were shaken in an atmosphere of 95 per cent. O_2—5 per cent. CO_2 for one and one quarter hours at 37° C. Two experiments were performed on newborn rat brain in a similar manner, except that slices of six brains were divided in two lots, one for the control and the other for incubation with thymus tissue.

After incubation the total Ach was obtained by treatment with HCl and the extracts assayed on the isolated heart of *Venus mercenaria*. In each experiment a careful comparison of the effects of the extracts of brain tissue incubated with and without thymus tissue was made and the actual amounts of Ach in the extracts estimated by comparison with known amounts of Ach.

RESULTS

The results are given in Table 1. The amount of

TABLE 1

EFFECT OF NEWBORN RAT THYMUS ON ACH SYNTHESIS BY RAT BRAIN SLICES

	Incubated without added thymus	Incubated with added thymus
	Total Ach, γ/gm	Total Ach, γ/gm
A. Adult brain slices		
Exp. 1	10	10
2	6–7	6–7
3	20	20
4	4–6	4–6
5	5	5
B. Infant brain slices		
Exp. 1	3	3
2	1	1

total Ach normally present in whole, adult rat brain is of the order of 1 to 1.5 gamma per gram; that in newborn rat brain of the order of 0.5 gamma per gram. It may be noted, therefore, that appreciable amounts of Ach were synthesized in all experiments. In each experiment, however, the amounts of Ach obtained from brain slices incubated with or without added thymus tissue are the same. It may be concluded that under the conditions of these experiments normal infant rat thymus neither accelerates nor retards the synthesis of Ach by brain slices.

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A FIBRINOLYTIC ENZYME IN MENSTRUATION AND LATE PREGNANCY TOXEMIA

THE demonstration[1, 2] that prothrombin and fibrinogen are lacking in menstrual discharge suggests that the blood in it has clotted and the clot dissolved. Evidence for the existence of lytic substances to account for its fluidity has been presented by many but has been inconclusive. Fibrinolytic action in the uterus is nevertheless a rational explanation and a proteolytic enzyme might, in addition, account for the great toxicity of the euglobulin fraction of menstrual discharge, which contains altered protein.[3] Such an enzyme might be a product of endometrial injury due to the withdrawal of hormonal support. Its physiological function might be lysis of damaged tissue, including clotted blood, for the purpose of elimination. The toxic by-product, as has been suggested,[4, 5] might be the final cause of menstruation through vascular injury. Since the hormonal situation in toxemia of late pregnancy is entirely analogous to that at the time of menstruation and the generalized vascular changes similar to the local ones in the menstruating endometrium, we have theorized that this disease might be due to a similar toxin.[5] We have been unable to find such a toxin in the circulating blood at the time of menstruation or toxemia. If a proteolytic enzyme, however, were associated with these states it might be demonstrable, since tests for it are so much more sensitive than our criterion for toxicity, which depends upon obtaining an amount lethal to an immature rat.

A simple method was employed in testing for fibrinolytic activity.[6] Fresh plasma from oxalated human venous blood has been our source of fibrinogen, each sample being tested before use for its ability to form a stable clot with thrombin.[7] Cell and platelet free serum was tested for fibrinolytic activity, always within 24 hours of collection.

Twelve specimens of fresh menstrual "serum" have been examined. All contained marked fibrinolytic activity. A typical protocol is presented in Table 1. There was evidence that the enzyme was even more concentrated in the endometrial "debris" than in the "serum."[8]

During menstruation the venous serum of 5 women

[1] H. D. Glueck and I. A. Mirsky, *Am. Jour. Obst. and Gynec.*, 42: 267, 1941.

[2] E. L. Lozner, Z. I. Taylor and F. H. L. Taylor, *New Eng. Jour. Med.*, 226: 481, 1942.

[3] O. W. Smith and G. V. Smith, *Proc. Soc. Exp. Biol. and Med.*, 44: 100, 1940, and 55: 285, 1944.

[4] G. V. Smith and O. W. Smith, *Am. Jour. Obst. and Gynec.*, 45: 15, 1943.

[5] G. V. Smith and O. W. Smith, *Jour. Clin. Endocrinology*, 1: 470, 1941.

[6] H. J. Tagnon, C. S. Davidson and F. H. L. Taylor, *Jour. Clin. Invest.*, 21: 525, 1942.

[7] The thrombin employed was prepared by the Department of Physical Chemistry, Harvard Medical School, Boston, Mass., from blood collected by the American Red Cross, under a contract recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[8] Since prothrombin is lacking in menstrual discharge,[1, 2] the formation of a clot when menstrual "serum" is added to oxalated plasma must be due to the formation of thrombin from the prothrombin of the oxalated plasma and the calcium of the "serum." No clot formed when washed endometrial "debris" was added to oxalated plasma unless calcium or thrombin was also added. Under the latter conditions the subsequent dissolution of the clot occurred with as little as 0.01 cc of "debris."

(10 specimens) was consistently fibrinolytic. A typical protocol is presented in Table 1. With 7 specimens from 4 of these women during the intermenstruum the tests were entirely negative. One woman, whose cycles are very irregular, had some of the enzyme in her serum on the 3 occasions during the intermenstruum when blood was taken, although fibrinolysis was minimal. Five other non-menstruating women gave negative tests, whereas the sera of 2 with abnormal uterine bleeding were fibrinolytic. Two specimens were tested 24 and 48 hours before the onset of flow. Both were positive.

TABLE 1

TYPICAL PROTOCOLS SHOWING THE FIBRINOLYTIC ACTIVITY OF SERUM

Material tested	cc*	Clot formation	Dissolution of clot†	Thrombin added at 1 hr.
	0	None in 1 hour	.....	Clot (none dissolved at 1 hr.)
	0.1	None in 1 hour	.....	Clot (completely dissolved at 1 hr.)
Menstrual "serum"	0.3	8 min.	Started at 15 min. +++ at 1 hr.	No clot
	0.8	5 min.	Started at 10 min. Complete at 1 hr.	No clot
	0	None in 1 hour	.....	Clot (none dissolved at 1 hr.)
Venous serum	0.1	None in 1 hour	.....	Clot (++ dissolved at 1 hr.)
First day of menstration	0.3	2 min.	Started at 30 min. ++ at 1 hr.	No clot
	0.8	1 min.	Started at 12 min. +++ at 1 hr.	No clot
	0	None in 1 hour	.....	Clot (none dissolved at 1 hr.)
Venous serum	0.1	20 min.	Started at 30 min. + at 1 hr.	No clot
Severe preeclampsia at 8 months	0.3	5 min.	Started at 15 min. ++ at 1 hr.	No clot
	0.8	1 min.	Started at 7 min. Complete at 1 hr.	No clot
	0	None in 1 hour	.....	Clot (none dissolved at 1 hr.)
Venous serum	0.1	None in 1 hour	.....	Clot (none dissolved at 1 hr.)
24* after operation	0.3	2 min.	Started at 8 min. ++ at 1 hr.	No clot
Pt.'s temp. = 100° F.	0.8	1 min.	Started at 5 min. ++ at 1 hr.	No clot
		A Typical Protocol from the Control Series		
Venous serum	0	None in 1 hour	.....	Clot (none dissolved at 1 hr.)
9th day of a 31-day cycle	0.1	None in 1 hour	.....	Clot (none dissolved at 1 hr.)
	0.3	12 min.	None in 1 hr.	.....
	0.8	5 min.	None in 1 hr.	.....

* Each tube (13 × 100 mm) contained 0.2 cc of oxalated plasma and enough physiological saline to give a total volume of 1 cc when the serum was added. After incubation for one hour at 37.5° C., 10 + U of thrombin were added.

† Unless the clot was completely dissolved, the degree of dissolution was graded as follows: + = less than 0.5; ++ = 0.5 to 0.8; +++ = 0.8 or 0.9.

The sera from 7 patients with late pregnancy toxemia, one with eclampsia and one flowing prior to miscarriage at 4 months were tested. All 9 specimens were markedly positive for fibrinolytic activity. A typical protocol is presented in Table 1. The eclamptic patient and 2 of those with toxemia were studied 2 weeks later, when they had been delivered and were well. No enzyme was demonstrable. The sera of 7 normally pregnant women were also negative.

Although the enzyme and toxin are both concentrated in the euglobulin fraction of menstrual discharge, we have evidence that they are not identical. First, the fibrinolytic activity of many venous sera was as great as that in menstrual "serum"; whereas we have never been able to demonstrate toxicity in the venous serum of toxemic or menstruating women. Secondly, samples of menstrual discharge that have lost toxicity on standing may completely retain their fibrinolytic activity.

It has seemed to us that pathological syndromes associated with cellular injury from any cause whatsoever might be the effect of the release of toxic by-products of proteolysis from the action of this enzyme. To weigh this theory we have made preliminary studies on the blood of a few women before and after surgery. Of 8 patients taken at random, all with gynecological complaints, the sera of 4 contained fibrinolytic activity before operation. From the other 4, whose preoperative tests had been completely negative, bloods were taken one to 3 days after operation. All showed fibrinolysis. A typical protocol is given in Table 1. It is suggested that injured tissue produces a proteolytic enzyme.[9] This idea has also been recently expressed.[10]

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[9] In a recent paper (*Archives of Pathology*, 39: 28, 1945), V. Menkin refers to unpublished data indicating fibrinolytic action in the injury factor of canine pleural exudate. We have previously suggested[3] that his so-called "necrosin" and our menstrual "toxin" may be identical, both being products of cellular injury.

[10] A. Mirsky and E. D. Freis, *Proc. Soc. Exp. Biol. and Med.*, 57: 278, 1944.