

the carcinogenic action of methylcholanthrene. 2. Administration of stilbestrol increased the incidence of uterine tumors. 3. The effect of testosterone upon tumors of the male adnexæ was contradictory.

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An Ether-Extractable Substance from Blood Serum and Buffy Coat Which Contracts Smooth Muscle.

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The presence in blood serum and platelets of substances which contract smooth muscle has been recognized for the past seventy years. Although numerous investigators have prepared active extracts of serum and platelets by the use of alcohol and acetone, few have employed water-immiscible solvents. T. F. Zucker and Stewart¹ and Janeway *et al.*,² using strips of ox carotid artery as an indicator, reported that only a small fraction of the total activity of serum was extractable by ether. Recently, Wu, Fang, and Tsai³ reported the separation of a fraction from dried rabbit serum by extraction with ether. This fraction was active on the rabbit intestine, but not on the vessels of the perfused rabbit ear, whereas rabbit serum was active on both of these indicators.* In the present paper,

the properties of an ether extractable fraction which appears to be identical with that obtained by Wu *et al.*³ will be described.

Methods. In the original method of Wu *et al.*,³ the serum was dried on a steam-bath before extraction with ether. We were unable to obtain consistent results with this somewhat drastic method, but found that an ether extract made from serum dried at room temperature with Na₂SO₄ was uniformly active.

Rabbit serum was ground thoroughly with 5 times its weight of anhydrous Na₂SO₄. The powder was then extracted 4 times with dry ether and the ether was evaporated on a steam-bath from the combined extracts. Approximately 0.1 mg of residue was obtained from 1 cc of serum, of which about one-half was soluble in water. Extracts were prepared in a similar manner from cat and human serum. The portion of the residue soluble in Locke solution will be referred to as "extract of dry serum." Solutions were made up so that 1 cc of extract represented from 1 to 10 cc of the original serum. This made it possible to compare the relative activity of serum and extract because the activity of both could be made approximately equal. The dosage values given in Table I represent the volume, not of the extract, but of the serum from which it was obtained.

The activity of an extract of the buffy coat of human citrated blood was also studied. The buffy coat was mixed with an equal volume of acetone and filtered. The filtrate was freed of acetone by vacuum distillation. The resulting aqueous extract was dried by mixing

¹ Zucker, T. F., and Stewart, G. N., *Zentralbl. f. Physiol.*, 1913, **27**, 85.

² Janeway, T. C., Richardson, H. B., and Park, E. A., *Arch. Int. Med.*, 1918, **21**, 565.

³ Wu, C. H., Fang, H. S., and Tsai, C., *Proc. Chinese Physiol. Soc., Chengtu Branch*, 1942, **1**, 83.

* One of us (M.B.Z.), unaware of the work of Wu *et al.*,³ had separated an active fraction from the buffy coat of human blood. The dry fraction was insoluble in ether. In doses of 0.5 to 2.0 mg it constricted the vessels of the perfused cat tail and stimulated the muscle of the rat and rabbit intestine. The recent visit of Dr. Tsai to this country presented an opportunity to compare the fractions studied in the Chinese and American laboratories. The present paper is a partial report of this joint study. Data on the fraction prepared by M.B.Z. and the comparison of the two fractions will be reported elsewhere.

TABLE I.
Comparison of the Action of Serum and Extracts of Dry Serum and Buffy Coat on Smooth Muscle.*

Material	Rat gut		Rabbit ear		Cat tail		Cat N.M.	
	Dose, cc	Effect	Dose, cc	Effect	Dose, cc	Effect	Dose, cc	Effect
Rabbit serum	0.04	++++	0.1	+++	0.1	++++	0.3	++++
Extr. dry rab. serum†	0.5	+++	2.0	0	1.0	++	2.0	++
Cat serum	0.04	++++	0.1	+++	0.1	++++	0.3	++++
Human serum	0.8	++	1.0	+	0.5	++	—	—
Extr. dry human buffy coat‡	0.4	+++	1.0	0	1.0	++	4.0	++++

* The data presented, with the exception of those concerning human serum, represent assays performed on the same indicators on a single day. They are typical of the results obtained in numerous experiments.

† The dosage represents the volume of serum from which the extract was prepared, not the volume of extract administered.

‡ One cc represents the extract from the buffy coat of 950 cc of blood.

it with 5 times its weight of Na_2SO_4 , and the powder extracted with ether in the same manner as was the serum. One cc of the final extract represented the buffy coat of from 500 to 1200 cc of blood.

As test objects, rabbit ileum, rat duodenum, cat nictitating membrane, and the vessels of the perfused rabbit ear and the cat tail were used. The ear and tail were perfused at room temperature at a pressure of 30 to 70 cm H_2O . The intestine was suspended under slight tension in a 20 cc bath maintained at 37°C. The maximum volume of test solution used was 1 cc, except in the tests on the previously denervated nictitating membrane, in which the solution in a 2 cc volume was injected peripherally through the cannulated carotid artery. The tests on each preparation were repeated several times in a different order. Control tests of Locke solution and of a blank Na_2SO_4 extract were frequently made and were uniformly negative.

Results. The extract of dry rabbit serum had between one-tenth and one-twentieth of the activity of the original serum on the rat duodenum, cat nictitating membrane and tail vessels (Table I). The substance was active on the rat intestine in a dilution of 1:500,000. The rabbit ileum was less sensitive to both serum and extract than was the rat duodenum. The extract had no effect on the vessels of the perfused rabbit ear, even in a dose equivalent to 7 cc of serum, whereas 0.1 cc of whole rabbit serum had a marked constrictor action.

Extracts of dry human and cat serum also showed about one tenth of the activity of the original serum on the rat intestine, but their

effect on the rabbit ear vessels was equivocal. The pharmacological properties of the extract of buffy coat were identical with those of the extract of dry rabbit serum.

It was found that an aqueous solution of the extract, when dried on a steam bath, yielded a residue insoluble in ether. This residue retained all of the activity of the original extract as tested on the rat intestine. It may be concluded, therefore, that the active material is soluble in ether only in the presence of certain of the lipids in serum and the buffy coat.

The active material in the extracts of dry serum and buffy coat resisted boiling in Locke solution for 10 minutes. The activity of these extracts, whether or not lipids were present, was markedly diminished by treatment with 0.1 N NaOH for $\frac{1}{2}$ to $1\frac{1}{2}$ hours at room temperature, whereas similar treatment with 0.1 N HCl had no effect.

Discussion. It should be noted that, although the extracts of rabbit serum and of human buffy coat stimulate the smooth muscle of the rat intestine, they have no effect upon the vessels of the rabbit ear. On the other hand, the ear vessels are strongly constricted by 0.1 γ of histamine,⁴ whereas the rat intestine is very insensitive to this drug. These observations indicate that the active constituent in the ether extracts cannot be histamine.

Rabbit serum constricts the blood vessels of both the rabbit ear and the cat tail. The fact that the extract of dry serum does not act upon the vessels of the rabbit ear indicates

⁴ Rothlin, E., *Biochem. Z.*, 1920, **111**, 299.

that rabbit serum contains active substances not present in the extract. It is relevant to consider the nature of these substances. The histamine content of rabbit serum (1 to 3 $\gamma/\text{cc}^{5,6}$) is so great that it may be responsible for a considerable part of the activity of serum on the rabbit ear vessels. Evidence for this was furnished by Tsai *et al.*⁷ who found that incubation of rabbit serum with crude histaminase reduced its vasoconstrictor activity by about one-third. As these workers pointed out, the residual activity indicates the presence of a non-histamine vasoconstrictor substance in rabbit serum. Such a substance must be responsible for the major part of the

activity of cat and human sera upon rabbit ear vessels, since these sera contain insufficient histamine (0 to 0.06 $\gamma/\text{cc}^{5,6,8}$) to account for an appreciable part of their vasoconstrictor activity.

Summary. A substance which stimulates smooth muscle was separated from dried rabbit serum and from the buffy coat of human blood by ether extraction. This substance is soluble in ether only in the presence of certain blood lipids. Aqueous solutions of the material stimulate the rat and rabbit intestine, cat nictitating membrane and tail vessels, but are devoid of effect on the vessels of the rabbit ear. The present findings, together with those of Tsai *et al.*,⁷ suggest that several vasoconstrictor substances are present in serum.

⁵ Code, C. F., *J. Physiol.*, 1937, **90**, 349.

⁶ Anrep, G. V., Barsoum, G. S., Talaat, M., and Wieninger, E., *J. Physiol.*, 1939, **96**, 130.

⁷ Tsai, C., Wu, C. H., Fang, H. S., and Chen, T. I., *Proc. Chinese Physiol. Soc., Chengtu Branch*, 1943, **2**, 1.

⁸ Barsoum, G. S., and Gaddum, J. H., *J. Physiol.*, 1935, **85**, 1.

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Further Studies on the Menstrual Discharge of Women.*

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Earlier studies on the menstrual discharge of normal women have been reported¹ with emphasis on its great toxicity, as little as 0.01 cc when fresh killing an immature rat within 48 hours. This paper summarizes certain corrections and additions that have evolved from continued investigation of this material.

It was stated that the toxin was most concentrated in the cells and debris of menstrual fluid. We have since found that the endometrial debris forms upon centrifugation, a middle layer between the serum and blood corpuscles. Even after being washed 3 to 6 times with saline, this layer is nearly 10 times

as toxic as the serum, whereas the washed red blood cells are practically harmless. The amount of endometrial debris varies, being least when the rate of catamenial flow is greatest; the toxicity of different collections during menstruation, whether serum or whole discharge is tested, varies in direct proportion to the quantity of endometrial debris. It would appear, therefore, that the toxicity is derived from the endometrial debris.

As stated, after fractional precipitation of whole discharge with $(\text{NH}_4)_2\text{SO}_4$ toxicity is most concentrated in the water-insoluble portion (after dialysis) of the englobulin precipitate. This precipitate, however, is apparently not a true englobulin, since it does not go into solution in the presence of electrolytes. When the whole discharge is not filtered before precipitation, the endometrial debris itself might be carried down and account for

* The Mrs. William Lowell Putnam Investigation of the Toxemias of Pregnancy, aided by grants from the Committee for Research in Problems of Sex, National Research Council.

¹ Smith, O. W., and Smith, G. V., *Proc. Soc. Exp. Biol. and Med.*, 1940, **44**, 100.