Quantitative Determination of Isozymes of Glutamic Oxalacetic Transaminase in Human Serum.* (29084)

WALTER D. BLOCK,† ROBERT CARMICHAEL, AND CHARLES E. JACKSON
Caylor Nickel Research Laboratories, Bluffton, Ind.

Glutamic-oxalacetic transaminase (GOT) concentrations in blood and serum are altered characteristically in a variety of disease states and, therefore, have assumed clinical importance in medicine(1). This enzyme recently has been shown to exist in various molecular forms, or isozymes, in mammalian tissues and sera(2-5). Present methods for studying these isozymes are inadequate to determine if abnormalities exist between normal serum and tissues and those from individuals with various diseases. Therefore, the following work was undertaken to develop a satisfactory quantitative method and to investigate isozyme forms and concentrations of GOT in human serum.

Materials and methods. Serum. Freshly separated serum, from 24 control subjects ranging in age from 10 to 70 years, was used. *Concentration of serum.* Four ml of serum were placed into Visking tubing (18/32″ diam.) and concentrated 4- to 6-fold by dialyzing for 16 to 18 hours at 0-4°C against a 15% solution of polyvinyl pyrrolidone (PVP). *Electrophoresis. Starch gel.* A modification of the procedure of Smithies was used(6). Gel prepared from hydrolyzed potato starch (Connaught Medical Research Laboratories, Toronto) and 0.023 M boric acid - 0.0092 M sodium hydroxide buffer, pH 8.9, was poured into a Lucite tray 2 cm wide and 42 cm long. A slit 0.25 cm wide was cut approximately one-third the distance from one end of the gel and filled with a slurry of 0.5 ml of concentrated serum and 0.3 g of unhydrolyzed potato starch. Horizontal electrophoresis was done at 10°C for 21-22 hours with a voltage of 4.5 V/cm using a 0.30 M boric acid - 0.06 M sodium hydroxide buffer, pH 8.2, as a bridge. The electrodes were reversed to permit better separation of the faster moving cathodic GOT isozymes. *Soluble starch* (purified potato starch). The procedure of Kunkel and Slater was used(7). *Determination of enzyme activity.* Total GOT activity of fresh and concentrated serum was determined by the methods of Steinberg *et al*(8), and Reitman and Frankel(9). After electrophoresis in starch gel of concentrated serum, the gel was cut horizontally into an upper and lower half. One-half was cut into a continuous series of 1 cm segments, and each segment placed into an individual centrifuge tube. Two ml of 0.1 M potassium phosphate buffer, pH 7.4, were added and the segment macerated with a narrow nickel spatula. Material adhering to the spatula was rinsed into the tube with 0.2 ml of buffer, and the suspension centrifuged at 3500 rpm for 15 minutes. The supernatant fluid was transferred to a 1 cm Beckman DU cuvette and assayed for GOT activity(8). The other half of the gel was segmented as above and each 1 cm segment suspended in 1 ml of substrate (2 mM α-ketoglutarate and 200 mM dl-aspartic acid in phosphate buffer, pH 7.4), macerated, the spatula rinsed with 0.2 ml of substrate, and the suspension centrifuged as described above. The supernatant fluid was assayed for GOT activity by the colorimetric method of Reitman *et al*(9). In addition, areas of GOT activity were determined in starch gel by the staining technique of Schwartz *et al*(10) modified so that a 0.1 M phosphate buffer, pH 7.4, instead of 0.067 M was used. After zone electrophoresis in soluble starch of concentrated serum, a continuous series of 1 cm starch plugs was removed. Each plug was placed into an individual centrifuge tube, GOT eluted using the appropriate procedure, and the relative concentration determined by the 2 photometric methods(8,9).

* Supported in part by U. S. Public Health Service grant.

† Address: Dept. of Dermatology, Biochemical Research Laboratories, Univ. of Michigan Med. Center, Ann Arbor.

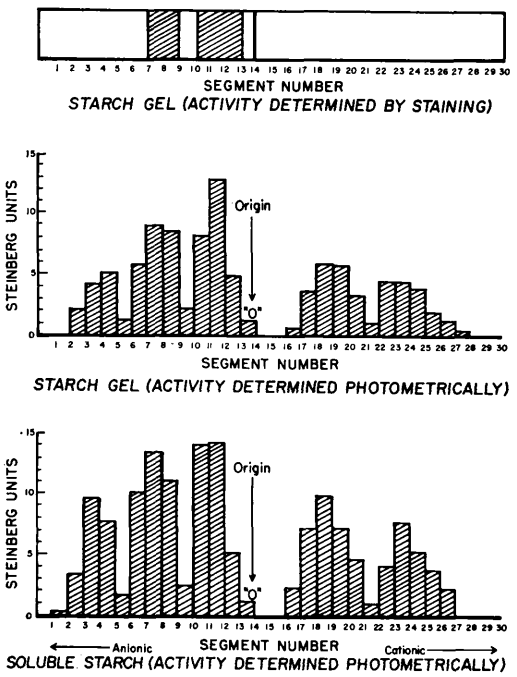

FIG. 1. Comparison between staining and photometric analysis for GOT activity after zone electrophoresis in starch gel and soluble starch of concentrated serum from control subjects.

Results. As the spectrophotometric method of Steinberg *et al*(8) and the colorimetric method of Reitman *et al*(9) gave essentially the same values for GOT activity, reference is made only to results obtained with the former method unless specified otherwise. Fig. 1 depicts results obtained when concentrated serum was subjected to starch gel and soluble starch electrophoresis and the zones of GOT determined by staining or by eluting the bands and analyzing the activity photometrically. Two anionic bands of activity could be detected by staining, no cationic bands were visible. If the GOT were eluted, 3 anionic and 2 cationic areas of activity could be detected in serum by the more sensitive and quantitative photometric assay regardless of whether starch gel or soluble starch electrophoresis was used. The bands were approximately in the same positions with either method, although the amount of GOT activity was greater and the areas defined more sharply with the soluble starch preparation. With the technique of elution used, approximately 93% of applied GOT activity was recovered from the soluble starch; 87% from the starch gel preparation.

Table I presents average relative percentage distributions of GOT activity in each anionic and cationic area after zone electrophoresis in starch gel of serum from 24 subjects, who were placed into one of 3 groups according to age. The anionic area C had the greatest GOT activity and area B the next, except in age group 50-70, where areas B and C had approximately equal activity. These 2 anionic areas, B and C, accounted for 48 to 55% of the total GOT activity. Concentrations of GOT in the cationic areas

TABLE I. Average Relative Percentage Distribution of GOT Isozymes After Zone Electrophoresis in Starch Gel of Concentrated Serum from Control Subjects.

Subjects				Areas of Activity									
				Anionic						Cationic			
				A		B		C		D		E	
No.	♂	♀	Range of age	T*	%†	T	%	T	%	T	%	T	%
				units‡		units		units		units		units	
7	3	4	10-30	10.8	10.4	24.6	23.6	27.0	25.9	21.1	20.3	20.4	19.9
7	5	2	30-50	15.0	14.7	21.7	21.5	26.5	26.3	20.0	19.8	17.7	17.5
10	4	6	50-70	20.4	13.3	42.2	27.7	41.1	27.0	27.2	18.6	20.8	13.6
Avg value for concentrated serum				15.4	12.8	29.5	24.3	31.5	26.4	22.8	19.6	19.6	17.0
Avg value for unconcentrated serum				3.2§		6.1		6.6		4.9		4.2	

* Total enzyme activity in each area.
† Total enzyme activity in each area × 100/total enzyme activity of all areas.
‡ Steinberg units(8).
§ Total GOT activity of fresh serum × percentage of activity in each area of concentrated serum subjected to electrophoresis.

D and E were approximately equal in the 2 younger age groups, and accounted for 37 to 40% of the total activity. This was not true for age group 50-70, as area D was more active than area E. On the whole, however, there did not appear to be any differences in percentage distribution of isozyme activity in serum that were consistent with the ages of the subjects. Therefore, the relative percentage distribution of activity in each area for all groups was averaged and this figure used to extrapolate the units of GOT that would be present in unconcentrated serum (Table I).

A series of replicate analyses was done to determine reproducibility of values for serum GOT isozyme activity obtained by the combination of starch gel zone electrophoresis and photometric assay. The average difference of the values from the mean was 9%, the range 0 to 20%. The thermostability of the five molecular forms of GOT found in serum after starch gel electrophoresis was studied by varying the temperature at which the supernatant fluid extracted from the gel was incubated with the buffered substrate(9). If the temperature were raised from 37° to 55°C, loss of activity ranged from 37% in area C to 60% in area A. A further increase in temperature resulted in a further loss of activity in all areas.

Discussion. GOT in human serum concentrated by dialysis against PVP could be separated into 5 molecular forms, 3 anionic and 2 cationic, by zone electrophoresis in either starch gel or soluble starch. Elution of these molecular forms separately and quantitatively from both of the starch preparations was possible with relatively little loss in activity. The slightly higher recoveries noted with soluble starch preparations probably resulted because the enzyme is easier to elute from this medium. The relative percentage distribution of GOT, however, was approximately the same as that found with starch gel, which is more adaptable for routine analytical work. A staining technique employing azoene fast violet B for determination of GOT active areas lacked sensitivity, and was not satisfactory. The molecular forms of serum GOT, unlike those of some other enzymes(11), were found to be thermolabile at temperatures over 37°C.

Summary. Three anionic and two cationic isozymes with GOT activity were found in serum from human subjects, and their relative concentrations determined. A specific and quantitative method involving zone electrophoresis in starch gel of serum concentrated against PVP, elution of GOT from the gel, and determination of GOT activity by photometric means is described.

1. Agress, C. M., *Am. J. Cardiol.,* 1959, v3, 74.
2. Fleisher, G. A., Potter, C. S., Wakim, K. G., PROC. SOC. EXP. BIOL. AND MED., 1960, v103, 229.
3. Augustinsson, K. B., Erne, K., *Experientia,* 1961, v17, 396.
4. Boyd, J. W., *Clin. Chim. Acta,* 1962, v7, 424.
5. Decker, L. E., Rau, E. M., PROC. SOC. EXP. BIOL. AND MED., 1963, v112, 144.
6. Smithies, O., *Biochem. J.,* 1959, v71, 585.
7. Kunkel, H. G., Slater, R. J., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 42.
8. Steinberg, D., Baldwin, D., Ostrow, B. H., *J. Lab. and Clin. Med.,* 1956, v48, 144.
9. Reitman, S., Frankel, S., *Am. J. Clin. Path.,* 1957, v28, 56.
10. Schwartz, M. K., Nisselbaum, J. S., Bodansky, O., *Tech. Bull. Med. Tech.,* 1963, v33, 101.
11. Wróblewski, F., Gregory, K. E., *Ann. N. Y. Acad. Sci.,* 1961, v94, 912.

Received December 30, 1963. P.S.E.B.M., 1964, v115.