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Effect of Certain Sulfur Compounds on Coagulation of Blood.

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The initial observation of Mueller and Sturgis[1] that cysteine inhibits the coagulation of whole blood *in vitro* has since been confirmed and elaborated by Carr and Foote,[2] who suggested that the delay in coagulation might be due to certain changes which they observed in the physical state of the fibrinogen. Kühnau and Morgenstern,[3] working with glutathione, also described inhibition of coagulation within a certain range of pH. Since the mechanism of the reaction has not been explained, the experiments reported here have been undertaken. Three lines of investigation have been carried out:

1. *In vivo experiments in human subjects.* Cysteine and methionine, ingested and injected intravenously in amounts varying from 1 to 3.5 gm., prolonged the bleeding time as indicated by the Ivy method from a 4 minute upper limit of normal to 15 minutes (methionine, 1.31 gm. intravenously) and delayed the coagulation time as shown by the 8 mm. tube method, from an 8 minute upper normal to 16 minutes (cysteine, 1 gm. intravenously and methionine, 1.31 gm. intravenously).

2. *In vitro experiments with whole blood.* Cysteine hydrochloride, taurocholic acid, taurine, methionine, glycine, alanine and cysteic acid, neutralized to pH 7, were added in graded amounts giving final concentration of from 4.4×10^{-5} *M* to 0.18 *M*, and the coagulation time was determined by the 8 mm. tube method. Glycine, alanine and cysteic acid did not retard coagulation. Methionine, in contrast to its behavior *in vivo,* also had no effect in delaying coagulation. Cysteine produced a significant effect at a 0.046 *M* concentration, with a coagulation time of 18 minutes. At 0.18 *M* concentration, the coagulation time was 53 minutes. Taurine showed a definite effect at 0.023 *M* concentration (16 minutes), with a 90 minute coagulation time at 0.18 *M* concentration. Taurocholic acid produced a significant effect at 0.011 *M* concentration (34 minutes),

[1] Mueller, J. H., and Sturgis, S., *Science,* 1932, **75**, 140.

[2] Carr, J. L., and Foote, F. S., *Arch. Surg.,* 1934, **29**, 277.

[3] Kühnau, J., and Morgenstern, V., *Z. physiol. Chem.,* 1934, **227**, 145.

with no coagulation during 24 hours in concentrations of 0.023 to 0.18 *M*.

3. *In vitro experiments with isolated components of the blood clotting system.* The components of the coagulation system were isolated by the following methods: Prothrombin was prepared from horse, rabbit and human plasmas by the method of Mellanby.[4] Fibrinogen was repeatedly salted out from horses' plasma with sodium chloride (Eagle[5]). The tissue factor was obtained from 2 sources, (a) platelet suspension (Eagle[5]), and (b) desiccated rabbit lung (Eagle[6]), by drying in the Flosdorf-Mudd desiccator.* Calcium was added as 1% $CaCl_2$. The pH was controlled at 7.0 ± 0.1 with brom thymol blue as indicator. From a stock solution of cysteine hydrochloride, freshly prepared immediately before using, and adjusted to a pH of 7.0 ± 0.1, a series of dilutions was made. In a typical experiment the following amounts of the various constituents were used: prothrombin solution, 0.8 cc.; $CaCl_2$ solution, 0.01 cc.; platelet suspension, 0.02 cc. or approximately 2 mg. of desiccated rabbit lung; fibrinogen solution, 0.8 cc. When cysteine was added to the system, 0.4 cc. were used and 0.4 cc. of physiological salt solution added to the controls.

Cysteine, added to the coagulation system before the formation of thrombin had an inhibiting effect similar to that in the whole blood experiments. This inhibiting effect was constant regardless of the length of time allowed for the formation of thrombin. When thrombin, formed by the interaction of prothrombin, tissue factor and calcium, was subjected to cysteine in the higher concentrations given above, only a slight inhibiting effect was produced.

Each of the elements was then subjected separately to the action of cysteine and subsequently freed from traces of cysteine by suitable methods. No effect on the rate of coagulation was produced by the action of cysteine on (a) fibrinogen, (b) platelet suspension, or (c) platelet suspension plus calcium. When prothrombin was acted upon by cysteine in 0.4 *M* concentration, the coagulation time was 540 minutes, in 0.2 *M* concentration the coagulation time was 28 minutes and in 0.1 *M* concentration, 5 minutes and 5 seconds. In the control tube coagulation occurred in one minute and 20 seconds. When sodium hypophosphite was employed in place of cysteine in

[4] Mellanby, John, *Proc. Roy. Soc. Lond.*, 1931, B, **107**, 271.

[5] Eagle, Harry, *J. Gen. Physiol.*, 1934-5, **18**, 531.

[6] Eagle, Harry, personal communication.

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equimolar concentrations, no inhibition of the coagulation was detected.

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Hypophysis-Thyroid-Gonad Relationship.*

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Thyroidectomy in male and female rats will increase the effectiveness of crude hypophyseal gonad stimulating extracts but the removal of this gland will not increase the effectiveness of pregnancy urine or pregnancy mare serum extracts (Smith,[1] Schockaert,[2] Fluhmann,[3] Leonard and Hansen[4]). Since Fluhmann[3] showed that thyroid feeding in rats decreased the effectiveness of both types of gonad stimulating hormones (hypophyseal and pregnancy urine), he suggested that the thyrotropic principle, present in the crude hypophyseal extracts, induced a hyperthyroid condition in the normal rats, which made the gonad stimulating hormone less effective. Pregnancy urine, not possessing a thyrotropic factor, was equally effective in normal and operated animals. This report will show that follicle stimulating hormone from hypophyseal or urinary sources and free of the thyrotropic hormone is more effective in the absence of the thyroid.

Immature female rats 24 to 26 days of age were thyroidectomized and injected with extracts of the urine of castrate women and of a man with a testis tumor, for 8 to 9 days. A similar number of control rats received only injections of the hormone. Another group of operated and control rats were treated with hypophyseal follicle stimulating hormone, prepared by Drs. Fevold and Hisaw,† for 5 to 6 days. None of the extracts were found to contain a thyrotropic factor when tested on young guinea pigs or hypophysectomized rats in the doses given. The ovaries and uteri were weighed at autopsy. More than 50 rats were used in these

* Aided by grants from the Rockefeller Foundation.

1 Smith, *Sex and Internal Secretions*, 1932, Ed. by Allen.

2 Schockaert, *Compt. Rend. Soc. Biol.*, 1931, **108**, 431.

3 Fluhmann, *Am. J. Physiol.*, 1934, **108**, 498.

4 Leonard and Hansen, *Anat. Rec.*, 1936, **64**, 203.

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