

acid-soluble phosphate increases up to maturity and then drops off again at an advanced age. The inorganic phosphate decreases progressively with advancing age. The change in the ATP of the liver parallels that of the total phosphate. The decrease in the total acid-soluble and ATP phosphate in the 400 to 500 g rats may be due to an increase in the fat content of the liver in these animals.

It is of interest that the livers of the younger animals contain a higher content of phosphorus in the barium residual fraction and a lower content in the alcohol precipitate than do the old ones. With increasing age, there is a shift

of phosphorus from the barium residual fraction to the alcohol precipitate.

No definite conclusions can be drawn regarding the significance of the phosphate changes which occur with age, until more information is available on the relationship of age to the metabolic activities of the liver.

Summary. Fasting causes a decrease in the total acid-soluble phosphate, adenosine triphosphate, and the glycerol phosphate fractions of the liver. With advancing age there is a shift of phosphate from the fraction containing phosphoglyceric acid to the fraction containing glycerol phosphate.

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Relation of Riboflavin Deficiency to Spontaneous Epidemics of *Salmonella* in Mice.

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In a previous publication¹ it was reported that vitamin A-deficient mice and rats are highly susceptible to a *Salmonella* infection. However, it was found that this increased susceptibility appeared only in the late stages of avitaminosis and that animals given adequate amounts of the vitamin, but the same caloric intake as those on the vitamin-free diet were equally susceptible. In this type of infection vitamin A was not the "anti-infection vitamin;" the same decreased resistance resulted from a reduced food intake.

Below we present results on the effect of riboflavin-deficient diets on spontaneous *Salmonella* epidemics in mice. Our stock mice became infected with *Salmonella* and occasionally single mice died from the infection without any serious epidemic outbreaks. Young mice, four weeks old, were divided into 3 groups. (1) Control group, received the following diet *ad libitum*: Casein (alcohol extracted), 15%; rice flour, 71%; olive oil, 10%; salt mixture, 4%. Each mouse received in addition 50 I.U.

vitamin A, 2 I.U. vitamin D twice a week, and 0.1 cc of a yeast extract daily. The yeast extract was irradiated in alkaline solution with a 200-watt lamp at a distance of 25 cm for 8 hours, then neutralized and 25 γ thiamin, 25 γ calcium pantothenate, and 25 γ riboflavin per ml added. (2) Riboflavin-deficient group, received the same diet as the controls, without riboflavin. (3) Paired-fed group, received the same diet as the control except that the daily amount of the basic ration was weighed out and equalled that eaten the previous day by the animals on the riboflavin-deficient diet.

Eight series of experiments have been carried out with consistent results. In general they fall into 3 main types.

In the first series mice from the mixed stock were divided into groups of 15, of approximately the same average weight. The animals were kept under observation 21-28 days during which mice which died were cultured. At the end of that period the remaining mice were killed and the liver, spleen, and mesentery gland cultured for *Salmonella*.

In the second series the mice were divided into groups of litter-mates. Each litter was

* Dr. I. J. Kligler died September 24, 1944.

¹ Kligler, I. J., Guggenheim, K., and Henig, E., *J. Hyg.*, in press.

TABLE I.
Relative Susceptibility of Mice Kept on Full, Riboflavin Deficient, and Isocaloric Diets to a Spontaneous Infection with *Salmonella*.

Exp.	Source of mice	Observation time before culturing	No. of mice	No.* died	No. alive and cultured	Total infected	Liver pos.	Spleen pos.	Spleen and liver pos.	Mesentery gland positive
I.										
Rib. Def.	Mixed stock	3-4 weeks	78	27	51	42	13	11	8	40
P.F.			69	17	52	26	4	5	4	26
Control			30	4	26	5	2	2	0	5
II.										
Rib. Def.	Litter mates	3-4 weeks	30	3	27	23	14	16	13	22
P.F.			30	1	29	20	11	13	11	20
Control			30	0	30	13	3	3	1	13
III.										
Rib. Def.	Litter mates	2 weeks	42	0	42	27	10	10	9	27
P.F.			42	0	42	20	8	7	7	20
Control			42	0	42	12	1	2	1	12

* Of the 52 mice which died it was possible to culture 39; all of them showed a general infection of *Salmonella*.

taken directly from the mothers and divided into 3 groups. The object was to obtain as nearly as possible a uniform distribution of initially-infected mice in each group. In all other respects the procedure was the same as in the first series.

The third series also consisted of litter-mates, but the mice were killed after 14-15 days instead of 3-4 weeks. In order to assure infection one mouse infected *per os* with 0.05 cc culture of *Sal. typhimurium* was introduced into each cage of 15, three days after the infection. The purpose of this series was to see whether different degrees of susceptibility existed in the early stages of avitaminosis, *i.e.*, after 2 weeks on the deficient diet.

The results are summarized in Table I. It is evident that the riboflavin-deficient mice had a markedly higher rate of infection than the controls; the fatality in the experiments of long duration was nearly 3 times as great and the liver and spleen infections in all survivors about 3-4 times as high. The paired-fed animals fared better consistently than the deficient ones, but came a close second to them. The riboflavin-deficient animals differ from those receiving a vitamin A-deficient diet in

that in the former loss of appetite sets in promptly after the first week and the low level of food intake remains constant throughout the period of observation, while in the latter the food intake remains almost normal for 2 or 3 weeks and only then drops sharply. It would seem that riboflavin deficiency acts more critically to the organism than vitamin A deficiency and causes a sharp loss of resistance within two weeks; in this case too, however, starvation as distinct from avitaminosis is also a determining factor in enhanced susceptibility. These findings are consistent with those of Wooley and Sebrell² with experimental nasal infections with pneumococci in riboflavin-deficient mice.

Summary. Controlled nutrition experiments with mice kept on a riboflavin-deficient diet show that these animals are much more highly susceptible to a spontaneous *Salmonella* infection than are control mice from the same stock and mothers. Isocaloric paired-fed controls given a full vitamin ration show an intermediate degree of susceptibility between the riboflavin-deficient and the controls.

² Wooley, J. G., and Sebrell, W. H., *Pub. Health Rep.*, 1942, **57**, 149.

¹ Reed, Kacene, R., Sapirstein, Leo A., Southard, F. D., Jr., and Ogden, Eric, *Am. J. Physiol.*, 1944, **141**, 707.

sed by neurovascular or other alterations which maintain blood pressure after the dis-