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Resistance of Rats to Inoculation with Corynebacterium Pathogenic in Pantothenate Deficiency. (20938)

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During the course of other work in this laboratory(1) an unusual infection was encountered in over 50% of rats dying in pantothenate deficiency. Discrete lesions in several of these animals yielded pure cultures of a corynebacterium, a group commonly considered to be non-pathogenic for rats. The present preliminary study was carried out in order to determine whether this organism was actually the causative agent of the observed lesions, and to test their reproducibility in both deficient and well-fed rats.

Methods and materials. The rats used in this study were of the more susceptible 13C and 14C strains described in the preceding paper(1). The same basal pantothenate-deficient diet was used, modified by the addition of 0.2% l-cystine and with "Vitamin Supplement II," omitting cod liver oil and ascorbic acid. Litter mate control rats received this basal diet to which 4 mg of calcium pantothenate per 100 g had been added. In addition to this, a group of young rats was given a natural food stock diet in the first experiment.

The mice used were random bred offspring of 3 strains which happened to be available: "Swiss" albinos, C57 blacks, and Strong's BrS. They were fed a pelleted diet (Derwood). The organism bears a close morphological resemblance to *C. diphtheriae*. Its characteristics were studied only to an extent which enabled us to recognize it when recovered from the lesions of spontaneous and experimental infections. Morphology, growth characteristics, fermentation reactions and pathogenicity have remained constant through subcultures and animal passages for nearly 2 years. For all animal inoculations, 0.15 cc of a 24 hour plain broth culture was used. Experimental animals and controls invariably received organisms from the same individual culture. Tissues were fixed in Zenker's solution, and paraffin sections were stained with hematoxylin and eosin, Gram's stain, and Mallory's trichrome.

Results. Spontaneous infection. The first experiment was designed primarily to obtain virulent cultures of the corynebacterium in question. Nine rats were placed on the pantothenate-deficient diet at 3 weeks of age. Of these, 4 developed the desired infection, dying, on an average, 68 days later. From them the

organism was readily cultured, and appeared in enormous numbers in direct smears from characteristic lesions.

As part of the characterization of the organism, the effect of intraperitoneal injection was determined on several small groups of mice. Out of 23 animals, there were 14 fatal infections.

Lesions. The following description is derived chiefly from our observations of the spontaneous disease, but is strengthened by the study of many experimentally produced infections. These latter, in deficient animals, differ from the former chiefly in the location of lesions, a matter influenced by the point of introduction of the organisms. In the spontaneous disease the lungs and pleura are the tissues most conspicuously affected, often with the pericardium, and by extension, the myocardium. Spleen, kidney and liver are less frequently and noticeably involved. Ulceration and necrosis in the mouth is sometimes extensive, and there may be cervical lymph node involvement. Rounded clumps of bacteria, staining blue in the routine hematoxylin and eosin sections are the most fundamental feature found in all lesions so far observed. They vary in size and number with the severity of the infection, and are often coated with a light layer of fibrin, particularly when on a serous surface. Generally a few polymorphonuclear leucocytes are in close contact with them. Deeper in the tissues, the bacterial clumps are more often associated with necrotic debris and exudate of which polymorphonuclear leucocytes in various stages of disintegration form a recognizable component. The foregoing form gross masses which if sufficiently large, appear opaque, somewhat granular, and practically white. The term caseous is apt. In fulminating cases these masses in the lung may be associated with extreme congestion, oedema, and focal hemorrhage. Macrophages are scattered through such exudate. In other cases, the bacterial and necrotic masses are enclosed and limited by densely packed mononuclears. The thickness of this mononuclear layer varies, and the tissue beyond it is generally clear of exudate. Sometimes the bacterial and necrotic masses are encapsulated by fairly cellular fibrous tissue, seeming to denote a relatively greater resistance on the part of the animal in question.

Inoculation of young rats on natural food stock diet. Eleven animals 3 and 4 weeks old received 0.15 cc of broth culture intraperitoneally as described above. They continued to gain weight in a normal manner. They were killed 13-14 days later. Of the 11 animals, 5 showed either no lesion, or a completely healed minimal one. Three had small subcutaneous abscesses at the site of injection, without other sign of disease; and 3 others had in addition to this, healing or healed local peritoneal involvement. Simultaneously with the above experiment, auxiliary data from animals on the same diet were obtained from 4 which were injected in the foot with the same dose of organisms. Here the gross development of resultant lesions could be more easily followed. The animals were somewhat older: 40 and 68 days. Swelling and redness with abscess formation soon developed, but at the end of a week three had almost healed, and the fourth showed some subsidence of the swelling. No sign of active disease was present at the time of autopsy a month later. Those that had recovered most promptly bore no trace whatever of infection. The fourth had an externally visible but completely healed scar at the site of injection. Nineteen additional rats on a completely supplemented purified diet served as littermate and time controls for the various pantothenate-deficient rats as discussed below.

Experiments on pantothenate-deficient rats. (Table I.) The effect of the corynebacterium culture upon young pantothenate-deficient rats was next determined. The experiments first performed were necessarily of an exploratory nature; they consisted of small groups of rats with variations in starting age, age at inoculation, and mode of inoculation. This has complicated our task of clear presentation, but the underlying phenomena appear unequivocal.

Two series of rats were run, starting at different ages. In *series A,* thirteen young rats with eleven littermate controls were placed on their respective diets at 3 weeks of age. The control diet contained a supplement of calcium pantothenate. This series was divided into 3 groups: Group 1 consisted of 5 animals with

TABLE I. Experiments on Pantothenate-Deficient Rats.

Series	Group	Days deficient*	Experimental	Control
A	1	16	1 severe infection 4 moderate or localized infection	4 complete recovery
	2	23	1 severe infection	1 " "
	3	40	7 " "	4 " " 2 minor residua
B	1	33	5 " "	5 complete recovery
	2	58	3 " "	2 " " 1 non-specific lesion
	3†	30	2 " " 1 localized infection	See text

* At time of inoculation.
† This group was inj. in the foot. All others inj. intraperitoneally.

four controls injected after 16 days on diet. One severe fatal (46 days after injection) corynebacterium infection occurred, showing wide dissemination in and beyond the peritoneum. The other animals surviving up to 68 days after injection had only moderate or localized peritoneal lesions, but even these manifestations were lacking in all four controls. Group 2, comprising a single pair injected after 23 days on diet showed in the deficient animal a severe widespread infection, fatal in 16 days, while the control was free of lesions. Group 3 included 7 animals with 6 controls. They were injected after 40 days on diet. All seven died of widespread corynebacterium infection which had centered in the peritoneal cavity. Two of these, surviving only 3 days had peritoneal surfaces that resembled sandpaper, the granules representing clumps of bacteria as described above. The average survival time here was about 13 days. Four controls showed no lesions at autopsy; and two, respectively, had one and two small peritoneal abscesses. It is to be noted that the latter control animal was killed according to policy only three days after injection when its littermate was found dead, whereas other controls had a longer period in which to effect total recovery.

Series B (Table I) was put on experiment at 4 weeks of age, and injected after a generally longer period of deficiency. Except for these differences, treatment of the animals was identical to that of series A. There were 8 experimental rats and 8 littermate controls, divided in the following manner: *Group 1* had in it 5 experimental animals and 5 controls. They were injected after 33 days on diet. Three of the deficient animals developed either focal or generalized peritonitis with spread of infection to other organs, and 2 had peritonitis alone. The 5 controls had no lesions. *Group 2* contained 3 animals with 3 controls. They had been on diet 58 days before being injected. The deficient animals all had focal peritonitis of varying severity, with spread of infection to other organs. Two controls had no lesions. The third control, killed 17 days after injection, had a single atypical non-specific inflammatory lesion in the peritoneal cavity. In *group 3* the injection site was the foot; the 3 animals of this group were injected after 30 days on the deficient diet. There were no littermate controls, but the rats on stock diet which were injected in the foot and described above may serve for comparison. Infection reached the lungs in one case, and the kidney, pelvis and ureter in another. It remained localized though active in the third.

Discussion. The Coryneform organisms(2) of no direct importance in human disease lack definitive classification and nomenclature despite their considerable role in infections of domestic animals. (See Merchant(3) and Brooks and Hucker(4).) The resultant confusion is particularly apparent when the diseases of laboratory animals are considered. The ability of the present organism to produce progressive or fatal disease when injected intraperitoneally in mice, and its failure to do so in well-nourished rats place it in a category

with organisms studied by Kutscher(5) in rats and mice, by Bongert(6) in these and other animals, and Reed(7) in mice. A corynebacterium has been recently encountered by Le Maistre(8) in cortisone treated rats whose lesions bear a resemblance to those of our animals.

The situation described in this report offers an unusually favorable opportunity for the study of mechanisms of natural resistance. Through control of diet, resistance and susceptibility can be produced at will in the same species, even within the same litter. The organism is easily recognizable on smears and is readily cultured. Its virulence may be verified in the naturally susceptible mouse. The disease itself may be studied as it occurs spontaneously, or as an experimentally induced infection.

Summary. A corynebacterium has been isolated from spontaneous "pseudotuberculous" lesions of pantothenate-deficient rats. In inoculation experiments the organism is not pathogenic for rats on a fully supplemented diet in doses which produce progressive disease in deficient animals. This break in resistance can be demonstrated by inoculation of the organism sometimes as early as 16 days after the rats have been placed on the deficient diet, before overt deterioration in physical condition has developed.

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Intracellular Localization of I^{131}-Tagged Lactogenic Hormone in Mammary Gland of the Rabbit.* (20939)

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The lactogenic hormone is believed to function. primarily, in the epithelial cell of the mammary gland in initiating and maintaining the synthetic activity of these cells. Its activity in this connection would involve the activation and maintenance of the enzyme systems involved in the synthesis of the various constituents of milk. The question arises as to the role of the various cell components in milk secretion(1). If it could be shown that the lactogenic hormone becomes associated in greater concentration with certain cell particulates, it would be presumptive evidence of the synthetic activity of such particulates and of the site of hormone activity in the cell.

Cox(2) and Sonenberg *et al.*(3) demonstrated that the lactogenic hormone tagged with I^{131} lost little biological activity and following intravenous injection could be found localized in various tissues. Stadie, Haugaard, and Vaughan(4) demonstrated the binding of I^{131} tagged insulin in diaphragm muscle. Crampton and Haurowitz(5) used tagged antigens in their study of antibody formation and used the procedure of centrifugal fractionation of tissue homogenates to determine the point of intracellular localization of the antigens. These methods have been applied to a study of the intracellular localization of lactogenic hormone in the mammary gland.

Methods and materials. Essentially, the method of Stadie, Haugaard, and Vaughn(4) was followed for the preparation of lactogenic-

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