THE EMERGENCE OF PSEUDOTUBERCULOSIS IN RATS GIVEN CORTISONE*

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PLATES 40 AND 41

(Received for publication, December 21, 1951)

The emergence of infection, either bacterial or viral in origin, in mammalian hosts following alterations produced by various biologic, chemical, and physical means, is a recognized phenomenon. In certain of the evoked bacterial infections, those following Roentgen ray irradiation, for example, the etiologic agent has usually been a microbial species which can readily be demonstrated to be a part of the normal bacterial flora of the host. In the present report, a situation is described in which a severe, fulminating infection regularly emerged in rats maintained under the influence of excessive amounts of cortisone. The etiologic agent of this infection was a microbial species not demonstrably a part of the usual bacterial flora of the rat, and of low virulence when artificially introduced into this host.

This extensive necrotizing disease process, similar in its gross appearance to tuberculosis, was observed in cortisone-treated rats during the course of an investigation of the effects of corticotropin and cortisone on the production and evolution of tuberculous lesions in animals of various species. The frequency and regularity of appearance of this unusual disease in rats, subjected to no other procedure than the administration of cortisone, made it seem of interest to characterize it further and to investigate the circumstances of its occurrence. The present report is concerned with this investigation.

Materials and Methods

Adult male albino rats (Wistar strain)† which weighed between 250 and 350 gm. were used throughout the investigation. The diet consisted of Purina dog chow‡ and water offered...
ad. lib., with a single feeding of cabbage and lettuce, each on separate days, once per week. Each animal was weighed weekly during a preliminary observation period of at least 2 weeks and throughout the subsequent period of investigation.

The animals were housed in two animal rooms hereafter designated animal room A and animal room B. With the exception of one group, all animals were housed in animal room A. This is a small oblong room equipped with ultraviolet ceiling lights which are kept in operation at all times except during the periods in which the animals are being handled. The only entrance into animal room A is through a small enclosed room similarly equipped with ultraviolet lighting. During the period of the present investigation this room also housed guinea pigs, the majority of which were infected with Mycobacterium tuberculosis (H37Rv). The animals were separated according to groups and maintained in adjacent cages containing a maximum of eight animals. One group of animals (III C) was housed in animal room B, which is an L-shaped room maintained for the housing of rabbits, guinea pigs, rats, and mice which have not been subjected to experimental infection. This room can be entered from a corridor or from the enclosed room adjacent to animal room A and is not equipped with ultraviolet lighting.

The tuberculous infection was produced by the intraperitoneal injection of an inoculum of Mycobacterium tuberculosis avium (Kirschberg) obtained from a 10-day old Tween80-albumin culture. Each inoculum was suspended in 1.0 cc. of Sorenson's phosphate buffer (pH 7.3) and contained an amount of diluted culture equivalent to 0.16 ml. of the original 10-day culture. (By turbidimetric measurement with reference to the previously established standard of this laboratory, it is estimated that each animal received approximately 2.5 million viable units of tubercle bacilli).

Corticotropin was administered subcutaneously in equally divided doses at 8 hour intervals. A total daily dose of 7.5 mg. or 22.5 mg. of hormone per animal was used. Cortisone acetate was administered subcutaneously at 12 hour intervals. A total daily dose of 10 mg. per animal was used throughout the investigation. Control animals received 0.2 ml. of 0.85 per cent sodium chloride solution subcutaneously at 12 hour intervals.

At the completion of an experiment the surviving animals were sacrificed by a blow on the head. The organs of each animal were examined and cultured on unenriched beef infusion agar, 2 per cent rabbit blood agar, Löeffler's serum agar, and oleic acid—albumin agar containing penicillin 25 units per ml. Duplicate smears of the gross lesions were stained for acid-fast bacilli by the Ziehl-Neelsen technique and for other microorganisms by the Gram-staining technique. Specimens of the tissues from each animal were obtained for histologic examination. The following tissue staining techniques and stains were used in the examination of these specimens: Ziehl-Neelsen, Van Gieson, Gram-Wiegert, hematoxylin and eosin, and Masson's trichrome.

Plan of the Investigation

Ninety-six hours after the initiation of subcutaneous administration of corticotropin, cortisone acetate, or sodium chloride, animals of certain groups were inoculated with M. tuberculosis avium by the intraperitoneal route. The

3 Tween 80 is the proprietary name of the polyoxyethylene derivative of sorbitan monololate, obtained from the Atlas Powder Company, Wilmington.
4 Armour standard LA-1-A, obtained through the courtesy of Dr. John R. Mote, Armour and Company, Chicago.
5 The cortisone acetate used in this investigation was obtained through the courtesy of Dr. J. N. Carlisle, Merck & Co., Inc., Rahway.
hormone or saline administration was continued for a total of 28 consecutive days at which time the surviving animals of all groups were sacrificed.

The grouping of the animals for the experiment is outlined in Table I.

**RESULTS**

*Weight.*—All animals which received sodium chloride or corticotropin, irrespective of whether inoculated with *M. tuberculosis avium*, showed no marked change in body weight during the investigation. All animals which received cortisone, however, steadily lost weight whether or not they had been inoculated with tubercle bacilli. As may be seen in Text-fig. 1 the weight loss during corti-

| TABLE I |
| Grouping of Animals in Experiment |

<table>
<thead>
<tr>
<th>Animal group No.</th>
<th>No. of animals</th>
<th>Inoculation</th>
<th>Agent administered</th>
<th>Individual dose</th>
<th>Interval of admin.</th>
<th>Total daily dosage of agent</th>
<th>Animal room in which experiment was performed</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>20</td>
<td><em>M. tuberculosis avium</em></td>
<td>Saline</td>
<td>0.2 ml.</td>
<td>12</td>
<td>0.4 ml.</td>
<td>A</td>
</tr>
<tr>
<td>II</td>
<td>10</td>
<td>Uninoculated</td>
<td>Corticotropin</td>
<td>7.5 mg.</td>
<td>8</td>
<td>22.5 mg.</td>
<td>A</td>
</tr>
<tr>
<td>C</td>
<td>20</td>
<td><em>M. tuberculosis avium</em></td>
<td>Corticotropin</td>
<td>7.5 mg.</td>
<td>8</td>
<td>22.5 mg.</td>
<td>A</td>
</tr>
<tr>
<td>A</td>
<td>10</td>
<td>Uninoculated</td>
<td>Cortisone</td>
<td>2.5 mg.</td>
<td>8</td>
<td>7.5 mg.</td>
<td>A</td>
</tr>
<tr>
<td>II</td>
<td>20</td>
<td>Uninoculated</td>
<td>Cortisone</td>
<td>5.0 mg.</td>
<td>12</td>
<td>10.0 mg.</td>
<td>B</td>
</tr>
<tr>
<td>C</td>
<td>20</td>
<td><em>M. tuberculosis avium</em></td>
<td>Cortisone</td>
<td>5.0 mg.</td>
<td>12</td>
<td>10.0 mg.</td>
<td>A</td>
</tr>
</tbody>
</table>

sone administration was extreme and frequently amounted to one-third the total body weight at the beginning of the experiment.

*Survival.*—All animals which received sodium chloride or corticotropin, irrespective of whether inoculated with *M. tuberculosis*, survived the experimental period with the exception of two animals sacrificed for histologic studies on the 23rd day after infection.

Thirty of the 50 animals which received cortisone died before completion of the experiment. The mortality rate was the same in the uninoculated groups (18 of 30) as in the groups which had been inoculated with *M. tuberculosis avium* (12 of 20). The earliest death occurred on the 6th day after the initiation of cortisone administration. The median and mean days of death for the uninoculated group were 22.5 and 22.4 days, respectively, and for the group inoculated with *M. tuberculosis*, 23.25 and 20.7 days, respectively. The mortality data are presented in Text-fig. 2.
Necropsy Findings.—

Group I: Rats Given Saline

On macroscopic examination at autopsy, no abnormalities were seen in the lungs, liver, heart, or kidneys in any of the 20 animals which had been inoculated intraperitoneally with *M. tuberculosis avium* and subsequently given injections of saline. The spleens were enlarged on the average one and one-half times normal size in all animals in this group but no discrete splenic lesions were observed in the gross.

All 20 animals had in the omentum multiple, pearly white, indurated nodules ranging from 1 to 10 mm. in diameter. The tracheobronchial lymph nodes were estimated to be two or three times normal size. Necrosis was not observed in any of the above lesions.

Microscopic examination of the sections of lung revealed the presence of moderate interstitial infiltration, predominantly by mononuclear cells, in all animals. In addition, epithelioid tubercles were scattered throughout all specimens. Acid-fast bacilli, with the morphologic characteristics of *M. tuberculosis avium*, were present in small numbers in all specimens.

Examination of the sections of omental nodules revealed many large tubercles composed of mononuclear cells and numerous intracellularly and extracellularly situated acid-fast bacilli. The individual tubercles were encircled by a connective tissue border as was the entire nodule. The tracheobronchial lymph nodes contained many well formed epithelioid tubercles without
marked necrosis but in which acid-fast bacilli were seen. Many tubercles, each containing several acid-fast bacilli, were present in all sections of liver and spleen. Microscopic examination of the sections of heart and kidneys revealed no lesions in any of the 20 animals.

The adrenal glands were normal in size, in gross appearance and on microscopic examination.

Text Fig. 2. 30 of the 50 animals which received cortisone died before completion of the experiment. All animals which received sodium chloride or corticotropin survived the experimental period with the exception of 2 animals sacrificed for histologic studies.

Group II: Rats (40) Given Corticotropin

Rats (10) Not Inoculated with Tubercle Bacilli (Group II A).—The macroscopic and microscopic examination of the tissues from the animals which had not received an inoculum of tubercle bacilli but which had received the larger of the two doses of corticotropin (22.5 mg. daily) revealed no abnormalities except for slight enlargement of the adrenal cortex.

Rats (30) Inoculated with Tubercle Bacilli (Groups II B and II C).—Except for slight hypertrophy of the adrenal glands in the animals which received corticotropin, there were no significant differences in the findings on macroscopic and microscopic examination between the group of saline-injected rats inoculated with tubercle bacilli and the similarly inoculated groups of animals which had received 7.5 mg. or 22.5 mg. of corticotropin daily.
In all inoculated animals given corticotropin there were nodules in the omentum and enlarged tracheobronchial lymph nodes. The appearance of the omental nodules and the tracheobronchial lymph nodes was similar in all respects to the gross appearance of the nodules and lymph nodes in the animals which received saline. Microscopic examination of the sections of tissue from all animals which received corticotropin revealed tuberculous lesions of similar structure in the same numbers and involving the same organs as in the tissues of the animals which received saline. An example of a characteristic tubercle of the lung may be seen in Fig. 1. In one animal the lower lobe of one lung appeared shrunken. Microscopic examination revealed only fibrous replacement of the lung parenchyma without evidence of an active inflammatory process.

Group III: Rats (50) Given Cortisone

In 41 of the 50 animals which had received cortisone, the lungs contained pale yellow necrotic lesions. A representative example of the gross appearance of the pulmonary lesions may be seen in Fig. 2. These lesions were present in 14 of the 20 rats inoculated with tubercle bacilli and in 27 of the 30 rats which had not been so inoculated.

Rats (30) Not Inoculated with Tubercle Bacilli (Groups IIIA and IIIB).—In 27 of the 30 animals which had not been inoculated with tubercle bacilli but which had received cortisone, multiple lesions of the lung and other viscera were visible on examination of the tissues. The lesions were usually distributed throughout all lobes and consisted of pale-yellow coalescent areas of semisolid necrosis. The lesions ranged in size from an approximate diameter of 1 or 2 mm. to confluent areas which involved all of one or more lobes. The borders of the lesions were sharply delineated from the surrounding pulmonary tissue which appeared normal on macroscopic examination. A granular, necrotic pleuritis involving both visceral and parietal pleural was present in all animals in which advanced or moderately advanced pulmonary lesions were present. Pericarditis was present in 15 of the 30 animals, presenting a granular thickened surface which was adherent to the myocardium. In addition, multiple grey-white necrotic lesions of the kidneys were observed in 19 of the 30 animals and similar lesions were noted on the surface of the liver in 15 animals.

Microscopic examination of the sections from lungs with lesions visible in the gross revealed large necrotic areas with amorphous debris obliterating the normal architecture of the lung. The lesions consisted of a central area of necrosis surrounded by structures with the appearance of microorganisms which were present both grouped in colonies or as individual cells (Figs. 3 a, 3 b, 3 c). A relatively narrow and ill defined area of cellular infiltration was present at the border of the lesions. Mononuclear cells were the predominant constituent of the infiltration with few polymorphonuclear leukocytes present. No epithelioid cell formation was observed and the cellular process did not give the appearance of delimiting the progress of the necrotizing lesions. In areas not directly adjacent to the lesions the lung parenchyma showed moderate infiltration with mononuclear cells. The pleural surfaces were covered in many areas by a homogeneous exudate containing many colonies and individual cells with the morphologic appearance of microorganisms. The cellular reaction in the lung parenchyma adjacent to these areas was not dense and consisted mainly of mononuclear cells.

Microscopic examination of the pericardium revealed inflammatory changes similar to those seen in the pleura. Stained structures presumed to be bacterial colonies similar to those seen in the lung lesions were readily discerned both in the pericardium and in the areas of myocarditis (Fig. 4). The myocardial cellular infiltration consisted of mononuclear and polymorphonuclear leukocytes.

The sections of kidney revealed the presence of multiple lesions of the parenchyma in all 27 animals with lung lesions visible in the gross. The renal lesions were situated mainly within glomeruli, encroaching upon and obliterating the normal architecture of the glomerulus. The
lesions contained only structures morphologically similar to microorganisms (Figs. 5 a and 5 b). The renal parenchyma about the involved glomeruli was, in most instances, devoid of cellular infiltration.

Necrotizing lesions were observed in the liver but not in the spleen on microscopic examination of the sections from these organs. Stained structures morphologically similar to microorganisms were, however, present in both organs. The normal architecture of the liver parenchyma surrounding the lesions was preserved but in the spleen there was a marked decrease in the lymphocytic elements with prominence of the fibrous stroma.

The possibility existed that the pulmonary lesions in the animals which received cortisone were related to their residence in animal room A which was also being used to house animals experimentally infected with *M. tuberculosis avium* and *hominis*. With regard to this possibility, it is worthy of emphasis that the 30 animals reported above represented two experimental groups (Groups III A and III B) which were housed in different animal rooms. Neither group was inoculated with tubercle bacilli. The 10 rats (Group III A) which had never been in animal room A or in contact with experimentally infected animals, were maintained in animal room B and given the same regimen of cortisone as the cortisone-treated animals in animal room A. 6 of the 10 animals died during the last 2 weeks of cortisone administration and an additional 2 animals were moribund when sacrificed. In 9 of the 10 animals the lungs and other viscera were the site of lesions, indistinguishable in the gross or on microscopic examination from those described in the similarly treated animals housed in animal room A.

**Rats (20) Inoculated with Tubercle Bacilli (Group III C).**—In 14 of the 20 animals which had been inoculated with tubercle bacilli and given cortisone multiple lesions of the lungs and other viscera developed. These lesions were indistinguishable on macroscopic and microscopic examination from those observed in the animals which had received cortisone but which had not been inoculated with tubercle bacilli. Moreover, the stained structures which appeared to be bacteria were present in comparable amounts, and in the same distribution in relation to the lesions as in the animals which had not been inoculated with tubercle bacilli.

Pericarditis and myocarditis were present in 11 of the 20 animals. Renal lesions visible in the gross were present in 11 animals. As in the animals which had not been inoculated with tubercle bacilli, glomerular lesions were seen on microscopic examination in all 14 of the animals with lung lesions visible in the gross. Necrotic lesions of the surface of the liver were present in 8 of the 20 animals.

**Extent of the Tuberculosis in the Rats Given Cortisone.**—In all 14 animals with the pulmonary lesions visible in the gross, the necrotizing process was so extensive that it was not possible to identify on microscopic examination of the tissues non-necrotic tubercles of the type observed in the animals which had been inoculated with tubercle bacilli and given saline or corticotropin.

Macroscopic examination of the lungs from the 6 animals inoculated with tubercle bacilli which did not develop the necrotizing pulmonary lesions failed to reveal abnormalities. Microscopic examination of the sections of lung from these animals, however, revealed moderate interstitial cellular infiltration of the alveolar septa by mononuclear cells and small collections of epithelioid cells, often containing acid-fast bacilli (Fig. 6). The estimated number of acid-fast bacilli present in these sections was comparable to that derived from examination of the tissue sections from the animals given corticotropin or saline.
Four of the 20 animals inoculated with tubercle bacilli and given cortisone developed multiple hard, pearly white, indurated, omental nodules which were approximately 1 to 3 mm. in diameter. On microscopic examination of the nodules, a central area of coagulation necrosis with numerous acid-fast bacilli at the periphery was noted. A relatively thin connective tissue border with scanty mononuclear and polymorphonuclear cellular infiltration surrounded the lesion. In addition, sections of omentum from the other 16 animals revealed numerous collections of intracellularly and extracellularly situated acid-fast bacilli without evidence of cellular reaction or formation of a limiting barrier.

The tracheobronchial lymph nodes were markedly reduced in size so that they were difficult to find in all 20 animals. Microscopic examination of the sections from tracheobronchial lymph nodes revealed a marked decrease in lymphoid elements and prominence of the stroma with acid-fast bacilli distributed throughout the tissue.

The size of the spleen was reduced in all 20 of the animals. Small collections of epithelioid cells, often containing acid-fast bacilli, were present in the sections of liver and spleen. In addition, acid-fast bacilli were distributed intracellularly and extracellularly throughout the tissues of both organs.

Microscopic examination of sections of pericardium, myocardium, and kidneys did not reveal lesions in the 6 animals without pulmonary lesions visible in the gross. The tissues from the remaining 14 animals of this group failed to reveal lesions with the characteristics of those caused by mycobacteria.

**Summary of the Lesions Observed in the Rats Given Cortisone.**—In 41 of the 50 animals given cortisone an extensive necrotizing disease developed irrespective of whether the animal had been inoculated with tubercle bacilli. Lung lesions were present in 41 animals, with a severe pleuritis in the 32 animals with multiple pulmonary lesions involving more than one lobe of the lung. Pericarditis and myocarditis were present in 26 of the 41 animals. Multiple lesions of the kidney were observed in 30 animals and lesions of the surface of the liver in 23 animals.

Microscopic examination of the tissues from the 41 animals revealed an extensive necrotizing disease characterized by numerous structures resembling microorganisms both grouped as individual cells and in colonies as well as by the lack of limiting reaction about the lesions.

The extent of the tuberculous process was difficult to evaluate in the lungs of the 20 animals inoculated with tubercle bacilli as only 6 animals failed to develop the necrotizing pulmonary disease described above.

**Tissues Stained for Bacteria.**—

Examination of the tissues from the animals of all three groups was undertaken using carbol-fuchsin stain by the Ziehl-Neelsen and Van Gieson techniques and Gram-Weigert stain. The microscopic examination of the tissues from the animals which were infected with tubercle bacilli and given sodium chloride or corticotropin revealed similar findings in both
groups of animals. Acid-fast bacilli, with the morphologic characteristics of *M. tuberculosis anum*, were present in the lungs, liver, spleen, and omentum in numbers approximately equal in both groups. Structures with the morphologic characteristics of microorganisms were not observed in the tissues of the animals given corticotropin which had not been inoculated with tubercle bacilli.

Examination of the tissues from the 41 animals given cortisone which developed pulmonary lesions visible in the gross revealed strikingly consistent findings, irrespective of whether the animal had been inoculated with tubercle bacilli.

Structures with the appearance of microorganisms both grouped in colonies and as individual cells, were present throughout the lung parenchyma and pleura but were most prominent at the immediate border of the areas of necrosis. The lack of phagocytic cellular reaction around these presumed bacterial colonies was particularly striking. With the Gram-Weigert stain the structures appeared to be Gram-positive bacilli. On preparations stained with hematoxylin and the Ziehl-Neelsen technique, the structures retained the carbolfuchsin stain in a variable degree. In some areas they presented the appearance of light-red staining bacilli; in others large aggregations of pink- or red-staining granules were visible. In still other areas the appearance of this structure was not definitely distinguishable from true acid-fast bacilli. Structures with the same staining characteristics were also observed in the necrotic lesions of the heart and kidney.

**Cultures.**

In Table II can be seen a summary of the specimens from which cultures were made and the results of the cultures. Of the 41 animals with pulmonary lesions visible in the gross, the lungs of 30 animals were used in an attempt to isolate the causative microorganism. A strain of microorganism with the bacteriologic characteristics of *Corynebacterium pseudotuberculosis murium* was isolated on blood agar medium from 28 of the 30 animals. Isolation of this strain of microorganism was attempted from the lungs of 9 animals, which received cortisone but in which the characteristic lesions of pseudotuberculosis were not detected on macroscopic or microscopic examination of the tissues, and was successful in 6 instances. All of the 6 animals were from a group (Group III C) which had been inoculated with tubercle bacilli. Therefore, *Corynebacterium pseudotuberculosis murium* was cultivated from the lungs of the animals given cortisone in 34 of the 39 animals in which cultivation was attempted.

Cultivation of this microorganism from the lungs, liver, spleen, and kidneys of 10 rats from the group of animals given saline and 10 rats from each of the groups of animals given corticotropin was attempted but in all instances the attempts were unsuccessful.

In addition, 10 albino rats which had been maintained in animal room A for 3 months were sacrificed and isolation of this strain of microorganism from the nasopharynx, trachea, lung parenchyma, kidney, liver, and spleen attempted. In all instances the attempts were unsuccessful.

Cultivation of microorganisms from the lungs of all animals used in the experiment was attempted on oleic acid-albumin agar. Microorganisms with
the morphologic and staining characteristics of \textit{M. tuberculosis avium} were isolated from all animals which had been inoculated with tubercle bacilli but from none of the other animals.

\textit{Characteristics of the Strain of Corynebacterium Isolated.}—The strain of Corynebacterium isolated from the animals which received cortisone had a cellular and colonial morphology which closely resembled \textit{C. diphtheriae}. The

\begin{table}
\centering
\begin{tabular}{|l|c|c|c|c|c|c|}
\hline
Animals from which specimens were obtained & Animals with pulmonary lesions & Animals without pulmonary lesions & Animals without pulmonary lesions & Animals with pulmonary lesions & Animals without pulmonary lesions & Animals without pulmonary lesions \\
& No. of animals & Total No. of lungs cultured & No. of lungs yielding \textit{Corynebacterium pseudotuberculosis murium} & Total No. of lungs cultured & No. of lungs yielding \textit{Corynebacterium pseudotuberculosis murium} & Total No. of lungs cultured \\
\hline
Normal rats & 10 & 0 & 0 & 0 & 10 & 0 & 10 \\
Rats given saline or corticotropin and inoculated with tubercle bacilli & 30 & 0 & 0 & 0 & 30 & 0 & 30 \\
Rats given corticotropin but not inoculated with tubercle bacilli & 10 & 0 & 0 & 0 & 10 & 0 & 10 \\
Rats given cortisone and inoculated with tubercle bacilli & 20 & 14 & 3 & 3 & 6 & 6 & 6 \\
Rats given cortisone but not inoculated with tubercle bacilli & 30 & 27 & 27 & 25 & 3 & 3 & 0 \\
\hline
\end{tabular}
\caption{Results of Attempts to Cultivate \textit{Corynebacterium pseudotuberculosis murium} from Pulmonary Tissue}
\end{table}

microorganisms were slender, frequently clubbed bacillary forms which exhibited a considerable degree of pleomorphism. The bacillary forms often contained numerous metachromatic granules. On solid media, small individual colonies which irregularly produced grey-white pigment were noted. In broth the microorganism grew with uniform turbidity and produced a heavy sediment but no pellicle. The strain did not form indol or hydrogen sulfide and gelatin was not liquified. Dextrose, saccharose, maltose, and salicin were fermented with the production of acid but no gas. Lactose and mannitol were not fermented. The strain of microorganism was Gram-positive and non-
motile. These were the characteristics of *Corynebacterium pseudotuberculosis murium*. The microorganism and the disease it caused in mice was first described by Kutscher (1). Both Kutscher (1) and Bongert (2) attempted to define the pathogenicity of this strain for various animal species but were successful in establishing infection only in the mouse.

Tests have been made of the pathogenicity of the strain of *Corynebacterium* isolated from lesions of rats given cortisone.

It has been possible to reproduce the disease in normal rats by the intracardiac injection of 1.0 cc. of a 10⁻⁴ saline dilution of an 18 hour broth culture of this microorganism. Only 2 of 5 rats thus treated, however, have died from the infection. These rats died 5 and 10 days subsequent to inoculation. The lesions observed in these animals were similar to those which occurred in rats given only cortisone (Fig. 7). The rats remaining alive were sacrificed and 2 were found to have intrathoracic abscesses and the 3rd had a kidney abscess. Corynebacteria were recovered from each of the three abscesses.

Five rats survived intracardiac inoculation of 1.0 ml. of a 10⁻⁴ saline dilution of broth culture. 10 rats were inoculated intraperitoneally with 1.0 ml. of a 10⁻² saline dilution of a broth culture and 10 rats received 1.0 ml. of a 10⁻⁴ culture dilution. All 20 of these animals survived.

Similar attempts were made to infect white mice with 0.2 ml. of various saline dilutions of an 18 hour broth culture. All 5 mice which received the 10⁻² saline dilution intravenously died with 72 hours subsequent to inoculation. All had granulomatous lesions in the liver, spleen, and kidneys. Of 5 mice which received the 10⁻⁴ saline dilution intravenously, 3 died in the 2nd week subsequent to inoculation. Abscesses of the liver and kidney were present in these animals. Of 18 mice inoculated intraperitoneally with 1.0 ml. of one of the above dilutions of culture, only 1 died and in none were pulmonary lesions demonstrated at autopsy. 3 of the mice had small intraperitoneal abscesses and 2 had abscesses of the kidneys. Corynebacteria were recovered from these lesions.

Attempts were also made to infect 4 guinea pigs and 4 rabbits with a 1.0 ml. intraperitoneal inoculum of either a 10⁻² or 10⁻⁴ saline dilution of an 18 hour broth culture. None of these animals died from infection due to corynebacteria.

**DISCUSSION**

An extensive necrotizing disease, characterized by multiple coalescent lesions in the lungs and similar, though smaller, lesions of the pericardium, pleurae, liver, and kidneys, developed in 41 of 50 rats which were given cortisone. The disease was the apparent cause of death in 30 of the 41 rats in which it appeared. 60 rats which received injections of sodium chloride or corticotropin failed to develop the disease as observed in the animals given cortisone.

Macroscopic examination of the tissues from the animals which received cortisone revealed pulmonary lesions which were strikingly similar in character with the lesions of a disseminated, far advanced tuberculous process. Although 20 of the 50 animals which received cortisone had been inoculated intraperitoneally with *M. tuberculosis avium*, the necrotic pulmonary lesions presented the same characteristics and occurred in the same incidence in the animals which had received no tubercle bacilli as in the animals inoculated with living
tubercle bacilli. The histologic appearance of the lesions was sufficiently distinctive so that they could be differentiated from the characteristic lesions caused by *Mycobacterium tuberculosis*. Moreover, a strain of *Corynebacterium* was cultivated from the lungs with necrotic lesions in the gross in 28 of the 30 instances in which isolation of this microorganism was attempted. Tubercle bacilli were, however, cultivated only from the lungs of the animals which had been inoculated with *M. tuberculosis avium*. Corynebacteria could not be cultivated from the tissues of 50 animals which did not receive cortisone.

The microscopic appearance of the pulmonary lesions in the animals which received cortisone had many distinguishing characteristics. The central necrotic area was surrounded by numerous Gram-positive, frequently bipolar and club-shaped rods which were present both in colonies or as individual cells, and around the lesion little cellular reaction was observed. The structures which resembled bacteria were frequently acid-fast and were present in the tissue sections from all 41 animals with pulmonary lesions visible in the gross. The morphologic and staining characteristics of these structures, although similar in many respects to those of avian tubercle bacilli, closely resembled those of the strain of *Corynebacterium* isolated from 28 lung specimens with lung lesions visible in the gross. Finally, it was possible to reproduce the lesions of this disease in normal rats by intracardiac injection of a large infecting inoculum of this strain of *Corynebacterium* grown in broth culture, although the strain cannot be said to have a high degree of pathogenicity for this species. The possibility has not been excluded, however, that the relative lack of virulence of this strain of microorganism for adult rats was a consequence of previous infection with corynebacteria.

It was not possible in the present experiments to establish unequivocally the pathogenesis of the pseudotuberculosis caused by *C. pseudotuberculosis murium*. Whether the animals which received cortisone were already harboring the microorganisms before receiving cortisone or whether the cortisone rendered them uniquely susceptible to air-borne infection with the microorganisms remains unanswered. Corynebacteria were not cultured from the nasopharynx, trachea, lung, liver, spleen, or kidneys of rats which had not been subjected to any experimental procedure but which were maintained in both animal rooms. Agar plates were exposed for various periods of time to the air of both animal rooms without evidence of growth of corynebacteria. Moreover, corynebacteria were not isolated from the lungs and other viscera of the animals which received sodium chloride or corticotropin for the 28 day experimental period. Corynebacteria were, however, isolated from the pulmonary tissues of 6 of the 9 animals which received cortisone but in which pseudotuberculosis failed to develop.

It seems probable that the animals which were receiving cortisone provided an unusually favorable environment for the multiplication of corynebacteria.
Lesions presumably caused by this microorganism have been observed during cortisone administration to animals known to be susceptible to corynebacteria. Antopol (3) has reported the appearance of granulomatous nodules in the liver, kidney, and spleen, from which Corynebacterium pseudotuberculosis murium was isolated, in 8 of 36 mice which had received large doses of cortisone. The lesions were not found in the control animals which had received sodium chloride.

Contamination of the cortisone by corynebacteria as a source of introduction of the microorganism would seem to be an unlikely possibility. A high concentration of preservative (1.5 per cent benzyl alcohol) was present in all cortisone used and the preparations of hormone were repeatedly sterile on culture.

On the basis of the present observation, therefore, the extent of permissible inference is that in the majority of the animals, the administration of cortisone transformed the animals into uniquely susceptible hosts for the propagation of Corynebacterium pseudotuberculosis murium and favored the development of lesions in the lungs and other viscera.

Differences between the effects of corticotropin and cortisone upon the resistance of certain hosts to infection have been recently noted by Shwartzman (4). Shwartzman has demonstrated enhancement of poliomyelitis infection in mice and hamsters by the administration of cortisone whereas in the same experimental situation corticotropin alone failed to alter the susceptibility in the animals. It was suggested that the failure of corticotropin to produce this effect might be due to the elaboration of a factor capable of reversing the enhancing effect of the cortisone produced by the adrenal cortex. Thus, it appears that despite the many similarities between the two hormones, their effects in terms of the resistance of certain animals to certain parasites may be distinctly different.

The marked difference in the behavior of the animals which received corticotropin and those which received cortisone in regard to the development of pseudotuberculosis was noteworthy. It is conceivable that this apparently different action of the two substances is merely a reflection of our present inability to judge comparable quantities of the respective drugs and the frequency of administration in terms of the physiologic effects produced. In this connection it is of interest that Selye (5) has recently reported the appearance of pulmonary lesions in the course of fatal systemic infections in female piebald rats which received large doses of either corticotropin or cortisone. The etiologic microorganism was not identified. In his experiments, the daily dose of corticotropin used was 12 mg. per animal administered in equally divided doses at 4 hour intervals. In the present study, no pseudotuberculous lesions developed in animals which received corticotropin administered in equally divided doses at 8 hour intervals. Moreover, a special attempt was made to exclude the possibility of a dosage effect masking any similarity in the action of cortico-
tropin and cortisone by the inclusion of a group of animals which received 22.5 mg. of corticotropin daily in divided doses at 8 hour intervals for a 28 day period. Even on such excessively large dose of corticotropin, there was no evidence of the development of pseudotuberculosis.

It is of considerable interest that the infection which emerged in the cortisone-treated rats was consistently caused by the same microbial species, Corynebacterium pseudotuberculosis murium. Under similar circumstances in the same host species, another host-parasite relationship, the experimental tuberculous infection, was not markedly altered by the administration of either hormone. The determining factors produced by cortisone administration, which in this instance regularly led to the development of infection due to one microbial species, were not specifically defined. It should be noted, however, that Michael and his associates (6), using a different strain of albino rats and a different type of mycobacteria, did not encounter pseudotuberculosis in cortisone-treated animals but noted the development of a fulminating rapidly fatal tuberculous process. The contrasting results of the experiments of Selye, Michael et al., and those of the present investigation emphasize the necessity for consideration of the strain of the animal species as well as the type of mycobacteria in the interpretation of results when the effects of hormone administration are to be evaluated.

Gundel, György, and Pagel described a disease which developed in albino rats maintained on a diet deficient in a substance designated as vitamin H (7). The pseudotuberculous lesions closely resembled those noted in the present investigation. Corynebacterium pseudotuberculosis murium was isolated from the tissues of the animals and considered to be the etiologic agent responsible for the disease. In both the present experiments and those of Gundel et al., a correlation existed between the development of lesions and the amount of weight lost by the animal. Irrespective of whether inoculated with tubercle bacilli, all animals which received cortisone showed marked weight loss. It should be noted, however, that the 9 animals which received cortisone but did not develop macroscopic lesions also lost weight to approximately the same degree as the other animals which received cortisone.

From the observations in the present experiment it appears that the possibility of the evocation of pseudotuberculosis, or the creation of a state uniquely favoring its development, must be taken into consideration in experiments concerned with the influence of cortisone upon experimental tuberculous lesions in rats and perhaps also in mice. The close resemblance between the two disease processes is not only confusing on macroscopic examination but the capacity of the corynebacteria to retain the acid-fast stain to a variable degree may easily lead to similar confusion on examination of stained sections of tissue. The possible implications of the presence of marked weight loss in both the animals in the present experiments and those of Gundel et al., make it advisable
that interpretation of results should be carefully considered when cortisone, or perhaps any procedure which causes marked loss of weight in rats, is included in an experimental situation in which the effects of inoculation with \textit{M. tuberculosis} are being studied.

**SUMMARY**

The emergence of a severe infection in albino rats during cortisone administration is reported. Evidence is present that the causative agent of the disease was \textit{Corynebacterium pseudotuberculosis murium}, a microbial species not demonstrably a part of the usual bacterial flora of the host. It has been possible to reproduce the disease in rats by a relatively large infecting inoculum of this strain of \textit{Corynebacterium}, but the susceptibility of normal rats to infection has been found to be low.

The disease occurred in 41 of 50 rats given cortisone and in 28 of 30 instances in which isolation of the etiologic agent was attempted, this strain of \textit{Corynebacterium} was recovered. The disease was characterized by widespread necrotizing lesions, with multiple coalescent lesions occurring in the lungs, and similar, though smaller lesions of the pericardium, pleurae, liver, and kidneys. In its gross appearance the pulmonary disease was similar to that of a disseminated, far advanced tuberculous process. The histologic appearance of the lesions, however, was sufficiently distinctive so that they could be readily differentiated from the lesions characteristically produced by \textit{Mycobacterium tuberculosis}. Moreover, the host-parasite relationship established by the experimental infection with avian tubercle bacilli, was not markedly altered by the factors which led to the emergence of the pseudotuberculosis.

In contrast to the high frequency of pseudotuberculosis in rats given cortisone, no instance of this disease has been encountered in a similar group of animals given large quantities of corticotropin.

The assistance of Miss Marguerite Leask in this investigation is gratefully acknowledged.

**BIBLIOGRAPHY**

EXPLANATION OF PLATES

PLATE 40

Fig. 1. Epithelioid tubercle in the lung of a rat inoculated with \textit{M. tuberculosis avium} and given corticotropin. Hematoxylin and eosin stain. × 100.

Fig. 2. Lesions of the lung of a rat which had received cortisone but was not inoculated with tubercle bacilli.

Fig. 3a. Lesions of the lung and pleura of a rat which had received cortisone but was not inoculated with tubercle bacilli. Gram-Weigert stain. × 2

Fig. 3b. Lesions of the lung of a rat which received cortisone but was not inoculated with tubercle bacilli. Gram-Weigert stain. × 100.

Fig. 3c. Lesions of the lung of a rat which received cortisone but was not inoculated with tubercle bacilli. Note colonies of microorganisms. Gram-Weigert stain. × 10.
PLATE 41

Fig. 4. Myocarditis and pericarditis in a rat which received cortisone but was not inoculated with tubercle bacilli. Gram-Weigert stain. × 100.

Fig. 5a. Lesions of the kidney in a rat which received cortisone but was not inoculated with tubercle bacilli. Gram-Weigert stain. × 100.

Fig. 5b. Lesion of the kidney in a rat which received cortisone but was not inoculated with tubercle bacilli. Gram-Weigert stain. × 450.

Fig. 6. Epithelioid tubercle in the lung of a rat inoculated with *M. tuberculosis avium* and given cortisone. At necropsy necrotizing pulmonary lesions were not observed. Hematoxylin and eosin stain. × 100.

Fig. 7. Lesions of the lung in a rat which received by the intracardiac route 1 ml. of a 10⁻² saline dilution of an 18 hour culture of *Corynebacterium pseudotuberculosis murium*.
(LeMaistre and Tompsett: Cortisone and emergence of pseudotuberculosis)