was made up in frog Ringer's solution in concentrations of 0.2, 0.4, 0.8, 1.6, 2.4, and 3.2 grams percent. Control eggs were covered with a mixture of one part frog blood to one part Ringer's. A third set of eggs were pricked in the absence of any solution. In this way it was possible to compare the effectiveness in producing artificial parthenogenesis of heparinated blood with the effectiveness of plain blood of the same concentration. After treatment the eggs were immediately transferred to pond water which had been boiled and filtered, and were kept at room temperature. Four to 5 hours later, counts were made of the number of divided cells and the total number of cells. No attempt was made to distinguish between normal and abnormal divisions.

The results are summarized in Table I, which shows the percent cleavage obtained when the eggs were pricked in the presence of blood and Ringer's fluid (0% heparin in column 1), and in the presence of blood and heparin of various concentrations (columns 2-7). In every case the percent of dividing eggs was lower when the heparinated blood was used than when the blood-Ringer's solution was used. In only one case, however, was the percent as low as in eggs treated by pricking alone. The values obtained for the experimental eggs were calculated as percent

† Hynson, Westcott and Dunning, lots 198 and 202.

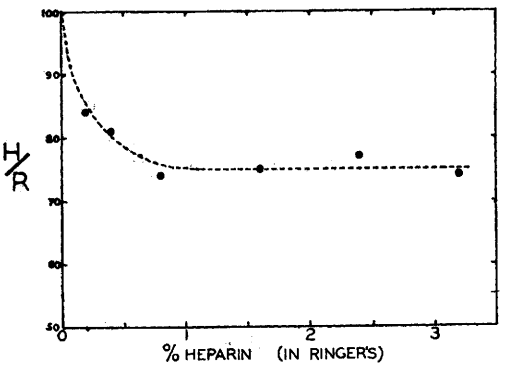

FIG. 1.
Inhibition by Heparin of Artificial Activation.

$$H/R = \frac{\%\ \text{Cleavage in Blood-Heparin Solution}}{\%\ \text{Cleavage in Blood-Ringer's Solution}} \times 100$$

of the blood-Ringer's controls, averaged for each concentration, and they were then plotted in Fig. 1. The 100% line represents the cleavages in the blood-Ringer's fluid. From this figure it can be seen that there is a definite inhibition by heparin of activation by pricking in the presence of blood. This lends further support to the colloidal theory of cell division.

Summary. Eggs of *Rana pipiens* were pricked in the presence of heparinated frog blood. The percent of dividing eggs was lower in every case than when the eggs were pricked in the presence of blood and Ringer's fluid

Received March 11, 1949. P.S.E.B.M., 1949, **71**.

17063. Failure of Rutin to Inhibit Hyaluronidase in the Albino Rat.

SAMUEL K. ELSTER. (Introduced by Joel Warren.)

From the Department of Chemistry and Physics, Army Medical Department Research and Graduate School, Army Medical Center, Washington, D.C.

Rutin, a naturally occurring flavanol glucoside, has been found to influence favorably various states characterized by an increased capillary fragility.[1] It has been demonstrated that hyaluronidase increased the speed of passage of fluids and the blue dye T-1824 across the capillary membrane in the albino rat.[2] Chambers and Zweifach[3] have considered that hyaluronidase accentuated capillary fragility rather than produced direct changes in capillary permeability. *In vitro,* rutin in high

1 Griffith, J. Q., Jr., Couch, J. F., and Lindauer, M. A., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 228.

2 Elster, S. K., Freeman, M. E., Dorfman, A., *Am. J. Physiol.,* 1949, in press.

3 Chambers, R., and Zweifach, B. W., *Physiol. Rev.,* 1947, **27**, 436.

concentrations effectively inhibited hyaluronidase, but was inactive in lower concentrations.[4] Levitan[5] tested this reaction *in vivo* in the albino rat, and concluded that rutin markedly inhibited the spreading activity of intradermally injected inked hyaluronidase.

A technic has been described[6] in which the activity of hyaluronidase was tested in the albino rat. Following the intravenous administration of hyaluronidase, changes in blood volume occurred. Edema of the extremities appeared and marked elevation of the blood hematocrit was noted. A substance may be tested for its ability to inhibit this reaction, if it does not of itself alter the fluid balance of the body. Thus, a substance may be considered a hyaluronidase antagonist if, after its administration, the hematocrit does not rise following the injection of the standard amount of enzyme.

This technic has been applied to the study of rutin in the body and its possible role in hyaluronidase inhibition.

Experimental. 136 male albino rats of the Sherman strain were fed Purina laboratory chow and water *ad lib.* The hyaluronidase was prepared according to the method of Freeman *et al.*[7] The enzyme was assayed[8] and measured 3000 turbidity reducing units (T.R.U.) per mg of nitrogen.

1) *The effect of the route and mode of administration of rutin on the hematocrit.* 64 animals, weighing 200-350 g were divided into 5 groups:

Group A—20 normal rats.

Group B—15 rats, 200 mg rutin suspended in 1 ml normal saline fed orally (via stomach tube) for 4 successive days.

Group C—9 rats, 200 mg rutin suspended in 1 ml normal saline injected intraperitoneally.

Group D—10 rats, 1 ml propylene glycol injected intraperitoneally.

Group E—10 rats, 200 mg rutin dissolved in 1 ml propylene glycol injected intraperitoneally.

All animals were bled from the aorta and sacrificed. The hematocrit was determined by centrifugation at 3000 rpm for 30 minutes.[9] Blood was drawn from Groups C, D and E 3½ hours following the injections and from Group B 1½ hours following the last gastric administration.

Marked changes were noted in the animals in Groups C, D and E. They were lethargic and their abdomens were protuberant. When their abdominal cavities were opened, several ml of free fluid were encountered. In Groups C and E, this fluid was light yellow; rutin was precipitated on the peritoneal surfaces of the organs. The abdominal fluid encountered in the animals of Group D was slightly cloudy and colorless. The intestines and peritoneum were inflamed and edematous. The most severe changes were noted in the animals of Group E, in which as much as 8 ml of fluid were removed from the abdominal cavity. The animals of Groups A and B appeared normal.

The changes in plasma volume are reflected in the hematocrit determinations (Fig. 1).

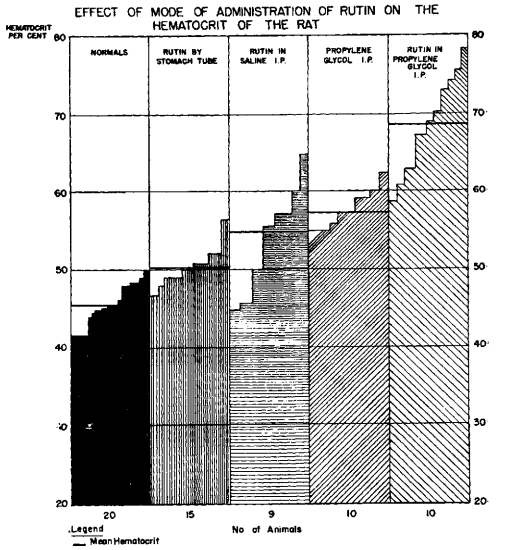

FIG. 1.

[4] Beiler, J. M., and Martin, G. J., *J.B.C.*, 1947, **171**, 507.

[5] Levitan, B. A., PROC. SOC. EXP. BIOL. AND MED., 1948, **68**, 566.

[6] Elster, S. K., Freeman, M. E., Anderson, P., *J. Lab. and Clin. Med.*, 1949, in press.

[7] Freeman, M. E., Anderson, P., Oberg, M., and Dorfman, A., 1949, in press.

[8] Dorfman, A., and Ott, M. L., *J.B.C.*, 1948, **172**, 367.

[9] Wintrobe, M. M., Clinical Hematology, Lea & Febiger, 1947, Philadelphia.

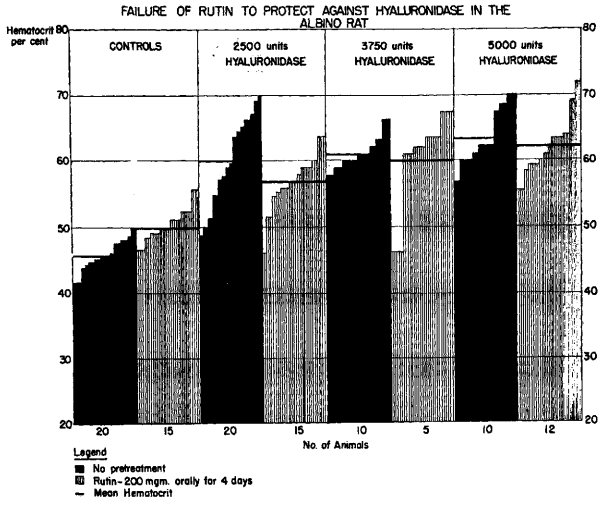

FIG. 2.

The mean hematocrit of Group A, 46.0%, was representative of normal values obtained for a similarly constituted normal population of 150 male rats. All other groups showed evidence of hemoconcentration. The mean hematocrit value for Group C was 54.4%, Group D 57.1% and Group E 68.5%. A few animals of Group C had a normal hematocrit, but none of Group D or E was normal. Group E had the greatest clinical effects and the greatest hemoconcentration. Every animal of this group had severe diminution of plasma volume. The hematocrits of the rats in Group B were slightly elevated; the mean value was 50.3%. Most animals of this group had little or no change of hematocrit and only one had a moderate increase (56%). Therefore, the oral route was selected as the best available means of rutin administration for the second part of the experiment, since it caused the least change in the hematocrit.

2) *The role of rutin in hyaluronidase antagonism in vivo.* 72 animals weighing 160-230 g were used. Thirty-two animals were fed 200 mg rutin in 1 ml saline by stomach tube for 4 successive days. On the last day 1½ hours following the gastric administration, 15 rats were given 2500 T.R.U., 5 rats were given 3750 T.R.U. and 12 animals were given 5000 T.R.U. hyaluronidase by the intravenous route. At the end of 30 minutes, they were bled from the aorta and the hematocrit of the blood determined. Forty animals were not pretreated with rutin; 20 rats were given 2500 T.R.U., 10 rats were administered 3750 T.R.U. and 10 were given 5000 T.R.U. hyaluronidase intravenously. These were bled and sacrificed 30 minutes after the injection and the blood hematocrit determined.

The animals that received hyaluronidase alone, developed edema of the extremities and face within 5-15 minutes; this was maximal in 30 minutes. In a previous communication[2] it has been reported that the edema began to recede in 2 hours and had disappeared completely in 24 hours. The hematocrits of the blood were markedly elevated (Fig. 2); the mean values were 60.0 to 62.9%. The group that was pretreated with rutin and then received hyaluronidase intravenously was indistinguishable from the previous group. Edema of equal intensity and duration appeared in the same areas. The hematocrits of these animals were elevated and the mean values were not significantly different from that group receiving hyaluronidase alone. Although the range of values was different, the mean hematocrit (57.1%) of the animals receiving 2500 units hyaluronidase and rutin was not statistically different from the mean (60.0%) of the group receiving only hyaluronidase. In the groups receiving the larger amounts of hyaluronidase, only one animal failed to respond with a rise of hematocrit.

Discussion. In the evaluation of the inhibitory action of rutin on hyaluronidase *in vivo,* the method of rutin administration must be considered. It has been demonstrated that 200 mg rutin suspended in saline, when given intraperitoneally to a rat, acted as an irritant and caused the formation of hydroperitoneum. Propylene glycol had a similar action. Rutin dissolved in propylene glycol was the most active and caused so much peritoneal fluid loss that marked hemoconcentration occurred. In the experiments of Levitan, 200 mg rutin dissolved in 1 ml propylene glycol were given intraperitoneally to rats weighing 250-400 g. Three and one-half hours later, testicular hyaluronidase and india ink were injected into the skin and the rats were sacrificed 22 hours after that. There was marked inhibition of the spread of the hyaluronidase-ink mixture, as well as a decrease in the spread of the control ink and saline. Propylene glycol alone, when given intraperitoneally, caused a smaller decrease in the spread of the hyaluronidase. Since both of these agents have been demonstrated to cause extreme hemoconcentration when given intraperitoneally, the validity of conclusions as to the efficacy of these drugs on hyaluronidase inhibition is open to extreme doubt. Duran-Reynals[10] has emphasized that "in the living animal a permeating effect on blood capillaries takes place which may contribute to the final phase of the spreading." In an animal in a condition of marked diminution of blood volume, the available fluid for the spreading reaction is greatly diminished and the conditions for spread are not optimal.

Rutin has been found to inhibit hyaluronidase *in vitro,* and this inhibition has been potentiated by Vitamin C. These studies have demonstrated that rutin, when administered orally, failed to modify the action of hyaluronidase on edema formation and elevation of hematocrit in the albino rat. Rutin in the body is presumed to decrease capillary fragility. However, the investigations performed at this laboratory have failed to produce evidence that hyaluronidase increased capillary fragility, as the edema is not accompanied by red blood cell extravasation.

Summary. 1. Hyaluronidase increased the diffusion of fluid across the capillary membrane and cause elevation of the blood hematocrit.

2. Rutin, in the amounts used in these experiments, failed to inhibit this reaction in the albino rat.

3. Rutin in propylene glycol, given intraperitoneally to the albino rat, caused the formation of hydroperitoneum and marked hemoconcentration. Propylene glycol given by the same route had a similar, although lesser, effect. When administered orally, rutin caused little elevation of the hematocrit.

The assistance of Miss Elaine Lowry is gratefully acknowledged. The author is indebted to Major Monroe E. Freeman, MSC, for the hyaluronidase preparations.

Received March 15, 1949. P.S.E.B.M., 1949, **71.**

[10] Duran-Reynals, F., *Bact. Rev.,* 1942, **6,** 197.

17064. Role of Potassium in Dialysing Fluid in Treatment with the Artificial Kidney.

J. WENER,* AND N. K. M. DE LEEUW.† (Introduced by J. S. L. Browne.)

From the McGill University Clinic and the Department of Surgery, Royal Victoria Hospital, Montreal, Canada.

Spontaneous elevations of serum potassium levels with electrocardiographic changes similar to those found in dogs with experimental anuria[1] have been demonstrated in man during the terminal stages of uremia,[2-6] indicating that hyperpotassemia may be an important

* C. Rutherfurd Caverhill Fellow in Medicine, McGill University.

† Archibald Fellow in the Department of Experimental Surgery, McGill University.

[1] Hoff, H. E., Smith, P. K., and Winkler, A. W., *J. Clin. Invest.,* 1941, **20,** 607.