Reports

Role of Enzyme Induction in Embryonic Development

Abstract. Measurement of tryptophan pyrrolase activity in embryos of *Rana pipiens* did not reveal significant amounts of constitutive enzyme. All attempts to induce enzyme formation in embryos by culture in tryptophan solution or in ovarian eggs by injection of tryptophan into the mature female were negative.

In 1958 Stearns and Kostellow (*1*) reported on tryptophan pyrrolase activity in dissociated embryonic cells of *Rana pipiens*. Enzyme activity was not detected in the intact embryo before hatching. In embryos dissociated into cell cultures at a stage prior to gastrulation and incubated in L-tryptophan, enzyme activity could be detected, reaching a maximum level between 8 and 10 hours of incubation. Of striking import was the observation that, before the onset of gastrulation, populations of presumptive endoderm cells demonstrated enzyme activity after tryptophan incubation. After gastrulation was complete, enzyme activity could be induced only in those cultures containing presumptive gut cells. From this work it may be concluded that tryptophan pyrrolase activity can be induced in cells lacking constitutive enzyme. These results have been frequently cited in support of the hypothesis that enzyme induction plays an important role in embryonic development.

In contrast to this observation is the report by Nemeth and Nachmias (*2*) and that by Auerbach and Waisman (*3*) that tryptophan pyrrolase is present in adult mammalian liver but is either absent or at very low levels in fetal liver. Nemeth (*4*) demonstrated that tryptophan pyrrolase activity rises to adult levels 24 hours after birth in the guinea pig and rabbit and 15 days after birth in the rat. Injections of tryptophan did not increase tryptophan pyrrolase activity in fetal liver, and the response to injection in all species developed simultaneously with the rapid increase of enzyme activity to adult levels.

In view of the conflicting results between the mammalian and the amphibian data we decided to repeat the work of Stearns and Kostellow using Friedberg and Eakin's method (*5*) of cutting embryos into halves and quarters to permit penetration of substrate. Accordingly, the jelly and vitelline membrane were removed from *Rana pipiens* eggs by the papain-thioglycolate method of Spiegel (*6*). Intact embryos, halves, and quarters were incubated for 12 hours in either Holtfreter's solution or 0.02*M* L-tryptophan in Holtfreter's solution. Cut embryos remained alive, as indicated by normal closure of the cut surface in both control and tryptophan media. After five washings with Holtfreter's solution, tryptophan pyrrolase activity was measured in 12.5-percent homogenates by the method of Knox and Auerbach (*7*). The results of seven experiments with 4200 embryos per experiment indicated no constitutive enzyme present in either the blastula, late gastrula, or early neurula stages. Of importance is the result that incubation in L-tryptophan failed to induce enzyme activity in these stages.

Stage 25 (*8*) embryos were cultured in either 10-percent Holtfreter's solution or 0.03*M* L-tryptophan (in 10-percent Holtfreter's solution) for either 6 or 24 hours. Measurement of tryptophan pyrrolase activity again revealed no constitutive enzyme, and no indication of activity was noted after tryptophan treatment.

Enzyme measurements were also made on ripe ovaries of adults, and no constitutive enzyme was detected. Attempts were made to induce enzyme formation by injecting mature females with 15 mg of L-tryptophan in 3.0 ml of 0.65-percent sodium chloride and by assaying ovarian homogenates 3, 6, and 9 hours after injection. No enzyme activity was detected. During this period, however, a 420-percent increase was noted in liver tryptophan pyrrolase activity (40.5 ± 4.90 versus a basal activity of 9.64 ± 1.26) (*9*). These results are in agreement with the results of Nemeth (*4*) on fetal and adult mammals. Tryptophan pyrrolase activity is absent during early stages of development and cannot be induced in the absence of significant amounts of constitutive enzyme.

The methods employed by Stearns and Kostellow may have increased the permeability of embryonic cells to the substrate, which may account for our failure to confirm their observations. A detailed report of their methods would permit the testing of this hypothesis. The work reported here, however, does not support the hypothesis that substrate induction of enzymes plays an important role in development (*10*).

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9. Activity is expressed in micromoles of kynurenine per gram (dry weight) per hour, plus or minus standard error of the mean.
10. This work was supported in part by U.S. Public Health Service research grant No. E-3030.

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Differential Thermograms of Polysaccharides

Abstract. Carrageenans and various other polysaccharides were characterized by differential thermal analysis in an atmosphere of air. The carrageenans, although isolated from different sources, had essentially the same thermographic characteristics. Of the other compounds studied, such closely related polysaccharides as amylose and amylopectin showed widely different thermal behavior. Thermographic replication was highly satisfactory.

The characterization of clay minerals by differential thermal analysis is a technique widely used in the past (*1*). Very little work, however, has been concerned with the use of this technique in the characterization of organic compounds. A survey of the possibility of using differential thermal analysis to characterize simple organic compounds —that is, organic acids and their derivatives—has been reported (*2, 3*). In an

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* **125**, 16 (1957)].

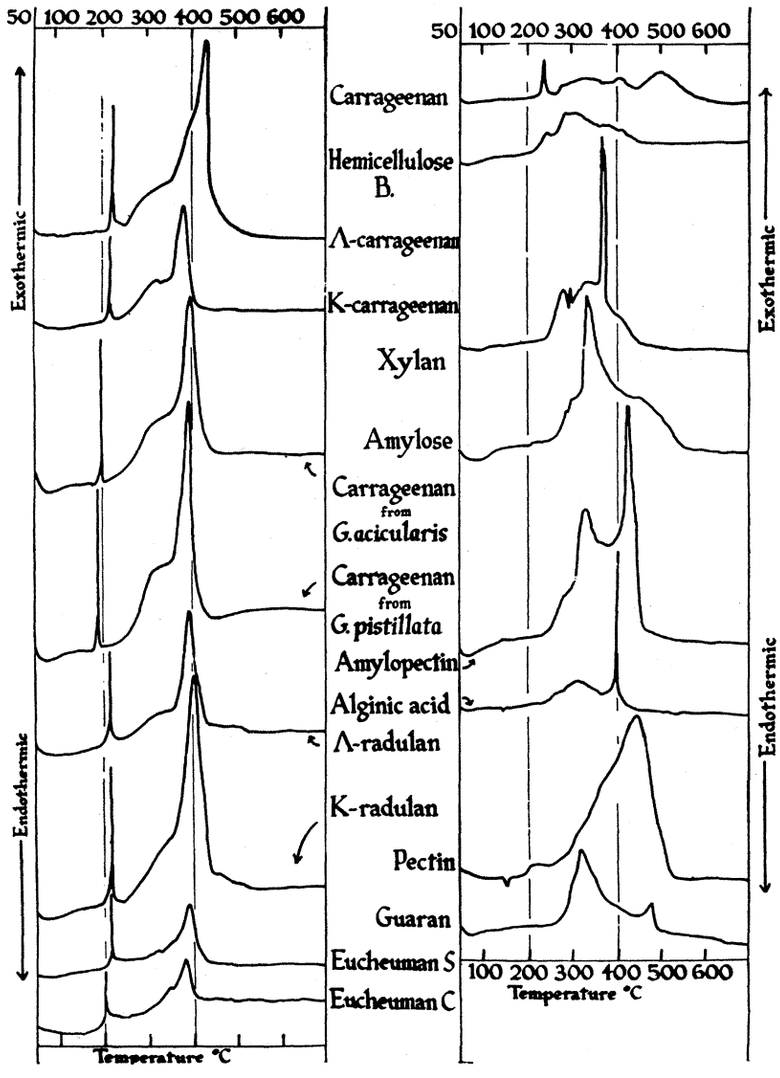

Fig. 1. Differential thermograms of polysaccharides determined in an atmosphere of air.

attempt to relate the thermograms of dextrans with their physical and chemical properties, Morita (*4*) concluded that supplementation of the differential thermal analysis data with viscosity and molecular weight determinations yielded valuable information concerning the constitution of these compounds. Endotherms in the 100° to 310°C region were related to the type of linkage encountered in these dextrans. Further variations were associated with solubility changes and unique viscosity properties.

The apparatus used in our experiments consisted of a furnace in which the rate of increase in temperature was adjusted to 10°C per minute. The temperature differential between the sample (polysaccharide) and a thermally inert standard (calcined alumina) was measured by a two-headed platinum-iridium differential thermocouple. The furnace temperature was measured by a platinum–platinum-10-percent rhodium thermocouple. The polysaccharide (*5*) was thoroughly mixed with three times its weight of calcined alumina prior to packing in the nickel sample holder. There was free access of air during all determinations. The data were automatically recorded on a Leeds and Northrup Speedomax type G recorder and plotted with furnace temperature as abscissa and differential temperature as ordinate.

The carrageenan thermograms shown in Fig. 1 (at left) have essentially the same shapes irrespective of their method of preparation. They each possess a sharp exotherm in the 200°C region and a large, broad exotherm starting at approximately 250°C and reaching a maximum in the 400°C region. The carrageenans are sulfated polysaccharides composed almost entirely of D-galactose residues and found in the cell wall of red marine algae. The exotherm at 200°C is not displayed by any of the nonsulfated polysaccharides. On the other hand, the thermogram (not shown) of agar, another sulfated polysaccharide, has a similar sharp exothermic peak at 265°C. It is therefore assumed that these peaks are a function of these sulfated esters. The thermograms of the carrageenan and of the hemicellulose B (Fig. 1, at right) suggest that these preparations are mixtures of compounds.

Amylose and amylopectin show essentially the same differential thermal curve up to a temperature of 380°C, with both possessing an exothermic reaction maximum at 330°C. At temperatures above 330°C, the branched amylopectin molecule shows a second, large exothermic reaction at 425°C. The exothermic reaction of the straight-chained amylose molecule above 330°C does not display such a large energy change on reaction. However, the temperatures at which thermal reactions cease are 570° and 470°C, respectively, for amylose and amylopectin, showing that amylose has a higher thermal stability when the two polysaccharides are heated in air.

The two uronic acid-containing polysaccharides—namely, alginic acid and pectin—display a small endothermic reaction at 145° and 155°C, respectively. These reactions are believed to be due to decarboxylation of the uronic carboxyl group. This is inferred from a similar reaction occurring with 3-indoleacetic acid to form skatole at 170°C (*3*).

At this time, little can be postulated concerning the thermal reactions of the xylan or the guaran. Duplication of the thermograms was highly satisfactory in all cases. Although the mechanism of these thermal reactions of polysaccharides cannot be completely elucidated, it is suggested that this technique may have application in the field of polysaccharide characterization and in general identification of organic compounds (*6*).

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5. We are indebted to Prof. R. L. Whistler of Purdue University and to Dr. L. Stoloff of Marine Colloids, Inc., Rockland, Me., for providing the polysaccharide samples used in this study.
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