nized against canine necrosin. Presumably because of this, we have gotten transferable protection against menstrual toxin with only 4 of 12 specimens of necrosin-immune rabbit serum.

The evidence presented in favor of the identity of menstrual toxin and Menkin's necrosin supports the hypothesis that the production of a specific toxic protein may be a universal result of cellular injury. On the basis of recent work,[8,10] we now have the concept that tissue damage releases a proteolytic enzyme and that enzymatic action yields the toxic protein.

Conclusion. Physical, chemical, and biological similarities, cross precipitin tests and experiments on transferable immunity indicate that Menkin's so-called necrosin of canine pleural exudate and the toxin of human menstrual discharge are identical.

[10] Mirsky, A., and Freis, E. D., PROC. SOC. EXP. BIOL. AND MED., 1944, **57**, 278.

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Evidence for Circulating Menstrual "Toxin" During Menstruation and Toxemia of Late Pregnancy.

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We have suggested that menstrual "toxin," a by-product of the change in the endometrium which follows withdrawal of hormonal support, may be responsible for the local vascular phenomena which characterize menstruation and that the same or a similar toxin from the uterus may cause the similar but generalized vascular damage in toxemia of late pregnancy, this disease also being associated with hormone withdrawal.[1] We have been unable to obtain from the circulating blood of menstruating or toxemic women a factor lethal to immature rats. The findings presented in the preceding paper,[2] however, have provided more sensitive tests for this material. Its pyrogenic and fibrinolytic activity may be demonstrated with minute amounts. Furthermore, the fact that canine pleural exudate contains an identical substance supplies a highly specific test in the form of cross precipitin reactions with canine "necrosin"-immune rabbit serum.

Both menstrual "toxin" and "necrosin," injected intravenously into the rabbit, cause a typical rise in rectal temperature, a maximum elevation of 2.5 to 5°F being observed 3 to 4 hours after injection.[2] In testing for pyrogenicity, readings are made just before and at hourly intervals for 7 hours after injection. In order to avoid any possibility of anaphylaxis, we plan to use an animal repeatedly at no more than 4-day intervals for a series of tests and then let it rest for at least 8 weeks. Half of a cc of unextracted serum from the venous blood of women was injected. Sixteen sera from normal nonmenstruating women were tested. The average rise in temperature was 0.9°F. In 10 of these there was no more than a 1° rise at any time. In the other 6 a 1.6° to 2.6° elevation occurred 1 to 2 hours after injection, but, unlike the curve after menstrual "toxin" or "necrosin," was declining by the time of the 3-hour reading. Thirteen tests were run using serum from patients normally pregnant between the thirty-fourth week and term. The average rise in temperature was 1.1°F. In all but 2, the tests were negative; that is, there was either no fever at all or the readings at 3 hours and thereafter were lower than those at 1 to 2 hours following injection. The sera from 2 of the normally pregnant patients

[1] Smith, G. V., and Smith, O. W., *J. Clin. Endocrinol.*, 1941, **1**, 470.

[2] Smith, O. W., and Smith, G. V., PROC. SOC. EXP. BIOL. AND MED., 1945, **59**, 116.

gave the response typical of the toxin; that is, an elevation of 2.5 to 3.4°F reaching a peak at the 3- to 4-hour reading. With 13 specimens taken during menstruation the average rise in temperature was 2.5°F. Nine elicited a maximum elevation of 1.7 to 4.8°F at 3 to 5 hours after injection. The other 4 failed to give the response typical of menstrual "toxin" and "necrosin." With 10 specimens from patients with pre-eclampsia or eclampsia, the average rise was 2.7°F. Seven gave a 2.4 to 4.5°F rise with the typical 3- to 4-hour peak and 3 gave no significant change in temperature. Sixteen of 23, or 70%, of the tests with sera of toxemic or menstruating women, therefore, were positive, whereas only 2 of 29, or 7%, of the control sera resulted in a similar response.

Menstrual "toxin" and "necrosin" are markedly fibrinolytic.[2] Evidence for fibrinolytic activity in the circulating blood at the time of menstruation, in late pregnancy toxemia, and in post-operative morbidity has been reported.[3] In these preliminary experiments, unextracted serum was employed and oxalated plasma used as the source of fibrinogen. We have since changed our technic, using a purified preparation of fibrinogen* as well as thrombin* and prolonging the period of observation to 24 hours at 37°C. We have also concentrated the sera by dialyzing 5.0 cc against running tap water for 5 hours, and taking up the euglobulin precipitate in 0.8 cc of saline for test. To this, 0.1 cc of an approximately 1% solution of fibrinogen and 0.1 cc (1 U) of thrombin have been added. With this technic, 6 sera from non-menstruating women and 6 from normal pregnancies have been tested. No measurable dissolution of the clot occurred in any, although there was slight retraction from the sides of the tubes by the end of the 24-hour period. Of 9 sera from the venous blood of menstruating women, 2 showed complete dissolution and the other 7 contained only a slight residual clot, 0.7 to 0.9 cc of fluid being present in each tube at the end of 24 hours. The results of 7 similar tests on the venous sera of 6 patients with late pregnancy toxemia and 1 with eclampsia were even more striking, complete dissolution of the clot being observed in every instance.

The identity of menstrual "toxin" and canine "necrosin" was established by the cross precipitin experiments with immune rabbit serum reported in the preceding paper.[2] If this same factor is present in the circulating blood of menstruating and toxemic women, their serum should show positive precipitin reactions with canine "necrosin"-immune rabbit serum. We have tested 9 sera from normal non-menstruating women (5 were pregnant), 4 from menstruating women, and 4 from patients with toxemia of late pregnancy against the sera of 3 rabbits injected repeatedly with canine "necrosin" to a precipitin titer of 1:1000 or over. Negative results were acquired with all 9 of the control sera, whereas the other 8 all gave titers of 1:1000 or over. Four of these positive sera (3 from menstruating women and 1 from a patient with pre-eclampsia) gave negative tests after standing 2 to 3 weeks in the refrigerator. This is in accord with the known lability of the toxic factor and further substantiates the specificity of this test. It would appear that a substance identical to menstrual "toxin" and canine "necrosin" is present in the circulating blood of menstruating and toxemic women.

One of our original purposes in looking for a substance similar to menstrual "toxin" in other than human material was to obtain, for possible clinical use, an immune serum from which antibodies to non-specific human proteins would be eliminated. The difficulties encountered in our attempts to protect rats against menstrual "toxin" with "necrosin"-immune rabbit serum have been reported in the preceding paper.[2] Of even greater prom-

[3] Smith, O. W., and Smith, G. V., *Science*, in press.

* The fibrinogen, thrombin, and immune serum globulins employed in these investigations were kindly supplied by Prof. E. J. Cohn and Dr. J. T. Edsall of the Department of Physical Chemistry, Harvard Medical School, Boston, Massachusetts. These products were prepared 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.

ise, however, from the therapeutic angle has been the finding that a protective substance is always present in the same material that contains the toxic factor and appears to be concentrated in the pseudoglobulin fraction. These fractions were prepared by half saturation of the material with $(NH_4)_2SO_4$ after removal of the euglobulins either by dialysis or by one-third saturation with $(NH_4)_2SO_4$. In the experiments reported, the equivalent of 2.5 to 5.0 cc of the original material was used for each test. Following the previously published technic,[4] we have been able to demonstrate consistently a significant prolongation of survival time of rats after the injection of a lethal dose of toxin by the simultaneous administration of the pseudoglobulin fractions of menstrual discharge (6 experiments), of canine pleural exudate (3 experiments), and of human pleural exudate from metastatic cancer of the breast (3 experiments, in all of which protection against death was complete). Furthermore, in 19 experiments with the pseudoglobulin fractions of venous sera collected during menstruation and in 7 with the pseudoglobulins of sera from patients with toxemia of late pregnancy, definite protective action was demonstrable, death being completely prevented in 5 instances when pseudoglobulins of venous sera from menstruating women were given. In 14 control tests, no protection against a lethal dose of menstrual toxin was afforded by the administration of pseudoglobulin fractions of sera from normal males, normal non-menstruating females or normally pregnant women or of the ascitic fluid from a patient with hepatic cirrhosis. Concentrated immune serum globulins from normal human blood* were also ineffective. The therapeutic possibilities of these findings in toxemia of pregnancy are apparent. Since the "toxin" appears to be a product of cellular injury from tissue damage other than that involving hormone withdrawal,[2] even more generalized clinical implications are involved. The nature of the protective substance remains obscure, but the fact that it is apparently found only in association with the "toxin" supplies further evidence in favor of the presence of this "toxin" in the circulating blood of menstruating and toxemic women.

Conclusion. Serum from the circulating blood of women during menstruation and toxemia of late pregnancy differs from that of normal non-menstruating and normally pregnant women and resembles menstrual "toxin" in that it is pyrogenic and fibrinolytic, gives positive precipitin tests with the serum of rabbits immunized against canine "necrosin," and contains a pseudoglobulin capable of prolonging the survival time of rats after a lethal dose of menstrual toxin. It is concluded that a factor identical to menstrual "toxin" is present in the circulating blood of menstruating and toxemic women. The protective factor is being further investigated as to its possible therapeutic applications.

We are indebted to Dr. William J. Mulligan of the Boston Lying-in Hospital for obtaining blood from pregnant patients and to the nurses of the Free Hospital for Women for donating their blood for the studies on non-pregnant women.

[4] Smith, O. W., and Smith, G. V., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 285.