

hypochromatic type of anemia is the time required not only to raise the erythrocyte count to normal but to replace the erythrocytes present in the circulation at the onset of treatment with new erythrocytes of normal hemoglobin content. This is in fundamental contradistinction to the situation in pernicious anemia.

At no time during the treatment with fetal liver was there any increase in the number of reticulocytes.

Conclusion: A procedure to accelerate the hemoglobin synthesis in secondary anemia through the feeding of fetal calf's liver has been carried out successfully.

#### 4086

#### Effect of Cholesterol Exposed to Roentgen Rays on Rachitic Rats.

WILHELM STENSTROM, ANNE LOHMANN AND H. T. HILLSTROM.

*From the Laboratory of Biophysics, University of Minnesota.*

Hess and Weinstock<sup>1</sup> state that cholesterol, which has been irradiated with roentgen rays, does not cure rickets, and also that the absorption spectrum in the ultra violet of cholesterol is not changed by this kind of radiation. On the other hand, Reinhard and Buchwald<sup>2</sup> found that a definite change takes place in the absorption if cholesterol is irradiated in chloroform solution. This change in the absorption was similar to the change that is produced by irradiation with ultra violet. If commercial cholesterol is exposed for a short time to ultra violet radiation from a mercury arc, it obtains a curative effect on rickets and there seems to be a correlation between the activity and the change in the absorption. It seems, therefore, that cholesterol in a chloroform solution might obtain anti-rachitic properties by being exposed to roentgen radiation.

After some preliminary tests with the production of rickets in rats, the following experiment was carried out. Through the courtesy of Dr. Cornelia Kennedy a litter of 8 rats was obtained after weaning, at the age of 28 days. The mother rat had been fed on Steenbock's diet<sup>3</sup> which is poor in vitamine A. The eight rats were put on Steenbock and Nelson's diet No. 2966<sup>4</sup> free from vitamine

<sup>1</sup> Hess, A. F., and Weinstock, M., *J. Biol. Chem.*, 1925, lxiv, 1923.

<sup>2</sup> Reinhard, M. C., and Buchwald, K. W., *J. Biol. Chem.*, 1927, lxxiii, 383.

<sup>3</sup> Steenbock, *Science*, 1923, lviii, 449.

<sup>4</sup> Nelson, E. M., and Steenbock, H., *Am. J. Physiol.*, 1925, lxxiii, 341.

D. Rats C and D were on this diet alone; A and B received 0.2 cc. of codliver oil per day; E and F received unirradiated cholesterol, and G and H received cholesterol which had been exposed to roentgen rays while dissolved in chloroform. The codliver oil was fed by means of a pipette. The cholesterol was administered by pouring 1 cc. of a solution of 0.6 gr. of Kahlbaum's cholesterol in 100 cc. chloroform on the day's ration and the chloroform evaporated off at room temperature. Any food remaining was added to the next ration.

The cholesterol solution to be irradiated was sealed in glass tubes about 15 cm. long and 1.5 cm. in diameter (15 cc. in each tube). As a rule, one tube at a time was placed inside the large metal cylinder that housed the roentgen tube and was left there until shortly before it was needed. The distance from the target to the center of the cholesterol container was 57 cm.; 180 to 200 kilovolts and 30 milliamperes were used, the only filter being the glass wall of the container. The time of exposure varied from 1 to 15 hours with interrupted radiation, the average time being 7 hours. The rats were kept 26 days, and at the end of this period roentgen pictures were taken which showed the typical rachitic picture in rats C to H inclusive. Sections were made of bones from all the rats, the frozen sections being made without decalcification and stained according to Von Kossa's technique with silver nitrate.<sup>5</sup> In rats C to H there was no evidence of calcification in the epiphyseal cartilage and in the greater part of the metaphysis. Both of these tests showed no difference in the intensity of the rickets produced in the last 6 rats. Rats A and B were normal in both of these tests. The results of the experiment are summarized in the following table:

TABLE I.

Rat	Addition to ration	Weight			Roentgen Plate	Calcium staining <sup>5</sup>
		1st day	14th day	26th day		
A	Codliver oil	59	72	82	—	—
B	" "	44	60	70	—	—
C	—	57	72	78	+	+
D	—	56	71	75	+	+
E	Unirradiated Cholesterol	56	70	77	+	+
F	" "	55	71	77	+	+
G	Irradiated Cholesterol	57	71	77	+	+
H	" "	61	78	86	+	+

— = No evidence of rickets could be noticed.

+ = Definite evidence of rickets present.

<sup>5</sup> Von Kossa, *Ziegler's Beiträge*, xxix, 190.

Our experiment thus shows that the roentgen radiation to which the solution of 0.6% cholesterol in chloroform was exposed did not induce any anti-rachitic potency to the cholesterol. The roentgen dose was neither exceedingly small nor exceedingly large. We therefore consider that enough change should have been produced to have had a noticeable effect on the rats if anti-rachitic properties were induced by irradiation with roentgen rays of a solution of cholesterol in chloroform, and doubt that the dose could have been large enough to both produce and destroy completely an antirachitic substance.

We wish to express our appreciation to Dr. Grace Medes for the advice received regarding the diet for the rats used in this experiment.

#### 4087

### A Streaming Potential Method of Measuring Electrokinetic Potentials of Proteins.

DAVID R. BRIGGS. (Introduced by R. A. Gortner.)

*From the Division of Agricultural Biochemistry, University of Minnesota.*

The sign and magnitude of the electrical charge on proteins has profound effects upon the physico-chemical properties of protein systems, and various methods have been utilized to evaluate these factors in a quantitative manner.

Most of these methods have utilized the technic of cataphoresis. Thus, Svedberg and Tiselius<sup>1</sup> photographed the migrating boundary between egg albumin and a buffer solution by the aid of the fluorescence produced by protein solutions in ultra-violet light. Loeb<sup>2</sup> coated particles of collodion with various proteins and observed with the aid of an ultra-microscope the direction and rate of migration in an electric field.

Freundlich and Abramson,<sup>3</sup> and Abramson<sup>4</sup> adsorbed protein on the surface of quartz particles and studied the influence of pH on the rate and direction of migration of these coated particles. They found that the quartz particles coated with protein behaved as pure protein particles.

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<sup>1</sup> Svedberg, Th., and Tiselius, A., *J. Am. Chem. Soc.*, 1926, *xlviii*, 2272.

<sup>2</sup> Loeb, J., *J. Gen. Physiol.*, 1923, *v*, 395.

<sup>3</sup> Freundlich, H., and Abramson, H. A., *Z. physik. Chem.*, 1927, *cxxviii*, 25.

<sup>4</sup> Abramson, H. A., *J. Am. Chem. Soc.*, 1928, *l*, 390.