Table 1. Effect of long-term pectin consumption on changes in body weight, food intake, cholesterol content of plasma and liver, and severity of atherosclerosis in adult cockerels fed on a diet containing nonnutritive fiber (cellulose) or pectin.

Measurements	Diet supplement	
	Cellulose	Pectin
Body weight (g) at start	2327 ± 49*	2240 ± 58
After 18 months	2511 ± 50	2317 ± 52
Food consumption (g/day)	118 ± 5	124 ± 4
After 18 months		
Plasma cholesterol (mg/100 ml)	96 ± 4	111 ± 6
Liver cholesterol (mg/g wet wt)	5.19 ± 1.3	5.87 ± .20
Abdominal aorta:		
Macroscopic score	2.04 ± .12	1.39 ± .08
Severe scores†	58	20
Weight-area ratio	53.3 ± 1.4	50.7 ± 1.5
Histological grading:		
Intima†	32	21
Localized fibrous plaques†	68	45
Media†	26	8

* Mean ± standard error for 24 and 20 birds surviving on the nonnutritive fiber and pectin supplemented diets, respectively. † Frequency of severe scores (%); severe scores were those with grade 2.0 or above; scoring was based on a 1 to 3 scale with 1 indicating essentially no lesions and 3 very severe plaques.

pectin (first group) or 5 percent nonnutritive fiber (second group). The animals, kept in individual cages, had continuous access to food and water and were maintained on these diets for 18 months. Body weights and food consumption were recorded at monthly intervals. Toward the end of the experimental period, blood was taken from a wing vein of each bird for determination of the cholesterol content of the plasma (*2*).

At the end of the 18-month period the surviving birds were killed; livers were removed for cholesterol determination, and aortas removed from the heart to the iliac bifurcation. After careful cleaning, each aorta was visually scored on a scale from 1 to 3 with 0.5-unit intervals in which a value of 1 indicated an essentially clean blood vessel, and a value of 3 represented an aorta practically occluded by plaques. After the macroscopic scoring, the aortas were weighed and the area was determined by planimetry to obtain the weight-area ratio (*3*). They were then examined histologically by techniques described previously (*4*), on the basis of (i) diffuse intimal proliferation, (ii) localized fibrous plaque formation, and (iii) medial lesions. Within each of these characterizations, lesions were graded as to severity on a 1 to 3 scale.

The pectin-fed birds had significantly ($p < .001$) fewer atherosclerotic plaques in the abdominal aorta than the control birds, measured both macroscopically and histologically (Table 1). Only the weight-area ratio did not show a significant difference, suggesting that this measurement is more a reflection of the diffuse intimal proliferation than of the localized plaque of which the macroscopic score appears to be more representative. In addition to the aortic measurements given in Table 1, three aortas from control birds only showed calcium deposition in localized fibrous plaques. No hypocholesterolemic action could be ascribed to the pectin feeding, in contrast to reports for the rat and for man (*5*). Indeed, the pectin-fed animals had significantly higher concentrations of cholesterol in the plasma and liver.

The birds fed with pectin consumed slightly more food throughout the experiment than did the control birds fed with cellulose (Table 1). The control birds, however, gained almost three times as much weight as did the pectin-fed birds, indicating that pectin reduced the utilization of nutrients.

Table 2 shows the results of a separate study in which analyses were made of the excreta of 10-week-old chickens fed pectin or cellulose in diets which contained 10 percent fat and 0.5 percent cholesterol. There was no difference in the moisture content of the excreta, although the excreta from the birds fed pectin gave the appearance of being diarrhetic. However, the pectin-fed birds lost three times as much lipid and almost twice as much cholesterol per gram of excreta as did the control birds.

Table 2. Moisture, total lipid, and cholesterol content of excreta from chickens fed pectin or nonnutritive fiber (cellulose).

Excreta measurement	Diet supplement	
	Cellulose	Pectin
Moisture (%)	73.4	73.5
Extractable lipid (mg/g dry excreta)	48.7	145.2
Cholesterol (mg/g dry excreta)	8.7	15.6

These results confirm our previous suggestion (*1*) that pectin may be efficacious in retarding spontaneous avian atherogenesis. The excreta analyses, in conjunction with the food intake and patterns of body weight, suggest two possible modes of action for pectin: it might accelerate food passage or interfere with the digestive processes so that less nutrients become available for absorption; or it might specifically interfere with the absorption or reabsorption of atherogenic substances (cholesterol). With regard to the first possibility we have recently shown that a restricted pattern of food intake was beneficial in retarding avian atherosclerosis (*6*). The higher plasma and liver cholesterol values of the pectin-fed birds make the second possibility appear unlikely.

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References and Notes

1. H. Fisher, H. S. Weiss, P. Griminger, *J. Atherosclerosis Res.* **3**, 57 (1963).
2. H. Fisher, H. Kaunitz, *Proc. Soc. Exptl. Biol. Med.* **116**, 278 (1964).
3. H. S. Weiss, P. Griminger, H. Fisher, *Anat. Record* **148**, 527 (1964).
4. W. G. Siller, in *Comparative Atherosclerosis: Spontaneous and Experimental* (Hoeber, New York), in press.
5. B. H. Ershoff, *Exptl. Med. Surg.* **21**, 108 (1963).
6. P. Griminger, H. Fisher, H. S. Weiss, *Life Sci.* **6**, 410 (1963).
7. Supported by grants-in-aid from NIH and the New Jersey Heart Association. We acknowledge the splendid help of Mrs. O. Donis, H. Lutz, and E. Borbely.
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Formation and Degradation of Cyclic Dextrins by Intracellular Enzymes of Bacillus macerans

Abstract. *The enzymes of* Bacillus macerans *that participate in the formation and degradation of Schardinger dextrins were shown to be intracellular.* Bacillus macerans *amylase converts starch into cyclic dextrins. A newly discovered enzyme, cyclodextrinase, catalyzes the degradation of cyclic dextrins.*

Bacillus macerans amylase, also known as cyclodextrin transglycosylase, is an enzyme that converts starch into Schardinger dextrins, a homologous series of cyclic oligosaccharides contain-

ing six or more glucosyl residues linked $1 \rightarrow 4$ as in amylose (*1*). This enzyme has also been reported to catalyze coupling reactions (*2*) whereby a linear series of malto-oligosaccharides are formed when α-dextrin (cyclohexaamylose) and a cosubstrate such as glucose or maltose are incubated with a crude enzyme preparation. In all previous work it has been considered to be an extracellular enzyme (*3, 4*). Several media have been described for the production of this enzyme, but so far the only suitable media contain either autoclaved potatoes or oatmeal plus 1 to 2 percent $CaCO_3$ (*1, 5*). *Bacillus macerans* usually must be grown at 37° to 40°C for 10 days to 2 weeks before the enzyme can be detected in the culture medium (*4, 6*).

During a study to develop a less complex medium for the production of this enzyme, we noted the disappearance of starch and the formation of cyclic dextrins in 18-hour culture filtrates. Attempts to detect enzyme activity in such filtrates, however, were unsuccessful, suggesting that *B. macerans* amylase is an intracellular enzyme.

Three assays are currently used in this laboratory for studies on the purification of these enzymes. The first is the Tilden and Hudson slide test (*5*) which is a specific semiquantitative test for the cyclic dextrins. The second is a modification of the dextrinogenic assay of Manning and Campbell (*7*). The assay mixture contains 5 ml of 3 percent Lintner soluble starch in 0.01*M* phosphate buffer at *p*H 6.7 and 0.1 ml of $5 \times 10^{-2}M$ $CaCl_2$, in each of two tubes. A third tube containing 5 ml of the buffer and 0.1 ml $CaCl_2$ serves as the reagent blank. Enzyme (1.0 ml) is added to one tube and the tubes are incubated for 5 minutes at 40°C, 2.0 ml of 1*N* HCl are added to each tube, and 1.0 ml of enzyme is then added to the starch control tube and to the reagent blank. Samples (0.1 ml) from each of these tubes are transferred to 25 × 150 mm test tubes containing 0.5 ml of 1*N* HCl; 0.1 ml of an iodine solution (0.1*N* I_2 and 0.1*M* KI), and 45 ml of water are added to each tube. Absorbancy is measured at 620 mμ in a Bausch and Lomb Spectronic 20 colorimeter. Activity is calculated as described by Manning and Campbell (*7*). One dextrinogenic unit is defined as that amount of protein which will hydrolyze 10 mg of starch per minute under these conditions. The third method is the saccharogenic assay of Fischer and Stein (*8*) modified as follows: the reaction mixture containing 1.0 ml of the 3 percent buffered starch, enzyme, and 0.02 ml of $5 \times 10^{-2}M$ $CaCl_2$ is incubated for 5 minutes at 40°C. One saccharogenic unit is defined as that amount of protein which will release 1.0 mg of reducing groups, as maltose, in 5 minutes under these conditions. Since the Schardinger dextrins have no reducing power (*1*) the dextrinogenic assay is a measure of *B. macerans* amylase, assuming that no α- or β-amylase is present. The saccharogenic assay measures the activity of the cyclodextrinase in crude preparations. The ratio of saccharogenic specific activity to dextrinogenic specific activity should become zero when the *B. macerans* amylase preparations are free of cyclodextrinase. The specific activity of the enzymes is expressed as units per milligram of protein.

The medium (*p*H 7.2) devised for enzyme production contained 2 percent soluble starch (Fischer), 0.5 percent casein hydrolyzate powder (Nutritional Biochemicals), 0.5 percent yeast extract (Difco), 0.5 percent Na_2HPO_4, 0.2 percent KH_2PO_4, and 10^{-3} *M* $CaCl_2$. Under the conditions of our experiments, *B. macerans* amylase and cyclodextrinase are intracellular enzymes (Table 1). These enzymes were not

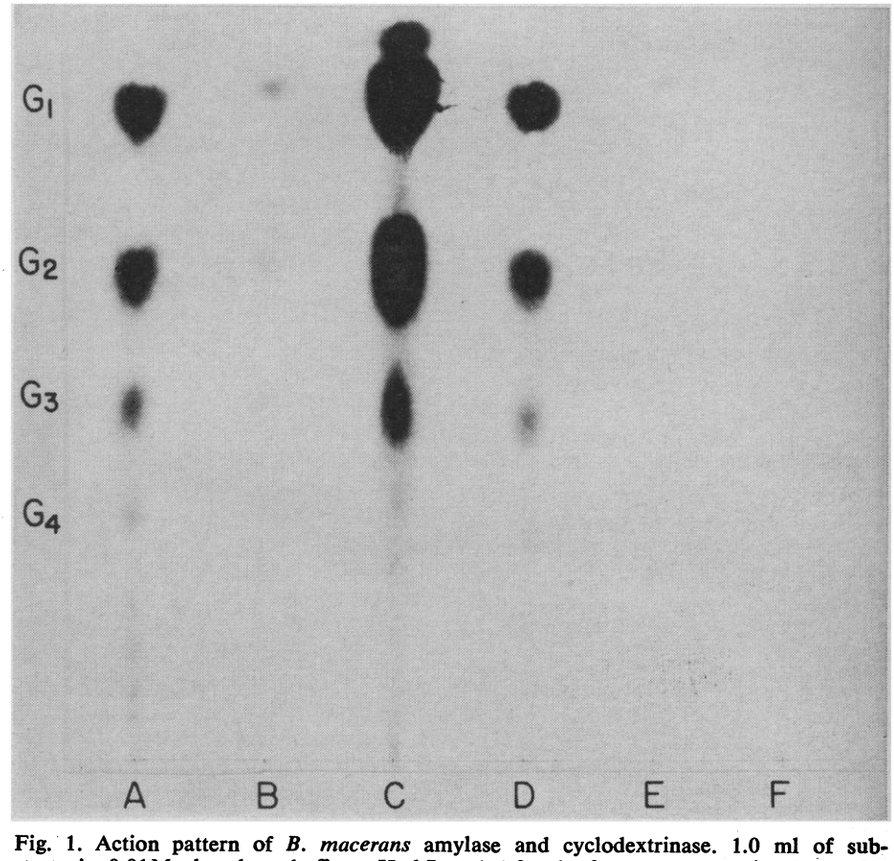

Fig. 1. Action pattern of *B. macerans* amylase and cyclodextrinase. 1.0 ml of substrate in 0.01*M* phosphate buffer, *p*H 6.7 and 1.0 ml of enzyme were incubated for 60 minutes at 40°C; the reaction was stopped with 0.5 ml of 1*N* HCl. Approximately 15 μl of each reaction mixture were spotted on 46 by 46 cm sheets of filter paper (No. 598, Schleicher and Schuell). The developing solvent was *n*-butanol-pyridine-water (6 : 4 : 3). The multiple ascending procedure of Pazur and French (*11*) was used with three ascents of 20 hours each. Reducing sugars were detected by the modified silver-dip method of Welker and Campbell (*12*). (*A*) 5 percent α-dextrin + crude cyclodextrinase; 6.5 saccharogenic units. The ratio of the saccharogenic specific activity to that of the dextrinogenic was 9.5. (*B*) 5 percent α-dextrin + partially purified *B. macerans* amylase; 9.3 dextrinogenic units. The ratio of the saccharogenic specific activity to that of the dextrinogenic was < 0.005. (*C*) Technical grade maltose (Pfanstiehl) containing glucose, maltose, and higher malto-oligosaccharides (*13*). (*D*) 3 percent Lintner soluble starch + cyclodextrinase. (*E*) 3 percent Lintner soluble starch + *B. macerans* amylase. (*F*) Control (starch + α-dextrin + both enzymes, unreacted). (G_1, G_2, G_3, and G_4) refer to glucose, maltose, maltotriose and maltotetraose.

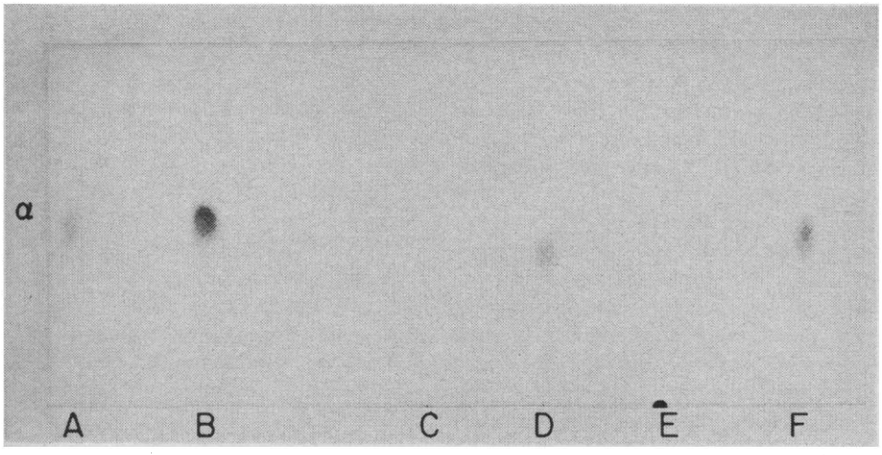

Fig. 2. Action pattern of *B. macerans* amylase and cyclodextrinase. Experimental conditions were identical to those of Fig. 1 except that the chromatogram was sprayed with 1.0*M* phosphate buffer, *p*H 6.7, air dried, and sprayed with an iodine solution (0.0035*M* I_2 and 0.25*M* KI) to detect α-dextrin. (*A*) α-dextrin + crude cyclodextrinase. (*B*) α-dextrin + partially purified *B. macerans* amylase. (*C*) Starch + cyclodextrinase. (*D*) Starch + *B. macerans* amylase. (*E*) Control (starch). (*F*) Control α-dextrin. (α) Refers to α-dextrin.

detected in 18- or 24-hour culture filtrates.

The action patterns of a crude preparation of cyclodextrinase and a preparation of *B. macerans* amylase (partially purified by manganese chloride, ammonium sulfate precipitations, and DEAE chromatography), are shown in Fig. 1. A linear series of malto-oligosaccharides was obtained when the crude dialyzed preparation of cyclodextrinase (which still contained some *B. macerans* amylase) was incubated with either α-dextrin (*9*) or with starch. With the partially purified, highly active preparation of *B. macerans* amylase, only small amounts of oligosaccharides were formed. This is presumably due to traces of cyclodextrinase that remained in the preparation. It should be noted that no cosubstrates were used in these experiments.

Figure 2 shows that α-dextrin was formed from starch by the *B. macerans* amylase preparation, but none was detected when the crude cyclodextrinase preparation was incubated with starch, even though oligosaccharides were formed (*10*).

It thus appears that at least two enzymes participate in the formation and degradation of Schardinger dextrins: (i) *B. macerans* amylase which forms Schardinger dextrins from starch and (ii) cyclodextrinase which degrades the cyclic dextrins to glucose, maltose, and malto-oligosaccharides. In addition, these enzymes are intracellular rather than extracellular. The detection of *B. macerans* amylase in culture filtrates in previous studies (*3, 4*) has probably resulted from cell lysis occurring during the long incubation periods.

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Table 1. Intracellular *Bacillus macerans* amylase and cyclodextrinase. *B. macerans* ATCC 8514 was grown at 38°C on a rotary shaker at 300 rpm in duplicate Fernback flasks, each containing 2 liters of the medium described or in medium containing 0.1 percent $CaCO_3$ in place of the phosphate and $CaCl_2$. The flasks were inoculated with a 1 percent inoculum of *B. macerans* in the same medium. After 18 or 24 hours incubation the cells were harvested by centrifugation, washed once with 20 ml of 0.01*M* phosphate buffer, *p*H 6.7, and suspended in 20 ml of the same buffer. The cells were broken either in a French pressure cell at 10,000 pounds per square inch or by sonic disintegration for 15 minutes in a Raytheon sonic oscillator. Deoxyribonuclease (10 μg/ml) and 1.0 ml of 0.05*M* $MgCl_2$ were added to the extract and, after stirring for 30 minutes at 30°C, cell debris was removed by centrifugation for 30 minutes at 17,500 rpm in a Serv-all centrifuge (SS-34 head, 0°C). The Tilden–Hudson test was positive in all cases.

Time of incubation (hr)	Medium	Cell-free extract	
		Dextrinogenic (units/ml)	Saccharogenic (units/ml)
18*	$CaCO_3$	12.5	122
18*	PO_4	12	28.2
24†	$CaCO_3$	7.7	50
24†	PO_4	4.3	50

* Cells were broken by sonic disintegration.
† Cells were broken in the French pressure cell.

References and Notes

1. For a detailed review see D. French, *Advan. Carbohydrate Chem.* **12**, 189 (1957).
2. D. French, M. L. Levine, E. Norberg, P. Nordin, J. H. Pazur, G. M. Wild, *J. Am. Chem. Soc.* **76**, 2387 (1954).
3. E. B. Tilden and C. S. Hudson, *ibid.* **61**, 2900 (1939); W. S. Hale and L. C. Rawlins, *Cereal Chem.* **28**, 49 (1951).
4. D. French, in *Methods in Enzymology, Preparation and Assay of Enzymes*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1962), vol. 5, p. 148.
5. E. B. Tilden and C. S. Hudson, *J. Bacteriol.* **43**, 527 (1942).
6. S. Schwimmer and J. A. Garibaldi, *Cereal Chem.* **29**, 108 (1952). These workers have reduced the incubation time to 12 to 15 hours with an oatmeal medium but this was done in a fermenter with an aeration rate of 150 liters of air per minute.
7. G. B. Manning and L. L. Campbell, *J. Biol. Chem.* **236**, 2952 (1961).
8. E. H. Fischer and E. A. Stein, *Biochem. Prep.* **8**, 27 (1961).
9. Supplied by D. French.
10. β-Dextrin (cycloheptaamylose) is not detectable with the spray used, therefore its absence cannot be excluded; J. Robyt and D. French, *Arch. Biochem. Biophys.* **104**, 338 (1964) reported that an enzyme preparation from *Bacillus polymyxa* degrades both Schardinger dextrins and amylose.
11. J. H. Pazur and D. French, *J. Biol. Chem.* **196**, 265 (1952).
12. N. E. Welker and L. L. Campbell, *J. Bacteriol.* **86**, 681 (1963).
13. ———, *ibid.* **86**, 687 (1963).
14. One of us (J.A.D.) was an NIH predoctoral trainee (2G-510) during the tenure of this research. Supported in part by NSF grant GB-4.

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Association of Rapidly Metabolized DNA and RNA

Abstract. *An association of rapidly metabolized DNA and RNA labeled with P^{32} has been found in peanut cotyledons by incorporation of either uridine-C^{14} or thymidine-C^{14} by peanut cotyledon slices. Chromatography of the extracted nucleic acids on methylated-albumin columns shows that the rapidly metabolized DNA and RNA are contained only in the first half of the DNA peak where RNA and DNA occur in equal amount. One-fourth of the radioactivity is recovered in ribonucleotides. Density-gradient centrifugation in cesium chloride indicates that the material containing the P^{32} has a higher density than most of the DNA.*

Mead (*1*) has isolated a DNA-RNA complex from *Drosophila melanogaster*. Other natural DNA-RNA complexes have also been described (*2, 3*). It is believed that a DNA-RNA complex is an intermediate in the transcription of the genetic information of the DNA (*4*). Konrad and Stent (*5*) were unable to find a putative DNA-RNA