17062. Effect of Heparin on Artificial Activation in the Frog Egg.*

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It is a well known fact that protoplasmic gelation precedes mitosis. The colloidal theory of cell division likens this protoplasmic gelation to blood clotting (see Heilbrunn[1] for discussion). It would be expected, therefore, on the basis of this theory that anticoagulants, such as heparin, might prevent protoplasmic gelation. Indeed, Heilbrunn and Wilson[2] have shown that this gelation in the Chaetopterus egg can be prevented by heparin and, moreover, that cell division is subsequently inhibited. Heparin also inhibits cell division in tissue cultures (Fischer[3]).

The most effective method of producing artificial parthenogenesis in the frog egg is by pricking the egg in the presence of blood or blood serum (Bataillon[4]). It was thought that the active principle in the blood might be a thrombin or thrombin-like substance which could initiate a protoplasmic gelation resulting in cell division. If this were true, anticoagulants should have some effect on parthogenesis in preventing the action of blood serum. The following experiments indicate that such is indeed the case.

Rana pipiens females were induced to ovulate by the implantation of pituitary glands, and the ovulated eggs were stripped in rows on to glass slides immediately before use. The eggs were covered with the blood mixture to be tested, and then pricked with a fine glass needle. For a discussion of the technic employed see Rugh.[5] Mixtures consisting of one part frog blood collected by heart puncture to one part heparin solution were employed in the above procedure. The heparin†

TABLE I.
% Cleavage of Eggs Pricked in 1:1 Mixtures of Blood and Heparin Made Up in Ringer's Solution.

Concentration of Heparin in g %						
0.0	0.2	0.4	0.8	1.6	2.4	3.2
68	60	—	—	—	—	—
41	—	25	19	—	—	—
63	—	—	54	42	—	—
46	38	40	43	24	—	—
62	56	55	56	46	—	—
70	—	—	51	50	—	56
67	—	—	—	66	—	63
72	—	—	69	72	—	65
61	—	—	44	48	—	44
64	—	—	40	43	46	34
49	42	48	—	—	—	—
61	—	45	38	45	48	32
76	63	74	—	—	69	62
77	72	67	—	—	65	55
77	—	—	—	—	66	—
67	61	65	—	—	63	60
59	39	23	—	—	31	—
68	68	54	—	—	60	—
51	29	—	—	—	26	—
43	—	—	—	—	32	—
64	—	—	36	45	—	38

* Aided by a grant from the U. S. Public Health Service, administered by L. V. Heilbrunn.

1 Heilbrunn, L. V., *An Outline of General Physiology*, 2nd ed., Philadelphia, 1943.

2 Heilbrunn, L. V., and Wilson, W. L., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 179.

3 Fischer, A., *Arch. f. path. Anat. u. Physiol.*, 1930, **279**, 94.

4 Bataillon, E., *Compt. rend. Acad. Sci. Paris*, 1911, **152**, 920.

5 Rugh, R., *Experimental Embryology*, New York, 1941.

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.

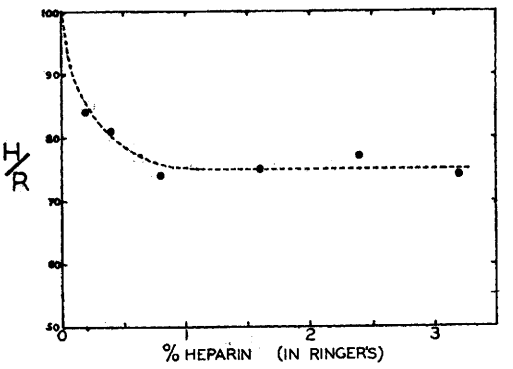

FIG. 1.
Inhibition by Heparin of Artificial Activation.

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

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.

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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.