Effect of Bacterial Polysaccharide on Cell Division[1]

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In 1943, Shear and his associates at the National Cancer Institute were able to extract, from the red pigmented bacterium *Serratia marcescens*, a polysaccharide which caused regression of tumors in mice and rats (*13*). This important discovery was followed by attempts to use the polysaccharide on human patients, but unfortunately it is more toxic for men than it is for mice (*9*). Accordingly, various workers have attempted to reduce the toxicity in one way or another (*11, 12*).

If progress is to be made, it might be helpful if we knew something about the action of the polysaccharide. In work done a year ago, we showed that heparin could prevent cell division in the egg of the worm *Chaetopterus* (*8*, compare also *3*). This fact was brought into relation to the theory advanced by one of us 20 years ago (*6*). According to this theory, initiation of mitosis is due to a protoplasmic clotting or gelation essentially similar to blood clotting. In all cells that have been investigated, the appearance of the mitotic spindle is preceded by a mitotic gelation. Heparin in dilute solution prevents this mitotic gelation and thus inhibits the division of the cell. In our paper we suggested that the bacterial polysaccharide of Shear might have an effect similar to that of heparin. If this were true, we would then have an explanation of the action of the polysaccharide.

It is logical to assume that the bacterial polysaccharide would act like heparin, for heparin is indeed a polysaccharide. Accordingly, we asked Dr. Shear for some of his bacterial polysaccharide and he was kind enough to supply us with enough for experimentation. Shear's polysaccharide resembles heparin in containing sulfur, although the amount of sulfur is much less than that contained in ordinary heparin compounds (*5*). Moreover, solutions of the bacterial polysaccharide give a beautiful metachromatic (red) color when tested with solutions of toluidine blue. This is a standard test for heparin, and although not conclusive, it gives strong indication of the presence of heparin or heparinlike substances. Also, the bacterial polysaccharide acts like heparin in preventing coagulation of the blood. In experiments with human blood plasma, Sylvia Most[3] found that the bacterial polysaccharide in high enough concentration can completely inhibit clotting. Actually, it is a much weaker anticoagulant than ordinary heparin; thus, for example, the potent heparin preparations of Hynson, Westcott, and Dunning are about 300 times as powerful as the bacterial polysaccharide.

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[3] Mrs. Most's work on the anticlotting activity of the bacterial polysaccharide will be published shortly.

In our experiments with heparin on *Chaetopterus* eggs (*8*), we had some difficulty in obtaining satisfactory results because heparin penetrates the eggs slowly. It is necessary to expose the eggs to heparin solutions for about 10 min before fertilization in order to insure an effect after fertilization. But if they are thus exposed, fertilization is ordinarily inhibited by heparin. We were able to overcome this difficulty by using excess amounts of sperm; with such excess sperm, a high percentage of the heparinized eggs could indeed be fertilized.

The bacterial polysaccharide seems to enter the *Chaetopterus* egg cells more rapidly than does heparin. Accordingly, we were able to introduce the eggs into solutions of the bacterial polysaccharide after fertilization and get a pronounced effect. In four experiments in which eggs were put into a 0.15% solution of bacterial polysaccharide (Shear's PP25 preparation) 2 min after fertilization, the percentage of cleavage varied from 0% to 5%, although control eggs in sea water showed almost 100% cleavage. The counts were made soon after the normal time of cleavage; some hours later a slightly higher percentage of cleavage could be noted in the treated cells. In four other experiments in which eggs were put into dilute solutions of bacterial polysaccharide (0.058%–0.125%) and 5 min later were fertilized with excess sperm, there were three instances in which none of the eggs cleaved at the regular time, and in the fourth instance only 1% of the eggs cleaved. In these experiments in which eggs were exposed to polysaccharide before fertilization, approximately 100% of the eggs were fertilized by excess sperm in three out of four instances; in the fourth experiment 57% of the eggs were fertilized. Similarly, Harding was able to show that bacterial polysaccharide tends to suppress the initiation of mitosis in frog eggs (*4*).

Thus, it is clear that bacterial polysaccharide acts like heparin in preventing cell division. Also, the two polysaccharides have a similar inhibiting effect on fertilization; this inhibition can in both cases be overridden by excess amounts of sperm. Moreover, the bacterial polysaccharide acts like heparin in preventing mitotic gelation. Protoplasmic viscosity tests were made at frequent intervals after the eggs were introduced into solutions of the bacterial polysaccharide. In all instances it could be shown clearly that the protoplasm of the treated eggs preserved its fluidity. Normally, prior to the appearance of the mitotic spindle, the viscosity of the protoplasm rises from a value of 7 to a value of 14 (*7*). In the eggs immersed in the bacterial polysaccharide solutions, the viscosity stays at about 7 during all the time that the control eggs are showing mitotic gelation.

We thus have a simple and clear interpretation of why it is that bacterial polysaccharide prevents cell division, an interpretation perfectly in line with earlier theory. It is our belief that in living cells there is antagonism between thrombin or thrombinlike substances which tends to induce protoplasmic clotting and anticlotting substances, including those of a heparinlike nature.

We believe that heparins and heparinlike substances of cells differ widely in their molecular size and in their

chemical composition. Perhaps those of smaller molecular size or those with less acidity, and consequently with fewer polar molecules, enter cells more rapidly. If a search could be made for all sorts of polysaccharides from many diverse types of cells, we might be able to find one which would cure not only mice but men.

Years ago, Goerner, basing his experiments on the theory proposed by one of us in 1928 (*2*), was able to show that heparin could destroy the tumor-producing potency of tumor cells *in vitro*. We have tried some experiments on the effect of heparin on rat tumors *in vivo*, but as yet have not had much success. The experiment is complicated by the fact that injection of heparin may produce what physicians call "heparin rebound" (*1*), a state in which the blood becomes more instead of less coagulable.

In line with our theory, there is a possibility that various other substances which prevent blood clotting may prevent cell division and be useful in cancer therapy. Actually, some authors have had success with anticoagulant dyes (*10, 14*). We ourselves have tried the effect of dicumarol on *Chaetopterus* cells and are planning to try it on cancer tissues. The action of dicumarol on *Chaetopterus* egg cells is very striking but somewhat complicated. We will report on it fully in a paper we expect to publish in *Protoplasma*, where we shall also present details of our work with the bacterial polysaccharide.

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Amino Acids in High and Low Protein Corn[1]

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Improvement in nutritive value of plant sources of food both for human and for animal consumption is a challenge to nutritionists and to plant scientists. Progress in research in this field has already led to the use of strains and varieties of plants having superior nutritive value with respect to certain nutrients.

Since the early work of Hopkins (*6*), a number of investigators have shown that there is a definite relationship between protein content and heredity in maize. In spite of these findings, introduction of hybrid corn has generally resulted in production of corn lower in protein content, emphasis being primarily on improved yield per acre. However, high yields and high protein content may not be entirely incompatible if full consideration is given to soil fertility level and density of stand in plants per acre, as well as to heredity.

Corn protein is not of the highest quality, owing to a deficiency of the amino acids lysine and tryptophan in zein, the protein of corn endosperm. The remainder of corn protein is of relatively good quality with respect to its amino acid pattern. The value of corn high in protein content is questionable since much of the increased protein may be in the form of zein, which is characteristically deficient in lysine and tryptophan. Increase in protein content, therefore, may not improve the amino acid pattern, and may even make the total corn protein more unbalanced with respect to the distribution of amino acids.

Doty and associates (*2*) published data which they interpreted as indicating that amino acid distribution in corn protein is heritable. Furthermore, they stated that the physicochemical nature of protein in the grain from two single cross hybrids was distinctly different, as shown by the fact that the sample which contained larger amounts of cystine, arginine, histidine, tryptophan, and tyrosine also contained a larger percentage of alkali-soluble nitrogen and a smaller percentage of alcohol-soluble nitrogen, i.e., the zein fraction.

It follows directly from the heritability of protein that the amounts of amino acids in corn are related to genetic constitution, but it is another question whether or not the amino acid distribution in high protein corn is any different from that in corn of low protein content.

Frey *et al.* (*4*) recently reported on the effects of selection upon protein quality in the corn kernel. In their experiments no improvement was made in the zein-protein ratio in one cycle of selection. These authors stated that the percentage of tryptophan is slightly and positively correlated with the percentage of total protein.

In experiments dealing with the heritability of various nutritive factors in corn, we studied the lysine, tryptophan, and methionine contents of nine different single crosses of inbred lines of yellow dent corn. Individual replicates of these varied in crude protein content from 8.48% to 14.12%.

Each single cross was planted at the same rate in plots two hills wide and ten hills long, each plot being replicated four times in a randomized block. Thus, seasonal, soil, and fertility conditions were uniform, being subjected only to such variation as was imposed by the plot location.

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[2] From Eastern States Farmers' Exchange, Buffalo, New York.