compound that is primarily protein in nature—that is, hemoglobin—with another substance found in the serum results in a profound change in the electrophoretic characteristics of the hemoglobin. This emphasizes the possibility that characteristic alterations in the electrophoretic pattern of serum proteins seen in certain diseases may be the result, at least in part, of complex formation occurring between normal serum proteins and other substances not ordinarily present in the serum.

References and Note

* John and Mary R. Markle Foundation scholar in medical science. This investigation was supported in part by research grant H1380 from the National Institutes of Health, U.S. Public Health Service.

1. A. H. Tuttle, *Am. J. Diseases Children,* in press.
2. K. Singer, A. I. Chernoff, L. Singer, *Blood* **6**, 413 (1951).

15 November 1954.

Hydrolysis of Amylotriose by Crystalline Salivary Amylase

John H. Pazur and Tania Budovich

Department of Biochemistry and Nutrition, University of Nebraska, Lincoln

In an earlier publication (*1*) it was shown that partially purified preparations of salivary amylase were capable of hydrolyzing the glucosidic bonds of amylotriose to yield glucose and maltose as the hydrolytic products. Whelan and Roberts (*2*) did not observe any hydrolytic products in digests of amylotriose (maltotriose) with highly purified salivary amylase, and they concluded that the enzyme was without action on this compound. In our laboratory, crystalline salivary amylase (*3*) has now been shown to effect a hydrolysis of amylotriose and of 1-C^{14}-amylotriose. The results of our studies are presented in this paper (*4*).

Four milligrams of amylotriose were dissolved in 0.05 ml of water and treated with 0.05 ml of a solution of crystalline salivary amylase. The enzyme solution was buffered to *p*H 6.8 with 0.1*M* phosphate and assayed 12 units (*5*) of amylase activity per milliliter. An aliquot of 0.01 ml of the digest was placed on a paper strip immediately after the addition of the enzyme and was heated at 100°C for 5 min to inactivate the enzyme. Subsequent samples were obtained at 6- and 24-hr reaction periods. The reducing sugars in the aliquots were separated in *n*-butyl alcohol-pyridine-water (6:4:3 by volume) solvent and located on the paper with copper sulfate reagent (*6*). A photograph of the chromatogram is reproduced in Fig. 1. Filtered saliva containing 12 units of amylase activity per milliliter was also tested on amylotriose under conditions identical to those of the preceding experiment. The finished chromatogram of this digest was the same as that reproduced in Fig. 1.

In order to determine which bond in the trisaccharide was susceptible to enzyme hydrolysis, 1-C^{14}-amylotriose was subjected to the action of the crystalline amylase. Four milligrams of 1-C^{14}-amylotriose (*7*) dissolved in 0.05 ml of water was treated with 0.05 ml of the solution of crystalline enzyme. Aliquots of the digest were analyzed for reducing sugars by paper chromatography at 0-, 6-, and 24-hr reaction periods. The areas at which reducing sugars appeared on the chromatogram were cut from the paper and counted for radioactivity in a conventional counting apparatus. The radioactivities of the various compounds are recorded in Table 1.

An examination of Fig. 1 shows that, under the conditions of our experiments, amylotriose was rapidly hydrolyzed to glucose and maltose by crystalline salivary amylase. In a 6-hr reaction period, considerable hydrolysis of the trisaccharide had already occurred, while in a 24-hr reaction period more than 75 percent of the trisaccharide was hydrolyzed to glucose and maltose. Comparison of the chromatogram in Fig. 1 with that obtained for the samples of amylotriose treated with crude saliva revealed that, at equivalent concentrations of amylase activity, the crystalline and crude enzyme preparations hydrolyzed amylotriose at the same rate. In addition, neither the crude preparation of saliva nor the crystalline amylase preparation exhibited any maltase activity. On the basis of these findings, it would appear that the hydrolysis of amylotriose is the result of amylase activity and not of the activity of maltase or some yet unidentified enzyme in saliva (*8*). The discrepancy in the results obtained by Whelan and Roberts (*2*) and by us may be due to differences in enzyme concentrations or in some other reaction condition.

The values in Table 1 for the radioactivities of the products from 1-C^{14}-amylotriose show that salivary amylase is capable of hydrolyzing the glucosidic bonds at the reducing and nonreducing end of the amylotriose molecule. Hydrolysis of the bond nearest the reducing end leads to the production of radioactive

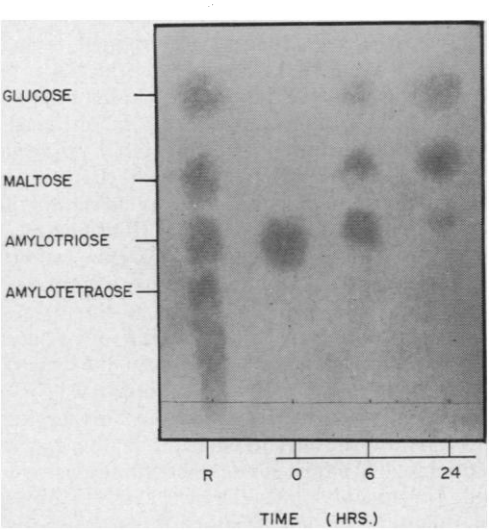

Fig. 1. A triple-ascent paper chromatogram of the digest of amylotriose and crystalline salivary amylase at several stages of enzymolysis: R, reference amylooligosaccharides.

Table 1. Radioactivities of products in the digest of 1-C^{14}-amylotriose with crystalline salivary amylase.

Compound	0 hr (counts/min)	6 hr (counts/min)	24 hr (counts/min)
Glucose	8	185	366
Maltose	4	156	226
Amylotriose	692	350	107

glucose and nonradioactive maltose, while hydrolysis of the bond nearest the nonreducing end leads to the formation of nonradioactive glucose and radioactive maltose. Since the radioactivity of the glucose produced from 1-C^{14}-amylotriose was consistently higher than that of the maltose, a faster rate of hydrolysis is indicated for the glucosidic bond nearest the reducing end. It is generally believed that alpha amylase action on starch and starchlike compounds proceeds from both the reducing and the nonreducing end of the molecule. These results support this type of action pattern for salivary amylase.

References and Notes

1. J. H. Pazur, *J. Biol. Chem.* **205**, 75 (1953).
2. W. J. Whelan and P. J. P. Roberts, *J. Chem. Soc.* 1298 (1953).
3. We are indebted to Jytte Muus, Department of Physiology, Mount Holyoke College, South Hadley, Mass. for the generous sample of crystalline salivary amylase. The sample had been recrystallized four times as described by Muus [*Compt. rend.* **28**, 317 (1953)].
4. Published with the approval of the director as Paper No. 690, Journal Series, Nebraska Agricultural Experiment Station.
5. R. M. Sandstedt, E. Kneen, M. J. Blish, *Cereal Chem.* **16**, 712 (1939).
6. D. French, D. W. Knapp, J. H. Pazur, *J. Am. Chem. Soc.* **72**, 5150 (1950).
7. J. H. Pazur, *ibid.* **77**, 1015 (1955).
8. D. J. Manners, *Ann. Repts. Chem. Soc.* **50**, 288 (1953).

26 January 1955.

Method for Tracing Dark Adaptation in the Pigeon

Donald S. Blough

Harvard University, Cambridge, Massachusetts

Animal subjects are not often used in psychophysical research, because they cannot follow complex instructions or report verbally what they see or hear. The method described in this paper represents an attempt to overcome these difficulties and to obtain with animals some of the efficiency and control that human subjects provide. The method owes much to the work of Skinner and his associates (*1*) and to Békésy's method of human audiometry (*2*). The procedure outlined here is designed for the study of dark adaptation in the pigeon (*3*), but, with modifications, it may be applied to a variety of animal discrimination problems.

Automatic apparatus is used. It includes the following items: (i) a light-tight adaptation box, containing pigeon, response keys, food magazine, and stimulus patch; (ii) a network of relays and timers that control the stimulus luminance and the presentation of food; (iii) a light source and an optical system, with a device that continuously records the stimulus luminance.

A panel divides the adaptation box into two chambers. The bird is trained to stand in one chamber and place its head through a round hole in the panel (Fig. 1). The bird faces a small window through which it views a stimulus patch, 1 cm in diameter, 4 cm beyond the frame of the window. The only light in the adaptation box comes from this stimulus patch. There are two small response keys, *A* and *B*, just below the window. Each peck on one of these keys momentarily opens a switch that is connected with the controlling relay network. When the bird is to be rewarded, a solenoid raises a magazine containing grain to an opening in the floor below the response keys.

The stimulus patch is illuminated from behind by a beam of light. A motor-driven optical wedge in the path of the light beam regulates the luminance of the patch. A shutter may be closed to black out the stimulus patch completely. The movements of both the wedge and the shutter are controlled through the relay network.

The pigeon's basic task is to peck key *A* when the stimulus patch is visible and to peck key *B* when the patch is dark. Training on this discrimination proceeds in several stages. When the bird becomes proficient at one stage, the next stage is introduced; 50 training hours may be needed before experimental data can be collected.

First, the hungry bird (70 to 80 percent of free-feeding cage weight) is trained to peck the two keys at random by the "response differentiation" technique described by Ferster (*4*). Next, the stimulus patch is illuminated, and the control circuit is so adjusted that a peck on key *A* closes the shutter, blacking out the patch. After a peck on key *A* has blacked out the patch, a peck on key *B* causes the food magazine to be raised within reach for about 5 sec. Pecks on key *B* are useless when the patch is lighted, and pecks

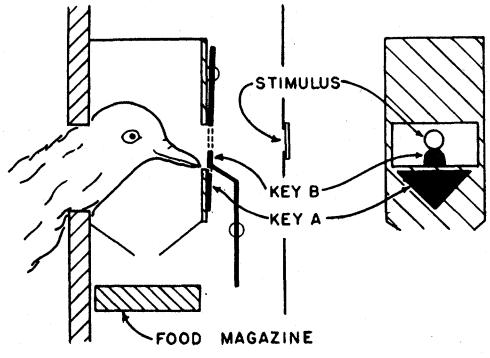

Fig. 1. Response chamber of the adaptation box. (Left) Side view, showing relative positions of pigeon, food magazine, response keys, and stimulus patch. (Right) Keys *A* and *B* and patch seen from the pigeon's position.