chick. This view is strengthened by the fact that a compound which is formed from aureomycin by treatment with dilute alkali is inactive for the chick and exhibits only a fraction of the antibacterial activity of aureomycin. These data further support the view that the antibiotic activity of a compound and its growth promoting activity for animals are related. The specific activity of each compound as regards its growth promoting effect is most probably correlated with the antibiotic spectrum of the compound and with the availability and stability of the substance in the intestinal tract of the animal. These factors could account in part for the fact that certain antibiotics are superior to others as growth stimulators.

Several substances when added to solutions of aureomycin stabilize the latter against inactivation by alkali. The exact mode of this action is unknown.

Summary. Alkali treated *Streptomyces aureofaciens* fermentation liquor contained considerable unchanged aureomycin. The pure antibiotic is inactivated by similar treatment. The product arising from mild alkaline inactivation of aureomycin was isolated and found to have essentially no antibacterial activity and no growth stimulating activity for the chick.

We are indebted to Dr. J. H. Williams, Director of Research, for his constant interest and advice. It is a pleasure to express our appreciation to Mr. L. M. Brancone and staff for the microanalyses, to Mr. Carl Wolf and Mrs. Angela Goldman for the preparation of the alkali inactivated aureomycin and to Mr. Peter Arnow for the chromatography of the various solutions.

Received February 15, 1951. P.S.E.B.M., 1951, v76.

Pathological Changes in Adrenal Cortex of Mouse During Experimental Meningococcal Infection.* (18593)

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Importance has been attached to hemorrhage(1,2) and other changes in the adrenal cortex, particularly the "tubular degeneration" of Rich(3), in patients dying from fulminating meningococcal sepsis. These reports suggested that similar changes might be reproduced in animals experimentally infected with meningococcus.

The following observations are limited to microscopic changes in the adrenal cortex such as changes in the amount and distribution of fat, hyperemia and inflammatory infiltration.

Methods and materials. The meningococcus employed was a type I strain originally isolated from the blood stream of a child acutely ill with epidemic meningitis. A 6-hour culture of the organism was suspended in 4% mucin‡ and mice were inoculated intraperitoneally with 1.0 ml containing from 10^2 to 10^5 organisms. The virulence of this strain had been increased by mouse passage so that < 10 meningococci in mucin produced sepsis which was fatal within 48 hours. Animals which were inoculated with 10^2 organisms and remained untreated were moribund at 24 hours and rarely lived for 48 hours. No

* This investigation was supported, in part, by the U. S. Navy, Office of Naval Research, and, in part, by the A. B. Kuppenheimer Foundation of the University of Chicago.

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‡ Granular mucin, type 1701-W, supplied by the Wilson Laboratories, Chicago, Ill. The method of preparation is described by Miller and Bohnhoff(4).

1. Moritz, A. R. and Zamcheck, N., *Arch. Path.*, 1946, v42, 459.

2. Daniels, W. B., *Arch. Int. Med.*, 1948, v81, 145.

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4. Miller, C. P. and Bohnhoff, M., *J. Inf. Dis.*, 1948, v83, 256.

comparison therefore could be made between penicillin-treated and untreated mice after 24 hours. Mice injected with 10^5 meningococci died within 20 hours unless they were treated not later than 6 hours post-infection. Treatment consisted of a single dose of 200 units of penicillin§ administered subcutaneously in 0.5 ml of saline 6 hours after infection. The heart's blood of untreated mice was cultured on a meat digest agar(5) and meningococci were recovered in pure culture after 24 hours' incubation under reduced oxygen tension. The strains of mice¶ used were equally susceptible to the strain of meningococcus employed. The mice were adult males, 6 to 8 weeks of age, weighing 20 to 24 g.

Autopsy, staining and fixing.‖ In order to avoid postmortem changes, animals were killed at various times after infection and the kidneys removed with the untouched adrenal glands attached. The combined specimen was fixed for 7 days in a modified Bouin's solution.** The adrenal gland, attached to the superior pole of the kidney, was frozen and sectioned on a Spencer freezing microtome. The technic gave sections through the same regions of each gland and avoided changes which might be produced by manipulation. This was important because the adrenal gland is extremely delicate, and the slightest pressure will cause artifacts to appear in the microscopic sections(6,7). Of the 350 pairs of adrenals studied, 130 were stained with hematoxylin and eosin-azure and 220 were stained for fat with Sudan III (saturated solution in equal parts of 50% alcohol and Carbitol††) and counter-stained with hematoxylin. Both staining methods were satisfactory for the study of cellular changes, but only the fat stain was adequate for distinguishing lipid vacuoles. For this reason, most of the sections were stained for fat.

Anatomy of the mouse adrenal cortex. The adrenal cortex of the mouse is divided into 3 zones (See Fig. 1): (a) the zona glomerulosa, rounded groups of cells averaging 8 to 12 cells in depth. This zone normally contains relatively less fat than the second zone. (b) the zona fasciculata, the thickest zone which consists of radial columns of cells, and contains most of the fat principally in its outer two-thirds. (c) the zona reticularis ("embryonic, boundary zone, fetal or x-zone"), the structure of which depends upon the age and sex of the animals(7-10).

Controls. Adrenals were prepared from mice injected with mucin, penicillin and saline as well as from normal healthy controls and from animals which had been deprived of food and water.

Microscopic study. Each adrenal cortex was examined for the following details: 1. Cellular changes: such as cellular degeneration, zonal degeneration of the type described by Humphreys and Donaldson(11), "tubular degeneration"(3) and increased mitoses which are frequently seen in the pathological adrenal cortex(9,12,13). Changes in thickness of the cortex described as occurring "under stress"(14,15) were observed but could not be evaluated with sufficient ac-

§ The penicillin was supplied by the Abbott Laboratories, Lederle Laboratories, Eli Lilly and Co., and Schenley Laboratories.

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¶ Maple Grove Rabbitry, Springfield, Mo. and CF No. 1 strain, Carworth Farms, New City, N. Y.

‖ This method of obtaining, fixing and staining was devised with the assistance of Mr. John H. Daniels of the Department of Medicine, University of Chicago.

** Fixative: 2 parts of glacial acetic acid; 75 parts saturated solution of picric acid; 25 parts of formaldehyde solution.

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†† Carbitol: Doethylene glycol monoethyl ether, a product of The Carbide and Carbon Corp.

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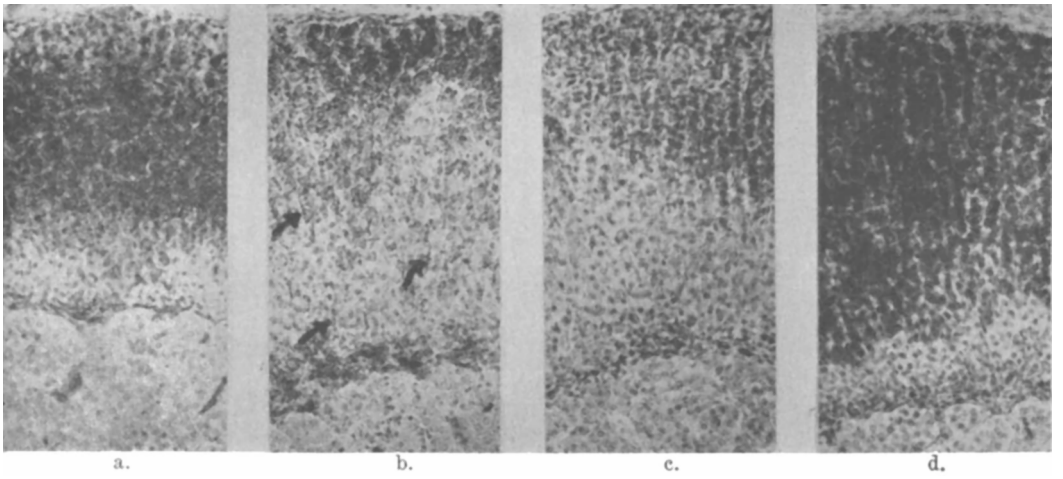

FIG. 1.

Sections of mouse adrenals showing depletion of fat in the zona fasciculata during meningococcal infection and its restoration after successful treatment with penicillin. All sections stained with Sudan III and counter-stained with hematoxylin eosin-azure. Mag. ×210.

(a) Normal control: Section shows, from above downward, the zona glomerulosa, the zona fasciculata, and the outer rim of the medulla.

(b) Untreated mouse: Killed 24 hr after infection with meningococcus. Blood culture positive. Adrenal cortex shows depletion of fat (compare with normal) and engorgement, hemorrhage, and inflammatory cell infiltration at cortico-medullary junction. Arrows indicate engorged capillaries.

(c) Penicillin treated mouse: Killed 24 hr after infection with meningococcus. Blood culture negative. Adrenal cortex shows more fat than untreated (b). Much of the light gray color in the inner half of the zona fasciculata represents fat in finely divided droplets. Less engorgement and inflammatory cell infiltration than (b).

(d) Penicillin treated mouse: Killed 48 hr after infection with meningococcus. Blood culture negative. Adrenal cortex shows a return to normal amount of fat.

curacy. 2. Areas of inflammatory cell infiltration. 3. Engorgement of capillaries and hemorrhage within the cortex and at the cortico-medullary junction. 4. Abnormalities in quantity and distribution of fat in the cortex. In estimating the amount of fat depletion and engorgement in adrenals from infected animals, allowance was made for normal variation as determined by examination of adrenals from control mice.

Results. The adrenal cortex of mice infected with meningococcus showed depletion of fat, engorgement and inflammatory cell infiltration. Most of the sections from infected mice showed generalized reduction of fat, most commonly seen in the zona glomerulosa and in the inner half of the zona fasciculata. Typical examples are shown in the microphotographs in Fig. 1 which includes a normal cortex for comparison. Moderate fat depletion occurred in untreated mice as early as one to 2 hours after infection and became conspicuous at 7 to 24 hours. Subsequent observations were possible only in the treated animals. Less than half of these mice at 28 to 48 hours showed depletion of fat, and only a few of them showed marked depletion, indicating that recovery was in progress.

In Table I the incidence of fat depletion and other changes is related to the course of the infection. It should be pointed out, however, that the degree of depletion, not recorded in the table, was most marked at those periods when it was found in the highest percentage of mice. It was of interest to note that in a few sections, the fat was concentrated in the zona glomerulosa and in a band in the zona fasciculata with or without a fat-free zone between the two. An occasional section showed fat only in the zona glomerulosa.

The adrenals of control mice receiving mucin, penicillin and saline showed no significant differences from the adrenals of normal stock animals. Engorgement was usually greatest at the cortico-medullary junction and areas of hemorrhage within the cortex

TABLE I. Changes in Adrenal Cortex of Mice Infected with Meningococcus.

Time after infection, hr	% of adrenals showing: Fat depletion		Engorgement		Inflammatory cell infiltration	
	UnR_x, %	R_x,* %	UnR_x, %	R_x,* %	UnR_x, %	R_x,* %
Uninfected controls	0	0	0	0	0	0
1–2	40	*	20	*	50	*
3–6	65	*	60	*	40	*
7–20	75	100	60	70	70	75
24	100	65	75	40	85	45
28–48	†	40	†	45	†	60

* 200 units of penicillin administered subcut. 6 hr after infection.
† No survivors among untreated mice.

were most frequently located in the zona fasciculata adjacent to the area of greatest cortico-medullary engorgement. Engorgement seldom extended more than half way into the zona fasciculata. Table I shows a comparison of the amount of engorgement in untreated mice and those treated with penicillin.

Inflammatory cell infiltration was greatest at or adjacent to the areas of greatest cortico-medullary engorgement, and the reaction seldom extended farther than the inner half of the zona fasciculata. (Table I). No other cellular changes: *e.g.*, mitoses, were observed in any of the sections.

Discussion. Fat depletion, engorgement and inflammatory cell infiltration occur in the adrenals of mice with experimental meningococcal sepsis. The extent of these changes was modified by treatment with penicillin which terminated the infection. Infected mice did not show increased mitosis, cellular degeneration or zonal degeneration which have been produced in animals by various infectious or toxic agents(3,9,11-13). They also lacked the tubular degeneration described by Rich in the adrenals of patients dying of fulminating meningococcal sepsis. It was not possible in these mouse adrenals to evaluate with sufficient degree of accuracy changes in thickness of the cortex such as had been reported in various inflammatory diseases (14).

Since the animals infected with meningococcus did not eat or drink, adrenals were examined in a small group of mice deprived of food and water for 48 hours. Fat depletion was not noted at 24 hours, but it was observed in a moderate degree at 48 hours. This degree of fat depletion at 48 hours was not so great as the depletion which occurred at 24 hours in infected untreated animals. No other abnormalities were seen in these adrenal glands.

Summary. Employing a technic which minimized the production of artifacts, a histological study was made of the adrenal glands of mice infected with meningococcus and also of appropriate controls. Depletion of fat, engorgement and inflammatory cell infiltration were demonstrated during the course of the infection. After successful treatment with penicillin the adrenal cortex showed a progressive recovery as manifested by reaccumulation of fat and reduction of hyperemia and inflammatory reaction.

Received February 16, 1951. P.S.E.B.M., v76, 1951.