does not need insulin to maintain its carbohydrate metabolism, implying thereby the existence of regulatory mechanisms which differ markedly from those of other species. That a similar situation may exist in the chicken is indicated by our experience with two chickens which, after alloxan administration, showed no abnormality of the blood sugar level.

Summary. Alloxan does not produce diabetes mellitus in the duck in spite of the fact that the drug does produce necrosis of the Islets of Langerhans.

We wish to acknowledge our appreciation of the cooperation and facilities extended by Dr. Kenneth G. Kohlstaedt, Director, and the staff of the Lilly Laboratory for Clinical Research, and our indebtedness to Dr. A. Nettleship for the histologic examination of the pancreases.

14971

Influence of Pantothenic Acid Deficiency on Resistance of Mice and Rats to Experimental Pneumococcal Infection.*

HARRY G. DAY AND L. S. MCCLUNG.

From the Chemical and Bacteriological Laboratories, Indiana University, Bloomington.

Several recent reports are concerned with the effects of pantothenic acid deficiency in animals on their resistance to various infectious agents. Becker, Manresa, and Johnson found that pantothenic acid increases the production of oöcysts by rats infected by *Eimeria nieschulzi*[1] and reduces the efficiency of ablastic action of pantothenic acid-deficient rats infected with *Trypanosoma lewisii*.[2] According to Trager[3] the survival of *Plasmodium lophuræ in vitro* is favored by the presence of pantothenic acid. Lichstein, Waisman, Elvehjem, and Clark[4] reported that young, pantothenic acid-deficient, Swiss mice show increased resistance to Theiler's encephalomyelitis virus, but little or no change in resistance to the Lansing strain of poliomyelitis. West, Bent, Rivera, and Tisdale,[5] using 15 animals, claimed that pantothenic acid deficiency greatly increased the resistance of the rat, infected by nasal insufflation, to a strain of Type I pneumococcus. Since these organisms require pantothenic acid for growth, West *et al.* postulated that the apparent increase in resistance was due to inability of the pathogenic organisms to secure sufficient pantothenic acid from the host rats to permit maximal growth. Robinson and Siegel[6] did not find a change in resistance to experimental pneumococcal pneumonia in 20 young adult rats restricted to a pantothenic acid-deficient diet as compared to the resistance of control animals which were fed the vitamin.

Wooley and Sebrell[7] reported that young mice deficient in riboflavin or thiamine are more susceptible to Type I pneumococcus by nasal insufflation than controls which received these vitamins. Robinson and Siegel,[6] using 20 young adult rats, appeared to confirm these findings for thiamine.

An important problem in investigations of

* Supported by a grant from the Graduate Research Fund of Indiana University. The authors are indebted to Miss Ruth Toabe and Mr. David K. Barnes for technical assistance.

1 Becker, E. R., Manresa, M., Jr., and Smith, L., *Iowa State Coll. J. Sci.*, 1943, **17**, 257; *Chem. Abstr.*, 1943, **37**, 1478.

2 Becker, E. R., Manresa, M., Jr., and Johnson, E., *Iowa State Coll. J. Sci.*, 1943, **17**, 431; *Chem. Abstr.*, 1943, **37**, 3801.

3 Trager, Wm., *J. Exp. Med.*, 1943, **77**, 411.

4 Lichstein, H. C., Waisman, H. A., Elvehjem, C. A., and Clark, P. F., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 3.

5 West, H. D., Bent, M. J., Rivera, R. E., and Tisdale, R. E., *Arch. Biochem.*, 1944, **3**, 321.

6 Robinson, H. J., and Siegel, H., *J. Infect. Dis.*, 1944, **75**, 127.

7 Wooley, J. G., and Sebrell, W. H., *U. S. Pub. Health Rep.*, 1942, **57**, 149.

TABLE I.
Effect of Varying Degrees of Pantothenic Acid Deficiency on the Resistance of Young Mice to Type I Pneumococcus.

Diet No.	No. of mice	Days on diet before inoculation	Wt increase per mouse, g	% dying during first 96 hr after inoculation	Avg survival time, hr
1*	10	7	2	70	38
2†	10	7	2	100	36
1	25	14	2	88	39
2	26	14	3	73	45
1	32	19	2	60	39
2	19	19	4	53	38
1	16	21	1	100	37
2	18	21	5	100	35
1	31	26	3	52	37
2	40	26	9	60	40
1	26	27	2	54	52
2	30	27	7	80	51
1	35	28	2	88	40
2	39	28	7	93	38
		Summary.			
1	175	22	2	71	41
2	182	22	6	77	41

* Diet 1: Pantothenic acid deficient.
* Diet 2: Control diet (calcium pantothenate added).

the effect of diet on resistance to pneumococcal infection is the mode of infecting the animals. Nasal insufflation is extremely tedious and secondary injuries to the animals may result. An additional disadvantage is the fact that all animals may not take into the lungs comparable numbers of organisms even though caution is observed in the administration of the infecting dose. Direct introduction of the organisms into the bronchial tree by injection into the exposed tracheæ, as practiced by Robinson and Siegel, is less likely to produce secondary injuries but it is obviously troublesome. In the investigation to be reported, all animals were inoculated by intraperitoneal injections. Although this may not be the ideal route for production of experimental pneumonia it can give rise to a lethal infection and has been used extensively with mice. For example, Buck and Schnitzer[8] inoculated their animals by intraperitoneal injection to study the synergistic effect of penicillin and anti-pneumococcus serum.

Experimental. Weanling piebald rats from our own colony and young white mice purchased from the Harlan Small Animals Industry, Cumberland, Indiana, were used.

The pantothenic acid-deficient diet (Diet 1) contained: casein (vitamin-free), 18; glucose (Cerelose), 68; hydrogenated vegetable oil (Crisco), 7; solvent-extracted wheat germ oil,† 2; percomorph liver oil, 2 drops; salts No. 12,[9] 4; and vitamin mixture, 1. The vitamin mixture‡ was composed of thiamine hydrochloride, 1 mg; pyridoxine hydrochloride, 1 mg; riboflavin, 2 mg; nicotinic acid, 10 mg; *p*-aminobenzoic acid, 20 mg; inositol, 20 mg; choline chloride, 120 mg; and glucose, 826 mg. The pantothenic acid-added diet (Diet 2) was identical to Diet 1 except that the vitamin mixture contained, in addition to the vitamins listed, 50 mg of calcium pantothenate, and a corresponding reduction in the content of glucose.

Sex and initial weight were considered in distributing the animals between Diets 1 and 2. Litter membership was also considered in the experiments with rats. This factor was not

[8] Buck, M., and Schnitzer, R. J., *Arch. Biochem.*, 1944, **5**, 153.

† The wheat germ oil was kindly furnished by Mr. Ezra Levin, VioBin Corporation, Monticello, Illinois.

[9] Jones, J. H., and Foster, C., *J. Nutrition*, 1942, **24**, 245.

‡ Most of the synthetic vitamins were furnished through the courtesy of Dr. D. F. Robertson, Merck and Co., Rahway, New Jersey.

controlled with the mice since the various litters could not be distinguished in the shipments received from the breeder. The mice were kept in groups of 5 in roomy screen-bottomed cages; the majority of the rats were kept in individual cages. Tap water and food were permitted *ad libitum*. By means of air conditioning equipment, the temperature of the animal room was not allowed to exceed 82°F.

Both rats and mice restricted to Diet 1 showed the typical effects of pantothenic acid deficiency. Animals fed Diet 2 grew well and appeared normal. The effects on growth are indicated in Table I.

At intervals ranging from 19 to 38 days after restriction to the experimental diets, the rats were injected intraperitoneally with 0.2 ml of a 10^{-2} dilution of Type I pneumococcus.§ The dilutions, prepared in physiological saline solution, were made from a 6-8 hour culture grown in heart infusion broth enriched with tryptone (1%), glucose (0.2%), and fresh rabbit blood (0.2 ml per 8 ml of medium). At intervals the culture was passed through mice to maintain the virulence. The mice were injected intraperitoneally at intervals of 7 to 28 days after restriction to the experimental diets. The inoculum was prepared as described above and the dosage was 0.5 ml of a 10^{-6} or 10^{-7} dilution. All animals were observed frequently during a 4-day period following inoculation. Heart blood cultures on blood agar were made from a large number of the animals which died within this period to confirm the fact that death was due to the inoculated organism. Pneumococci were present in all samples which were plated.

The results of the experiments on rats may be summarized as follows: A total of 29 (74.3%) of the 39 rats on Diet 1 (pantothenic acid-free) died whereas 25 (69.3%) of the 36 animals on Diet 2 (control) succumbed. Although the percentage figure of the animals dying which were on the experimental diet is slightly higher than the value for those on the control diet, it is doubtful that the difference is statistically significant. Certainly, in so far as these data may be interpreted, there is no marked change in the susceptibility to the pneumococcus in rats showing pantothenic acid deficiency.

With respect to the mice, 125 (71%) of 175 on the pantothenic acid-deficient diet succumbed in contrast to 141 (77%) of the 182 on the control diet (Table I). This does not appear to be a significant difference. However, as shown in Table I, the animals were inoculated at intervals ranging from 7 to 28 days after being started on the test diets. Although there is no survival difference between the experimental and control mice inoculated between the 7th to 21st days (77% fatalities in the pantothenic acid-deficient group as compared with 78% in the controls), it may be significant that in the groups of mice restricted to the diets 26 to 28 days before inoculation 66% of the pantothenic acid-deficient animals died whereas 77% of the controls failed to survive.

Summary. A study of the resistance to the intraperitoneal injection of Type I pneumococci of 39 rats showing pantothenic acid deficiency in contrast to 36 controls fed pantothenic acid failed to reveal a significant change in the susceptibility of the experimental group. Similarly, no marked change in susceptibility was observed in 175 mice showing pantothenic acid deficiency in contrast to the survival percentage of 141 controls receiving the vitamin (normal animals). However, the difference is great enough to warrant the conclusion that acute pantothenic acid deficiency in mice may slightly increase the resistance to Type I pneumococcus. Certainly the results give no indication of decreased resistance to this organism in pantothenic acid deficiency.

§ This culture was received through the courtesy of Dr. H. M. Powell of the Eli Lilly Research Laboratories, Indianapolis, Indiana.