have been responsible for the poor action of streptomycin against these particular gram negative organisms.

The strains of *S. aureus* and *S. typhimurium* were resistant to the sulfonamides employed. *Str. agalactiae* and *C. pyogenes* were susceptible to sulfapyridine, sulfone, sulfadiazine and sulfamerazine. *In vivo* study is necessary to prove these drugs of value as adjuncts to penicillin in the treatment of chronic streptococcic mastitis and so-called "summer mastitis." *E. coli* was sensitive to sulfapyridine, sulfamethazine, sulfadiazine, and sulfamerazine among which sulfamerazine and sulfamethazine showed great activity. *Br. abortus* was susceptible to sulfamethazine, sulfadiazine, and sulfamerazine. *Ps. aeruginosa* was inhibited by sulfamerazine and sulfapyridine. Against the strain of *Ps. aeruginosa* employed, sulfadiazine was the most effective antibacterial agent.

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The Effect of Heparin on Cell Division.*

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When a living cell is incited to divide, its protoplasm first undergoes a sharp increase in viscosity. There is indeed what may be called a mitotic gelation and this gelation precedes the appearance of the mitotic spindle. Once the spindle is formed, the protoplasm reverts to its original fluid state. This sequence of stages in the protoplasm of a dividing cell has been described by all authors who have used objective methods for the determination of protoplasmic viscosity, (for discussion, see Heilbrunn;[1] Heilbrunn and Wilson[2]).

Since 1928,[1] it has been maintained that the mitotic gelation is similar to the gelation produced in protoplasm by so-called stimulating agents, and that this fundamental gelation reaction of protoplasm is in many respects similar to the gelation that occurs in vertebrate blood when it clots. As a matter of fact the protoplasm of all types of living cells is rich in thrombin or thromboplastic substances, substances which affect not only protoplasm but blood as well (compare Heilbrunn *et al.*[3] and references cited there).

If this line of reasoning is correct, it might be thought that substances which inhibit blood clotting would also prevent protoplasmic clotting in general and mitotic gelation in particular. Clearly, then, a substance like heparin is of interest in this connection.

Perhaps the best type of cell in which to observe protoplasmic clotting is the egg of the sea-urchin Arbacia. When this cell is torn or broken, as the protoplasm begins to flow out, the cell seals itself in a reaction like that which occurs when blood flows from a vessel.[4] This reaction has been called the surface precipitation reaction. Years ago, one of us attempted to show that heparin might have some effect on this surface precipitation reaction. However, no effect could be observed. Because of this failure to demonstrate an effect of heparin on the surface precipitation reaction, no further studies were made. Moreover, it was hardly to be expected

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1 Heilbrunn, L. V., *The Colloid Chemistry of Protoplasm*, Berlin, 1928.

2 Heilbrunn, L. V., and Wilson, W. L., *Biol. Bull.*, 1948, **95**, 57.

3 Heilbrunn, L. V., Harris, D. L., Le Fevre, P. G., Wilson, W. L., and Woodward, A. A., *Physiol. Zool.*, 1946, **19**, 404.

4 Heilbrunn, L. V., *Arch. f. exp. Zellforsch.*, 1927, **4**, 246.

that heparin could act on the interior of a cell, for the heparin molecule is a large one and it scarcely seemed plausible to suppose that heparin could enter a cell in sufficient amount to affect clotting processes in the interior protoplasm.

Fortunately for us, some months ago, one of our beginning graduate students, Mr. Julius H. Jacobson, was less certain of failure and in some preliminary experiments was able to show that heparin tended to prevent clotting in the protoplasm of paramecium. Following this lead we took courage and began to wonder whether heparin might not have an effect on the mitotic gelation as it occurs in marine eggs.

In a recent paper[2] we have shown the exact course of the mitotic gelation in the egg of the worm Chaetopterus and the time sequence of this gelation in relation to the morphological changes which take place during cell division. Hence it was logical to attempt a study of the effect of heparin on various aspects of mitosis in the Chaetopterus egg.

Unfortunately, no very pure preparations of heparin were available to us. We began work with two different lots of the Hynson, Westcott and Dunning preparation. In preliminary experiments with sea-urchin (Arbacia) eggs, we found that one of these lots (no. 194) was apparently contaminated with thromboplastin or thromboplastic substance. When sea-urchin eggs are placed in oxalate solutions and then broken by pressing down on the coverslip over them, no surface precipitation reaction occurs and instead of the protoplasm sealing itself as it does when calcium is present, it flows out in a steady stream. On the other hand, if to the oxalate solution an extract of injured living material is added, the protoplasm even in the absence of calcium does show a surface precipitation reaction.[3] Thus the protoplasm of the sea-urchin egg can be used as an indicator for the presence of clotting (thromboplastic) substances. A drop of Arbacia eggs in sea-water was placed on a slide; to this was added a drop of 0.45 molar potassium oxalate and a drop of 0.25% solution of heparin from lot 194 of Hynson, Westcott and Dunning. After waiting a minute for the calcium of the sea-water to precipitate out, the eggs were crushed. A definite surface precipitation reaction occurred. The experiment was repeated many times, always with the same result. The result indicates the presence of a protoplasmic clotting substance in the heparin. An alcoholic extract of the heparin was prepared. This was evaporated to dryness and the dry residue dissolved in distilled water. The protoplasmic clotting substance was present in the alcoholic extract. Years ago, in preparing heparin from lung tissue, Charles, Fisher and Scott[5] found that it was difficult to separate the heparin from a substance in lung tissue which favored blood clotting; this clotting substance was soluble in alcohol. The presence of a clotting substance in lot 194 of the Hynson, Westcott and Dunning heparin was also indicated by the fact that dilute solutions (0.1 - 0.02%) also caused a well-marked increase of the protoplasmic viscosity of the protoplasm of sea-urchin eggs exposed to these solutions for 2 hours. Viscosity tests were made with the centrifuge method.

Fortunately, another lot of the Hynson, Westcott and Dunning heparin was free from the clotting substance. This was lot 198. Solutions of it did not induce a surface precipitation reaction in the presence of oxalate nor did they cause increase in protoplasmic viscosity. Accordingly, in our experiments on the effect of Hynson, Westcott and Dunning heparin on Chaetopterus eggs, we were always careful to use lot 198.

When Chaetopterus eggs are placed in dilute heparin solutions and are inseminated some minutes later in the usual way with dilute suspensions of spermatozoa, they typically do not cleave. Much of this effect is due to an inhibition of fertilization by the heparin. Unfortunately, fertilization in the Chaetopterus egg is not accompanied by any marked cortical change, so that it is not possible to tell immediately whether or not sperm entrance has been effected. However, at about 9 minutes after fertilization (at 21°) the first polar body is given off, so that a count of

5 Charles, A. F., Fisher, A. M., and Scott, D. A., *Proc. and Trans. Roy. Soc. of Canada*, 1934, 3rd ser., **28**, sec. V, 49.

polar bodies can indicate whether or not sperm entrance has occurred. Because the polar bodies of Chaetopterus are rather small, it is not easy to make polar body counts. We made counts in two ways. If the eggs are undisturbed during the count, ordinarily only about a third of the polar bodies are visible, so that a count which showed approximately 35% of the eggs with polar bodies would indicate that all of the eggs had been fertilized. If the eggs are rolled during counting, as high as 90% of the eggs could be seen to have polar bodies. Ordinarily, we did not roll the eggs, and in calculating percentages of eggs with polar bodies, we assumed that a 35% count indicated that 100% of the eggs had polar bodies.

By making counts of polar bodies we were able to determine with some certainty whether or not sperm entrance had occurred in any given case. After some disappointing experiments, we were fortunate to find that by increasing the concentration of sperm we could override the inhibiting effect of heparin on fertilization. In some cases in which sperm entrance did not occur (as judged by the polar body test) we were able to induce sperm entrance by adding more sperm. As an example of the effect of sperm concentration, the following experiment may be cited. A drop of dry sperm, obtained by cutting a worm, was diluted with 10 ml of sea-water to form a sperm suspension. Varying amounts of this sperm suspension (1, 10, 20 drops) were added to eggs which had been for 10 minutes in a 0.05% solution of heparin in sea-water. The results are shown in Table I. In this table percentages of eggs with polar bodies are calculated as noted above. It is clear that higher concentrations of sperm cause an increased percentage of sperm penetration.

If sperm penetration has occurred and then the eggs do not cleave, it is obvious that heparin has prevented the division of the cell. Table II shows a series of 10 experiments. In all of these experiments, control eggs inseminated in sea-water showed approximately 100% fertilization and cleavage. The experimental eggs were inseminated after 10 minutes immersion in 0.05% heparin solutions. Polar body counts showed the approximate percentage of eggs in which sperm penetration had occurred, calculated as before. Of these fertilized eggs, only a relatively small percentage showed cleavage. From the table it is obvious that dilute solutions of heparin can block mitosis in the Chaetopterus egg.

TABLE I.
Effect of Increasing Concentrations of Sperm on Sperm Entrance Into Heparinized Eggs as Indicated by Polar Body Formation.

No. of drops of sperm suspension added	% of eggs with polar bodies
1	3
10	51
20	57

TABLE II.
Effect of Heparin on Cleavage.

% of eggs with polar bodies	% of eggs cleaved	% of fertilized eggs cleaved
100	27	27
86	8	10
74	22	37
51	25	49
57	10	18
51	36	71
63	6	10
100	13	13
91	42	46
100	61	61

In Table II the first 8 experiments were done with Hynson, Westcott and Dunning heparin (lot 198). In the final 2 experiments, Hoffmann-LaRoche heparin was used. Other experiments in which eggs were exposed to heparin for longer times indicated that Hoffmann-LaRoche heparin is weaker in its effects than Hynson, Westcott and Dunning heparin. Fortunately, however, the Hoffmann-LaRoche heparin does not appear to contain any thromboplastic substance.

The effect that heparin has on cell division is not due to a killing action; at any rate the effect is, to some extent at least, reversible. Thus in one experiment in which eggs treated with heparin for 10 minutes before fertilization showed 36% cleavage, washing the eggs several times in sea-water caused the percentage of cleavage to increase to 55%.

Apparently, heparin acts by preventing the mitotic gelation which normally occurs in the early stages of mitosis. In the Chaetopterus

egg, during the mitosis which causes the division of the single egg cell into two blastomeres, the protoplasmic viscosity rises sharply just before the mitotic spindle is formed. This is clearly shown in the graph published recently.[2] Before the mitotic gelation, the protoplasmic viscosity has an arbitrary value of 7; this rises to 14 and then after the spindle is formed it drops to 7 again. In the heparinized eggs, such a mitotic gelation does not occur. Five tests of the protoplasmic viscosity of fertilized heparinized eggs showed in every case a viscosity of 6 or below. These tests were all made from 70-90 minutes after fertilization, that is to say, after ample time had been allowed for the mitotic gelation to occur. Thus beyond much doubt heparin prevents mitosis by inhibiting the mitotic gelation.

The fact that heparin can prevent cell division is not a new observation. Years ago Fischer[6] showed that heparin could prevent the division of cells in tissue culture. Presumably in these cells also heparin acts by preventing protoplasmic clotting. The concentration of heparin that Fischer used in his later experiments is identical with that we found best for Chaetopterus eggs. However, Fischer apparently used a purer preparation of heparin than the one we had at our disposal.

An important fact to remember is that heparin is a normal constituent of the blood and perhaps also of the cells of higher animals. It has been stated that in radiation sickness there is an increased amount of heparin in the blood.[7] Conceivably some of the effect of roentgen rays on cancer might be due to the presence of this heparin and its effect on cell division. Also it should be noted that the bacterial polysaccharide which in minute amounts causes regression of tumors in rats and mice is a hemorrhagic agent.[8] Inasmuch as excessive doses of heparin are known to induce hemorrhage,[9] the bacterial polysaccharide may perhaps be related to heparin. It may also be possible that the injection of one type of heparin or another, or the injection of other types of substances which impede blood clotting, like injection of dicumarin, may eventually prove to be of some help in the prevention of excessive cell division such as occurs in cancer.

Recent experiments in our laboratory by Drusilla Harding have shown that heparin can prevent cell division in the frog egg. These experiments will be published before long. We are also experimenting on the effect of injecting heparin and other anti-coagulants on sarcoma in mice.

[6] Fischer, A., *Arch. f. path. Anat. u. Physiol.*, 1930, **279**, 94; *Protoplasma*, 1936, **26**, 344.

[7] Allen, J. G., and Jacobson, L. O., *Science*, 1947, **105**, 388.

[8] Shear, M. J., and Turner, F. C., *J. Nat. Cancer Inst.*, 1943, **4**, 81; Shear, M. J., Perrault, A., and Adams, J. R., *ibid.*, 1943, **4**, 99; Hartwell, J. L., Shear, M. J., and Adams, J. R., *ibid.*, 1943, **4**, 107.

[9] Erschler, I. L., and Blaisdell, I. H., *J. Amer. Med. Assn.*, 1941, **117**, 927; Richmond, E. L., *ibid.*, 1941, **118**, 609; Keyes, J. W., and Shaffer, C. F., *ibid.*, 1942, **119**, 882; Sollman, T., *A Manual of Pharmacology*, 3rd ed., Philadelphia, 1948, p. 425.