On the surface was planted a square of *M. anthracotoxicus*. Surrounding this square is a zone in which no anthrax colonies have developed, owing to the diffusion of an inhibitory substance from the micrococcus.

There were other publications on the same subject about this time; but the work of Doehle, who published his thesis in 1889, is of outstanding interest, as it contains the earliest illustration of antibiotic action that I have encountered (Fig. 2). Doehle mixed anthrax bacilli with melted gelatin and poured the mixture into a dish and then planted in a square on the surface a certain micrococcus which he had isolated and called "*Micrococcus anthracotoxicus*." After incubation the square where the micrococcus had grown was surrounded by a clear zone in which the anthrax bacilli were completely inhibited. This technique and Garré's differ little, if at all, from those employed by some workers at the present day. Doehle hoped to use his organism for the protection of animals against infection by *B. anthracis*, but his results were indecisive.

Non-specific Immunity

While these clear-cut bacteriological observations had definitely shown that there were micro-organisms which produced substances harmful to the growth of some pathogenic bacteria another approach to bacteriotherapy was being made, which had as its basis the idea of a non-specific immunity produced by one type of organism against another. Clinicians had for long believed that an attack of erysipelas was beneficial in certain chronic infections, and in 1883 Fehleisen deliberately treated a case of lupus by injecting streptococci obtained from a patient with erysipelas. In 1887 Emmerich made observations on anthrax infection which showed that an injection of streptococci enabled a substantial proportion of rabbits to survive a subsequent dose of anthrax bacilli large enough to kill all the control animals. Moreover, the intravenous injection of streptococci into rabbits already showing "clinical" anthrax prevented the death of 60%. Emmerich demonstrated that there was no antagonism between the two organisms *in vitro* on the surface of a solid medium, so he ascribed his results to stimulation by the cocci of the body cells, which were then able to demolish the bacilli. This work was confirmed by several people, some of whom used other organisms than the streptococcus and in some cases killed the cultures before injection.

A paper by Bouchard in 1889 started work on another organism which still continues even at the present time. He published results showing that the inoculation of *Ps. pyocyanea* conferred a considerable degree of protection against *B. anthracis* infection in rabbits, though in guinea-pigs the results were not so good. This same organism was actually injected by Rumpf in 1893 for the treatment of typhoid fever in man, but the good results which he claimed were not confirmed by Kraus and Buswell (1894) or Presser (1895).

Local Application of Bacterial Products

During this period a number of authors contributed to the further accumulation of knowledge, but from a clinical point of view the observations of Honl and Bukovsky (1898) are of most interest. These Russian investigators described in enthusiastic terms the results of the local application of "proteins" from cultures of *Ps. pyocyanea*. They thought that they might accelerate the healing of ulcers by applying the proteins or products of micro-organisms isolated from the ulcers, but gave no details of how they made their preparation. They treated by local application more than a hundred infected ulcers of the leg and similar cases, some of which were so bad that amputation was under consideration, and they were very pleased with their results.

Unconnected with this work, Emmerich and Löw in 1899 introduced the first antibacterial extract into medicine. This extract, which they called pyocyanase, was capable of lysing suspensions of *B. anthracis in vitro* in a short time, and was also bactericidal to *Bact. typhosum, C. diphtheriae*, staphylococcus, and *P. pestis*. It was prepared from old cultures of *Ps. pyocyanea*. They performed therapeutic experiments on animals with the substance, but their views as to the rationale of the treatment are not by any means clear. Apparently they became confused between their knowledge of its antibiotic effect and their wish to bring it into line with the prevailing views on immunity.

Following Emmerich and Löw's work there was a remarkable outburst of energy both in the laboratory and in the clinic. Laboratory work fully confirmed that the extract was bactericidal, but controversy raged over the nature of the substance. Emmerich and Löw maintained that it was an enzyme (that is, a protein), while other workers found good evidence that it was a "lipoid" (that is, soluble in certain organic solvents)—a view which is confirmed by recent work.

A few years after the beginning of the century the idea of using it in the clinic by injection was replaced by that of using it as a local antiseptic. There can be no doubt that it was bactericidal, and Emmerich and Löw also thought that it was more harmful to bacteria than to animal cells. This important

idea of differential toxicity, which so largely governs the choice of antiseptics to-day, must have been widespread at that time, for Escherich wrote in 1906: "The resumption of these endeavours first became possible when the march of science made known to us a substance which possessed a high bactericidal capacity without at the same time harming the tissues as do previously known antiseptics. This material is the bactericidal substance from the autolysis of bacteria, discovered by Emmerich and Löw." In 1913, in a review of the position, Sonnenberger echoed these views, for he wrote: "The use of pyocyanase as a therapeutic agent is based on exact theoretical considerations. These are backed by clear-cut experiments both *in vitro* and *in vivo*. Its harmlessness within the limits of its usual employment is well established. Its capacity for healing in a large number of diseases has been proved in the clinic. Pyocyanase may be considered an important addition to therapeutics. Its widespread use is recommended within the limits given in this work." This material was very extensively used in the clinic. Many papers deal with its employment as a local antiseptic in diphtheria both for acute cases and for carriers. Meningococcal carriers were also, it was claimed, successfully treated, and the suggestion was made that it might be useful as a prophylactic spray during epidemics. It was used for conjunctivitis and other eye infections, for injection into abscesses, for the treatment of infection of the accessory nasal sinuses and diseases of the mouth such as Vincent's angina. It was injected into the trachea and even injected intrathecally in cases of meningitis. Many of these procedures have their parallel in recent uses of penicillin. It was used in veterinary medicine for streptococcal mastitis by injecting it into the udder, much as gramicidin and penicillin are used at the present day, and in addition both chemical and pharmacological examinations on a considerable scale were done to determine its properties. Thus it can be said with some truth that there is little that has been done with penicillin which was not attempted with the earlier antibiotic so far as the means then available allowed.

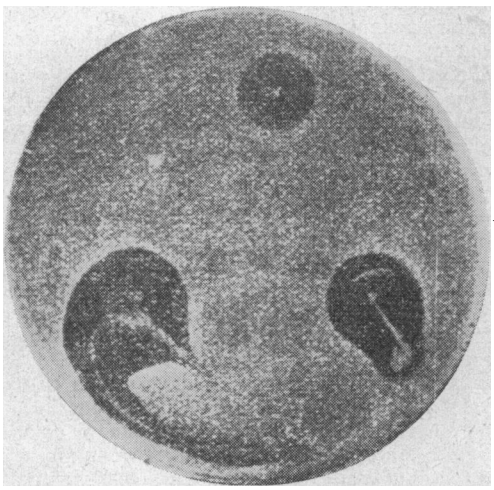

FIG. 3 (from Lode, 1903).—This shows a plate thickly sown with *M. tetragenus*. Colonies of an accidental air contaminant —a coccus—have been planted at three places, and around them are zones in which the growth of *M. tetragenus* is inhibited.

While this work was being actively pursued other investigations of great interest were recorded. In 1903 Lode wrote of an accidental contaminant he had found while preparing a plate of *M. tetragenus* for class demonstration purposes. This contaminating organism, which was a Gram-positive coccus, was subcultured and shown to produce a diffusible inhibitory substance (Fig. 3). It strongly inhibited anthrax bacilli and *Staph. aureus*, but not *Bact. coli* or Friedländer's bacillus. Lode did considerable work on the production of the antibiotic in fluid cultures, and showed that it was bactericidal. It was not an enzyme, though it was thermolabile, being inactivated slowly by heat. It could be dried by vacuum distillation and was soluble in alcohol but not in ether. Neither the micro-organism nor its metabolic products were toxic to animals, but unfortunately in artificially produced infection in mice the product had no chemotherapeutic effect. Nevertheless, this work is remarkable for the comprehensive and painstaking investigation which it displays.

In 1904 Frost published an important paper in which he described as many as seven different technical procedures for the investigation of bacterial antagonism both on solid and in liquid media. One of his most interesting techniques was to interpose a collodion membrane between culture fluid containing the organism he was trying to inhibit and culture fluid containing the organism which he suspected of producing an antibiotic. Fig. 4 is his illustration of this technique.

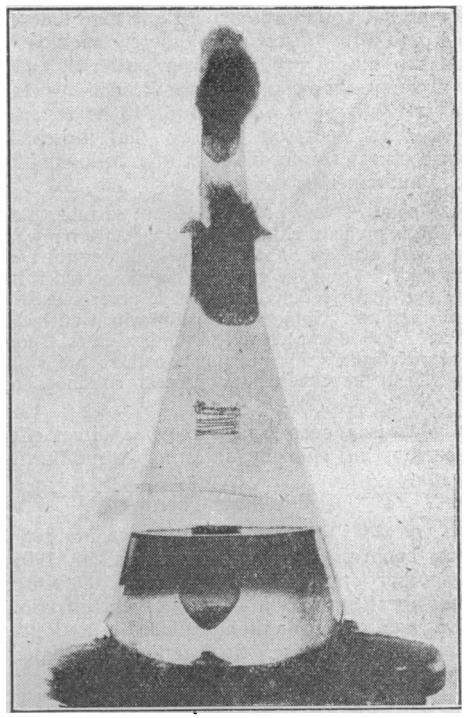

FIG. 4.—Frost's (1904) collodion sac for the study of bacterial antagonism. One organism is sown in the broth in the sac and another in the broth outside it in the flask. Inhibitory substances can pass through the collodion membrane, but the bacteria are kept apart.

The First Crystalline Antibiotic from a Mould

Somewhat before this time the first example of a crystalline antibiotic obtained from a mould was recorded. The mould was a *Penicillium* and the antibiotic is now called mycophenolic acid. Gosio, who did this work in 1896, showed that the crystalline material inhibited the growth of anthrax bacilli, but regretted that he was unable to perform any animal experiments owing to lack of material.

Chemotherapeutic Experiment

Nicolle demonstrated in 1907 the existence of bactericidal substances produced by *B. subtilis*; but of more immediate clinical interest are observations published by Rappin in 1912 in a journal which probably circulates little outside France. He found that *B. subtilis*, *B. mesentericus*, and *B. megatherium* acted strongly on *B. tuberculosis in vitro* and that the effect could be produced by filtered broth cultures. He went on to say:

"After having established a long time ago that these diastases [as he believed the active substances to be] were very active I naturally went on to consider whether they might not have important effects on the evolution of the tuberculous process. Nearly two years ago I started an investigation with the object of verifying this hypothesis. Several guinea-pigs which had previously been infected with tuberculosis were injected with filtered cultures of *B. mesentericus*, and I possess to this day one of the first series of animals which, treated in that way, lived more than 21 months after a virulent inoculation, with every appearance of perfect health. Another which died after 9 months had put on weight regularly and shown no sign of illness, and no lesion was seen at necropsy except a swelling at the site of inoculation and one in the opposite side. It is a very important fact that scrapings from these swellings inoculated into another animal have not so far

produced in it any sign of a reaction. When this experiment was repeated on other guinea-pigs similar results were obtained, and I have several animals which were treated in this way at varying intervals after infection with tuberculosis which are apparently in good health, while the controls have died with the classical lesions of experimental tuberculosis. These results have been obtained with a relatively small number of injections. Some animals received only two injections of filtered *mesentericus* broth in doses of one to two cubic centimetres given subcutaneously, and others the same amount into the peritoneal cavity. Neither group appeared to be disturbed by the injections, and there were no reactions."

Vaudremer wished to dispute the question of priority with Rappin, for he had found about the same time that *Aspergillus fumigatus* would also "digest" the tubercle bacillus. It is now known that this mould produces four powerful antibiotics, at least one of which—helvolic acid—has been shown to be active *in vitro* against *M. tuberculosis*. In 1913 he reported animal experiments, and went on to say that he had injected *A. fumigatus* filtrates into man, in the following terms (the italics are Vaudremer's):

"From the point of view of the treatment of tuberculosis in man any conclusion is premature. *Since 1910 we have treated more than 200 patients with extracts of A. fumigatus* in several hospitals and sanatoria in Paris. From the cases we have observed it is justifiable to conclude that the injections, which have never caused any febrile reaction, are harmless. Sometimes healing which we had not hoped for has taken place unexpectedly. In other cases there has been transient improvement, but unfortunately there are still many instances in which the tuberculous process continues to take its course."

He had obviously been trying to get a direct effect on the tubercle bacillus *in vivo* by injecting a mould metabolism solution.

Replacement Therapy

It will be recalled that Cantani had tried in 1885 to replace the tubercle bacillus with *Bact. termo*, and in 1909 this idea received another application. Schiøtz, a Danish physician, noticed that a patient with a staphylococcal infection, wrongly diagnosed as having diphtheria and placed in a diphtheria ward, did not develop the disease. Schiøtz then deliberately sprayed suspensions of staphylococci into the throats of carriers with, he claimed, good results. Many other people tried a similar procedure at different times, and some claimed that benefit was obtained—at any rate, none mentioned any disastrous infection from the staphylococcus. It may sound a somewhat absurd and empirical procedure in the absence of bacteriological information, but it is, as a matter of fact, true that many strains of staphylococcus will inhibit the growth of the diphtheria bacillus —as was shown, for example, by Dujardin-Beaumetz in 1932. In 1915 Colebrook suggested using the pneumococcus in the same way to combat the meningococcus in carriers, following on an *in vitro* observation of antagonism. Later still, in 1921, the pneumobacillus of Friedländer was suggested by Papacostas and Gaté for the treatment of diphtheria carriers.

The best-known example of replacement therapy, however, was furnished by the suggestion of Metchnikoff for the displacement of harmful gut organisms by the Bulgarian bacillus, which was later to give place to *B. acidophilus*. There is a very considerable literature on this subject, into which we will not go except perhaps to call attention to the fact that the lactic acid bacillus was used for the treatment of various infections in addition to those of the intestine. Newman in 1915, in this country, was treating cases of cystitis by injecting lactic acid bacilli into the bladder, and many authors treated infections of the nose, mouth, and sinuses, including diphtheria. Cultures of the bacilli found application in the treatment even of puerperal fever, breast abscesses, and other infections. The good clinical effects were ascribed to the production of lactic acid, which stopped the growth of the pathogens.

Another interesting replacement was suggested by Nissle in 1916. He maintained that certain strains of *Bact. coli* were antagonistic to other and more harmful organisms and that these antagonistic strains should be established in the gut: he even went so far as to say that newborn infants should be deliberately given these desirable strains. He put on the commercial market a preparation of living *Bact. coli* called "mutaflor," which apparently had a considerable sale. Here again there is pictorial evidence that such antagonistic strains of *Bact. coli* exist; for Gratia, who had described such an antagonism in 1925, figured this organism in 1932 (Fig. 5).

In connexion with the idea of "fighting" one intestinal bacterium with another, an interesting line of reasoning was displayed by Nitsch (1908). Apparently at that time two towns in France, Lyons and Versailles, were notorious for their freedom from epidemics of cholera. Nitsch reasoned that the only thing of which all the inhabitants of Versailles partook in common was air, and so it occurred to him that bacteria in

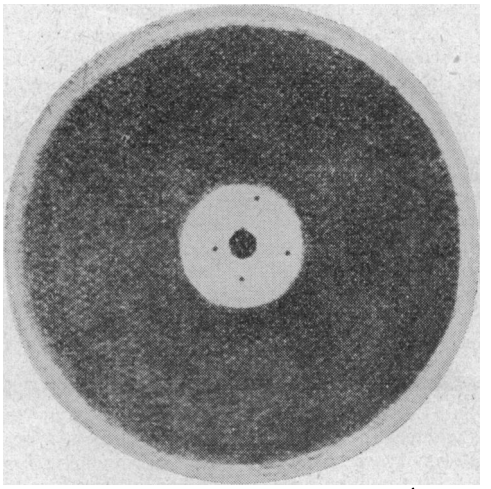

FIG. 5.—Gratia's (1925 and 1932) demonstration of the inhibition of one strain of *Bact. coli* by another. The strain sown over the plate is inhibited round the central colony of the other strain except for a few resistant colonies.

the air might inhibit the growth of the cholera organism and if they were swallowed would exert their antagonistic effects in the intestine. On putting this to the proof he found 11 strains of air bacteria out of 220 from various parts of Versailles and 4 out of 253 from Paris which when cultured at 37° C. inhibited the growth of *V. cholerae*. He obviously tested them on solid media, for he described sterile zones round the colonies varying in radius from a few millimetres to a centimetre, while many others had slighter effects. Most of the antagonistic organisms were like streptococci. He thought the next stage was to see if the faeces of the good inhabitants of Versailles harboured these organisms, but apparently this was never done —possibly Nitsch went home to Poland. Another Pole, Choukevitch (1911), carried on the observations with three of Nitsch's Versailles strains—Gram-positive organisms whose colonies had the appearance of streptococci when grown on agar. He confirmed that if a streak of one of these organisms was planted on an agar surface and *V. cholerae* was planted at the same hour on the following day, growth of the vibrios did not take place within a distance of 1 cm. from the antagonist. He found that the inhibition was not due to acid, that the inhibitory substance developed in broth as well as in solid media, and that it was thermolabile and not capable of filtration. He attempted to establish the antagonists in the intestines of newborn rabbits—animals which Metchnikoff had shown to be susceptible to cholera—but he was not able to demonstrate any protection against ingested cholera organisms, perhaps because he could never show that the cocci had established themselves. This episode is of considerable interest in that it was another early attempt to utilize for medical purposes organisms which produced one or more antibiotics.

Some New Ideas and Procedures

The nineteen-twenties saw the introduction of some new ideas, new procedures, and new antagonistic organisms. In 1921 Lieske published a monograph on the actinomyces, the great majority of which are harmless soil saprophytes. He wrote: "It is easily shown that actinomycetes secrete a specific substance acting extracellularly. There is a fairly wide zone round an actinomycete colony where other organisms are killed. This inhibition is not simply due to exhaustion of the medium by the actinomycete, for colonies of bacteria which

have already grown are lysed and disappear entirely." He studied particularly the effects on killed bacteria, many species of which were lysed, but he also saw lytic and inhibitory effects on live bacteria. He had in mind the possibility of making use of this property in therapeutics in a similar way to that in which pyocyanase had been used but nothing came of this.

Later Gratia and Dath confirmed his observations; they found a streptothrix which was particularly active, and examined many sources for antagonists (Fig. 6). They wrote: "We have

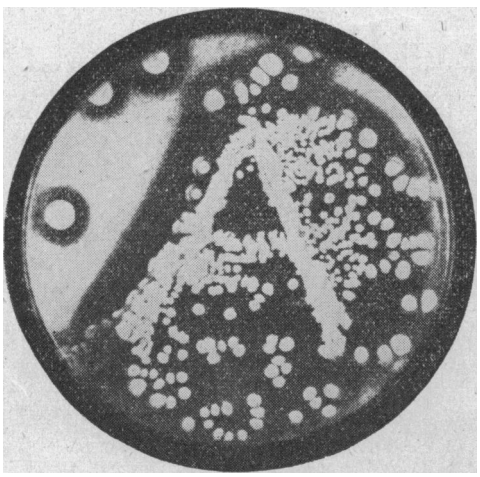

FIG. 6.—Demonstration by Gratia and Dath (1924 and 1934) of the lytic power of a streptothrix. The gelatin contained a heavy emulsion of staphylococci. After 3 days, as shown, there was lysis of the staphylococci round the streptothrix (planted in the shape of an A), and after 7 days the plate had cleared completely.

exposed plates of 2% gelatin in tap-water, incorporating a suspension of a sensitive microbe, such as the cholera bacillus, to air, and to such vehicles as tap-water, drain-water, mud, etc. By this means we have isolated active colonies not only of the usual streptothrix but of other moulds and also of various saprophytic bacilli, both Gram-positive and Gram-negative. As we see it these are examples of a general phenomenon in which a great variety of agents act against many saprophytic and pathogenic bacteria. Among these agents the streptothrices are the most widespread and the most active." Gratia proposed to use the lytic powers of his streptothrix for the purpose of preparing vaccines. He dissolved certain organisms, such as the staphylococcus, by means of the product of the streptothrix later called actinomycetin, and produced what was known as a mycolysate. This mycolysate was used for immunizing purposes with, it was claimed, results which were better than those obtained by ordinary vaccines. This idea, however, was not even then new, for Nicolle had performed experiments on animals in 1907 in which he showed that immunity could be produced by using the products of *B. subtilis*. Working in 1923, 1924, and 1925 with the same group of spore-forming organisms, Kimmelstiel, Much, and Sartorius had described the production of a lysin from certain *B. mycoides* strains which they named *B. cytolyticus*. By means of this organism lysates of numerous pathogenic organisms were made and produced commercially under the name of "sentocym," and there are clinical reports of their efficacy in such conditions as *Bact. coli* infection of the urinary tract. As well as using it for producing vaccines, Much had the idea of employing the lytic substance as a chemotherapeutic agent, for he wrote:

> "The material for injection obtained by the activity of *B. cytolyticus* contains, firstly, a solution of a particular bacterium, that is a colloidal dispersion of the bacterial content—the lysate. Secondly, it contains the dissolving agent—the lysin. This lysin acts in three ways. Firstly, the bodies of the highly reactive *B. cytolyticus* (Much) form a powerful unspecific therapeutic stimulus. Secondly, the lysin acts on the infecting bacteria in the body as a lytic agent and so frees the body directly from the bacteria. We are dealing here, therefore, with a real 'bacteriolytic' power, which is quite different from the hypothetical power of a serum. This power is clearly and unambiguously shown *in vitro*. Thirdly, products from the bacterial bodies destroyed *in vivo* by the lysin are added to assist the stimulating action of the lysate."

In 1925 Zukerman and Minkewitsch found on a plate of pseudo-diphtheria bacilli an accidental contaminant which was antagonistic to their growth. This is yet another example of an accidental contaminant which was noticed because of its inhibitory properties and subsequently isolated and examined. The organism was *B. mesentericus vulgatus*, and it showed antagonism only to diphtheria and pseudo-diphtheria bacilli, not to staphylococci, *Bact. coli*, *Bact. typhosum*, or a number of dysentery bacilli. Just as pyocyanase and the substance of Lode had selective antibacterial action, so this material showed the phenomenon to a very marked degree. The antibacterial substance was demonstrated to be mainly bactericidal—a 1% solution of a broth filtrate being potent enough to kill *B. diphtheriae* in 24 hours. Experiments were done with animals to see if the filtrate, the activity of which was heat-stable, was capable of affording protection to guinea-pigs against *C. diphtheriae*; but no sure results could be obtained, and the authors did not report them in detail. In 1925 Rosenthal also contributed work on the bacteriolytic powers of *B. scaber*, another spore-forming organism.

In 1929 Fleming noticed an accidental contaminant—a mould this time—which was also lytic to certain pathogenic organisms. The substance it produced was found to appear in the broth and to belong to the slow-acting type of antiseptic. It had marked differential powers of inhibiting bacterial growth. The crude metabolism liquid was shown not to be more toxic to animals than broth in which nothing had grown. No experiments were performed on experimental infection in animals, but the suggestion was made that the substance would be useful for dressing septic wounds in man, and indeed some were so treated without, however, any very striking results. The clinical possibilities of this antibiotic were not pursued at that time.

About that time, too, the explanation was forthcoming of why pyocyanase had largely dropped out of use in the clinic, as Lode complained when he wrote in 1929, referring to the views of Sonnenberger (1913): "In spite of these recommendations papers on therapy with pyocyanase have almost disappeared from recent literature. This is unjustifiable, so experienced clinicians like Herzog the otologist inform us, for the base of suppurating or very dirty wounds is cleaned quickly without damage to the tissues." There is no doubt that active pyocyanase had often been prepared in the laboratory, and probably commercial preparations had been active at first; but Wagner in 1929 and Kramer in 1935 showed that what activity commercial pyocyanase possessed at that time was almost entirely due to its contained phenol—a good example of what may happen to commercial biological preparations without the necessary strict control. How powerful active pyocyanase can be is shown by the recent work of Rake *et al.* (1943), who found that it inhibited the growth of *Str. pyogenes* at a dilution of 1 in 24,000.

In the nineteen-twenties another idea which has never been thoroughly examined was introduced by Schiller (1924a, 1924b, 1925a, 1925b, 1927; Schiller and Giltscher, 1928). In a series of papers he brought forward evidence that yeasts and possibly other organisms could be made to produce substances that were antagonistic to certain bacteria by growing them on media in which the only source of nitrogen was the bodies of the bacteria it was desired to antagonize. He claimed that in such experiments yeasts developed a thermolabile inhibitory substance which might be of value in therapeutics. He called the phenomenon "induced" antagonism, in contradistinction to the "natural" antagonisms we have so far been considering, and it seems likely that much more might be done to develop this line of research.

During the nineteen-thirties examples of antibiotic effects continued to be recorded and some suggestions made for therapeutic employment of the products; for example, Weiland in 1936 described the action of *B. mesentericus* against diphtheria bacilli and proposed that culture material from this organism would be at least as good as pyocyanase.

The Position in 1939

We may perhaps summarize the position at the end of the nineteen-thirties thus. A very large number of observations on antibiosis had been recorded from 1877 onwards—some of

the most striking have been considered, but there were a great many more—and from all this work certain proposals had emerged for the use of micro-organisms in therapeutics:

1. The replacement of a pathogenic organism by another and less harmful organism—for example, "*Bact. termo*" to replace the tubercle bacillus in the lungs; staphylococci to replace diphtheria bacilli, and pneumococci to replace meningococci, in the throat; lactic acid bacilli and *Bact. coli* to replace organisms infecting the intestine.

2. Artificial immunization by one organism to protect against infection by another.

3. The use of lytic substances from one organism for the preparation of soluble vaccines of other species—for example, Much's sentocym and Gratia's mycolysates.

4. The use of soluble bacterial or mould products by parenteral injection to treat established diseases—for example, pyocyanase in anthrax, and Vaudremer's extracts of *Aspergillus fumigatus* and Rappin's extracts of *B. subtilis* in tuberculosis. These were examples of true chemotherapeutic use.

5. The use of soluble bacterial or mould products as a topical application for the treatment of local infections—for example, pyocyanase and penicillin broth.

The Crystalline Polypeptides

In spite of an enormous bulk of work extending over 70 years, much of which has not been considered here, up to the end of the nineteen-thirties no antibiotic substance from a mould or bacterium had found an undisputed and permanent place in medicine. But in 1939 important papers were published by Dubos in which he described the isolation of a very active selective antibiotic from a spore-forming soil bacillus, *B. brevis*. The work embodied in these and subsequent papers comprised systematic and detailed investigations, both chemical and biological, and reached a new level in the scientific investigation of antibiotics. The active substance, now called tyrothricin, was separated into two crystalline polypeptides—gramicidin and tyrocidine—the first of which was particularly active against Gram-positive organisms such as pneumococci and streptococci. With this material animal experiments were performed, and it was shown that, although it was very toxic when introduced intravenously, it protected mice infected with pneumococci intraperitoneally to a high degree when small amounts of the drug were introduced into the peritoneal cavity even some hours later than the infecting dose of organisms. The work was then carried to the clinic, and it was clearly established that in some ways this antibiotic was superior to any known chemical antiseptic for local application to sites infected with susceptible organisms. A very considerable literature has now collected on the subject, some of it reminiscent of early pyocyanase work, and it seems probable that gramicidin will not occupy a permanent place in medicine only because of the discovery of less toxic substances soon after its introduction. Such, however, is the repercussion of one branch of science on another that *x*-ray analysis of these crystalline polypeptides and others like them may help to elucidate the structure of proteins. Already much interesting chemical and biochemical work has been done on these substances.

Penicillin

We have seen that the idea of using an antibiotic for parenteral injection into an infected animal had been brought forward for pyocyanase by Emmerich and Löw, for the products of *B. subtilis* by Rappin, for *B. mycoides* by Much, and for *Aspergillus fumigatus* by Vaudremer, but no antibiotic had been unequivocally shown to possess curative properties when so used. In 1940 the first observations on penicillin from Oxford were published (Chain *et al.*) which distinguished clearly this antibiotic from any of the preceding ones, for up to this time the real significance of penicillin had escaped detection. There are a large number of substances of both synthetic and natural origin which kill bacteria. Unfortunately most of them also damage animal tissues. Lister clearly realized this in the early days of antiseptic surgery. As his biographer, Godlee (1917), wrote:

"The antiseptic system aimed at destroying the germs by chemical agency, either before they gained access to a wound at the time of its infliction, or so soon afterwards that they had no chance of developing and multiplying; and, this end having been attained, it further aimed at preventing by means of chemical antiseptics the access of germs to the wound until the process of healing was complete. The problem as thus stated sounds simple, and so it appeared to be in the early days. But, even from the first, one great complication was apparent. Lister recognized the power of the living tissues to control the growth of micro-organisms, and the importance of not interfering with the vitality of the tissues by any harmful agency whatever. Therefore he felt bound to reduce the strength of the chemical substances which he employed to the minimum consistent with efficiency."

It has been seen how pyocyanase was used as a local antiseptic, and later it was proposed that penicillin broth could be used in the same way. The experimental work at Oxford disclosed, however, that an extract containing penicillin could be made which was of such incredibly low toxicity that it could be injected intravenously or by other parenteral routes in amounts far greater than those required for therapeutic effect without producing any sign of toxicity (Abraham *et al.*, 1941). It could thus circulate in the blood and body fluids in sufficient quantity to destroy sensitive bacteria in combination with the natural

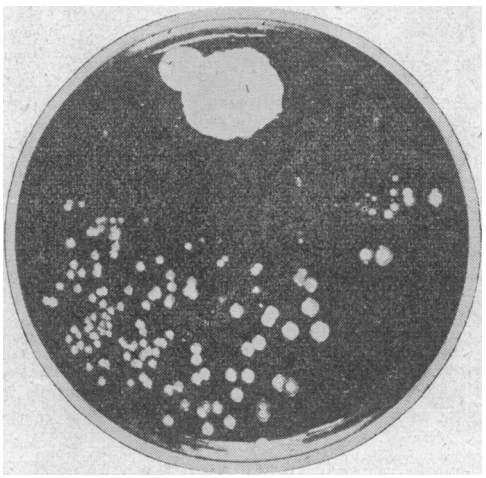

FIG. 7.—Photograph of a culture-plate showing the dissolution of staphylococcal colonies in the neighbourhood of a penicillium colony. (Alexander Fleming, *Brit. J. exp. Path.*, 1929, **10**, 226.) (Reproduced by permission of the Editors of the *British Journal of Experimental Pathology*.)

body defences without the least damage to those defences or to other tissues.

This clear-cut experimental demonstration of its chemotherapeutic action was the starting-point of the serious endeavour to apply penicillin in the clinic. By the time these observations were made—in 1940 and 1941—it had already been clearly established that synthetic chemicals of the sulphonamide group could be absorbed into the blood stream in sufficient concentration to deal in a spectacular manner with many infections—for example, lobar pneumonia and meningococcal meningitis and certain streptococcal infections such as puerperal sepsis. It was natural to suppose that these compounds would be of the greatest use in war surgery, and they were employed in large amounts both by mouth and by local application to wounds throughout the war. Probably the sulphonamides have been instrumental in diminishing generalized sepsis by preventing the spread of streptococci from wounds into the blood stream, but it was evident fairly early in the war that their use was not the final solution of the treatment of the suppurating wound or even for prophylaxis. The main reason for this is probably that there exist many inhibitors of the antibacterial action of the sulphonamides and that some of these are present in pus and in the breakdown products of tissues. It was therefore of the greatest good fortune that it had been discovered by 1941 that extracted penicillin was fully active in the presence of pus and tissue breakdown products, for it was the knowledge of this fact in conjunction with knowledge of the diffusibility and lack of toxicity of the extracts which made much of the subsequent work to bring the substance into clinical use appear worth the great effort necessary. From the results of experimental work it seemed more than likely that penicillin could be applied with success to septic infections involving pus formation either by injecting it into the blood stream or by local administration. These expectations have been fully borne out by subsequent clinical investigations, which have shown clearly that infection by sensitive organisms can be checked and overcome. An

additional encouragement lay in the fact that the staphylococcus was highly sensitive to penicillin, thus bringing into its therapeutic field a group of infections which were outside the range of any of the sulphonamides then known.

In the use of penicillin in war surgery there has been an evolution. Attention was at first directed to the relatively small proportion of wounds which had become severely and chronically infected, as it was thought that here the small amounts of penicillin then available would have the greatest effect. But the slow and sometimes disappointing progress in these deeply infected wounds drew attention to the fact that the use of penicillin in warfare involved not one consideration but two—not only the best methods of application but also the best time at which to make the application. So a move was made to the forward hospitals, and wounds of a few days' standing, even though septic, were treated by appropriate surgery, including suture, and penicillin applications, and in this way an attempt was made to prevent serious and chronic sepsis ever developing. Later, when penicillin became more plentiful —at the time of the invasion of North-West Europe—it was possible to give penicillin prophylactically soon after wounding. Though many factors have contributed to the excellence of the surgical results obtained in the later years of the war few will, I think, now dispute that penicillin has played an important part.

You would not wish me to describe again the reasoning on which the procedures for the use of penicillin in surgery were founded, but on this occasion it may be appropriate to point out that the position has now been reached when a very great measure of success can be claimed in the treatment and prophylaxis of sepsis for a combination of aseptic methods—that is, of methods not associated with chemicals and of antiseptic methods which involve their use. The really important point with regard to the latter is the recognition that there exists an antiseptic beyond the dreams of Lister—an antiseptic which can be applied through the blood stream as well as by appropriate local means, one immensely powerful against many pathogenic organisms but at the same time harmless even to very delicate tissues, and one fully active in surroundings where the sulphonamides fail.

An Outburst of Research

With the demonstration that at least one of the antibiotics was not simply an antiseptic, as had previously been supposed, but a chemotherapeutic agent in the sense in which that word is now used, a great outburst of research has taken place all over the world. Workers who had abandoned the field years ago again became interested, and it was not long before the air was filled with the cries of those wishing to join the new experimental gold rush and to secure what had previously been abandoned. In these days it is uncommon to peruse a medical journal without finding a record of some new organism with antibiotic powers or the re-examination of one already known. One of the most piquant has recently appeared: a mould isolated from human hair in New York has been found to produce a red dye which is active against *Bact. coli*. What a chance for journalists, who have already shown something of their imaginative powers in connexion with penicillin, to wax lyrical and romantic if this substance should ever be of therapeutic importance and it should be disclosed that it was a woman's hair ! I cannot help feeling that Lister would be mightily amused to see the army of eager enthusiasts now stretching out suppliant Petri dishes in the hope that they will receive some miraculous therapeutic manna. Some workers think that the energy spent in the search for new antibiotics would be more profitably spent in investigating more minutely the action of those already known, with the object of understanding bacterial metabolism better. This may be true, but, on the other hand, the search for more antibiotics with a chemotherapeutic action may be of service to surgery ; for, successful as the application of penicillin has been not only in war but also in civilian surgery, it cannot be said that all forms of sepsis can yet be controlled. Penicillin is ineffective against the Gram-negative bacilli, such as *Ps. pyocyanea*, *Bact. coli*, and *Proteus*, and these organisms can still cause mild suppuration in wounds free from streptococci and staphylococci. There is in addition the possibility of resistant strains arising, and though this has so far happened relatively rarely with penicillin, alternative drugs which will do for penicillin-resistant strains what the latter has done for some sulphonamide-resistant strains would be a valuable acquisition. The search, perhaps less urgent now the war has ended, goes on, and already a most interesting compound, streptomycin, has been isolated by Waksman and his collaborators (Schatz, Bugie, and Waksman, 1944) from an actinomycete—one of the group of organisms which, it will be recalled, was first shown to produce antibiotics by Lieske in 1921. Streptomycin is a base which is remarkably non-toxic to animals and can be given in large doses in much the same way as penicillin, and it is active against some of the Gram-negative organisms such as *Bact. coli* and *Ps. pyocyanea*. It has been shown, in mice, to have true chemotherapeutic action against infection by *Ps. pyocyanea* and other Gram-negative organisms, and it has been tried clinically—though with no clear-cut success—in typhoid fever and other infections. It has also some effect, both *in vitro* and experimentally *in vivo*, against the tubercle bacillus. As yet little has been reported of its use in surgery. Besides this most interesting compound this group has furnished streptothricin (Waksman and Woodruff, 1942), similar to but more toxic than streptomycin, and proactinomycin, a substance isolated by Gardner and Chain (1942) from an accidental contaminant. This last substance acts against Gram-positive organisms, and has recently been shown to have chemotherapeutic properties against peritoneal infection by the streptococcus when given by mouth (Florey, Jennings, and Sanders, 1945). It may be a measure of the progress in the chemotherapeutic field that this fact has not excited us greatly, for eleven years ago, before the discovery of the sulphonamides, it would have been considered almost miraculous. Some recent unpublished work which the authors permit me to mention illustrates clearly how very careful one has to be in assessing the powers of antibacterial substances. These three substances from the actinomycetes are all bases, and Dr. Abraham and Dr. Duthie find that their antibacterial power is considerably altered by relatively small changes in the reaction of the medium in which they act. Thus at *p*H 8 their activity is many times greater than at *p*H 7, and at *p*H 7 more than at *p*H 6. When it is realized that autolysing tissues and pus are quite acid it becomes clear that *in vitro* observations carried out in media at *p*H 7.4 or thereabouts may give far too optimistic a picture of what these substances are capable of achieving in the body.

Another development which illustrates fully the great importance of the biochemist in this field of work has recently been furnished by Doisy and his colleagues (1945). Biochemists and bacteriologists working together have isolated four crystalline substances from the organism on which so much sustained work was done in the early days—*Ps. pyocyanea*. These substances, which the workers call Pyo I, II, III, and IV, act powerfully against Gram-positive organisms and appear to have little toxicity to animal tissues, but experiments with infected animals have not yet been reported. It would be intensely interesting if this organism which has been worked on for so long should at last yield a substance of permanent value in therapeutics.

The field for research is obviously vast, and at the moment much attention is being applied to the possibility of discovering an antibiotic which will be effective against tuberculosis. That such a possibility exists no one will deny, but the urge to fame is such that I greatly fear there will be many unreliable and premature publications on this subject. The exploration of the possibility that skin fungal infections and protozoa may be susceptible to some antibiotics has only just begun—with what prospects no one can yet say.

Conclusion

I hope I have been able to make clear that the present happy position in the prevention and treatment of sepsis has not been reached by a flash of insight on anyone's part, but that we at Oxford made a choice from among the many antibiotics known and had the great good fortune to be able to show for the first time that an antibiotic could also be a chemotherapeutic agent.

One great lesson which a survey of work on antibiotics should have taught us is that real and continuous progress and understanding can be obtained only by the collaboration of people

with various outlooks and experimental accomplishments, and, in particular, that little progress can be made without first-class biochemical work. The other important point is that steady and rapid progress is possible in the clinic only when the clinician has firmly based experimental foundations on which to work. It is easy to exaggerate the importance of antiseptic drugs in surgery, and I have indeed noticed signs of restiveness among surgeons when misguided people have wished to attribute all their success in dealing with septic conditions to penicillin. I have always maintained that the introduction of such drugs demands better, not worse, surgery, and a better understanding of inflammatory processes. And it is, I think, quite clear that penicillin and substances like it will only be used with maximum effect if surgeons have a real appreciation of the properties of the drugs—not only what they can do, but also what they cannot do and the reasons for their limitations.

Perhaps I may close with some words of Lister's written in 1867: "And I may take this opportunity of warning some of your readers that they must not expect carbolic acid to act like a charm; but that, whether they employ this agent or some other of analogous properties, it is only by the light of sound pathology, and strict attention to practical details, that they can hope to attain in their full measure the magnificent results which the antiseptic treatment is capable of affording."

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NEUROLOGICAL COMPLICATIONS OF INFECTIVE HEPATITIS

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Cerebral changes in fatal cases of infective hepatitis have often been recorded. They have usually taken the form of coma, delirium, convulsions, and incontinence—symptoms which are common to the clinical picture of acute hepatic necrosis of any origin. Other neurological findings are, however, less well recognized, and it is the purpose of this paper to discuss various signs observed independently in cases of infective hepatitis by four persons in different parts of Assam and Bengal during 1944.

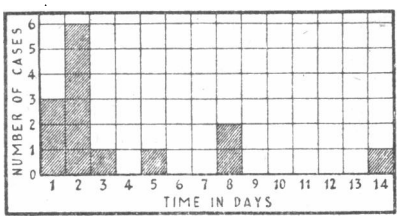
The neurological findings have been protean, but it is possible to dissect out four main groups. First, the picture commonly found in the fatal case, which is too familiar to need detailed description. Secondly, a symptom-complex comprising generalized or localized muscular rigidity with increased tendon-jerks, sometimes but not invariably associated with extensor plantar responses; in addition chloreiform movements have been observed, and in one case a Parkinsonian tremor. Thirdly, a group of cases with large focal haemorrhages into nervous tissue, which may or may not produce localizing signs. Fourthly, those cases showing signs of peripheral neuritis for whose direct relation to the onset of infective hepatitis there is good evidence.

The Common Type

This group may be illustrated by a "type" case:

S., aged 28, developed anorexia, followed by jaundice and bile-stained urine, on Dec. 19, 1944. He remained deeply jaundiced, and on Jan. 3, 1945, became drowsy and apathetic. The liver dullness was diminished, but there were no objective signs in the nervous system. On Jan. 4 he became alternately stuporous and maniacal. The urine contained no leucine or tyrosine crystals, and the cerebrospinal fluid, under a pressure of 100 mm., contained 60 mg. of protein per 100 c.cm. He died on Jan. 5. Necropsy showed characteristic changes of acute hepatic necrosis in a liver that weighed 750 g. The only demonstrable change in the brain was that of congestion of small vessels in the white matter.

There is nothing unusual about this case, but it illustrates well the short time that often elapses between the first onset of mental change and death. A histogram of this interval in 14 cases is shown in the accompanying figure.



Interval between onset of mental changes and death in 14 fatal cases of infective hepatitis

Some degree of mental depression is very common in infective hepatitis. Any increase in this depression or any behaviour change should be treated as a danger sign and as an indication for such protective therapy as is available.

The Striatal and Pyramidal Group

Nine cases have been observed in this group. The distribution of the relevant physical signs is shown in the accompanying Table. The following are brief histories of Cases 4 and 6.

Case 4.—A British private aged 30 was admitted on May 25, 1944, complaining of anorexia and dark urine for two weeks and jaundice for 10 days. On examination he was afebrile and deeply jaundiced.