loss of collagenous fibers, as seen for example in periodontitis, results from primary alteration by the inflammatory response and secondary attack by bacterial proteases.

Summary. Treatment with ethylene oxide rendered reconstituted acid-soluble collagen readily digestible by combined pure cultures of human oral strains of fusiform bacilli, diphtheroid bacilli, streptococci, and veillonellae. The fact that only a few of these strains individually attacked such altered collagen to a slight extent indicates digestion by synergistic and symbiotic reactions between members of the oral microbiota. Concomitantly with increased digestibility from treatment with ethylene oxide, reconstituted collagen exhibited marked morphological changes as revealed by electron microscopy.

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Received October 29, 1959. P.S.E.B.M., 1960, v103.

Separation of 2 Glutamic-Oxalacetic Transaminases by Paper Electrophoresis.* (25469)

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The enzyme glutamic-oxalacetic transaminase (GOT) has attracted considerable interest recently because its concentrations in blood and tissue are altered characteristically in a number of disease states. Methods of purification devised up to now(1,2) have produced no indication that this enzyme may occur in more than one form. We observed that crude GOT in an extract of cardiac muscle from dog, when chromatographed on certain ion exchangers, behaves as a mixture of 2 enzymes. When CM-cellulose was employed with phosphate buffer of pH 7.0 and $\Gamma/2 =$ 0.01 or less, one component was adsorbed and could be eluted at higher ionic strength and lower pH, while the other was adsorbed very little if at all. With DEAE-cellulose the behavior of the 2 fractions was reversed. These findings suggested that the 2 GOT's may have opposite electrical charges at pH 7. To test this assumption, the paper electrophoresis of crude preparations of GOT was investigated.

Methods and materials. Tissues were minced and extracted in a blendor with 3 volumes of cold water. The centrifuged extracts were concentrated several fold by ultrafiltration at 0°C. The solutions were recentrifuged at 30,000 g. They were then applied, in proportions of 10 to 40 μl, containing about 1 to 5 mg of protein, to the paper strips in a Spinco electrophoretic apparatus Model R. Phosphate buffer of pH 7.4, $\Gamma/2 = 0.075$, was used, often with the addition of one per cent bovine serum albumin as a stabilizer of enzymatic activity. Stabilization could be achieved also by applying quantities of tissue extracts corresponding to the larger amount of protein. Current was 5 milliamperes, temperature 8°C,

* This investigation was supported in part by Research Grant from Nat. Inst. of Arthritis and Metab. Diseases, P.H.S.

† The Mayo Foundation, Rochester, Minn., is part of Graduate School of Univ. of Minnesota.

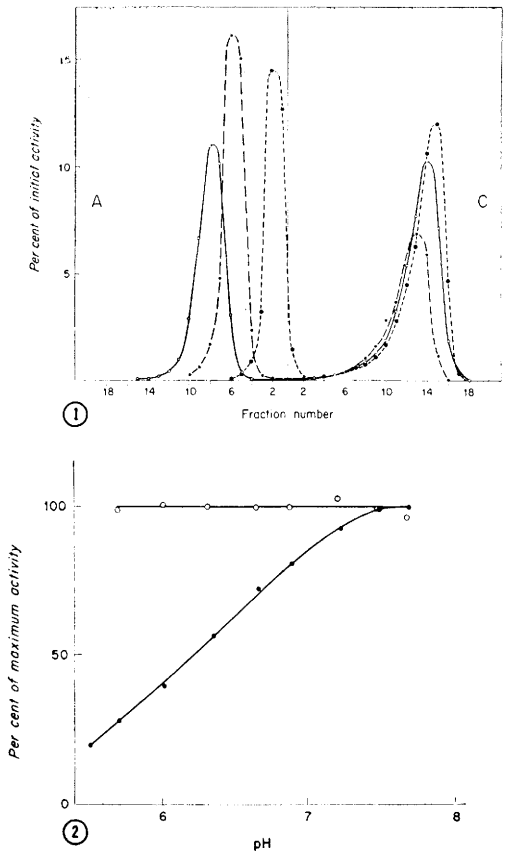

FIG. 1. Distribution of GOT activity following paper electrophoresis of crude heart extracts of man (○——○), pig (●– – –●), and dog (●·–·–●). Preparations were applied at center line. A and C represent, respectively, anode and cathode.

FIG. 2. Dependence on pH of the GOT activity (●, anionic; ○, cationic) of dog heart fractions.

time 45 hours. After electrophoresis the strips sometimes were dried partially in air (up to 1 hour), which did not cause loss of activity. They were cut into sections 5 mm wide, each of which was eluted with 2 ml of 0.9% NaCl. The various fractions were tested for GOT activity by the spectrophotometric method of Karmen at 38°C(3). Enzymatic activities were expressed as percentages of total activity applied to the paper. With the precautions specified, recoveries ranged from 70 to 80%. For study of the dependence of GOT activity on pH and on concentrations of α-ketoglutarate and L-aspartate, use was made of electrophoretic fractions at the peak of the bands. The pH and substrate concentrations listed were those of the final test mixtures. For the differential test the conditions were pH 6.0 with 5×10^{-3} moles/l of aspartate and pH 7.4 with 10^{-1} moles/l of aspartate. In both tests the α-ketoglutarate was 2×10^{-2} moles/l, and the buffer concentration and all other conditions were those of the standard test at pH 7.4. Because of the difference in substrate concentrations, the results in the special test at pH 7.4 were about 16% higher than in the standard test.

Results and comment. Electrophoretic patterns of GOT in heart extracts of man, pig, and dog are shown in Fig. 1. In each instance there were 2 separate active fractions of which one migrated toward the anode, the other toward the cathode. In the anionic activity the human transaminase always moved the fastest, the canine transaminase the slowest. On the other hand, differences in the rates of migration among the cationic enzymes are not necessarily significant. The ratios of the sum of cationic to the sum of anionic activities were 1.15, 0.72 and 1.37, respectively, for human, porcine, and canine heart. These patterns were obtained with 1 to 2 mg of tissue protein in the presence of serum albumin. In the absence of albumin the cationic bands were reduced to less than half, while the anionic bands were not affected. Increasing the amount of tissue protein applied to the paper was found to have the same beneficial effect as the presence of albumin in the buffer.

The 2 bands of GOT activity behave differently toward changes in pH shown in Fig. 2 for dog heart. The activity of anionic GOT, relatively low at pH 6 and less, rises to a maximum near pH 7.7, while the cationic GOT has a constant activity over this pH range. The former curve corresponds to that obtained with human serum(3) and with purified enzyme from pig heart(1).

The dependence of the 2 enzymatic activities on substrate concentrations was investigated at pH 6.0 and 7.4. The Michaelis constants determined from Lineweaver-Burk plots are summarized in Table I. The constants for L-aspartate at optimal concentration of α-ketoglutarate show striking differences, indicating that cationic enzyme has much greater affinity than the anionic for this substrate. On the other hand the latter enzyme

TABLE I. Michaelis Constants for 2 GOT Fractions from Canine Heart.

GOT fraction	pH	Km for α-ketoglutarate, moles/l*	Km for L-aspartate, moles/l†
Cationic	6.0	1.08×10^{-3}	2.48×10^{-3}
	7.4	$1.18 \times$ "	$.70 \times$ "
Anionic	6.0	$.17 \times$ " ‡	$38.4 \times$ " §
	7.4	$.57 \times$ "	$11.9 \times$ "

* With 66.7×10^{-3} moles/l of L-aspartate.
† " $13.3 \times$ " " " α-ketoglutarate.
‡ Inhibition above 4×10^{-3} moles/l of α-ketoglutarate.
§ Km = 21.9×10^{-3} moles/l at 3.33×10^{-3} moles/l of α-ketoglutarate.

showed greater affinity for α-ketoglutarate than the former, but was inhibited at pH 6.0 by concentrations above 4 x 10^{-3} moles/1.

On the basis of these data a differential test at these 2 pH values was devised for the purpose of calculating the proportion of the 2 GOT activities in unfractionated extracts. At pH 7.4 the sum of both activities was measured with an excess of both aspartate and α-ketoglutarate; at pH 6.0 cationic GOT was determined in almost complete exclusion of anionic GOT by using a high concentration of α-ketoglutarate and a low concentration of aspartate. The activity quotients (activity at pH 6.0 divided by activity at pH 7.4) are summarized in Table II. They were 0.415 to 0.472 for cationic fractions of cardiac muscle and liver from different species, and 0.023 to 0.027 for anionic fractions. The quotients found in crude extracts of heart (Table III) indicate a distribution comparable to that which the electrophoretic separation of similar extracts had shown (Fig. 1). It is of interest

TABLE II. Activity Quotients (Results at pH 6.0/Results at pH 7.4) of Cationic and Anionic GOT.

Source		Quotient: Cationic GOT	Quotient: Anionic GOT
Heart	Dog	.452	.026
	Man	.465	.026
	Pig	.415	.023
Liver	Dog	.472	.027

that the ratio between activities in canine and human liver is similar to the ratio between activities in the 2 kinds of heart, while serum from 3 patients with myocardial infarction and one with acute viral hepatitis gave indications for only small portions of cationic transaminase (Table III).

We have also studied the electrophoretic behavior of glutamic-pyruvic transaminase in heart and liver. There was no indication, however, for the presence of more than one component.

TABLE III. Activity Quotients (Results at pH 6.0/Results at pH 7.4) of Extracts and Serums, and Calculated Portions of Cationic Transaminase.

Source		Quotient	Cationic GOT, %
Crude extracts			
Heart	Dog	.281	60
	Man	.241	49
	Pig	.203	46
Liver	Dog	.255	51
	Man	.248	51
Serums			
Myocardial infarction		.057	7
		.067	9
		.074	11
Infectious hepatitis		.053	6

We are presently engaged in purification of cationic GOT.

Summary. By use of paper electrophoresis of crude extracts of heart and liver, 2 fractions with GOT activity were obtained, one of which migrated toward the anode and showed the known characteristics of serum GOT, while the other migrated toward the cathode and exhibited different affinities for both α-ketoglutarate and L-aspartate.

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Received October 30, 1959. P.S.E.B.M., 1960, v103.