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Inorganic Blood Phosphate of Rats on Rachitic and Non-rachitic Diets.

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This investigation of the inorganic blood phosphate was undertaken in order to determine its value as an early diagnostic sign of rickets. The blood phosphate was determined in rats receiving a control diet, the McCollum rachitic diet No. 3143, and the rachitic diet plus various substances which were being tested for anti-rachitic properties. The fluctuations in the blood phosphate of rachitic and non-rachitic rats due to age, anesthesia and injury were also investigated.

The blood was drawn from anesthetized rats by cardiac puncture, and except in case of the very young rats determinations were made on individual samples. The latest Briggs' modification¹ of the Bell and Doisy phosphate method was employed.

Deep anesthesia increased the inorganic blood phosphate rapidly. Light anesthesia for 7 to 15 minutes increased it only 2 to 6 per cent. The struggling and injury involved in stunning and bleeding the rats, which are methods used by other investigators, caused much greater increases in blood phosphate than any degree of anesthesia necessary to draw blood from the heart.

Values on control diet: The youngest rat upon which phosphate was determined was 6 weeks old. This gave a value of 8.6 mg. per 100 cc. of blood. During the latter part of the growth period the blood phosphate remained above 6 mg. After 3½ months it gradually declined to 4 to 5 mg., showing a reduction of phosphate with age.

Values on rachitic diet: Diet 3143 lowered the blood phosphate of mature rats from 4.2 to 1.8 mg. in 15 to 20 days, and of young rats from 6.25 mg. to 2.3 mg. in a similar period.

Effect on blood phosphate of curative measures:

Irradiation: Irradiation of mature rats on diet 3143 maintained the blood phosphate at a higher level than was the case in the non-irradiated animals, although there was a distinct tendency for this value to decrease. Irradiation of young, rapidly growing rats had no appreciable effect on blood phosphate.

Cod-liver oil: Two per cent cod-liver oil incorporated with the

rachitic diet raised the blood phosphate of a mature rat from 1.8 to 3.3 mg. in 4 days, and, 5.2 mg. in 15 days after the addition was made. The blood phosphate of young, growing rats was raised from 2.3 mg. to 4.3 mg. in two weeks and to 5.4 mg. in five weeks. The value for control rats the same age was 6.25 mg. These results agree with those reported by Robison and Soames.²

Irradiated cholesterol: The daily administration of 2.5 to 5 mg. of irradiated cholesterol entirely prevented rickets, but had only a slight influence on blood phosphate. Young rats receiving non-irradiated cholesterol showed a similar slight increase in phosphate.

Both irradiated and non-irradiated cholesterol maintained the blood phosphate of mature rats at the normal level, 4.4 to 5.4 mg.

The liquid NH_3 soluble fraction of irradiated cholesterol and of the non-saponifiable portion of cod-liver oil, when fed in amounts of 0.2 and 0.3 mg. daily, entirely prevented rickets, but had no influence on blood phosphate. The Ca values for these same rats were 10.77 and 11.9, and, the products of $(\text{Ca}) \times (\text{P})$ were 24.7 and 27.37 respectively, both within the rachitic range given by Howland and Kramer.³

Control diet after rachitic diet: When animals, which had been on a rachitic diet 2 months, were returned to a control diet, the blood phosphate was doubled in twenty-four hours and reached the high values of 8 to 10 mg. in one week. The increase in blood phosphate after the change from rachitic to control diet seems to be roughly proportional to the severity of the rachitic condition. After a period of 2 to 3 weeks the inorganic blood phosphate of these rats began to decrease, until, at the end of 6 weeks it had reached a normal value of 4 to 5 mg.

The results of this study indicate that while cod-liver oil raises the inorganic blood phosphate almost to normal, direct irradiation of, and feeding irradiated cholesterol to young rats, had very little effect on blood phosphate. It is possible to obtain low phosphate values, and a low product of $(\text{P}) \times (\text{Ca})$ in animals, which so far as roentgenograms can demonstrate, show a perfect calcification of the bones. The inorganic blood phosphate is influenced by many factors aside from the rachitic condition, many of which we cannot explain at present. We have in mind an investigation of the different forms of blood phosphate and other factors, which may help explain some of these phenomena.

¹ Briggs, A. P., *J. Biol. Chem.*, 1924, lix, 255.

² Robison, R., and Soames, K. M., *Biochem. J.*, 1925, xix, 153.

³ Howland, J., and Kramer, B., *Am. J. Dis. Child.*, 1921, xxii, 105.