critical step in the process. The final yield of avidin from the original egg white is approximately the same by both methods (*i.e.*, 38% shown in Table I and 35% for E.S.W.).

Although distilled water was used in the dialysis, it would be more convenient to use *cold* running tap water if water of good quality and low in iron is available.

Summary. 1. Preparation of avidin concentrates by precipitation of the active fraction during a 2-day static dialysis (in distilled water) of a 1% saline extract of an acetone coagulation of dried egg white solution at pH 5.1 has been described. 2. The activity of these preparations (1.5 units/mg) averages 20% that of "pure" avidin‖ (8.0 units/mg) and the yield is about one g of such material from 800 g of dried egg white. 3. About 100 lb of dried egg white has been processed by the method described.

‖ "Pure" avidin—considered as 8 units/mg (Ref. 3) and having electrophoretic characteristics of a single species of molecule.

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Received March 2, 1954. P.S.E.B.M., 1954, v85.

Pantothenic Acid Deficiency and Loss of Natural Resistance to a Bacterial Infection in the Rat.* (20937)

THEODORE F. ZUCKER AND LOIS M. ZUCKER.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.

This report deals with a consequence of pantothenic acid deficiency which, while alluded to by others, has not been subjected to detailed study. Many sudden deaths were encountered due, apparently, to an acute respiratory infection of a type we had not previously encountered in any rats. Dr. Nelson† (personal communication) obtained from some typical lesions pure cultures of a corynebacterium which reproduced a similar disease picture in mice. He called attention to the fact that this type of disease ("pseudotuberculosis"—for description see Dingle(1)) is known to occur spontaneously in mice, and that the corynebacterium responsible for it is generally held to be non-pathogenic for rats. This indicated that pantothenate deficiency abolished the species-determined natural resistance of the rat to this organism. The data given below were obtained some time ago, but publication was delayed awaiting the findings of Seronde(2) which verify the observations on the spontaneous disease by means of inoculation studies.

Methods and materials. The basal diet had the following composition, in g/100 g of diet: vitamin "free" casein (Labco) 18.0; glucose (Cerelose) 71.85; salt mixture (modification of Wesson salt mixture with 1.0% Ca and 0.55% P in the resulting diet) 6.1; cottonseed oil 2.0; cellulose (Celluflour) 2.0; l-cysteine hydrochloride 0.05.‡ Incorporated

* This investigation was supported in part by a grant from the National Vitamin Foundation. Vitamins were generously supplied by Hoffmann-La Roche.

† We take this opportunity to express our thanks to Dr. John B. Nelson of the Rockefeller Institute for his kind cooperation. Out of his wide experience with respiratory infections in laboratory animals he has been of frequent assistance in our struggles with the knotty problem of lung infections in the rat.

‡ As an antioxidant. By this addition, and by the use of ferrous iron ($FeSO_4$) and cuprous copper (Cu_2O) in the salt mixture, keeping quality of the diets is much improved. An unpleasant odor fails to develop, and carotene color persists.

in the oil were 5 mg alpha tocopherol and 1500 international units of vit. A activity as carotene. The cellulose was the vehicle for the vit. B factors and also 0.5 mg 2-methyl-naphthoquinone. Two vitamin supplements were used. Supplement I contained, per 100 g diet, 1 mg thiamine and pyridoxine hydrochlorides, 2 mg riboflavin, 4 mg niacin. Supplement II contained, in addition to the above, 0.2 mg pteroylglutamic acid, 0.02 mg biotin, 0.0012 mg B_{12}, 20 mg inositol, 10 mg p-aminobenzoic acid, 100 mg choline chloride; also separately by mouth, 1 drop cod liver oil twice a week, 10 mg ascorbic acid 5 times a week. Further dietary modifications involved the amino acid supply. In some of the youngest age groups a comparison was made between 18% casein, 18% casein plus 0.2% l-cystine, and 30% casein. The rats were housed in screen bottomed cages with ½-inch mesh. Our rat stock was derived from the colony of Dr. Sherman, in the Chemistry Department of Columbia University. For a number of years (10-14 generations), they have been selectively bred in this laboratory for body size, resulting in a small, an intermediate, and a large rat. The largest averages consistently 50% heavier than the smallest from weaning time on. Throughout, selection by progeny testing was also made for lungs showing minimal incidence of grossly visible lesions at approximately one year of age, with the result that the common enzootic lung disease as described by Nelson(3) has been diminished to 1/4 to 1/5 of the original incidence(4). The rats on which the present report is based were of the intermediate and large sized strains (13C and 14C).

Results. Our attention was first arrested by unexplained sudden deaths in rats put on the deficient diet at 3 weeks of age. Such deaths occurred as early as 21 days after the regime was started. Further observations are recorded below under various pertinent headings.

TABLE I. Incidence of Spontaneous Corynebacterium Infection in Pantothenate Deficient Rats.

Initial age (wk)	Vit. suppl.*	N†	% affected Mean	% affected Range‡
3– 4	I	67	61	38–62 (6)
	II	20	55	
5– 6	I	17	59	
15–90	I	158	1	
	II	28	0	

TABLE II.
Mortality in Pantothenate Deficient Rats.

Initial age (wk)	N†	Days on exp. for 50% mortality Mean	Days on exp. for 50% mortality Range‡
3	48	49	42– 54 (3)
4	39	67	60– 75 (3)
5	9	67	
6	16	93	90– 96 (2)
8	8	126	
10	12	143	
15–17	10	125	
35–50 ♂	12	104	
35–90 ♀	147	102	99–110 (7)

* Suppl. I is the simplified supplement of 4 B vitamins. II provides all known factors except pantothenate.

† Somewhat different experimental groups are involved in the two tables, so the N values are not the same. Table I excludes rats on which there were not satisfactory gross autopsies. Table II excludes experimental groups in which many rats were killed before spontaneous death.

‡ This is the range of mean values for groups of eight or more rats. The figure in parentheses is the number of such groups.

Description of the disease. There was little warning of the presence of the infection except occasional respiratory noise or embarrassment. Characteristic lesions at autopsy were serofibrinous pleural and pericardial exudate associated with small white abscess-like nodules. On section of the lungs these were found scattered through the parenchyma, and intervening tissue showed congestion and edema. Similar nodules not infrequently involved the liver, kidney and spleen.

Incidence in various age groups. Table I records the incidence of the disease as judged by gross autopsy findings in groups of rats started on the deficient diet at ages as early as 3 weeks and as late as 90 weeks. Apparently rats put on the deficient diet later in life do not develop the disease. Whether this is due to the smaller pantothenate requirement as the rat matures or to the development of a defense mechanism independent of pantothenic acid remains to be seen. The deaths in young rats which were not accompanied by gross signs of infection may be referable to some as yet unknown early results of the de-

ficiency, or perhaps to an exotoxin produced by the corynebacterium (see Topley and Wilson(5), pp 461 and 471). The shortest experimental period leading to death with signs of corynebacterium infection was 21 days.

Mortality. The ability to survive on a pantothenate deficient diet first increases with increasing starting age, and then decreases (Table II). The life of the adult rat is probably shortened by the severe perforating duodenal ulcers which are commonly found (Berg *et al.*(6)). This lesion, which is particularly prominent in adults, offers an argument against the idea that adults do not develop an extreme deficiency state.

General condition of the susceptible rats. It has frequently been pointed out (*e.g.* Schneider(7)) that increased susceptibility to infection is of doubtful significance in nutrition studies if it appears only when the animal has become greatly debilitated, with much loss of weight, severe inanition, or with falling body temperature. In the present work the animals on the whole remained active and in good condition until shortly before death. During the development of the deficiency state no differences were observed in daily measurements of rectal temperature between experimental and control animals. How fulminating the progress of the infection may be is illustrated by a rat which was active and had a normal temperature the day before death, and was active on the morning of its death. In the afternoon it became prostrate and its temperature fell to 97° at 2 P.M. and 96° at 4 P.M. It was dead at 5 P.M. A blood culture was positive for corynebacteruim.

Growth. Our growth data on young rats are similar to those in the graph recorded by Krehl *et al.*(8), concerning which they say: "Inanition, so often a factor in dietary deficiency states, was not a serious problem in these studies." The initial moderate growth is followed by weeks of essentially constant body weight. We found no precipitate weight losses up to 24 hours before death. Like the drop in rectal temperature, such weight loss is attributable to the infection.

Dietary modifications. The various modifications described above produced no significant differences in the results. We have illustrated this point in Table I by separately listing the results with the 2 vitamin supplements. The simplified supplement is like that of Daft *et al.*(9) used in the study of blood dyscrasias before pure folic acid was available. When granulocytopenia, a condition known to favor certain types of infection, had developed, many animals died suddenly even though the granulocytopenia was cured by pantothenate administration. Their later results indicate that the granulocytopenia was due to lack of intestinal folic acid synthesis. Our observations as well as those of others(10,11) confirm the absence of granulocytopenia in pantothenate deficiency if folic acid is given. The intervention of infection such as here described would explain their experience.

Discussion. If the question is raised as to why an apparently obvious cause of death such as described here has not received more attention, several considerations are pertinent. In the first place, respiratory disease is so universally enzootic in rat colonies that many investigators may consider lung infections troublesome but not relevant to experimental procedures. Furthermore there are rat strains much less susceptible, when made deficient, than those on which the present observations are based. Our small-sized strain (9B) which was not employed in these experiments has been found definitely less susceptible. While Nelson(12) noted the occurrence of corynebacterium saprophytic in the upper respiratory passages of normal rats, it is not known how universally such organisms are distributed in laboratory environments. On the other hand, Supplee *et al.*(13) and Daft *et al.* (9) have discussed sudden unexplained deaths, and Salmon and Engle(14), Jürgens and Pfaltz(15), and Krehl *et al.*(8) noted greater than usual incidence of respiratory infections in pantothenate deficient rats. Krehl *et al.*(8) said of their deficient animals used for further experiments: "All animals were carefully chosen so as to be free of respiratory infection to which the pantothenic acid deficient rats seem to be quite susceptible." These remarks, as well as the rather extended discussion of Daft *et al.*(9) show that the kind of phenomenon here described

is not restricted to one laboratory.

While the rat is held to be not susceptible to corynebacterium (notably including *C. diphtheriae*), 2 exceptions to this general statement have been reported. In one of György's earliest papers on vitamin H deficiency (anti-egg white factor, biotin), Gundel, György, and Pagel(16) described in rats a corynebacterium infection similar to ours. The diet composition was not given. LeMaistre and Thompsett(17) found that in rats with very large daily injections of cortisone a spontaneous disease emerged closely resembling that here described, and attributable to a corynebacterium similar to ours. The possible relation between these 3 unusual phenomena remains to be investigated. It is however unlikely that any simple relation such as high cortisone activity in the deficient animals is responsible. LeMaistre and Thompsett had to use cortisone doses large enough to produce weight losses of 100 g or more. Winters *et al.*(18) have produced good evidence for *lowered* adrenal activity in pantothenate deficiency.

Striking as the acute infectious death due to a nutritional deficiency is, there is so far no evidence that these findings have any bearing on practical nutrition problems. In this connection, however, the observations of Luecke (R. W. Luecke, personal communication) on the appearance of corynebacterium infection in pantothenate deficient suckling pigs are of interest. The dams were on purified pantothenate deficient diets. Our isolated observations (unpublished) on the appearance of the infection in rats kept for several months on a diet containing a small, but insufficient, amount of added pantothenate, as well as the very early susceptibility after only 16 days on a completely deficient diet reported by Seronde(2), justify at least an awareness of further possibilities. Luecke(19) has also shown that practical pig rations (*i.e.*, composed of natural feeds) may give rise to deficiency signs which are abolished by pantothenate.

An easy explanation of the loss of resistance would be available, in agreement with Culbertson's(20) scheme for the rat's defense against *Trypanosoma cruzi*, if it were shown that the defense in the case of the normal rat is through the very rapid formation of antibodies, and if the effect of pantothenate deficiency falls in line with Axelrod's(21) observations on low antibody (hemagglutinin) formation. It should be noted that Axelrod's observations on pantothenate deficiency do not agree with those of Stoerk(22), who found no lack of antibody in this deficiency. Axelrod offers as explanation the weaker antigen stimulus of the sheep cells Stoerk used as against the human cells which were the basis of his observations. If this difference in antigen can affect the result to such an extent, then certainly antibody response to invading organisms is not immediately deducible from hemagglutination studies.

Furthermore, when one surveys the data which have led to suggestions as to the mechanisms of natural resistance, it becomes apparent that there is no one universal mechanism of defense (see Raffel(23), pp 3-23). For the present, at least, it will be necessary to consider each case separately with regard to host species or even strain, the nature of the invading organism and other pertinent conditions, such as the age and the particular nutritional condition under study.

Summary. Pantothenate deficient rats may lose the innate species determined resistance to infection with a type of corynebacterium so far known to be pathogenic only for mice.

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Received March 10, 1954. P.S.E.B.M., 1954, v85.

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

JOSEPH SERONDE, JR. (Introduced by T. F. Zucker.)

From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York City.

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