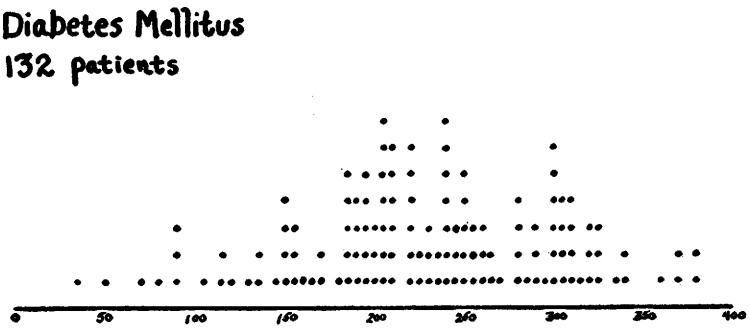

FIG. 3.

so closely parallels that of the larger unselected group of patients that one can infer no particular unsaturation of Vitamin B_1 in clinical diabetes mellitus.

11368

Menstrual Discharge of Women. I. Its Toxicity in Rats.*

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In an effort to find a factor to which the local processes resulting in menstruation might be attributable, 37 specimens of menstrual discharge, each varying from 30 to 120 cc in amount, donated by 5 normally menstruating, parous women, have been studied. They have been collected by means of soft rubber cups.† During collection each portion has been placed in the refrigerator immediately upon removal. For the most part, the experiments to be reported have been performed upon the whole specimen after pooling the various portions. For control experiments, whole venous blood, drawn during the first day of menstruation and citrated or mixed with sufficient distilled water to prevent clotting and kept in the refrigerator, has been used in similar or larger amounts.

Menstrual discharge has been found to be highly toxic to rats, their resistance being markedly affected by hormonal conditions.

* 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.

† The Hy-Kup Distributors (National), Indianapolis, Indiana.

Toxicity varies with different specimens and is more concentrated in the cells and debris than in the "plasma." Whole menstrual discharge given subcutaneously in 0.1 to 1.0 cc amounts (depending upon the specimen) twice daily to normal mature females, starting when they are in preëstrus, often results in death within 48 hours. Within 24 hours or less of the first injection the animals are apprehensive and "hunched up"; their fur is ruffled; water intake increases; the nose and inner canthi of the eyes become encrusted with blood and there is firm edema of a wide area around the site of injection. Death is not ushered in by convulsions. At autopsy, the adrenals are of a dark reddish-brown color, the liver is usually dark and mottled and the lungs are congested, as compared with controls. Occasionally blood is found in the urinary bladder. Under the microscope the lungs show edema and capillary hemorrhage, the kidneys varying degrees of parenchymatous degeneration, congestion and often capillary hemorrhage. The liver shows some degeneration. The most consistent picture is seen in the adrenal cortex, diffuse or focal hemorrhage and dissolution of cells in either or all zones. In the adrenal cortices of rats that have died early, hemorrhage may be the only finding. With increased length of survival, dissolution to actual necrosis of cells is also seen, along with increased vacuolization.

Animals in which injections are started during the beginning of postestrus are most likely to survive and become comparatively resistant to continued injections. On exploration after the third or fourth day, their ovaries contain large, red corpora lutea. The subcutaneous edema becomes replaced by brawny induration, so that in less than 15 days the stiffness and fixation of the pelt prohibit further injections.

If each dose of menstrual discharge is accompanied by the subcutaneous injection of 1 to 2 r.u. of a native estrogen (we usually have used 0.05 γ of estradiol), the animals almost invariably die within 48 hours of the first injections, regardless of the time of the cycle at which administration is started. Clear "plasma" from the discharge is usually innocuous in normal mature female rats in fairly large amounts (1 to 2 cc twice daily) at any stage of the cycle unless estrogen is concomitantly given, in which case the typical rapid death ensues. In control experiments with venous blood or serum with or without estrogen, the only deleterious effect noted was subcutaneous induration when more than 2 cc of whole venous blood was administered daily.

The most consistently lethal effect has been noted in 19- to 24-

day-old female rats. Thirty-five of the 37 specimens so tested have produced death, when given twice daily, in less than 48 hours from the first injection, the total dose varying with different specimens between 0.01 and 0.8 cc. The only 2 specimens non-lethal in 0.8-cc amounts in immature females were from the same donor and had been collected during the first day of the period when flow was profuse and contained little or no debris. In this individual, specimens during the last days of flow contained much brown debris and were very much more toxic, a single injection of 0.01 to 0.1 cc being usually lethal to immature female rats. Two to 4 cc of whole venous blood in 4 doses over 2 days is easily tolerated by immature female rats. Spayed immature female rats, and male rats, both immature, mature and castrated mature, have a relatively greater resistance to the discharge, and mature females, spayed more than 4 weeks previously, have tolerated even larger amounts. The administration of estrogen to spayed females does not render them more susceptible to the discharge. In 2 experiments, a mash of 6 to 7 fresh mature rat ovaries injected simultaneously with each dose of the discharge and estrogen produced the typical rapid death in spayed females; the control spayed females receiving the same amounts of the same discharge and estrogen survived.

From these observations, it would appear that the greatest susceptibility to the menstrual toxin depends upon the presence of the ovaries and that corpora lutea afford partial protection, but that the administration of estrogen overrides this protective action.

The toxicity of the discharge is destroyed by heat, ethyl alcohol, acetone or acid, in amounts sufficient to precipitate the proteins. It is diminished by raising the pH to over 8 or by allowing the specimens to become putrid either by standing at room temperature for a few days or in the refrigerator for at least 3 weeks. Dried rapidly *in vacuo* over $CaCl_2$, powdered, sealed and refrigerated, the material retains its toxicity for at least 8 months. The toxin is not soluble in the usual lipoid solvents and is nondialysable. After fractional precipitation with $(NH_4)_2SO_4$, it is found in greatest concentration in the water insoluble portion (after dialysis) of the englobulin precipitate.

Mature female rats have been completely protected against a lethal dose of menstrual discharge plus estrogen by the preliminary and simultaneous administration of large amounts of progesterone, to a total of 15 to 30 mg. Chorionic gonadotropin, if given so as to produce a good luteinizing response by the time a M.L.D. of the discharge is injected, protects immature female rats, but not if

estrogen be given with the discharge. Chorionic gonadotropin does not save mature females from the combined injection of discharge and estrogen. Desoxycorticosterone acetate (10 to 20 mg) does not prevent death from a M.L.D. of discharge in immature females or from discharge plus estrogen in mature females even when its administration is begun 2 days before injecting the toxin. Adrenal cortical extract (Eschatin, P. D. & Co.) in 1-cc doses twice daily for 2 days before and during the day of injection of twice the M.L.D. of discharge completely protected an immature female; a littermate control receiving the same amount of the same discharge on the same day died in 20 hours.

Immature and mature female rats may be rendered resistant to several times the M.L.D. of discharge alone or discharge plus estrogen by injection of sublethal doses twice a week for 2 to 3 weeks. Such immunity has been maintained for as long as 4 months. Mature female rabbits are extremely susceptible to the discharge alone, 1 to 3 subcutaneous injections of 1 cc usually resulting in death within 48 hours. By extreme caution, 1 rabbit was made to survive 14 days of small injections. Its serum, in 5 1-cc amounts over 2½ days prior to the injection of twice the M.L.D. of a specimen, completely protected an immature female rat. A pseudoglobulin fraction of this same serum also protected immature female rats against lethal doses of 2 other samples of discharge from 2 different donors.

Only by preliminary treatment were we able to protect rats against the menstrual toxin with progesterone, adrenal cortical extract or immune serum. After the discharge had been administered, even massive amounts of any of these failed to prolong the period of survival.

In an attempt to determine whether the toxicity of menstrual discharge is due to a specific toxin or simply to products of bacterial action or protein decomposition, the following experiments were run. Sterile citrated whole venous blood was incubated for 48 hours after inoculation with discharge. This material was toxic to immature rats but not lethal, even in amounts 3 to 5 times the M.L.D. of fresh discharge. The striking edema which characterizes the reaction to menstrual discharge was lacking in rats injected with inoculated venous blood. Furthermore, it was found that sterile venous blood alone, after 48 hours at incubation temperature, is as toxic as inoculated material. These experiments, therefore, gave no conclusive evidence either for or against the specificity of menstrual toxin. Discharge collected on the fourth day of flow in a sterile cup following a douche with 2 quarts of water killed an

immature female rat in 30 hours. This material was given in a single injection of 0.25 cc within 40 minutes from the time it passed the cervix. Its toxicity would seem to rule out bacterial activity or any protein decomposition other than what might occur in the uterus. The strongest indication of a specific toxin lies in the repeatedly confirmed observation that 1 to 2 cc twice daily of specimens of whole discharge or "plasma," which alone in these amounts are non-toxic to normal mature female rats, are lethal within 72 hours from the first injection when estrogen is simultaneously administered, whereas such is not the case with venous whole blood or serum.

Conclusion. The menstrual discharge of women with normal cycles is highly toxic to rats through the production of vascular damage. The possibility that this toxicity is accountable to protein decomposition has not been conclusively ruled out, although the marked effect of hormonal conditions upon resistance appears to argue against this. The greatest susceptibility requires the presence of the ovaries. Functional corpora lutea afford partial protection, but the administration of estrogen overrides this action. Protection may be rendered by pretreatment with large amounts of progesterone, adrenal cortical extract or "immune" rabbit serum. The toxin appears to be intimately associated with a large moleculed protein material.

11369 P

Menstrual Discharge of Women. II. Its Progesterone-Stimulating Effect in Mature Rats.*

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Early in the study of the toxicity of the catamenial discharge[1] it was noted that mature female rats, with previously regular cycles, which survived the first 2 or 3 days of injections went, within 72 hours, into constant diestrus on continued injections and reverted

* 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.