has prevented the testing of sera from infected gerbilles.

The possibility of naturally occurring infections of these species with *R. orientalis* has been discounted since this disease has never been known to occur in Egypt.

As already indicated, these rodents have been shown to be susceptible to epidemic and murine typhus infection. The finding described herein, namely, that they are also susceptible to tsutsugamushi infection enhances their value as experimental animals. Since infection results in death of these rodents, they are particularly suitable for titrations of tsutsugamushi inocula, thus making possible their use in vaccine assay, in neutralization tests, and in the trial of therapeutic drugs. They are satisfactory animals for strain maintenance and yield a rich source of infectious material. Their value in strain isolation cannot be estimated as no attempts have yet been made with them. They can, of course, receive a greater amount of inoculum than may be placed in the anterior chamber of a rabbit's eye. Furthermore, it was found that a much higher percentage of successful transfers resulted from rabbit-to-gerbille than from rabbit-to-rabbit passages. This made the gerbilles much more useful, both as for certainty of infection and as a richer source of infectious material for further passage.

It is not intended to imply, however, that these animals possess any great superiority over laboratory-bred white mice for the study of tsutsugamushi disease. Rather, *G. pyramidum* and *G. gerbillus* were discovered to be suitable substitute animals in the absence of a supply of white mice.

*Summary.* Experimental evidence indicates that the two rodents, *Gerbillus gerbillus* and *Gerbillus pyramidum* are readily susceptible to large doses of scrub typhus rickettsiæ. One hundred percent of the animals succumbed to the disease when inoculated by the intraperitoneal, subcutaneous, or intravenous route. Smears of the peritoneal and pleural exudates showed varying numbers of minute phomorphic rickettsiæ within the cytoplasm of the cells. It was found that a much higher percentage of successful transfers resulted from rabbit-to-gerbille than from rabbit-to-rabbit passages. For this reason gerbilles were found to be much more useful both as for certainty of infection and as a richer source of infectious material for further passage than rabbits. They are suitable substitute animals in the absence of a supply of white mice.

The author wishes to express his appreciation to Lt. Col. M. H. P. Sayers, R.A.M.C., for making available to them the Imphal and Calcutta strains; Major M. T. Parker, R.A.M.C., for the Imphal 8 and Imphal 13 strains; and Dr. S. R. Savoor for the Ceylon strain.

The assistance of Sgt. J. Dworkowitz and Corporals D. Hogan, R. Stearman, and R. Cassell in these studies is gratefully acknowledged.

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## Evidence that Menstrual "Toxin" and Canine "Necrosin" are Identical

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The similarities between the toxic material in menstrual discharge[1] and the more recently described injury factor, termed "necrosin," of pleural exudates[2] suggested to us that these substances might be identical.[3] It is merely for the sake of brevity that either is referred to as a single substance, since neither has been

[1] Smith, O. W., and Smith, G. V., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 100.

[2] Menkin, V., *Science*, 1943, **97**, 165.

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

purified to any degree, but the further investigations herein reported establish many points of similarity and give evidence that both contain an identical toxic protein.

*Materials.* Menstrual discharge has been obtained from normal women by means of rubber cups. Emphasis has been placed on refrigerating and using it as soon as possible after collection.

Pleural exudate from dogs following the injection of turpentine has been our source of necrosin.[4] Dr. Menkin kindly supplied us with a limited amount for our earlier experiments, the rest having been prepared by us. The fluid from the first tap, 24 hours after turpentine, usually clots soon after removal. At the 48-hour tapping it may or may not clot. When clotting occurs the pH is around 7 or over. After 48 hours the exudate does not clot. The pH of exudates that fail to clot is under 7, usually 6.4 to 6.8.

*Similarities between menstrual "toxin" and canine necrosin.* 1. Death of 19- to 21-day-old male or female rats within 48 hours after subcutaneous injection has been our criterion of toxicity. The toxicity of menstrual discharge is most concentrated in the endometrial debris which separates as a middle layer, upon centrifugation, between the red blood cells and the "serum." It is proportional to the amount of debris which is greatest when the rate of flow is slow and the pH of the fluid around 6.8.

The toxicity of whole canine pleural exudate differs greatly with different specimens and is rarely as marked as in menstrual discharge. The most toxic exudates are usually obtained 48 to 96 hours after the injection of turpentine when the fluid is thin and cloudy, does not clot, and has a pH around 6.8 or under. After centrifugation, the cellular debris which settles is about 10 times as toxic as the supernatant fluid. Toxicity is proportional to the amount of this debris. In both exudate and discharge, then, the toxicity is most concentrated in, and presumably derived from, tissue elements.

2. If, after centrifugation, the clear "serum" of menstrual discharge and the supernatant exudate are treated by methods which precipitate the euglobulins, the toxicity is concentrated in this fraction, indicating that both toxin and necrosin either are proteins of large molecular size or associated with such proteins. They are, however, atypical, compared with the euglobulin fraction of normal venous serum, being insoluble in physiological saline solution. With some specimens the water-insoluble portion after dialysis of the euglobulin precipitate from ammonium sulphate treatment forms a heavy suspension in physiological saline solution which is not thrown down when centrifuged at 3,500 r.p.m. We have noted this more often in preparing necrosin than menstrual toxin. When the supernatant fluid is clear, it is practically non-toxic.* Throughout this communication the terms menstrual "toxin" and "necrosin" refer to a suspension in physiological saline of the water-insoluble portion after dialysis of the euglobulin precipitate.

3. The toxicity of both discharge and exudate is greatly diminished or destroyed by heat, exposure to air, and changes in pH. Partially purified preparations, even in the dry state, are particularly labile. They may lose toxicity in the process of drying, even during dry-freezing. Even if ammonium sulphate is added to fresh materials to one-third saturation and they are refrigerated in this state until use, the toxicity is much diminished and sometimes entirely lost after 2 to 4 weeks. This lability has hampered attempts at purification, standardization, and immunization.

4. The pathological findings in a rat that has received a lethal dose of necrosin are

[4] Menkin, V., *Arch. Path.*, 1943, **36**, 269.

* Menkin has recently reported[5] further purification of necrosin by removing from the ammonium sulphate euglobulin precipitate that portion which is insoluble in water in the presence of sulphate ions. In our experience there is no insoluble portion at this stage except when whole exudate and discharge are processed, in which case the material which does not dissolve if water is added consists entirely of debris. Menkin has stated that this residue, after repeated washings with distilled water, is pyrogenic but non-toxic. Our only explanation for this is that the washing of the debris with ordinary distilled water, which is acid, greatly reduces its toxicity.

[5] Menkin, V., *Arch. Path.*, 1945, **39**, 28.

entirely similar to those described following the injection of menstrual toxin.[1] Furthermore, Dr. Menkin, while in this laboratory, found that the intracutaneous injection of our toxin in the rabbit elicited the same local response as necrosin.[2]

5. Menstrual discharge and necrosin[2] both hasten the clotting of blood.

6. We have amply confirmed Menkin's observation[6] that canine necrosin, given intravenously, is pyrogenic in the rabbit, very small quantities causing a 2.5 to 5°F rise in rectal temperature about 3 hours after injection. Menstrual toxin gives the same type of curve, even in the minute amounts that must be used to avoid killing the animals. The pyrogenic factor of both exudate and discharge is concentrated in the euglobulin fraction. The induction of fever is apparently not due to a foreign or non-specific protein reaction or to the intravenous injection of a suspension. It is equally marked when filtered menstrual serum or exudate is given and has failed to occur with saline suspensions of dried human placenta or preparations of exudate which had lost toxicity in the process of drying.

7. Menkin has referred to unpublished data indicating the presence of proteolytic activity in the necrosin fraction of canine pleural exudate.[5] Using a simple technic,[7] we have consistently been able to demonstrate complete dissolution of a fibrin clot with menstrual toxin[8] and have found 3 different active preparations of canine necrosin to be as markedly fibrinolytic as the human material. The enzyme is not demonstrable in the euglobulin fraction of normal human serum. Specimens that have lost toxicity may completely retain fibrinolytic activity, indicating that the toxin and enzyme are not the same.

*Identity of menstrual toxin and canine necrosin.* 1. It has been shown that repeated injections of menstrual toxin[3] or necrosin[2] into rabbits induce precipitin antibodies. Since, of course, neither material is a pure protein, the antibodies may have been non-specific. If, however, the serum of a rabbit immunized against menstrual toxin precipitated canine necrosin and *vice versa,* the evidence for an identical factor in these materials of different origin in different species would be quite definite. We have found this to be the case.

The sera of 3 rabbits with precipitin titers against menstrual toxin of 1:1000 or over consistently gave equally high titers against 8 samples of active canine necrosin but negative tests against 8 other preparations of canine necrosin in which no toxicity was demonstrable. Moreover, the sera of 10 rabbits immunized against canine necrosin to a titer of 1:1000 or over gave equally strong reactions with 12 specimens of active menstrual toxin but negative tests with 3 non-toxic samples.

For further controls, 9 sera from normal women and 2 from a normal male were tested against 3 of the canine necrosin-immune rabbits. All were negative. Control tests with normal canine serum against that of menstrual toxin-immune rabbits introduced an enigma. With 5 of 11 sera from 8 apparently healthy dogs, positive precipitin reactions were obtained. These same canine sera gave negative tests against normal rabbit serum, normal human serum, and that of a rabbit with a titer of 1:100,000 against normal human serum, thus appearing to rule out natural antibodies as an explanation. Obtaining unexpected positive tests with normal canine serum makes us wonder whether or not dogs may not often have the injury factor in their circulation, *e.g.,* from heartworms,[9] which are a common affliction of experimental dogs in this vicinity.

2. We have shown that the serum of rabbits immunized with menstrual discharge prolongs the survival of immature rats given a lethal dose of menstrual discharge.[3] This serum has also been found to delay significantly death from canine necrosin. The protective factor is concentrated in the pseudoglobulin fraction. To obtain even this partial protection required a precipitin titer of at least 1:1,000,000 in the immunized rabbits. We have not yet been able to induce so high a titer in rabbits immu-

[6] Menkin, V., *Federation Proc.,* 1944, **3**, 32.

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

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

[9] Phillips, J. H., *Am. J. Hygiene,* 1939, **29**, 121.

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.