(stages 19, 21, 23, and 25) following various exposure periods from 5 minutes to 1 hour. The time lapse for complete muscular immobilization varied from 30 to 80 seconds, and the recovery time ranged from 4 to 14 minutes, depending upon the duration of anesthetization and the stage of development considered. Here too it will be noted that recovery time consistently was increased only in the case of stage 25.

Summary and Conclusions. (1) The anesthetic M.S.222 in 1/3,000 concentration in spring water has no effect on the motility or the fertilizing power of frog spermatozoa. (2) Frog's eggs fertilized in this anesthetic and allowed to remain for 1 hour will develop as well as do the controls. Exposure for longer periods will show an increasing percentage of abnormal embryos. (3) Embryos that show muscular activity (stage 19 and older) are completely immobilized (except for ciliary activity) within 80 seconds at ordinary laboratory temperatures, some embryos becoming quiet within 30 seconds. Recovery rate (*i.e.*, the return to muscular activity) ranges from 4 to 14 minutes, depending more on the stage of development than on the duration of anesthetization. (4) Prolonged exposure to the anesthetic produces abnormal embryos if the exposure is made prior to neurulation and kills the embryos at stage 25. This latter instance may be due to the change in the respiratory mechanism at that time. (Urodele larvæ do not show this reaction, and may be anesthetized at least up until metamorphosis.) (5) The anesthetic has no effect on ciliary action but is quick in its effect on muscular activity; it is non-toxic in the dilution of 1/3,000 in spring water for periods adequate for surgical operations; and recovery time is uniformly brief. M.S.222 has proven to be a very satisfactory anesthetic for amphibian embryos.

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Effect of Starvation and Ageing on the Acid-Soluble Phosphate Components of the Liver.

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In contrast to the abundance of information on muscle phosphates, comparatively little is known regarding the acid-soluble phosphates of the liver. In connection with a tracer study of the phosphate metabolism of the liver with P^{32}, a comparison was carried out of the distribution of the acid-soluble phosphate fractions of the liver of the rat under certain extremely varied conditions of life. The analytical procedures are described elsewhere.[1]

Effect of Starvation. The distribution of the different phosphorus fractions in the livers of rats fasted for 12, 24, 48, 72, and 144 hours are recorded in Table I. For purposes of comparison, the table contains the phosphorus partition in the liver of rats who had access to food until they were sacrificed. The results in Table I, in general, are in agreement with the findings of Rapoport, Leva, and Guest.[2]

Effect of Age. That the acid-soluble phosphates of the liver changed with age was observed by Kay.[3] We considered it of value to investigate the nature of these changes more thoroughly.

For the purposes of this study, several litters of stock rats were sacrificed at different ages, beginning with 21-day-old (several days prior to weaning) and the phosphate content of the various acid-soluble fractions determined The results are given in Table II.

From Table II it will be noted that the total

[1] Kaplan, N. O., and Greenberg, D. M., *J. Biol. Chem.*, in press.

[2] Rapoport, S., Leva, E., and Guest, G. M., *J. Biol. Chem.*, 1943, **149**, 57, 65.

[3] Kay, H. D., *J. Biol. Chem.*, 1932, **99**, 85.

TABLE I.
Effect of Time of Fasting on Distribution of Acid-Soluble Phosphate Fractions of Rat Liver.*

		Barium insoluble					Barium soluble		
Hrs of fast	Animals No.	Total acid soluble P	Inorganic P	Labile ATP P	Non-labile ATP P	Residual† P	Hg‡ ppt P	Pb§ ppt P	Alcohol‖ ppt P
0	10	100.8 ±1.6	21.2 ±0.37	15.6 ±0.45	7.8	9.6 ±0.29	11.6 ±0.32	7.9 ±0.26	30.2 ±0.88
12	12	95.1 ±1.3	24.0 ±0.45	12.0 ±0.32	6.0	9.0 ±0.37	10.6 ±0.70	7.1 ±0.55	27.7 ±0.80
24	5	91.1 ±1.7	26.3 ±0.60	10.2 ±0.47	5.1	8.5 ±0.40	10.8 ±0.60	7.2 ±0.48	25.0 ±0.75
48	4	89.0 ±1.6	26.2 ±0.72	8.4 ±0.44	4.2	9.0 ±0.73	10.5 ±0.56	6.8 ±0.39	24.0 ±0.97
72	6	83.9 ±2.6	27.7 ±0.94	7.4 ±0.27	3.7	8.0 ±0.33	9.9 ±0.33	7.2 ±0.42	21.3 ±0.88
144	4	81.5 ±2.1	29.0 ±0.75	7.2 ±0.61	3.6	8.4 ±0.58	11.4 ±0.62	6.0 ±0.50	20.7 ±0.86

* All values are in mg P per 100 g fresh liver. Measure of variability is standard error of the mean.
† This fraction consists largely of phosphoglycemic acid.
‡ This fraction contains nucleotides other than adenosine triphosphate.
§ This fraction mainly contains inorganic phosphate that has escaped previous precipitation.
‖ This fraction consists largely of glycerol phosphate but it also contains some hexose monophosphates.

TABLE II.
Influence of Age on Liver Acid Soluble Phosphates.*

				Barium insoluble				Barium soluble		
Age of rat in days	Wt of rat in g		Total acid soluble P	Inorganic P	Labile P in ATP	Non-labile P in ATP	Residual P	Hg Ppt P	Pb Ppt P	Alcohol Ppt P
18	35†	a	85.5	31.8	7.2	3.6	17.2	11.3	4.7	18.9
19	42†	b	82.0	28.2	7.5	3.8	13.3	11.8	5.5	17.9
23	55	c	88.4	27.0	9.1	4.6	14.2	11.7	5.8	20.8
23	68	b	92.2	27.6	7.2	3.6	12.4	10.2	6.0	22.8
30	79	c	87.0	26.2	10.2	5.1	13.2	10.4		17.5
32	82	a	91.1	27.7	9.8	4.9	11.7	10.0	7.0	21.2
40	112	c	92.4	26.1	9.2	4.6	10.8	9.9	8.3	22.8
44	130	b	94.1	28.7	9.4	4.7	9.8	9.5	5.5	24.4
45	96	a	95.5	26.4	11.3	5.7	9.8	8.5	6.7	23.3
50	138	c	97.5	23.8	11.8	5.9	7.8	11.7	6.7	25.9
60	152	a	91.2	24.8	11.4	5.7	8.7	10.5	5.1	27.8
60	174	b	100.0	26.3	11.9	6.0	9.1	10.5	9.1	26.8
65	204	c	95.9	22.8	13.6	6.8	8.1	11.3	5.9	28.5
75	225	a	93.0	24.4	13.0	6.5	7.4	9.2	6.8	26.0
75	275	b	98.1	25.2	13.9	7.0	9.3	9.7	6.0	27.8
100	319	b	90.9	23.3	11.4	5.7	8.5	9.0	7.4	26.7
180+	402		82.6	22.4	10.5	5.3	7.9	8.5	5.5	24.4
180+	510		78.5	22.5	9.1	4.6	7.3	8.9	6.1	22.0

* All values are in milligrams per 100 g fresh liver. For significance of the different fractions see 1 and Table I.
† Unweaned rats.
All animals were fasted for a period of 12 hours before sacrificing. Rats listed with same letter are litter mates.

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 publication[1] 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.