CORYNEBACTERIAL PSEUDOTUBERCULOSIS IN MICE

II. Activation of Natural and Experimental Latent Infections*

By ROBERT M. FAUVE,‡ M.D., CYNTHIA H. PIERCE-CHASE, Ph.D., and RENE DUBOS, Ph.D.

(From The Rockefeller Institute, and The Pasteur Institute, Garches, France)

Plates 26 to 29

(Received for publication, April 10, 1964)

Corynebacterial pseudotuberculosis, a naturally occurring murine disease, often interferes with results obtained during the course of experimental studies. The infection commonly exists undetected in a latent form in mice and rats. It has been evoked into active disease, however, on numerous occasions when animals have been submitted to unfavorable physiological conditions such as those produced by pantothenic acid deficiency (1, 2), x-irradiation (3), cortisone administration (4–6), or by other infectious diseases, for example salmonellosis (7), and infectious ectromelia (8). Isolation of *Corynebacterium kutscheri* from normal animals has not been reported, but this agent can be recovered from the lesions when the latent infection has been activated.

As reported in the preceding paper, various inbred strains of mice exhibit striking differences in their response to experimental infection with *C. kutscheri* (9). Mice of the Swiss Lynch strain undergo rapidly fatal pseudotuberculosis, whereas animals of the C57Bl/6 strain survive infection without any residual pathologic or bacteriologic evidence of disease.

Early in the course of investigations of these differences in susceptibility, it was discovered that administration of cortisone to so-called normal (*i.e.* non-experimentally infected) mice of the C57Bl/6 strain evoked progressive and fatal corynebacterial pseudotuberculosis; evidence of the fact that these animals were latently infected with *C. kutscheri*. The present report deals with this phenomenon as observed in mice harboring latent corynebacteria acquired either naturally or experimentally. It will be shown, furthermore, that an avirulent organism appears to be converted into virulent *C. kutscheri* when cortisone treatment transforms latent infection into active disease.

Materials and Methods

Mice.—The strains of mice and the sources from which they were obtained are referred to individually in Table III and were, with one exception, the same as described in the preceding report (9). Unless otherwise indicated, all animals were maintained under the same conditions

* This investigation was supported in part by a research grant (AI-3790) from The National Institute of Allergy and Infectious Diseases, National Institutes of Health, United States Public Health Service, Bethesda.

‡ Present address: Institut Pasteur, Garches, Seine et Oise, France.

as reported previously. Mice obtained from the NCS colony raised at The Rockefeller Institute (10, 11) were used to establish the NCS (Institut Pasteur colony). The history and maintenance of this latter colony are presented elsewhere (12, 13).

Cortisone Injection.—Hydrocortone acetate (obtained from Merck, Sharp and Dohme, Rahway, New Jersey) in the form of a saline suspension containing 25 mg hydrocortisone acetate per ml was used throughout except for experiments with NCS mice (Institut Pasteur colony) which were carried out with a similar preparation of hydrocortisone acetate obtained from Roussel Laboratories, Paris. The suspension was injected subcutaneously with a sterile syringe and 27 gauge needle (changed for each animal) into the lower dorsal area previously shaved and cleaned with iodine.

Examination of Diseased Animals.—All mice were examined in the gross for the presence of typical corynebacterial lesions. Impression smears were fixed with absolute methanol and stained by Giemsa technique. Tissue sections were fixed in Bouin's fluid, embedded in paraffin, and stained with hematoxylin and eosin. Specimens, removed aseptically, were cultured on appropriate media. PF agar and broth and, on occasion, veal infusion medium, were used as in the previous study (9). Truche agar and broth medium (14) consisting of 4 per cent peptone No. 2 I.B.F.,[1] 0.5 per cent NaCl, and 0.2 per cent glucose at pH 7.8 were used for experiments conducted on NCS mice (Institut Pasteur colony). The bacterial cultures isolated from animals treated with cortisone were identified as *C. kutscheri* according to the characteristics reported previously (9).

Isolation of Streptomycin-Resistant Strain of C. kutscheri.—The stock laboratory strain of *C. kutscheri* (CK) (9) which is sensitive to streptomycin was inoculated into Truche broth. After incubation for 5 hours at 37°C, 2.5 mg of didromycine, (dihydrostreptomycin sulfate obtained from Specia, Paris) was added, giving a final concentration of 50 μg/ml; incubation was continued for 24 hours. Truche agar containing 50 μg/ml of streptomycin was then inoculated with 0.5 ml of the broth culture. After 48 hours of incubation, a single colony was transferred into streptomycin broth which was then incubated for 24 hours. An NCS mouse (Institut Pasteur colony) was inoculated intravenously with 0.2 ml of culture and sacrificed 2 hours later; some of the liver homogenate was plated on streptomycin agar. One of the colonies thus obtained was subcultured on agar and used for the stock strain of streptomycin-resistant *C. kutscheri* (CKsr). Twenty-four hours before being used as an infecting inoculum, the stock strain was transferred into streptomycin broth.

Biochemical Tests.—The biochemical properties of the bacterial strains were determined by the methods described in the previous publication (9).

Serologic Techniques.—Rabbit antisera against CK and CKsr were prepared according to the technique used by Wittler (15). Agglutination tests were carried out with serial saline dilutions of immune sera against formalized antigens adjusted to OD 0.1 at 660 mμ.

Acid soluble extracts (M protein-like materials) were prepared according to the Lancefield technique (16). Qualitative precipitin tests were performed in capillary tubes (17). Immunodiffusion tests (Ouchterlony) were conducted in Petri dishes with wells cast by temporary placement of glass rings on a basal layer of agar while the upper layer was being poured (18)

EXPERIMENTAL RESULTS

Activation by Cortisone of Laient Infection with Corynebacterium kutscheri in C57Bl/6 Mice.—When cortisone was administered to C57Bl/6 mice infected 3 months previously with *C. kutscheri* and to a control group of non-infected mice, deaths from corynebacterial pseudotuberculosis occurred in both groups. This finding indicated that the so-called normal animals harbored *C. kutscheri* in a latent form and, furthermore, that cortisone had activated the infection.

[1] I.B.F., Industrie biologique française.

The lethal disease produced in both groups by cortisone was similar to that occurring in susceptible strains of mice experimentally infected with *C. kutscheri*. Within several days after cortisone administration, mice exhibited ruffled fur and abnormal posture and gait; death occurred within 6 to 15 days after injection. Autopsy revealed abscesses in the kidneys, liver, lungs, and heart (Fig. 1 *a*). As in the case of the experimental infection (9), the spleen appeared essentially normal. The lesions were heavily infiltrated with leucocytes of the

TABLE I

Effect of Cortisone Dosage upon Activation of Latent Infection with C. kutscheri in C57Bl/6 Mice

Cortisone	No. mice		Death after injection*
	Males	Females	
mg			*day*
Single dose			
10	9		8, 10, 10, 10, 10, 10, 14, 16, 17
		10	6, 8, 10, 10, 10, 10, 10, 14, 14, 17
5	6		5, 7, 14, 17, S,‡ S
		6	17, S, S, S, S, S
1.25	6		S, S, S, S, S, S
		6	S, S, S, S, S, S
Divided dose			
7.5§	6		28, S, S, S, S, S
		6	7, 8, 9, 14, 15, 24

* Gross lesions and smears positive for *C. kutscheri* were observed in all mice which died.
‡ S, Sacrificed at 28 days. No gross pathology recognized.
§ 2.5 mg every other day.

mononuclear type, and *C. kutscheri* were seen both intra- and extracellularly. Giant cells and epithelioid cells were absent. *C. kutscheri* was isolated in pure culture from all lesions, and proved to be fully virulent, even after repeated broth transfers, when tested without cortisone in susceptible strains of mice.

The following experiments were carried out to investigate more fully the factors necessary for activation of corynebacterial pseudotuberculosis in normal animals of the C57Bl/6 strain.

Titration of cortisone dosage for optimum activation:

Mice of each sex, 6 to 8 weeks of age, were divided into three groups and given a single subcutaneous injection of 10, 5, or 1.25 mg of cortisone. Animals in another group received three injections of 2.5 mg, one every other day, a total of 7.5 mg having been thus administered (Table I).

A single injection of 10 mg of cortisone resulted in death of all animals and was chosen as the standard dose for activation experiments. (A number of pilot experiments were carried out to determine whether host resistance could be decreased by other methods such as starvation, pantothenic acid deficiency, water deprivation, maintenance at 4° C, adrenalin or endotoxin administration, thorotrast injection, splenectomy, partial hepatectomy, and unilateral ligature of the renal vein. All these procedures failed. X-Irradiation activated the latent infection in only a small percentage of animals.)

Occurrence of latent infection:

In order to eliminate the possibility that infection could be spread by direct contact after cortisone administration, ten mice (12 weeks old at the time of the experiment) were housed individually in separate cages and compared with ten other mice of the same age in groups of 5 per box; all animals received 10 mg of cortisone (Table II).

TABLE II

Effect of Housing upon Activation by Cortisone of Latent Infection with C. kutscheri in C57Bl/6 Mice

Type of housing	No. mice		Day of death after injection
	Males	Females	
5 per box	5		6, 7, 14, 14, 14
		5	14, 14, 14, 17, 17
1 per metal cage	5		6, 6, 6, 8, 10
		5	6, 6, 6, 7, 10

Each mouse received 10 mg cortisone subcutaneously.

Death from corynebacterial pseudotuberculosis occurred in all animals. Thus, disease developed from the activation of latent organisms within each host, rather than as a result of cross-contamination.

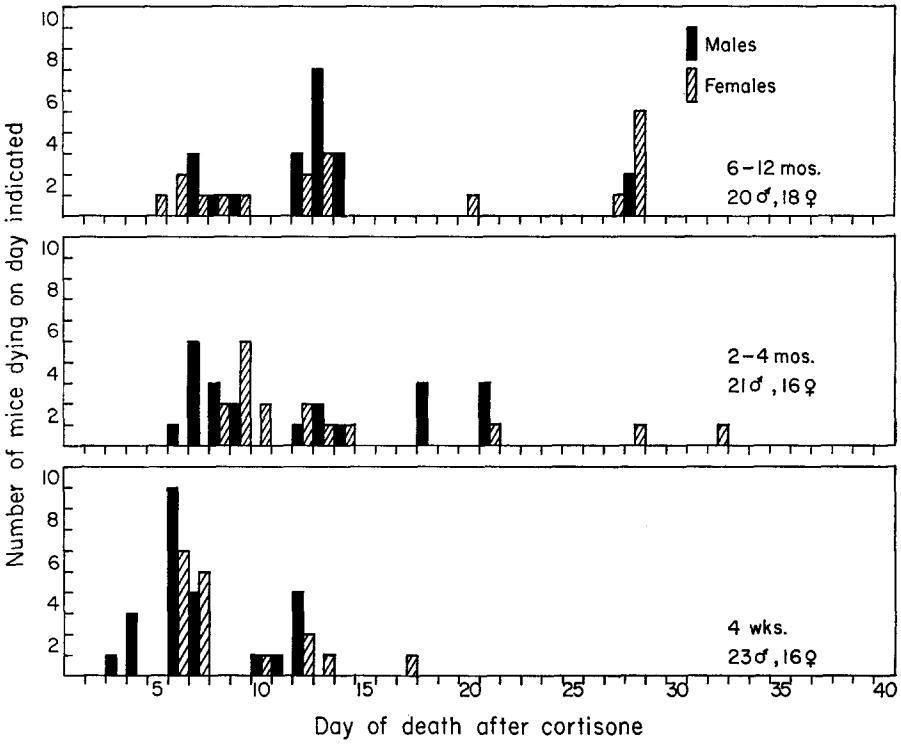
Influence of age and sex of mice on corynebacterial activation by cortisone:

Male and female mice of various ages were injected with 10 mg of cortisone. In Text-fig. 1, the animals are arranged in three general age groups; 4 weeks old (23 males, 16 females), 2 to 4 months old (21 males, 16 females), and 6 to 12 months old (20 males, 18 females).

Cortisone treatment caused lethal corynebacterial pseudotuberculosis in all mice, animals treated when 4 weeks of age dying somewhat earlier than those in the older age groups. Sex had no influence on the final outcome of infection.

Activation of corynebacterial infection by cortisone in mice of various strains and different sources:

Mice from 21 colonies representing 11 genetically different strains were compared with regard to activation of corynebacterial pseudotuberculosis by cortisone administration. The majority of animals were 6 to 8 weeks of age when tested. The findings for each mouse strain are summarized in Table III, taking advantage of the fact that, as shown in the preceding experiments, neither sex nor age influenced the final outcome of corynebacterial disease induced



TEXT-FIG. 1. Corynebacterial activation in C57Bl/6 mice of different ages treated with cortisone.

by cortisone. The presence of gross lesions consisting of renal, hepatic, cardiac, and pulmonary abscesses (Fig. 1 *a*), as well as positive smears and cultures, verified that cortisone evoked latent *C. kutscheri* into activity. Corynebacterial infection of the joint (Fig. 1 *b*) was a rare finding but is known to occur both in natural and experimental murine infections caused by various corynebacterial species (7, 19, 20).

Extensive corynebacterial pseudotuberculosis developed in mice of the C57Bl/6, DBA/2, and RIII strains. The failure of animals in some C57Bl/6 and DBA/2 colonies to develop the acute disease following cortisone administration may perhaps be explained by the antibiotic treatment of mouse stocks,

TABLE III

Occurrence of Corynebacterial Activation in Cortisone-Treated Mice of Different Strains

Strain	Source	No. tested	No. dead	CK
C57Bl/6	Dr. C. J. Lynch, The Rockefeller Institute	110	110	+
"	Dr. C. Haagensen, College of Physicians and Surgeons, Columbia University, New York	39	36	+
"	Roscoe B. Jackson Memorial Laboratories, Bar Harbor	55	17	±
"	Millerton Farms, Millerton, New York	20	20	±
"	Dr. G. L. Wolff, Institute for Cancer Research, Philadelphia	24	22	±
"	Dr. J. J. Bittner, University of Minnesota, Minneapolis	17	4	0
DBA/2	Dr. C. J. Lynch, The Rockefeller Institute	37	29	+
DBA/212	Dr. A. Goldfader, Francis Delafield Hospital, New York	5	5	+
DBA/2	Roscoe B. Jackson Memorial Laboratories	30	30	±
RIII	Dr. C. Haagensen, College of Physicians and Surgeons, Columbia University	5	5	+
CF_1	Carworth Farms, New City, New York	17	15	0
CF_1 (SPF)	" "	20	16	0
CFW	" "	22	19	0
NCS	The Rockefeller Institute	20	3	0
"	Pasteur Institute, Garches, France	20	0	0
BSVS	Dr. H. A. Schneider, The Rockefeller Institute	10	2	0
BRVR	" " " " " " "	9	9	0
A/Jax	Roscoe B. Jackson Memorial Laboratories	80	34	0*
Princeton	Dr. J. B. Nelson, The Rockefeller Institute	34	8	0
Swiss Lynch	Dr. C. J. Lynch, " " "	35	8	0
Swiss R/J	Roscoe B. Jackson Memorial Laboratories	100	9	0

CK (*Corynebacterium kutscheri*).

+, gross pathology, smears and cultures positive.

±, gross pathology and positive cultures rare; in general, organ smears showed pleomorphic bacilli resembling CK, but cultures were negative.

0, gross pathology, smears and cultures negative for CK. Cause of death undetermined.

* Extensive abscess formation in livers from which *E. coli* was isolated in pure culture

which is practiced by many breeders. On the other hand, the high degree of resistance to experimental *C. kutscheri* infection exhibited by all colonies of these two strains (9) might be a factor in preventing the disease elicited by cortisone from evolving fatally.

Attempts to isolate a specific etiologic agent responsible for death of mice among the other strains were unsuccessful except in the case of A/Jax animals

which uniformly exhibited liver abscesses from which pure cultures of *Escherichia coli* were isolated.

Among the many strains or colonies of mice in which cortisone did not evoke

TABLE IV

Recovery of Bacteria from Incubated Organ Homogenates of Cortisone-Treated DBA/2 Mice

Time of sacrifice after cortisone	No. mice	Organ homogenates				
		Spleen	Kidney	Liver	Lungs	P.P.*
day						
Control (no cortisone)	1	0	0	A_1	A_0	A_1
	2	0	A_2	C	A_0	A_1
	3	A_1	0	0	0	A_1
1	1	0	0	A_0	A_0	A_0
	2	0	0	A_0	A_0	A_1
	3	0	0	A_0	A_0	A_1
3	1	0	0	A_0	A_0K_0	C
	2	0	0	A_1	A_0K_1	A_0
	3	0	K_1	A_1	A_1	A_1
	4	0	K_2	C	C	C
4	1	A_1	A_1	A_0	A_0K_0	A_0
	2	K_0	K_0	K_0	K_0	A_0
	3	C	0	K_0	K_0	C
	4	0	0	0	A_1	C
5	1	K_1	K_1	A_1	A_1	A_2
	2	K_1	K_0	K_0	A_0	A_2
	3	K_0	K_0	K_0	K_0	A_2
	4	K_0	K_0	K_0	K_0	K_0
	5	K_0	K_0	K_0	K_0	K_0

0, No growth.

A, Avirulent organisms; K, *C. kutscheri*; Subnumerals (0, 1, 2) indicate incubation time (days) of homogenates before plating.

C, Contaminants

* P. P., Peyer's patches.

corynebacterial infection, the A/Jax, Princeton, Swiss Lynch, and Swiss R/J animals were shown to be highly susceptible to experimental infection with *C. kutscheri*. This correlation indicates that such mice were free of latent corynebacteria.

Bacteriologic Studies in Mice Naturally Harboring Latent C. kutscheri and Treated with Cortisone.—The foregoing experiments demonstrate that cortisone

treatment evokes acute disease in animals harboring latent *C. kutscheri*. Bacteriologic studies were undertaken to follow the fate of latent organisms during the course of this activation. Mice from those colonies of C57Bl/6 and DBA/2 strains proven by cortisone administration to be latently infected were chosen for this purpose.

Preliminary observations on peritoneal cells, collected in gelatin-Hanks' solution (21), from normal C57Bl/6 mice revealed the presence of *C. kutscheri*–like organisms within the macrophages, but only after the cell suspension had been incubated for 48 hours. Although attempts to cultivate the organisms were unsuccessful, this finding suggested the necessity of preincubating host tissues if detectable bacilli are to be obtained.

Detailed studies carried out upon both C57Bl/6 and DBA/2 strains of mice yielded similar results such as illustrated in the following typical experiment:

Female mice, 6 weeks old, of the DBA/2 strain received subcutaneously 10 mg of cortisone (0.4 ml). Control animals received 0.4 ml of saline. The cortisone-treated animals were sacrificed at various times for bacteriologic examination (Table IV). The mice were killed with chloroform, the abdominal skin was washed with 70 per cent ethanol and reflected, and the spleen, kidneys, liver, and lungs were removed aseptically with separate sets of instruments for each organ. Each specimen was placed in 5.0 ml gelatin-Hanks' solution. Peyer's patches, dissected from the small intestines, were collected in 1.0 ml of gelatin-Hanks' solution. Homogenization with teflon grinders was carried out according to a technique previously described (22). The homogenates were immediately plated onto both PF agar and Mueller's tellurite-serum agar and replated after incubation periods of 24 and 48 hours at 37°C. A similar procedure was carried out for the control group of non-cortisone-treated animals.

The results obtained after incubation of the agar plates for 24 to 48 hours are summarized in Table IV. In many instances, the preincubation of organ homogenates before plating allowed the isolation of colonies not obtainable from direct culture of the suspension. *C. kutscheri* (to be designated hereafter as K) was not obtained from control animals nor from mice sacrificed one day after cortisone injection. Both groups, however, yielded small translucent colonies (to be designated as A) similar to those described previously (9). The comparative morphology of K and A can be seen in Fig. 2. Cultures from mice having received cortisone previously for a period of 3 or 4 days yielded both K and A colonies. The decrease in the numbers of A colonies obtained coincided with an increase in the numbers of K colonies. *C. kutscheri* (K) was obtained from all mice sacrificed 5 days after cortisone treatment. Indeed, K colonies were isolated from 19 of the 25 organ homogenates and A colonies from the 6 specimens which were K negative. Potassium tellurite was reduced during growth of both A and K colonies.

Repeated subcultures in veal infusion broth of type A colonies obtained from liver homogenates of normal C57Bl/6 or DBA/2 mice retained their distinctive colonial morphology when plated onto solid medium. They were completely

avirulent as determined by inoculation of undiluted broth cultures into Swiss Lynch mice, a strain of mice shown previously to be free of latent corynebacteria and to be highly susceptible to experimental infection with *C. kutscheri* (9).

A few preliminary experiments in which cortisone was administered to Swiss mice injected with type A organisms resulted in activation of corynebacterial pseudotuberculosis from which virulent *C. kutscheri*, type K organisms, were obtained. The culture thus isolated was identical with the laboratory stock

TABLE V

Activation of Corynebacterial Pseudotuberculosis by Cortisone in NCS Mice Harboring a Latent Infection Experimentally Induced by a Strain of Streptomycin-Resistant C. kutscheri

Location of lesions	Length of infection before cortisone		
	30 days	60 days	90 days
Kidney	5*	5	5
Liver	2	3	4
Heart	1	3	4
Lungs	0	2	1
Skin	1	0	0
Joint	1	0	0
Total No. mice	5	5	5
Total No. dead	5	5	5

For each group tested, 5 control animals survived cortisone without evidence of disease.
* Figures refer to incidence and location of lesions in each group of 5 female mice.

strain of *C. kutscheri* and remained stable without showing any evidence of A type colonies upon repeated subculture. Further experiments are required, however, to define the exact conditions necessary for the conversion of the avirulent type A organisms into the virulent type K during cortisone activation of disease. The comparative properties of the two bacterial strains, A and K, are described more fully in a later section of this report.

Experimental Induction of Latency with a Streptomycin-Resistant Strain of C. kutscheri and Its Subsequent Activation.—The foregoing experiments have dealt with a naturally occurring form of *C. kutscheri.*

In order to define the conditions under which *C. kutscheri* infection evolves from the latent into the active state, an effort was made to establish latency experimentally with genetically marked organisms that could then be distinguished from bacteria normally present in the host.

NCS mice (Institut Pasteur colony), known to be free of *C. kutscheri* infection at the time of the experiments, were maintained under sanitary conditions designed to prevent recontamina-

tion. They were given the diet, Rat 98 C.N.R.Z.,[2] *ad libitum* as well as water containing a final concentration of 0.03 N HCl. A detailed description of the history and maintenance of this NCS colony has been published elsewhere (12, 13). Fifteen female mice, 5 weeks old and weighing between 23 and 25 gm, received subcutaneously 6000 infecting units of streptomycin-resistant *C. kutscheri* in 0.2 ml physiologic saline. A control group of 15 females of the same age and weight received saline only. The mice were kept in groups of 5 animals per cage. After 30, 60, or 90 days, all animals received subcutaneously 10 mg of cortisone. The results are summarized in Table V.

All mice previously infected died from severe corynebacterial pseudotuberculosis following cortisone administration; none of the controls so treated died. Autopsy findings revealed extensive abscesses occurring uniformly in the kidneys, less often in the liver, heart, and lungs. In only one case did skin lesions develop at the site of cortisone injection. As with naturally occurring latent infections, involvement of the joint (Fig. 1 *c*) was a rare finding. Corynebacteria were seen in impression smears and were cultured from all lesions. The *C. kutscheri* so isolated exhibited the same streptomycin resistance as the original infecting culture, and was virulent for mice not receiving cortisone.

These results show that (*a*) latent infection actually was produced in NCS mice, and (*b*) cortisone converted the latent infection into active disease just as observed in the case of mice naturally harboring latent corynebacteria (Tables I to III and Text-fig. 1). The pathologic findings in the experimentally infected and "activated" animals were the same as in cortisone-treated mice with naturally occurring latent infections. Figs. 3 *a* and 3 *b* present a section of kidney showing medullary abscesses and an occasional cortical abscess. A section of heart muscle (Figs. 4 *a* and 4 *b*) shows myocardial abscesses in which most of the inflammatory cells are of the mononuclear type.

Bacteriologic Studies on Mice with Experimental Latent Infection Induced by Streptomycin-Resistant C. kutscheri (CKsr).—As shown in Table IV, small translucent colonies, termed A, could be cultured from organ homogenates of normal mice which harbored naturally acquired *C. kutscheri* in a latent form. Following treatment of the animals with cortisone, the colony type isolated from them shifted from A to K. There was evidence that the A type colony was an avirulent variant form of *C. kutscheri.* The following experiment was therefore instituted to determine whether mice surviving infection with virulent, streptomycin-resistant *C. kutscheri* (CKsr), in which latency had been thereby induced experimentally, would yield the variant form of *C. kutscheri* bearing the "sr" marker.

Fifteen female mice from the NCS (Institut Pasteur) colony were inoculated with CKsr, and a control group received saline, under the same conditions described previously (Table V) with the exception that no cortisone was administered. All animals were sacrificed, the organs were homogenized in gelatin-Hanks' solution containing 50 μg/ml of streptomycin, and dilutions were plated onto agar containing the same streptomycin concentration in order to detect

[2] C.N.R.Z., Centre National de Recherches Zootechniques.

streptomycin resistance. Each liver homogenate was plated immediately and again after incubation for 24 and 48 hours. The results are summarized in Table VI.

The bacteria isolated from the liver homogenates of infected animals were all of the "A" type and they bore the streptomycin-resistance marker, "sr," of the infecting virulent CKsr. Liver homogenates of control animals did not yield microbial growth at any test period. As shown previously, preincubation of the liver homogenates increased the percentage of positive type A cultures obtainable. All animals tested at 30 or 60 days' postinfection yielded positive cultures; 3 out of 5 tested at 90 days were positive.

The streptomycin-resistant type A organisms (Asr) were shown to be avirulent by intravenous inoculation of normal NCS mice with $3 \times 10^{8-9}$ viable

TABLE VI

Latency Induced Experimentally in NCS Mice
Recovery of Avirulent (Streptomycin-Resistant) Bacteria from Incubated Homogenates of Liver Obtained from NCS Mice Surviving Infection with Streptomycin-Resistant Strain of C. kutscheri

Preincubation of homogenate	No. mice	Length of latent infection before liver culture		
		30 days	60 days	90 days
hrs.				
0	5	2*	0	0
24	5	3	3	1
48	5	5	5	3

* No. of mice yielding positive cultures from liver homogenates. Negative cultures were obtained from five control animals sacrificed at each testing period.

units in 0.2 ml broth culture. All animals remained healthy. Cortisone injected 1 month later, in the amount of 10 mg per mouse, caused within 8 to 16 days the death of all animals from acute corynebacterial pseudotuberculosis. The culture of *C. kutscheri* isolated from the lesions was fully virulent and more importantly was also streptomycin-resistant. Identical results were obtained in three separate experiments conducted on groups of 5 female mice.

Thus, the use of a strain of *C. kutscheri* possessing an induced resistance to streptomycin as a genetic marker has made it possible to follow the fate of the bacteria *in vivo*. Latency has been induced with this tagged strain and only its "avirulent" form isolated. Injection of the avirulent streptomycin-resistant form into normal NCS mice caused no obvious lesions until cortisone was administered; after this treatment, streptomycin-resistant virulent organisms were recovered.

Differential Characteristics of Virulent C. kutscheri (K) and Avirulent Organisms (A).—

Morphology: The marked differences in colonial and cellular morphology are shown in Fig. 2. The large, pearly white colonies (K), are made up of cells exhibiting pleomorphism, palisade

formation, and metachromatic granules typical of corynebacteria. They stand in sharp contrast to the small translucent colonies (A) composed of short, fat, regular shaped bacilli without the characteristic diphtheroid pattern.

Cultural requirements: Growth of both types of organisms occurred in various infusion

TABLE VII

Biochemical Reactions of C. kutscheri and Type A Organisms

Biochemical tests	K	A	Ksr	Asr
Fermentation				
Dextrose	+*	+	+	+
Levulose	+	+	+	+
Maltose	+	+	+	+
Mannose	+	+	+	+
Salicin	+	+	+	+
Sucrose	+	+	+	+
Adonitol	−	−	−	−
Arabinose	−	−	−	−
Dulcitol	−	−	−	−
Galactose	−	−	−	−
Glycerin	−	−	−	−
Inositol	−	−	−	−
Inulin	−	−	−	−
Lactose	−	−	−	−
Mannitol	−	−	−	−
Melizitose	−	−	−	−
Melibiose	−	−	−	−
Raffinose	−	−	−	−
Rhamnose	−	−	−	−
Sorbitol	−	−	−	−
Sorbose	−	−	−	−
Starch	−	−	−	−
Trehalose	−	−	−	−
Xylose	−	−	−	−
K. tellurite reduction	+	+	+	+
Catalase activity	+	−	+	−
Urease activity	+	−	+	−

K, *C. kutscheri*; Ksr, *C. kutscheri* (streptomycin-resistant); A, type A (avirulent organisms); Asr, type A (avirulent organisms, streptomycin-resistant)

* Production of acid but no gas in fermentation reactions.

media, but type A cultures grew more slowly. Addition of 1 per cent glucose to the media and incubation in the presence of 5 per cent CO_2 greatly enhanced the growth of type A. The transformation of type A into type K colonies was never observed on solid medium inoculated from broth cultures of A nor was the reverse, K to A, shown to occur, although occasional bacterial cells similar to the type A organisms were seen in smears of aged broth cultures of K.

Biochemical characteristics: The various fermentation and enzymatic tests employed (Table VII) yielded the same results with both strains except in the case of urease and catalase activity. K cultures were urease and catalase positive; A cultures, negative to both tests.

Immunological properties: Agglutination tests with anti-K immune serum and the homologous antigen resulted in strong agglutination at a very high titer (1:64,000). A very weak and easily dissociable agglutination at a titer of 1:10,000 occurred with anti-K serum against antigen prepared from type A organisms. Heat- and acid-soluble extracts (M-like proteins) prepared from the streptomycin-resistant A and K strains were tested against anti-K serum by precipitin and immunodiffusion techniques. Extract Ksr yielded positive results; extract Asr failed to react.

Virulence: Both K and Ksr were highly virulent for mice known to be free of latent corynebacteria, whereas A and Asr were avirulent. Cortisone treatment of such mice having received Asr resulted in acute corynebacterial pseudotuberculosis from which Ksr could be isolated in pure form. Under similar conditions, type A organisms occasionally produced acute disease from which K organisms were recovered.

DISCUSSION

Microbial disease of endogenous origin following administration of cortisone has been observed repeatedly in man and experimental animals. Because of its obvious clinical importance, this phenomenon has directed attention to the widespread occurrence of latent infections and to the fact that these can be converted into overt disease by many unrelated factors which depress the general resistance of the host. In fact, it is almost certain that non-specific activation of latent infectious processes constitutes today one very important cause of microbial disease in man, particularly in communities in which acute infections have been brought under control.

Three fundamental questions have to be answered before the problem posed by latent microbial diseases can be formulated in a manner useful for the understanding of their pathogenesis. In what form do the microorganisms persist *in vivo* during the period of latency? What are the mechanisms which hold their multiplication in check, yet fail to destroy them? What are the processes through which latent infection is converted into overt disease? It is very probable, needless to say, that the answers to these questions will differ depending upon the species of infectious agent under consideration and the kind of stress which evokes it into activity. The most that can be done in this early stage of analysis is therefore to observe in some detail the peculiarities of a few representative models. The observations on murine corynebacterial pseudotuberculosis reported in the present papers seem to have merit in this regard because they concern a disease of widespread occurrence in nature, and of practical importance in experimental work.

Corynebacterial pseudotuberculosis has often been reported to occur in rodents placed under conditions of stress. For example, x-irradiation, pantothenic acid deficiency, and cortisone administration are among the many factors which have been found to elicit the "spontaneous" appearance of the disease in apparently normal animals (1–6).

The findings reported from several laboratories in different parts of the world demonstrate that rodents carrying corynebacteria in a latent form are widely distributed geographically (20, 23–25). It has been a common experience that the evocation

of these organisms into activity by various kinds of physiological disturbances often complicates the results of experimental manipulations. The present study has been focused on the course of infection caused by *Corynebacterium kutscheri* in mice, especially under the influence of cortisone. The findings, reported in this and the preceding paper, and their possible implications, can be considered under the following headings.

1. Comparative Susceptibility of Various Strains of Mice to Experimental Infection with C. kutscheri.—It was found that certain strains of mice are exquisitely susceptible to experimental infection with *C. kutscheri* whereas others are, in contrast, extremely resistant. A standard infective dose (0.2×10^{-4} ml culture) caused death of all mice of susceptible strains, but allowed survival of all mice of resistant strains. The difference between the two classes of animals was observed regardless of sex or age. Mice of the Swiss Lynch strain which proved highly susceptible, and of the C57Bl/6 strain which proved highly resistant, were selected for further study because both are highly inbred and their genetic constitution is well defined.

It is of particular interest that animals of the C57Bl/6 strain were uniformly highly resistant, regardless of the sources from which they were obtained. These mice, injected intravenously with *C. kutscheri*, allowed an initial bacterial proliferation resulting in extensive abscess formation, particularly in the kidneys. However, this infection soon subsided leaving no pathologic or bacteriologic evidence of residual disease. The resistance of the C57Bl/6 animals could, nevertheless, be overcome by increasing the infective dose. For example, all mice died following injection of 0.2×10^{-1} ml of culture, but their greater resistance was apparent even then, since their death was much more delayed than that of Swiss Lynch mice infected with the same dose. It is worth mentioning that animals of the C57Bl/6 strain in which the dominant mutant "ragged" gene is carried in heterozygous condition were found to be as uniformly resistant as the homozygous C57Bl/6 stock.

The extreme susceptibility of mice of the Swiss Lynch strain to intravenous infection with *C. kutscheri* was evident from the fact that an injection of 0.2×10^{-6} ml of culture was lethal.

The extent of bacterial multiplication *in vivo*, determined in different organs at various intervals after infection, further emphasized the contrast in host response between the Swiss Lynch and C57Bl/6 strains of mice. In Swiss Lynch mice infected with 0.2 ml of 10^{-4} culture dilution, there was rapid multiplication of *C. kutscheri* in the kidneys, lungs, and liver and also, but to a small extent, in the spleen. Animals infected with this dose died uniformly within 3 to 11 days (the majority within 4 to 7 days). Parallel groups of C57Bl/6 mice did not yield culturable *C. kutscheri* from lungs or spleen, and, although a few typical colonies were occasionally obtained from homogenates of liver, virulent corynebacteria did not multiply in this organ. The multiplication that took place initially in the kidneys reached a maximum at 6 days' postinoculation, coincidentally with the appearance of renal abscesses; but the infectious process was not progressive; no gross lesions were seen, nor were colonies of *C. kutscheri* obtained from any organ of animals sacrificed 16 days after inoculation. Thus, the resistant C57Bl/6 strain is capable not only of retarding bacterial multiplication but also of eliminating virulent corynebacteria so thoroughly that ordinary cultural techniques fail to detect their presence. The small translucent colonies observed during the course of these studies are dealt with later on in this discussion.

All tests reported above were carried out with one single strain of virulent corynebacteria. In an attempt to enlarge the significance of the findings, four additional strains isolated in other laboratories from spontaneous mouse infections were tested with regard to their virulence for Swiss Lynch and C57Bl/6 mice. With each of these cultures, as with our own stock strain of *C. kutscheri*, Swiss Lynch animals proved to be susceptible to infection, and C57Bl/6 mice, resistant.

2. Latent Corynebacterial Infection and Its Activation by Cortisone.—Administration of large amounts of cortisone (10 mg per mouse) consistently evoked corynebacterial pseudotuberculosis in animals of the strains, C57Bl/6, DBA/2, and RIII. In contrast, cortisone treatment did not have this effect in other mouse strains such as, Swiss Lynch, Swiss R/J, Princeton, and A/Jax. The conclusion seems inescapable, therefore, that normal C57Bl/6, DBA/2, and RIII mice were latently infected with *C. kutscheri*, whereas normal Swiss Lynch, Swiss R/J, Princeton, and A/Jax mice were free of this infection.

There was a striking correlation between resistance to experimental infection with *C. kutscheri* and development of corynebacterial pseudotuberculosis following administration of cortisone. In other words, it appears that the mice which normally carried *C. kutscheri* in a latent form, belonged to the strains exhibiting resistance to experimental infection with this organism. A similar situation has been observed with rats as shown by the resistance to superimposed infection exhibited by animals from a strain in which pantothenic acid deficiency evoked corynebacterial disease (26).

3. Mechanisms through Which Cortisone Activates Latent Corynebacterial Infection.—That host resistance to a variety of infectious agents can be decreased by administration of cortisone has been repeatedly documented with regard to both experimental infections and activation of latent diseases (27, 28). As shown in the present report, activation of latent corynebacterial infection in mice of the proper strains could be brought about consistently by administration of large doses of cortisone. The doses required were indeed so large that many physiological functions were thereby affected. For this reason, it would not be useful to discuss the changes in the response of the mouse that were accountable for the phenomenon. On the other hand, a number of unexpected bacteriological observations suggest that latency, and its evocation into active disease, may involve changes in the infectious agent (29).

Small translucent colonies (hereafter called type A) differing markedly from those of typical corynebacteria, were occasionally isolated in considerable numbers from homogenates of liver and lungs of non-cortisone-treated C57Bl/6 mice which had been previously inoculated with *C. kutscheri*. On repeated subcultures these colonies remained stable with regard to morphology, and even large inocula from such cultures proved completely avirulent for Swiss Lynch mice. There is evidence that the A organisms represent avirulent variants of *C. kutscheri*.

Type A organisms could be isolated from normal (*i.e.* non-experimentally infected) mice of the C57Bl/6 and DBA/2 strains both before and after cortisone administration. Colonies of virulent *C. kutscheri* (hereafter called type K) were never obtained from normal animals before cortisone treatment. They could be recovered, however, from animals 3 days after treatment. As active infection progressed, the numbers of avirulent organisms, type A, decreased while those of virulent corynebacteria, type K, increased. These bacteriologic studies were greatly facilitated by preincubation of the

organ homogenates for 1 to 2 days before plating, a procedure which increased the incidence of positive cultures.

It is worth mentioning in passing that incubation of virulent *C. kutscheri* with normal liver tissue *in vitro*, or incubation of liver homogenates from infected mice yielding type K colonies, did not change the virulent organisms into avirulent forms.

As already mentioned, cultures of type A obtained from normal C57Bl/6 or DBA/2 animals remained stable upon repeated transfers and were completely avirulent for susceptible Swiss Lynch mice. Occasionally, however, cortisone treatment of such mice after injection of type A organisms resulted in lethal corynebacterial pseudotuberculosis from which type K organisms were recovered; these were fully virulent as shown by injection of small inocula into Swiss Lynch mice not receiving cortisone.

These findings constitute presumptive evidence for the view that corynebacteria persist in an altered form during latency and revert to virulent organisms as a result of modification of the host by cortisone. This hypothesis receives further support from the results of studies on experimental induction of latency. For these studies a virulent culture of *C. kutscheri* was rendered resistant to streptomycin. A sublethal inoculum of this resistant strain (hereafter called CKsr) was injected into NCS mice, known to be susceptible to experimental infection with and not to be carriers of corynebacteria. This procedure established a latent infection with CKsr which could be evoked into overt disease by administration of cortisone. Homogenates of organs taken from NCS mice during the period of latency, before cortisone administration, yielded small translucent colonies of avirulent organisms (Asr) which had the streptomycin resistance label of the virulent strain injected for the experimental establishment of the latent state. Colonies of typical corynebacteria were recovered from the lesions of the overt disease resulting from cortisone treatment and had the full virulence (as well as streptomycin resistance) of the parent strain, CKsr.

Even more suggestive were the results of experiments designed to establish the state of latency with the avirulent culture, Asr. Injection of large amounts of this culture into normal NCS mice produced no evidence of disease. However, when the animals were treated with cortisone 1 month later, they developed fatal corynebacterial pseudotuberculosis. The cultures recovered from the lesions were still streptomycin-resistant and, in addition, were now fully virulent and identical with CKsr.

Taken together, all these findings suggest that, under ordinary conditions, the normal defense mechanisms of the host tend to suppress the virulent form of *C. kutscheri*, but that this microbial species can persist and establish latent infection as an avirulent form which is capable of reverting to the virulent state when the animal is treated with cortisone.

4. Persistent Forms of C. kutscheri.—Atypical colonial morphology corresponding to L forms have been observed in corynebacteria (30, 31), and certain members of the corynebacterial species have been shown to exhibit a complex life cycle (15, 22). In addition, there are reports describing the close association of diphtheroids with pleuropneumonia-like organisms (PPLO) isolated from pathologic material (33). Transitional forms, similar to but not identical with L forms, have also been isolated from patients during antimicrobial treatment, and there is some evidence that these forms are related to the corynebacterial culture isolated before treatment (34). Thus, it is likely that corynebacteria exist in a variety of forms particularly when associated with disease processes.

The small translucent colonies, types A and Asr, described in the present reports are distinctly different from those produced by L forms. They bear a strong morphological and colonial resemblance to the variant "G" or minute forms isolated from a culture of *C. diphtheriae* obtained from a patient receiving antitoxin treatment (35). Although the phylogenetic origin of type A organisms has not so far been traced by their isolation *in vitro* from cultures of *C. kutscheri*, nonetheless streptomycin-resistant type A organisms occur *in vivo* when animals are inoculated with a streptomycin-resistant strain of *C. kutscheri*. Furthermore, type A organisms, though lacking urease and catalase activity, share with virulent *C. kutscheri* many other biochemical properties. The relation of urease activity to virulence has not been studied here, but its correlation with the affinity of virulent corynebacteria for the kidney has been noted by other workers (36).

The avirulent (A) differs from the virulent (K) form also in certain immunological characteristics. In particular, type A organisms lack an acid-soluble antigen (M protein-like?) which seems to be a surface component of the virulent corynebacteria. Preliminary as they are, the immunological observations made so far point to the likelihood that the phenomenon of latency depends upon structural alterations in the parasite which account for its persistence *in vivo*; on the other hand these alterations seem to be so readily reversible that the organism can reacquire full virulence and multiply extensively when the resistance of the host is lowered.

Infection Immunity and Endogenous Disease.—

There is no evidence, of course, that the factors which condition corynebacterial latency, and its conversion into overt pseudotuberculosis, operate in other infectious processes. Nevertheless, the phenomena which have been described and discussed in the present papers justify a few statements which have general applicability.

It had been assumed at the beginning of the study that the differences in response of various strains of mice to corynebacterial infection had a genetic basis. A similar contrast in host response to this agent has been observed between two genetically different strains of rats (37, 38). The correlation between genetic constitution and resistance is very striking, and indeed there is reason to believe that it constitutes more than an artifact. The findings reported in the present papers have revealed, however, that the mouse strains which are resistant to infection with *C. kutscheri* are also carriers of this organism. This association seems to illustrate the following statement: "It should also be borne in mind that some differences that have been attributed to genetic causes may be due to parasites. Because of the limited number of parents, there is a relatively high probability that an inbred line will become uniformly infected, particularly with parasitic organisms that are transmitted from mother to offspring" (39). It is even conceivable that specific mechanisms, such as immunological tolerance, could account for the state of resistance referred to as "infection immunity." Mechanisms of immunity do not rule out the possibility, however, that genetic factors are involved in the induction of corynebacterial latency and in its persistence through succeeding generations.

The correlation between resistance and carrier state was of great interest to immunologists around the turn of the century. It was then called "premunition" by the French workers (40) and "infection immunity" by the English (41). These two expressions have now all but disappeared from the immunological literature, yet infection

immunity is almost certainly of very wide occurrence and of great practical importance (42). Moreover, it consitutes a challenging theoretical problem because nothing is known of its mechanism.

Infection immunity implies persistence of the pathogen in the tissues. Increasingly, during the past few decades, evidence has accumulated that pathogens can exist *in vivo* in forms which differ from those by which they are recognized under usual conditions *in vitro*. For example, they can persist in atypical forms which are resistant to immune processes and to antibacterial drugs; recognition of the L and related forms in many different bacterial species constitutes another kind of evidence on this point. The isolation of type A colonies described in the present paper and the demonstration that they are probably avirulent variants of *C. kutscheri* is further documentation of this phenomenon. It is apparent, therefore, that the discovery and analysis of the latent state associated with infection immunity will require an awareness of the existence of atypical forms and, in many cases, the development of new methods for their cultivation and identification. Indeed, the use of media containing 0.3 M sucrose has permitted the isolation of *Streptococcus faecalis* persisting as protoplasts in the kidneys of rats treated with penicillin after experimental infection with the bacillary form (43).

Whereas latency often, if not always, results in increased resistance to superinfection, this advantage is gained at the cost of the danger that the latent state can be activated into overt disease. There is overwhelming evidence that a very large percentage of microbial disease in our communities has such an endogenous origin. Here again, corynebacterial pseudotuberculosis provides an enlightening model. Its study has revealed that the problem of endogenous disease must be considered from two entirely different points of view. One concerns the pathogen itself, the form in which it persists, and the conditions which make it shift reversibly from the latent to the active state. The other concerns the host. The paradoxical situation is that the internal environment of the healthy host prevents proliferation of the virulent form of the pathogen, but cannot eradicate its latent, inactive forms. This paradox is well illustrated by the latent *C. kutscheri* infection, since it appears that the avirulent variants are the ones which persist *in vivo*, being replaced, however, by virulent forms when physiological disturbances render the host susceptible to the disease, pseudotuberculosis.

SUMMARY

Latent corynebacterial infection occurs naturally in many strains of mice. It can be evoked into the active disease, pseudotuberculosis, by a single injection of 10 mg of cortisone.

The cortisone effect was tested in 21 colonies, representing 11 genetically different strains of mice. Animals of the C57Bl/6, DBA/2, and RIII strains were shown to be latently infected with *Corynebacterium kutscheri* by the fact that they developed fatal pseudotuberculosis following cortisone treatment.

Virulent *C. kutscheri* could not be isolated from homogenates of organs obtained from latently infected animals before cortisone administration; however, these homogenates yielded small translucent colonies of avirulent organisms. Recovery of these atypical colonies was facilitated by preincubating the organ homogenates before plating. The organisms constituting such colonies differed morphologically and immunologically from *C. kutscheri*, but had similar biochemical properties with the exception that they lacked urease and catalase activity.

Mice treated with cortisone yielded both the avirulent bacteria and virulent *C. kutscheri*. The latter was the predominant organism present in the organs at the height of infection.

Injection of avirulent organisms into Swiss Lynch mice, which are normally free of latent corynebacteria, occasionally established a latent infection which could be converted into corynebacterial pseudotuberculosis by cortisone. Cultures of fully virulent *C. kutscheri* were then obtained from the lesions.

Latency was produced experimentally with a streptomycin-resistant strain of virulent *C. kutscheri* (CKsr) derived from the stock culture. When sublethal doses of CKsr were injected into NCS mice (Institut Pasteur colony), they induced a latent infection characterized by the presence of avirulent organisms possessing the streptomycin resistance marker. These were isolated in the form of small translucent colonies from the livers of the infected animals. Administration of cortisone to these animals subsequently evoked active infection from which virulent CKsr could be obtained.

Injection of the avirulent streptomycin-resistant organisms into normal NCS mice established a latent infection which could be uniformly converted into corynebacterial pseudotuberculosis by cortisone. The virulent *C. kutscheri* obtained from the lesions bore the genetic marker of streptomycin resistance, thus being identical with CKsr.

Except for streptomycin resistance, the avirulent organisms isolated from the experimentally induced latent infections were identical with those found in the naturally occurring latent infections.

These results suggest that *C. kutscheri* can persist *in vivo* in an avirulent form which is resistant to the defense mechanisms of the host, and can thus establish a latent infection. Treatment of the animal with cortisone results in the conversion of the avirulent form into virulent *C. kutscheri*, and of the latent infection into active corynebacterial pseudotuberculosis.

The findings are discussed with regard to their relevance to infection immunity, and to the conversion of latent infection into overt disease.

The authors gratefully acknowledge the technical assistance of Mrs. Junia G. Hedberg, Mrs. Merle S. Brock and Miss Jane Slattery and express appreciation to Dr. Clara J. Lynch for generously supplying mice from her inbred strains.

BIBLIOGRAPHY

1. Gundel, M., György, P., and Pagel, W., Experimentelle Beobachtungen zu der Frage der Resistenzverminderung und Infektion, *Z. Hyg. u. Infektionskrankh.*, 1932, **113,** 629.
2. Zucker, T. F., and Zucker, L. M., Pantothenic acid deficiency and loss of natural resistance to a bacterial infection in the rat, *Proc. Soc. Exp. Biol. and Med.*, 1954, **85,** 517.
3. Schechmeister, I. L., and Adler, F. L., Activation of pseudotuberculosis in mice exposed to sublethal total body radiation, *J. Infect. Dis.*, 1953, **92,** 228.
4. Antopol, W., Glaubach, S., and Quittner, H., Experimental observations with massive doses of cortisone, *Rheumatism*, 1951, **7,** 187.
5. LeMaistre, C., and Tompsett, R., The emergence of pseudotuberculosis in rats given cortisone, *J. Exp. Med.*, 1952, **95,** 393.
6. Speirs, R. S., Effect of oxytetracycline upon cortisone-induced pseudotuberculosis in mice, *Antibiotics and Chemotherapy*, 1956, **6,** 395.
7. Wolff, H. L., On some spontaneous infections observed in mice. I. *C. kutscheri* and *C. pseudotuberculosis*, *Antonie van Leeuwenhoek, J. Microbiol. and Serol.*, 1950, **16,** 105.
8. Lawrence, J. J., Infection of laboratory mice with *Corynebacterium murium*, *Austral. J. Sc.*, 1957, **20,** 147.
9. Pierce-Chase, C. H., Fauve, R. M., and Dubos, R., Corynebacterial pseudotuberculosis in mice. I. Comparative susceptibility of mouse strains to experimental infection with *Corynebacterium kutscheri*, *J. Exp. Med.*, 1964, **120,** 267.
10. Nelson, J. B., and Collins, G. R., The establishment and maintenance of a specific pathogen-free colony of Swiss mice, *Proc. Animal Care Panel*, 1961, **11,** 65.
11. Dubos, R. J., and Schaedler, R. W., Some biological effects of the digestive flora, *Am. J. Med. Sc.*, 1962, **244,** 265.
12. Fauve, R. M., L'emploi, en pathologie expérimentale, de souris bactériologiquement "propres." La souche NCS, *Méd. et Hyg.*, 1963, **21,** 178.
13. Fauve, R. M., Résistance cellulair à l'infection bactérienne. I. Résistance opposée au développement de *Corynebacterium kutscheri* après son ingestion par des macrophages de souris d'origines différentes, *Rev. franc. Etudes clin. et biol.*, 1964, **9,** 100.
14. Milieux de Culture, (M. Cassagne, editor), Éditions de la Tourelle, Saint Mandé, 1961, **1,** 156.
15. Wittler, R. G., Cary, S. G., and Lindberg, R. B., Reversion of a pleuropneumonia-like organism to a corynebacterium during tissue culture passage, *J. Gen. Microbiol.*, 1956, **14,** 763.
16. Lancefield, R. C., The antigenic complex of *Streptococcus haemolyticus*. II. Chemical and immunological properties of the protein fractions, *J. Exp. Med.*, 1928, **47,** 469.
17. Swift, H. C., Wilson, A. T., and Lancefield, R. C., Typing group A hemolytic streptococci by M precipitin reactions in capillary pipettes, *J. Exp. Med.*, 1943, **78,** 127.
18. Kabat, E. A., and Mayer, M. M., Experimental Immunochemistry, Springfield, Charles C Thomas, 1961, 85.

19. Fischl, V., Koech, M., and Kussat, E., Infektarthritis bei Muriden, *Z. Hyg. u. Infektionskrankh.*, 1931, **112,** 421.
20. Friedlander, H., Habermann, R. T., and Parr, L. W., Experimental arthritis in albino rats produced by a strain of corynebacterium, *J. Infect. Dis.*, 1951, **88,** 290.
21. Battisto, J. R., and Chase, M. W., Immunological unresponsiveness to sensitization with simple chemical compounds, *J. Exp. Med.*, 1963, **118,** 1021.
22. Pierce, C. H., Dubos, R. J., and Schaefer, W. B., Multiplication and survival of tubercle bacilli in the organs of mice, *J. Exp. Med.*, 1953, **97,** 189.
23. Hojo, E., On the *Bacillus pseudo-tuberculosis murium* prevalent in mouse, *Jap. J. Exp. Med.*, 1939, **10,** 113.
24. Bicks, V. A., Infection of laboratory mice with *Corynebacterium murium*, *Austral. J. Sc.*, 1957, **20,** 20.
25. Vallée, A., and Levaditi, J.-C., Abcès miliaires des reins observés chez le rat blanc et provoqués par un Corynebacterium aérobie voisin du type *C. kutscheri*, *Ann. Inst. Pasteur*, 1957, **93,** 468.
26. Seronde, J., Jr., Resistance of rats to inoculation with corynebacterium pathogenic in pantothenate deficiency, *Proc. Soc. Exp. Biol. and Med.*, 1954, **85,** 521.
27. Shwartzman, G., editor, The Effect of ACTH and Cortisone upon Infection and Resistance, New York, Columbia University Press, 1953.
28. Thomas, L. T., Cortisone, ACTH, and infection, *Bull. New York Acad. Med.*, 1955, **31,** 486.
29. McDermott, W., Microbial persistence, *Yale J. Biol. Med.*, 1958, **30,** 257.
30. Dienes, L., and Sharp, J. T., The role of high electrolyte concentration in the production and growth of L forms of bacteria, *J. Bact.*, 1956, **71,** 208.
31. MacPherson, I. A., and Allner, K., L forms of bacteria as contaminants in tissue culture, *Nature*, 1960, **186,** 992.
32. Pease, P., and Laughton, N., Observations on corynebacteria and related PPLO organisms, *J. Gen. Microbiol.*, 1962, **27,** 383.
33. Smith, P. F., Peoples, D. M., and Morton, H. E., Conversion of pleuropneumonia-like organisms to bacteria, *Proc. Soc. Exp. Biol. and Med.*, 1957, **96,** 550.
34. Wittler, R. G., Malizia, W. F., Kramer, P. E., Tuckett, J. D., Pritchard, H. N., and Baker, H. J., Isolation of a corynebacterium and its transitional forms from a case of subacute bacterial endocarditis treated with antibiotics, *J. Gen. Microbiol.*, 1960, **23,** 315.
35. Hobby, G. L., A study of variation in *Corynebacterium diphtheriae*, *J. Infect. Dis.*, 1935, **57,** 186.
36. Tobin, A. J., Jr., and Morse, E. V., The pathogenicity of *Corynebacterium pseudotuberculosis* for laboratory white mice, *Cornell Vet.*, 1957, **47,** 413.
37. Seronde, J., Jr., Zucker, L. M., and Zucker, T. F., The influence of duration of pantothenate deprivation upon natural resistance of rats to a corynebacterium, *J. Infect. Dis.*, 1955, **97,** 35.
38. Seronde, J., Jr., Zucker, T. F., and Zucker, L. M., Thiamine, pyridoxine and pantothenic acid in the natural resistance of the rat to a corynebacterium infection, *J. Nutrition*, 1956, **59,** 287.

39. Russell, W. L., Inbred and hybrid animals and their value in research, *in* Biology of the Laboratory Mouse, (G. D. Snell, editor), New York, Dover Publications, Inc., 1956, 347.
40. Sergent, E., Latent infections and premunition. Some definitions of microbiology and immunology, *in* Immunity to Protozoa, A Symposium of the British Society for Immunology, (C. C. Garnham, A. E. Pierce, and I. Roitt, editors), Oxford, Blackwell Scientific Publications, 1963, 39–47.
41. Wilson, G. S., and Miles, A. A., Topley and Wilson's Principles of Bacteriology and Immunity, Baltimore, Williams and Wilkins Co., 1946, **2,** 1165–1166.
42. Meyer, K. L., Latent infections, *J. Bact.*, 1936, **31,** 109.
43. Guze, L. B., and Kalmanson, G. M., Persistence of bacteria in "protoplast" form after apparent cure of pyelonephritis in rats, *Science*, 1964, **143,** 1340.

EXPLANATION OF PLATES

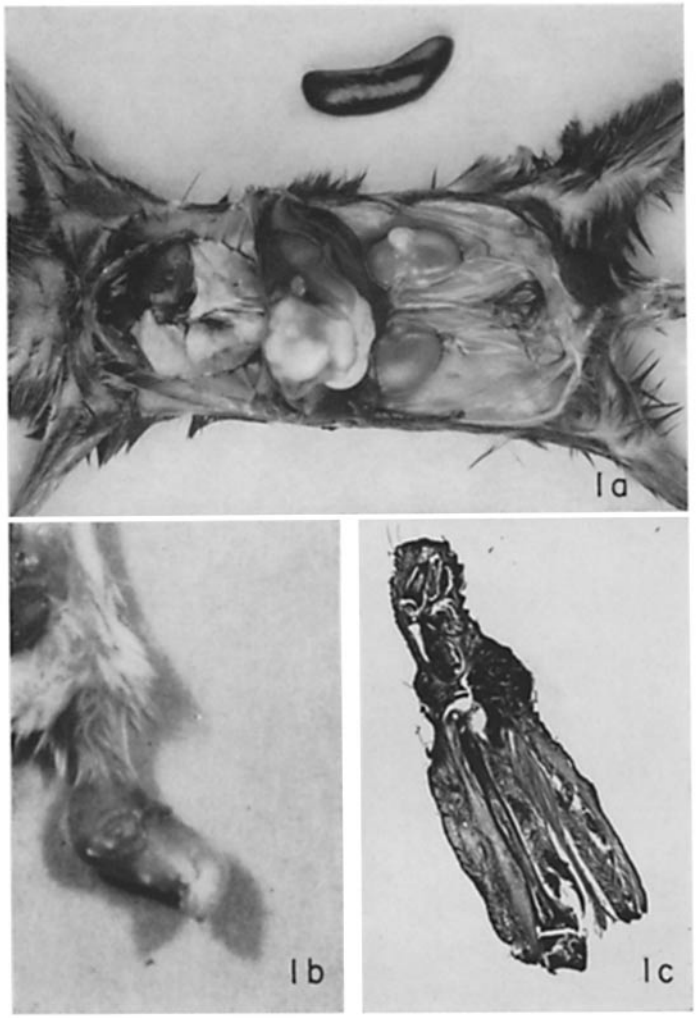
PLATE 26

FIGS. 1 *a* to 1 *c*. Activation by cortisone of latent corynebacterial infection.

FIG. 1 *a*. C57Bl/6 mouse (naturally acquired latency). Gross lesions observed at death 8 days after cortisone. × 1.5.

FIG. 1 *b*. RIII mouse (naturally acquired latency). Appearance of joint 12 days after cortisone. × 2.

FIG. 1 *c*. NCS, Institut Pasteur colony, mouse (experimentally induced latency). Section of infected joint from animal dying 10 days after cortisone. × 5.4.



(Fauve *et al.*: Corynebacterial pseudotuberculosis in mice. II)

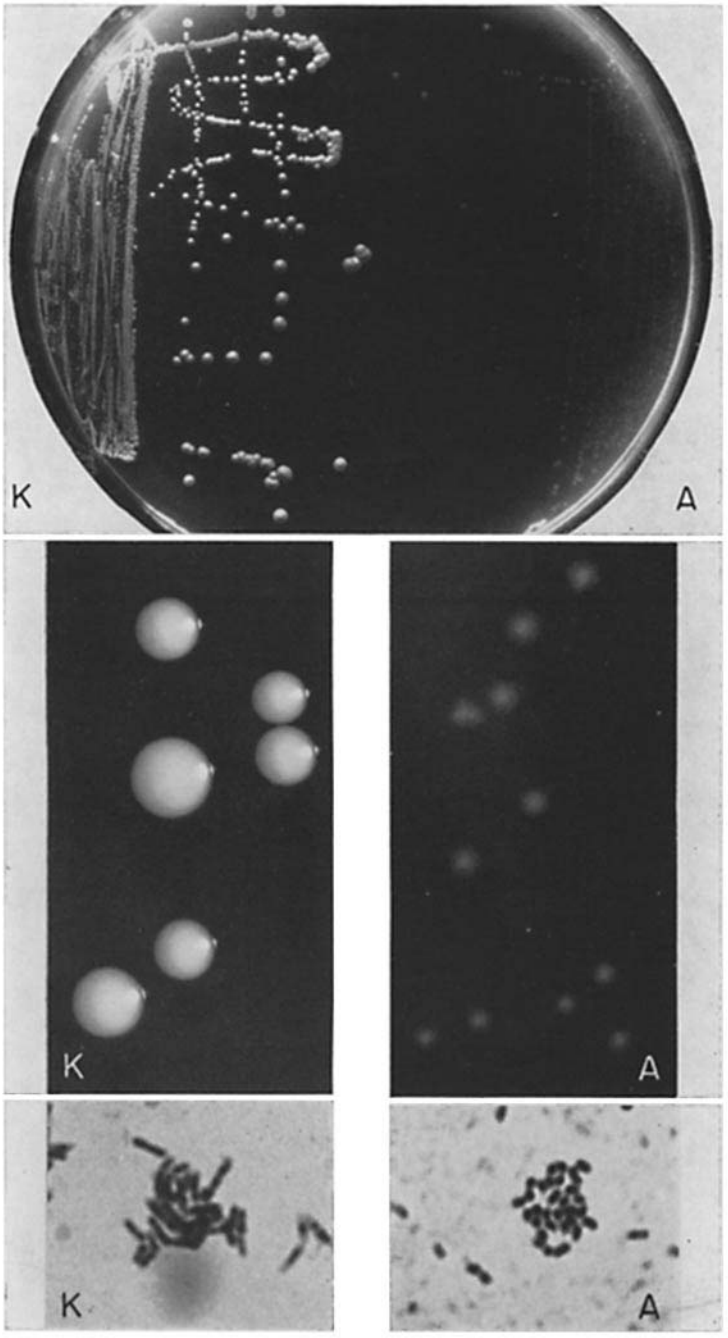
PLATE 27

FIGS. 2 *a* to 2 *c*. Morphology of bacteria recovered from homogenates of organs. K virulent *C. kutscheri*; A, avirulent organisms.

FIG. 2 *a*. Colonies on PF agar. Actual size (incident light).

FIG. 2 *b*. Colonies on PF agar. (Incident light.) × 7.

FIG. 2 *c*. Cellular morphology of colonies on PF agar. Giemsa stained preparation. × 2000.



2 *a*

2 *b*

2 *c*

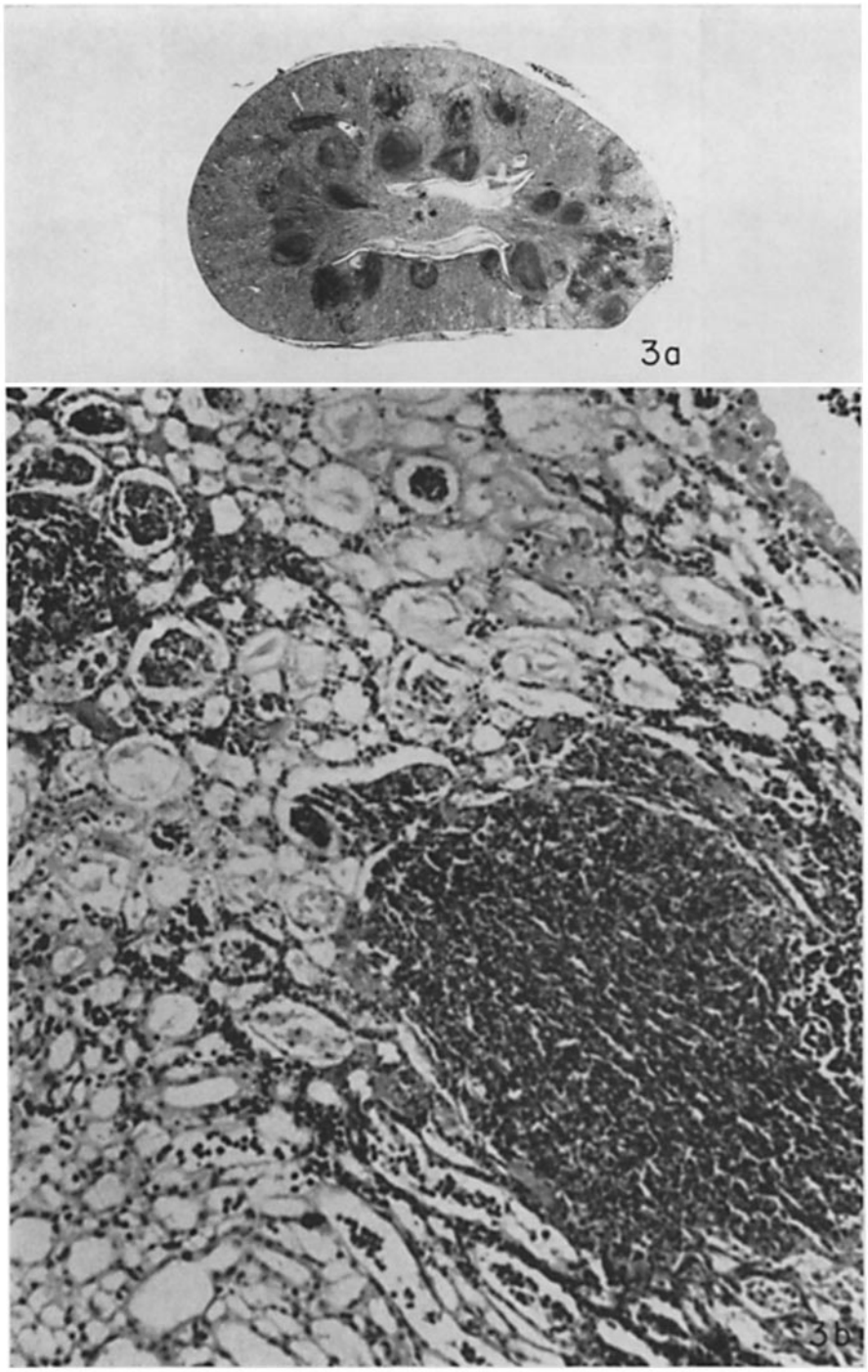
(Fauve *et al.*: Corynebacterial pseudotuberculosis in mice. II)

PLATE 28

FIGS. 3 *a* and 3 *b*. Activation by cortisone of experimentally induced latent corynebacterial infection.

FIG. 3 *a*. NCS (Institut Pasteur colony) mouse. Section of kidney showing medullary and occasional cortical abscesses. Hematoxylin and eosin stain. × 5.

FIG. 3 *b*. Same at higher magnification. × 200.



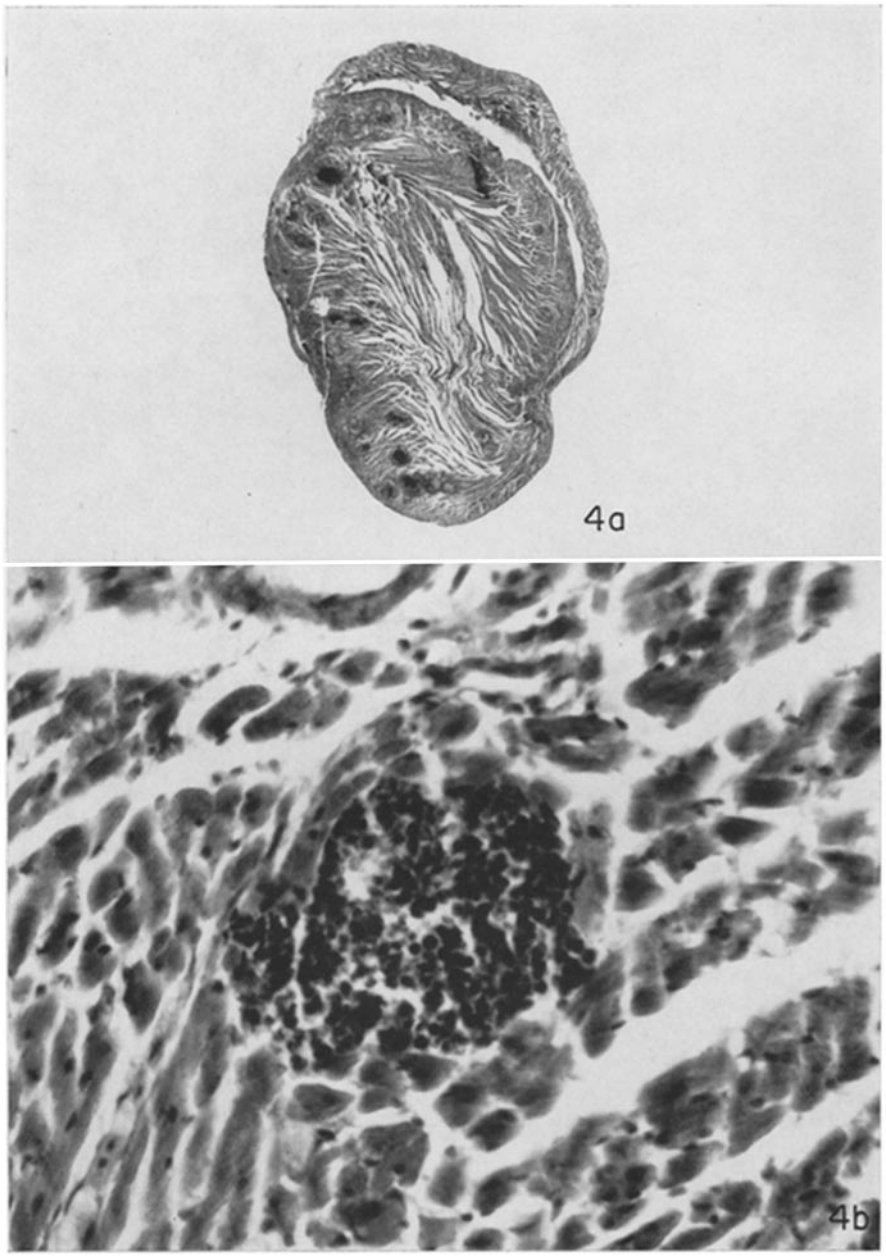
(Fauve *et al.*: Corynebacterial pseudotuberculosis in mice. II)

PLATE 29

FIGS. 4 *a* and 4 *b*. Activation by cortisone of experimentally induced latent corynebacterial infection.

FIG. 4 *a*. NCS (Institut Pasteur colony) mouse. Section of heart muscle with myocardial abscesses. Hematoxylin and eosin stain. × 10.

FIG. 4 *b*. Same at higher magnification demonstrating inflammatory cells predominantly of the mononuclear type. × 400.



(Fauve *et al.*: Corynebacterial pseudotuberculosis in mice. II)