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The Shortening of the Coagulation Time of the Blood by Irradiated Ergosterol.

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(Introduced by Evarts A. Graham.)

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This is a report of further studies on the effect of irradiated ergosterol on the mammalian thrombocyte counts begun by Phillips and Robertson[1] in this laboratory. They found that the thrombocyte count was markedly increased by the administration of irradiated ergosterol. In view of these findings and the results obtained by F. D. Gunn,[2] who produced a distinct rise in the number of thrombocytes by exposing young rabbits to radiations from the mercury vapor lamp, it was reasonable to expect a corresponding decrease in the clotting time.

In our experiments the Sooy and Laurens[3] method was used for counting thrombocytes as described by Phillips and Robertson.[1] For coagulation tests a modification of Boggs'[4] method was used, of which a brief description is here given: A small metal box with a glass bottom was covered by a tightly fitting glass cone. A round drop of blood was placed upon the small end of the cone and quickly inverted into the box. The instrument was placed on the stage of the microscope and the edge of the drop examined with the 16 mm. objective. A small stream of air was then directed on the edge of the drop at 15 second intervals. Instead of blowing directly into the chamber as described by Boggs, the air was passed through a wash bottle that was immersed in a water bath to keep the temperature and the humidity of the air constant. Coagulation was assumed to be completed when the corpuscles moved en masse, and sprang back to their original position.

White rats of various weights and unknown age were selected at random. Ten such rats were fed 3 minims of viosterol* daily with a medicine dropper for 9 days. Four rats were fed 3 minims for 2

[1] Phillips, R. A., and Robertson, D. F., *Trans. Soc. Exp. Biol. and Med.*, 1929, xxvi, 639.

[2] Gunn, F. D., PROC. SOC. EXP. BIOL. AND MED., 1926, xxiv, 120.

[3] Sooy, J. W., and Laurens, H., PROC. SOC. EXP. BIOL. AND MED., 1924, xxii.

[4] Boggs, T. R., *Internal. Clin. Phila.*, 1908, i, 31-39.

* A preparation of irradiated ergosterol furnished to us through the courtesy of Mead, Johnson and Company.

days. Four rats were fed 3 minims for one day. Adequate control rats were used with each series, and these failed to show any noteworthy change. A total number of 18 animals were followed for 12 days and on each animal thrombocyte counts and determinations of coagulation time were made daily.

The following results were obtained: The normal coagulation time varied between 1 minute and 45 seconds and 2 minutes and 45 seconds, with an average of 2 minutes and 10 seconds. The normal thrombocyte and red blood cell counts for the white rats agreed with those of Cramer, Drew and Mottram,[5] *viz.*, average red blood corpuscles were 8,500,000 to 10,000,000 per cm. and the average thrombocyte count from 500,000 to 700,000.

Each animal receiving viosterol showed a marked decrease in the coagulation time which occurred simultaneously with the marked increase in the thrombocyte count. The results of Phillips and Robertson[1] were confirmed in that the thrombocyte count was doubled in 48 hours. The highest counts recorded, which were as high as 3,000,000 from a normal count of 600,000, were obtained on the fifth through the seventh day, after the initial dosage. The lowest coagulation time (15 seconds to 30 seconds) occurred also on the fifth through the seventh day. This is readily observed in Table I, which represents 5 typical animals.

TABLE I.

Animals	No. of Doses Administered	Normal Thrombocyte Count	Highest Thrombocyte Count	Normal Clotting Time	Lowest Clotting Time	Day of Occurrence
Rat I	Continuous	612,500	2,250,000	2:00 min.	45 sec.	5th
Rat II	Continuous	664,500	3,132,000	1:45 "	15 "	5th
Rat III	2	712,000	2,345,000	2:15 "	30 "	7th
Rat IV	1	661,000	2,466,000	1:45 "	45 "	5th
Control	0	560,000	654,000	1:45 "	1:45 min.	.....

The series of rats which received only one or 2 doses showed approximately the same results as those which received continued doses. The continued dosage tended to produce a hypervitaminosis, as shown by a diarrhea, dullness, sluggishness, emaciation and a loss of hair, whereas, the smaller number of doses gave no evidence of hypervitaminosis.

It is believed that the series of animals presented was large enough to prove definitely that the coagulation time of the blood can be shortened, at least in normal rats, by feeding irradiated ergosterol.

[5] Cramer, Drew and Mottram, *Proc. Royal Soc. London*, 1922, xciii, 449.

We hope to demonstrate by further experiments that the coagulation time which is prolonged in obstructive jaundice can be shortened sufficiently to reduce the operative risk.

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Comparison of Effects of Various Preparations of Anterior Pituitary Gland on Thyroid of Guinea Pig.

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In a former communication,[1] we reported that the thyroid gland of guinea pigs which received injections of acid or alkaline extract of the anterior pituitary gland within a short time showed such a remarkable hypertrophy that it closely resembled the thyroid seen in pronounced cases of Graves disease. These findings were in marked contrast to the fact that the feeding of Armour's tablets of anterior pituitary prevented or greatly inhibited compensatory hypertrophy in thyroidectomized guinea pigs.

We now report further observations on the effect of the anterior pituitary gland.

Preparation of Extracts. The anterior pituitary of cattle was freed completely from other parts of the hypophysis, then dried and powdered. Five grams of the dried powder were extracted with 100 cc. of 0.5% acetic acid or with 100 cc. of 0.1% sodium hydroxide for a period of 24 hours, in ice chest. The fluid was separated from the residue by filtration, neutralized to pH 7.8 (Phenol Red), refiltered to remove a protein precipitate which falls out at the isoelectric point. The filtrate was then passed through a Seitz bacterial filter to render it sterile.

Experimental. Silberberg,[2] in the course of experiments carried out in this laboratory observed that there was a distinct hypertrophy in the thyroid gland of the guinea pig as early as one day after the injection of 1 cc. of acid extract of the anterior pituitary. Following this, we made a comparative study of the action of acid and alkaline extracts on the thyroid gland and also on the sex organs. One group of animals was injected daily with 1 cc. acid extract, and another

[1] Loeb, Leo, and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1929, xxvi, 860.

[2] Silberberg, Martin, PROC. SOC. EXP. BIOL. AND MED., 1929, xxvii, 166.