Effect of Carbon Tetrachloride on Serum Glutamic-Oxalacetic Transaminase Isoenzymes* (32796)

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Several investigators reported (1–4) that human and animal sera contain several glutamic-oxalacetic transaminase (GOT) isoenzymes with the same catalytic action. For instance, when starch gel electrophoresis was used for fractionation, extracts of rat liver and heart contained four isoenzymes, whereas the rat SGOT could be separated into two components (5, 6). In this laboratory extensive studies were carried out to investigate the GOT isoenzyme patterns in various clinical and experimental conditions. The purpose of these studies was to explore whether further diagnostic information would be obtainable by fractionating GOT into its isoenzyme components. During the course of these studies it also became evident that the mechanism per se responsible for increased SGOT is obscure, and basic data necessary for any kinetic interpretation of the isoenzyme changes are unavailable. Therefore, experiments were designed to obtain some of these data along with the isoenzyme findings. This paper wil present the effect of carbon tetrachloride-induced liver injury upon the SGOT isoenzyme pattern, in an attempt to relate the results to the basic mechanism of transaminase rise following hepatic damage.

Materials and Methods. Starch block electrophoresis was chosen as the method of fractionation (7). Hydrolyzed starch (Connaught Med. Research Laboratories, Toronto, Canada) was first washed with distilled water, then equilibrated with a buffer solution (0.02 *M* phosphate, pH 7.2). The supernatant was decanted and the sedimented slurry was poured and compressed into a 15 × 30 cm plastic tray. A slit 0.25 cm in width was cut off in the center, closer to the anode, and filled with strips of thick filter paper saturated with the sample. The plastic tray was placed horizontally on the electrophoretic apparatus and the ends of the block were connected with the electrode vessels by means of filter paper strips. The electrophoresis was carried out at 20°C for 20 hours with a voltage of 320 V, using the same phosphate buffer as for the starch. Then the entire starch block was sliced into 6-mm segments and each segment placed into an individual centrifuge tube. The GOT activity was assayed with a slight modification of the colorimetric method of Babson (8). Briefly, 1 ml of buffered GOT substrate was added to each tube, shaken vigorously, and centrifuged at 3500 rpm for 15 min. The clear supernatants were transferred quantitatively into assay tubes, incubated for 60 min at 37°C and after addition of the diazonium salt the color intensity was measured spectrophotometrically at 530 mμ.

Hepatic injury was induced in male and female rats, weighing around 250 gm, by administering carbon tetrachloride. All rats in this study were selected from our own Wistar colony and appeared healthy. Chemically pure carbon tetrachloride (Fisher Scientific Co., New York) was mixed with an equal volume of paraffin oil, and 0.2 ml per 100 gm of body weight was administered intraperitoneally. Rats injected with only paraffin oil served as controls. Blood samples were obtained by cardiac puncture under ether anesthesia. The rats were sacrificed by rapid exsanguination. After death the tissue specimens were removed without delay. Approximately 1 gram of liver tissue was gently homogenized in 10 ml of ice cold saline, using a glass tissue grinder. The suspension was cooled in the freezer, then centrifuged to remove particulate matter. The supernatant was diluted 1:20 with buffered solution of 3% bovine albumin and applied onto the starch block immediately to begin the electrophoretic fractionation.

Results. (a) Baseline values. In the serum of the intact rat we found five distinct GOT isoenzymes. Figure 1 shows a typical elution

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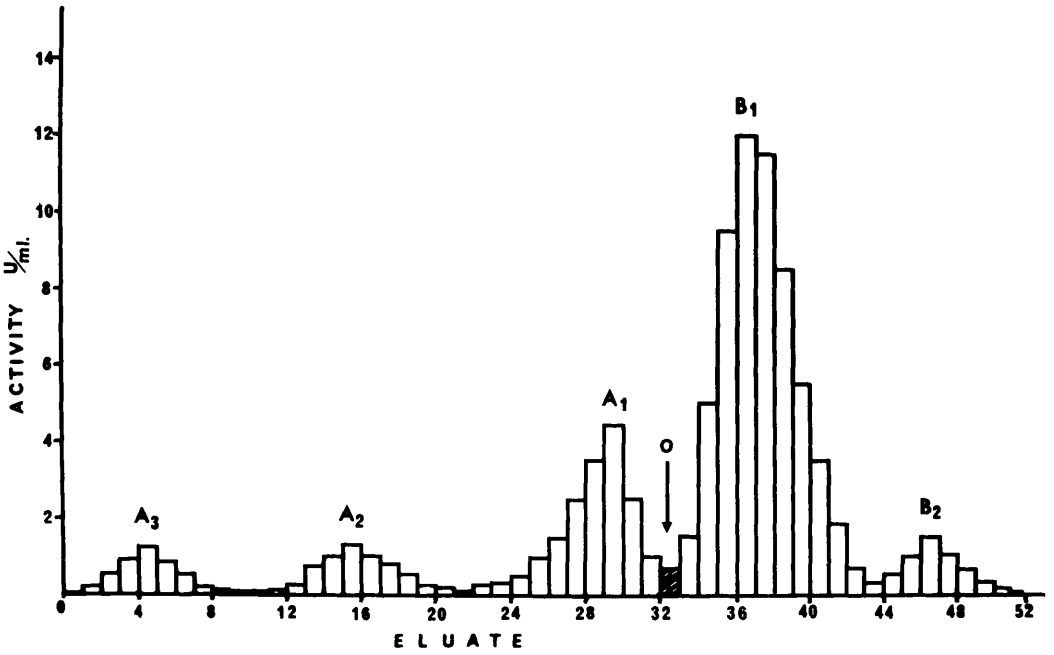

FIG. 1. GOT activity of eluate after starch block electrophoresis. Rat serum was placed at → O. Cathode is to the left.

pattern with the five fractions. Three fractions, A_1, A_2, and A_3, were detected in the cationic area, and two, B_1 and B_2, in the anionic area. The fractions A_3 and B_2 migrated faster whereas the A_1 and B_1 remained closest to the area of application. Most of the activity was recovered in the A_1 and B_1 fractions. Little activity was found on the application point (0).

The relative proportions of these five isoenzymes in a group of intact, apparently healthy rats are shown in Table I.

(b) Experimental liver injury. Carbon tetrachloride administration caused a rapid rise in the SGOT activity. Moreover, the relative distribution of the isoenzymes showed a shift (Fig. 2). Fraction A_2 increased disproportionately, whereas the increase of A_3, A_1, and B_2 was relatively less. The distortion of the distribution pattern was most prominent on the first days after CCl_4 injection, and decreased gradually toward the normal proportions later. Control rats receiving only paraffin oil showed SGOT isoenzyme patterns similar to those of the intact rats. (Table II).

Fractionation of the supernatant of the liver homogenate revealed a GOT isoenzyme profile that was qualitatively similar, but quantitatively quite different from that of the serum (Fig. 3). About one third of the activity appeared in the A_2 position and half of the activity was in the B_1 area.

Discussion. In laboratory medicine, assay of SGOT is an everyday procedure in suspected hepatic and myocardial diseases, yet the mechanism causing the rise in SGOT enzyme level has still been inadequately studied. One may consider three mechanisms (9): (a) mi-

TABLE I. Relative Distribution of GOT Isoenzymes in Intact Rat Sera.

Subject (rat)	Total activity (U/ml)	Cathode			Anode	
		A_3 (%)	A_2 (%)	A_1 (%)	B_1 (%)	B_2 (%)
1	117	3.6	4.4	18.3	69.5	4.2
2	136	3.1	4.9	15.7	72.6	3.7
3	98	2.8	5.2	13.4	75.2	3.4
4	120	4.3	3.8	16.8	70.3	4.8
5	104	4.6	3.4	17.6	69.2	5.2
6	143	2.5	5.6	14.0	74.8	3.1
7	112	3.8	4.2	16.3	71.3	4.4
8	140	2.7	5.4	15.3	73.4	3.2
Mean:	121	3.4	4.6	16	72	4
SD:	±16.8	±0.7	±0.8	±1.5	±2.3	±0.8

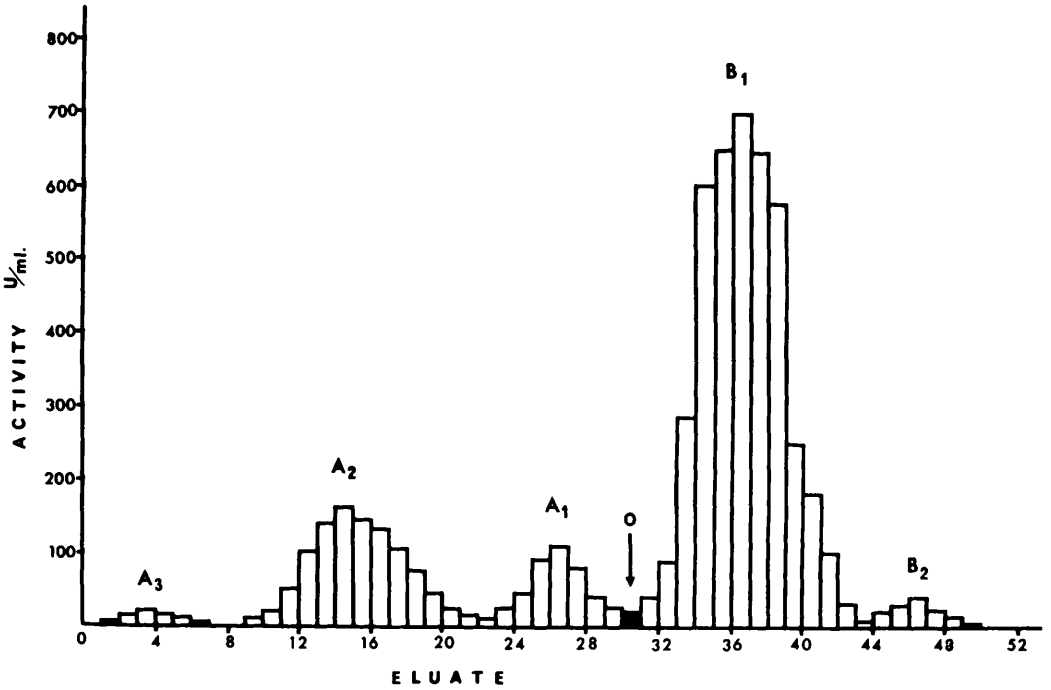

FIG. 2. Altered pattern of SGOT isoenzymes in a rat injected with CCl_4 one day previously. Fractionation techinque is same as in Fig. 1. Note prominent A_2 fraction.

tochondrial disruption in the injured cells followed by direct release of mitochondrial enzymes into the circulation; (b) "leaching" of (cytoplasmic and mitochondrial) enzymes through the damaged cell membrane; and (c) increased *de novo* synthesis by the injured liver cells, resulting in a higher equilibrium level. It should be kept in mind that the serum level of any component is generally regulated by (i) influx rate and (ii) elimination by inactivation, excretion, etc.

In our studies the supernatant of liver homogenate contained the same five fractions as the serum (Fig. 3) but in different proportions. In relative terms, A_2 was much greater in the liver homogenate. We assume that our method of homogenization disrupts subcellular structures and the supernatant is a true representation of the hepatocellular GOT enzymes, cytoplasmic as well as mitochondrial. If this assumption is valid, we can conclude that the level of A_2 is relatively higher in the liver cell than in the serum. The difference between the liver isoenzyme pattern and serum may be due to shorter intravascular life of A_2 isoenzyme (10). Alternatively, some of the SGOT isoenzymes may originate from sites other than the liver.

TABLE II. Average Distribution of GOT Isoenzymes in Sera of CCl_4 Treated Rats.

Treatment and time (hours)	No. of rats	Total activity (U/ml)	Cathode						Anode			
			A_3		A_2		A_1		B_1		B_2	
			%	Units	%	Units	%	Units	%	Units	%	Units
Paraffin oil controls	3	124	3.0	4	5.2	6	18.1	23	70.2	87	3.5	4
CCl_4 24	3	5380	1.3	70	18.7	1006	11.6	624	67.6	3637	0.8	43
CCl_4 48	3	3097	1.2	37	19.4	601	10.5	325	67.9	2104	1.0	30
CCl_4 72	3	246	1.7	4	6.8	17	22.4	55	66.4	163	2.7	7

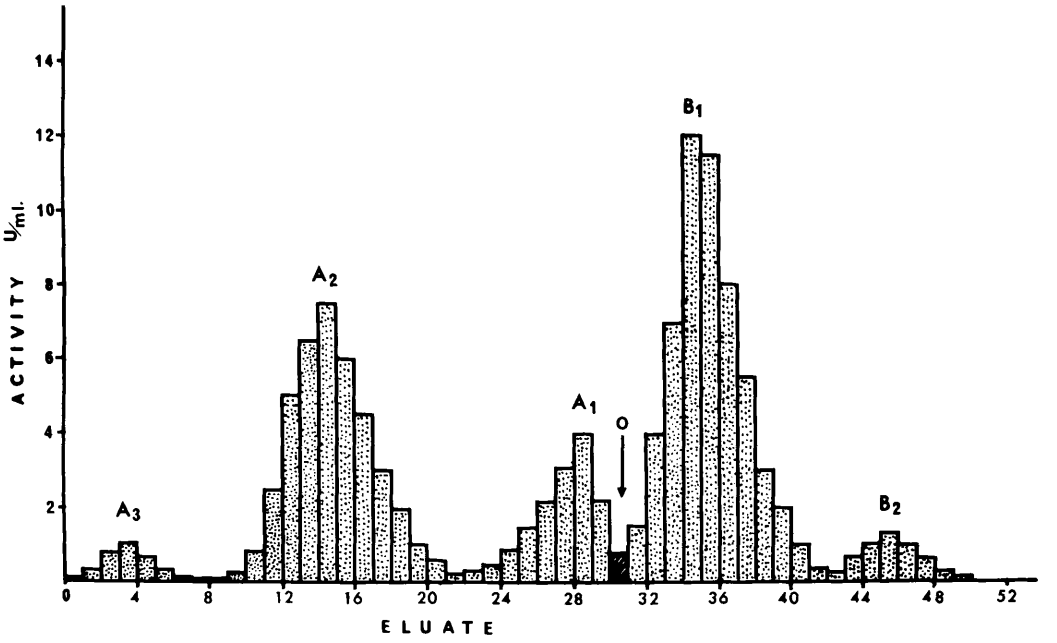

FIG. 3. GOT isoenzymes in supernatant of rat liver homogenate. Fractionation technique is same as in Fig. 1.

The results with carbon tetrachloride offer some further insight. The increase of A_2 fraction was relatively greater. The intrahepatic and serum isoenzyme profiles become nearly identical. Presumably the cationic fractions represent mitochondrial GOT (5, 11) whereas the anionic GOT has been interpreted as cytoplasmic in origin. Our results indicate a general increase of all five isoenzymes following carbon tetrachloride injury, but the A_2 isoenzyme, in particular, increased. This seems to be in agreement with the claim that carbon tetrachloride injury involves the mitochondria in particular (12). The full explanation of the altered SGOT profile is still lacking. The shift may reflect a change in the proportions of influx of the five isoenzymes, approaching the proportions of the liver homogenate. Our experiments do not exclude the other possibility, viz., that the clearance mechanism of the circulating A_2 isoenzyme is impaired resulting in piling up of this particular fraction in the serum. The kinetics of blood clearance should be studied in an isolated fashion to settle this issue.

The studies reported here suggest, however, that the elevation of SGOT after CCl_4 treatment is not simply a change in the balance of production of this enzyme, since the relative distribution profile in the serum became altered. In addition, it appears unlikely that the origin of the rising SGOT is preferentially due to mitochondrial disruption, since both B fractions were also elevated. Lacking more specific kinetic data (blood clearance, etc.), these studies allow only the general conclusion that apparently both cytoplasmic and mitochondrial release might be the most plausible explanation of the rise in GOT activity following carbon tertachloride poisoning in the rat. Our findings seem to support only partially the interpretation of Kawasaki *et al.* (13) that CCl_4 injury causes release of hepatic cytoplasmic GOT since the "mitochondrial" GOT increases even higher. This discrepancy may well be due to the difference in the fractionation techniques. Finally, it should be mentioned that our method resulted in only three GOT components in human serum. Therefore, the animal experiment data presented here should not be extrapolated to the human without further studies.

Summary. Starch block electrophoresis of rat serum resulted in five glutamic-oxalacetic transaminase (GOT) isoenzyme fractions. Carbon tetrachloride-induced liver injury was

associated with marked rise of all five fractions but to a different degree. The cationic A_2 fraction increase was greater. This altered isoenzyme distribution resembled the pattern of GOT isoenzymes in the liver homogenate. The implication of these findings with respect to the transfer of the intracellular hepatic GOT into the serum is considered.

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Regulation of Erythropoiesis. XXI. The Effect of Erythropoietin on the Stem Cell* (32797)

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It is generally appreciated that the recognizable elements of the bone marrow are not self-sustaining but are supported by a precursor compartment which is pluripotential, giving rise to erythroid, myeloid and megakaryocytic elements. In significant measure this hypothesis rests upon the observation of chromosomal abnormalities in radiation chimeras (1) and the presence of a pH chromosome in erythroid, myeloid and megakaryocytic elements in patients with chronic myelogenous leukemia (2). More recently a question has been raised as to whether there is, in addition, a committed precursor compartment intermediate between the primitive pluripotential cell and the differentiated or recognizable bone marrow elements. Support for the latter thesis derives from a number of observations: (i) in studies in vincristine-treated rats megakaryocytic differentiation appeared to continue although erythroid differentiation could not be achieved even with massive doses of erythropoietin (EP) (3); (ii) thymidine labeling data on megakaryocytes pointed to a difference in generation time between the precursor cell of the megakaryocyte and that for the erythroid elements (4, 5); (iii) the effect of colchicine on the hematopoietic recovery of mice (6); and (iv) observations of the effect of hypoxia on the pluripotential stem cell (7). In the latter studies Bruce and McCulloch observed that in the mouse the primary erythroid response to hypoxia was splenic and occurred within 2–3 days. This initial response was followed rather than preceded by a decrease in the numbers of splenic colony-forming units (CFU) or pluripotential cells. These observations were interpreted as evidence for an intermediate erythropoietin-sensitive precursor compartment which was not transplantable and served as a buffer between the pluripotential and the differentiated compartments. The slow decline in colony-forming units in the spleen of the hypoxic mice was suggested to be the consequence of a depletion of the pool of erythropoietin-sensitive cells with subsequent dif-

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