

We may conclude then that glucose metabolism is constantly modified by at least 2 hormonal mechanisms. The agent of the first of these is insulin, powerful but acting relatively a short time (2 to 3 hours for a given medium dose). The other is a mechanism having a longer duration. The insulin mechanism may be considered as taking care of immediate needs, responding very quickly to eating of food or rise in blood sugar but having a relatively transitory effect for one given dose. The other mechanism is more slowly produced but acts during a prolonged period.

*Summary.* The rate of utilization of glucose by the eviscerated preparation is affected by feeding and fasting prior to operation. The tissues of the fed animals utilize glucose at a rate double that of the fasted.

This increase cannot be due to insulin.

We wish to acknowledge our appreciation to the Eli Lilly Company for the gift of insulin used in these experiments.

#### 9594

### Recovery from the Anemia Caused by a Diet Deficient in Vitamin K.

SIDNEY A. THAYER, R. W. MCKEE, D. W. MACCORQUODALE AND EDWARD A. DOISY.

*From the St. Louis University School of Medicine, St. Louis, Missouri.*

The purpose of this investigation was to ascertain whether the restoration to normal of the clotting time of chicks on a diet deficient in Vitamin K was accompanied by a cure of the existing anemia. This anemia has been observed by Dam,<sup>1</sup> and Holst and Halbrook,<sup>2</sup> but thus far no extensive investigations on the regeneration of hemoglobin have been made. The changes toward normal produced by the administration of minute quantities of a Vitamin K concentrate were so rapid that it seemed desirable to report our results.

The chicks used in our experiments were one-day-old white Leghorns hatched from eggs laid by hens which had been fed a diet relatively free from Vitamin K. They were raised in battery brooders and had free access to food and water. The basal diet used in these experiments was that of Almquist<sup>3</sup> with only minor

---

<sup>1</sup> Dam, H., *Biochem. Z.*, 1929, **215**, 475.

<sup>2</sup> Holst, W. F., and Halbrook, E. R., *Science*, 1933, **77**, 354.

modifications. Our active extract was prepared from alfalfa meal by an adaptation of the methods of Dam and Schönheyder<sup>4</sup> and of Almquist.<sup>3</sup>

Blood was drawn from the brachial vein, samples being taken for the determination of clotting time, hemoglobin, and the erythrocyte count. The clotting time (Schönheyder<sup>5</sup>) was determined by allowing the blood to flow directly into a small porcelain dish. Zero time was taken as the time when the first drop fell into the dish. When the blood no longer flowed upon tilting the dish it was considered to be clotted. The blood was observed for 300 minutes, if it failed to clot in a shorter time. The hemoglobin was determined by the method of Palmer<sup>6</sup> in which the hemoglobin is converted to carbon monoxide hemoglobin and compared with a standard solution of carbon monoxide hemoglobin in a colorimeter. The erythrocyte count was made in the conventional manner.

The first group of 12 normal chicks was placed on a normal diet consisting of a commercial preparation (Startena—Ralston Purina Mills) for 35 days. From this group we obtained the normal values (Table I). The second group of 12 chicks was kept on the basal diet No. 12 supplemented with 0.5% dehydrated alfalfa meal for 35 days. The third group of 21 chicks was placed on the basal diet No. 12, five of them being kept on the diet for 27 days (Sub-Group A, controls) and 16 for 35 days (Sub-Group B, controls). All of this group of chicks were given 0.35 cc. of oleum sesami daily for the last 3 days. The fourth group of 21 birds was kept on the basal diet No. 12, five of them for 27 days (Sub-Group A, treated) and 16 for 35 days (Sub-Group B, treated). On the last 3 days the birds received 0.2 cc. oleum sesami containing 1.0 mg. of an alfalfa extract 4M8a daily. This solution was administered from a 1.0 cc. tuberculin syringe connected to a tube 9 cm. in length and 1.0 mm. in diameter. The tube was inserted directly into the crop, and the solution of the extract was washed through the needle with an additional 0.15 cc. of sesame oil. The results indicate that oleum sesami is a suitable vehicle in which to administer the active extracts since the controls not receiving Vitamin K showed the profound anemia and prolonged clotting time.

It is clear from the results obtained with the second group of birds that 0.5% alfalfa meal adequately protects the birds from

---

<sup>3</sup> Almquist, H. J., *J. Biol. Chem.*, 1936, **114**, 241.

<sup>4</sup> Dam, H., and Schönheyder, F., *Biochem. J.*, 1936, **30**, 897.

<sup>5</sup> Schönheyder, F., *Biochem. J.*, 1936, **30**, 890.

<sup>6</sup> Palmer, W. W., *J. Biol. Chem.*, 1918, **33**, 119.

TABLE I.

Diet	No. of Chicks	Clotting time in minutes			Erythrocytes per cu.mm. in millions			Hemoglobin gm./100 cc.		
		Min.	Max.	Aver.	Min.	Max.	Aver.	Min.	Max.	Aver.
Commercial feed (Startena), normal chicks	12	1.0	6.0	3.60	2.57	3.60	2.98	8.16	11.25	9.49
Diet 12 plus 0.5% dehydrated alfalfa leaf meal	12	1.5	6.0	3.5	2.30	3.07	2.67	7.47	9.72	8.92
Diet 12 (Controls)										
Sub-Group A	5	13	>300	78	1.80	2.01	1.94	6.91	8.77	7.79
Sub-Group B	16	14*	>300	266	0.90	2.10*	1.50	2.52	9.18*	5.68
Diet 12 plus 3 mg. of alfalfa extract 4M8a										
Sub-Group A	5	1	2.5	1.60	2.40	2.87	2.65	7.50	9.24	8.26
Sub-Group B	16	0.75	5.0	2.48	2.14	3.30	2.71	5.50	9.49	8.25

\*These values were obtained from the same bird.

Vitamin K deficiency. With one exception all of the birds in the third group developed the deficiency to a marked degree. The clotting time was prolonged and the anemia severe, in one case the red cell count being as low as 900,000 per mm.<sup>3</sup> and the hemoglobin 2.5 g. per 100 cc. of blood.

The 5 birds kept on the diet for 27 days showed an average clotting time of 78 minutes. Of the birds kept on the diet for 35 days only 2 showed clotting time of less than 300 minutes; however, one of these showed almost normal values for clotting time and hemoglobin. Even under extreme conditions an occasional bird fails to develop severe deficiency.

The birds of the fourth group receiving the extract containing the antihemorrhagic factor for only 3 days showed values for erythrocytes and hemoglobin which were approximately normal, and an average clotting time of 2.3 minutes. The return to normal accomplished by the administration of minute quantities of Vitamin K for 3 days shows that the hematopoietic tissues must have been functioning at an amazing rate.

In a recent examination of a standard grade of fish meal Almquist and Stokstad<sup>7</sup> obtained no evidence of the presence of toxic substances which prolong clotting time or produce anemia. According to these investigators<sup>8</sup> the anemia of chicks on a diet deficient in Vitamin K "must be regarded entirely as a consequence of hemorrhage." While there is no doubt that hemorrhage is a very important factor in the anemia it is possible that other factors play a rôle. Since it is conceivable that an examination of the hematopoietic tissues may throw additional light on the anemia, Dr. G. O. Broun is conducting a study of the bone marrow.

*Conclusions.* The administration of Vitamin K to chicks showing a prolonged clotting time and a profound anemia restores both of these to normal values. Minute amounts of Vitamin K permit complete recovery from the anemia within a period of 3 days.

We wish to acknowledge financial assistance from the Theelin Fund administered by the Committee on Grants for Research of St. Louis University.

---

<sup>7</sup> Almquist, H. J., and Stokstad, E. L. R., *Poultry Science*, 1937, **16**, 261.

<sup>8</sup> Almquist, H. J., and Stokstad, E. L. R., *J. Nutrition*, 1937, **14**, 235.