GLUTAMIC-OXALOACETIC TRANSAMINASE ACTIVITY OF SERUM IN MICE WITH VIRAL HEPATITIS*,‡

By CHARLOTTE FRIEND, PH.D., FELIX WRÓBLEWSKI, M.D., AND JOHN S. LA DUE, M.D.

(From the Sloan-Kettering Institute for Cancer Research, New York)

(Received for publication, August 17, 1955)

The finding of striking elevation of the serum activity of glutamic-oxaloacetic transaminase (SGO-T) during the introductory clinical phase of acute infectious hepatitis (1) prompted the present study of the alterations of SGO-T during the course of mouse hepatitis. The purpose of this study was: (*a*) to ascertain the relationship, if any, between SGO-T and the course of experimental hepatitis; and (*b*) to determine whether there was any correlation between the SGO-T activity, the degree of viremia, and the histologic hepatic changes resulting from mouse hepatitis.

Materials and Methods

F_1 hybrids of C58 × BALB (F_1) cross and Princeton Rockefeller Institute (PRI) mice were inoculated with a filtrate of the hepatitis virus which had been isolated from leukemic mice (2).

At designated periods following inoculations of the mice, groups of three animals were bled from the brachial artery in such a manner that muscle trauma was reduced to a minimum. A control group of untreated mice was bled at the same time. The SGO-T activity of each serum was determined and the average for the group of three was calculated. The virus content was determined on the residual pooled blood of each of the groups. Similarly, the livers and spleens of each group were pooled, homogenized, and made up to 10 per cent in saline. The virus content of each mixture was determined. Sections of liver were obtained for histologic study.

Weanling PRI mice are extremely sensitive to mouse hepatitis (2) and were therefore used for the titration of virus content of material from infected mice. The LD_{50} was calculated by the method of Reed and Muench (3).

Serum glutamic-oxaloacetic transaminase (SGO-T) was determined spectrophotometrically (4). One unit is designated as a change in optical density of 0.001/ml./minute at wave length 340 mμ. The micromolar extinction coefficient of $DPNH_2$ is 2.05. The normal range of SGO-T for the mouse was 50 to 125 units per ml. serum.

* This work was supported in part by grants from The National Cancer Institute of the United States Public Health Service; the American Cancer Society; and the Damon Runyon Memorial Fund for Cancer Research.

‡ The authors wish to acknowledge the technical assistance of Messrs. Martin Podgainy and Herman Steiniger.

RESULTS

A group of PRI weanlings and a group of F_1 adults were inoculated intraperitoneally with 0.1 ml. of 10^{-1} dilution of virus filtrate. At 8 hours, at 24 hours, and thereafter at daily intervals, three mice in each group were bled and their livers and spleens collected as described under Methods. Virus titrations were made of the viscera and of the blood; SGO-T activity was measured in the serum. Table I summarizes the data obtained from this experiment.

The PRI mice showed an SGO-T peak on the 4th day, but could not be followed longer, since there were no survivors on the 5th day. There is an apparent relationship between the SGO-T activity and the level of the blood virus titer.

TABLE I

SGO-T Activity in Mice Inoculated Intraperitoneally with 0.1 ml. 10^{-1} Dilution of Hepatitis Virus

Time after inoculation	Virus titer				SGO-T activity	
	Blood*		Liver and spleen*		Serum‡	
	PRI	F_1	PRI	F_1	PRI	F_1
					units	*units*
8 hrs.	<1.0	<1.0	2.5	1.5	133	147
24 "	6.5	NV	4.5	4.5	130	103
48 "	3.0	1.0	3.0	4.0	373	352
72 "	3.0	3.0	5.0	5.5	2191	627
4 days	6.0	5.0	8.0	3.5	3862	1816
5 "	—	3.0	—	3.0	—	4366
6 "	—	NV	—	2.5	—	737
7 "	—	"	—	<2.0	—	193
9 "	—	"	—	NV	—	195
11 "	—	"	—	"	—	142

NV, no detectable virus.

* Values of pooled samples from three animals.

‡ Average of three individual determinations.

With F_1 mice, which are more resistant to the disease than the PRI mice, the full course of mouse hepatitis was observed from the time of injection throughout the infection and into the recovery period. The peak of SGO-T activity was reached on the 5th day following virus inoculation and gradually returned toward normal during the ensuing 6 days. Again, in this instance the relationship between the SGO-T activity and the virus content of blood is apparent.

Microscopic examination of the livers of the mice of both groups revealed no histological abnormalities 24 hours postinoculation. In the PRI mice focal necrosis was apparent by the 2nd and 3rd days, and marked diffuse necrosis was present by the 4th day. In the F_1 mice extensive and diffuse necrosis was seen on the 5th and 6th days. After this time most of the acutely ill mice had either died or had been sacrificed. By the 7th day the remaining mice in the group

were beginning to recover and in these animals areas of focal necrosis were seen only occasionally.

In an attempt to prolong the incubation period and diminish the severity of the infection, a more dilute virus inoculum was administered to F_1 mice. In this experiment 0.1 ml. of 10^{-3} dilution of mouse hepatitis was given intraperitoneally to each mouse. Generally, all the animals survived the infection with this reduced amount of virus despite considerable virus multiplication, as evidenced by high virus titers in the blood and livers and spleens during the first few days postinoculation. Table II summarizes the results of this experiment. The peak SGO-T was considerably lower, rising only to 520 units. Here again the rela-

TABLE II

SGO-T Activity in F_1 Mice Inoculated Intraperitoneally with 0.1 ml. 10^{-3} Dilution of Hepatitis Virus

Time after inoculation	Virus titer		SGO-T activity
	Blood*	Liver and spleen*	Serum‡
			units
24 hrs.	0.5	4.0	103
48 "	4.5	6.5	163
72 "	5.8	5.0	236
4 days	6.0	4.5	520
5 "	NV	2.0	316
6 "	"	1.0	272
7 "	"	NV	125
9 "	"	"	97
11 "	"	"	99

NV, no detectable virus.

* Values of pooled samples from three animals.

‡ Average of three individual determinations.

tionship between the blood virus titer and the SGO-T activity was apparent. Histological examination of the livers of these mice showed focal necrosis. The diffuse necrosis noted in the mice infected with the larger inoculum was not observed. The other organs and tissues of the mice showed no significant pathological lesions. It is of interest to note that regardless of whether the virus is administered intraperitoneally or subcutaneously (5) the virus appears in the liver and spleen first and in greater quantity than in the serum. It is shortly after virus is released into the blood as a result of destruction of liver cells that the SGO-T activity reaches a peak.

If elevation of the SGO-T results from damage to liver cells permitting the escape of the enzyme into the blood stream, trauma to liver tissue should also result in an increase in the SGO-T. The following experiment was designed to explore this hypothesis.

A group of mice, serving as controls, was subjected to a sham operation involving abdominal incision, manual manipulation of the liver, and closure of the abdominal incision. A second group of animals was subjected to the same procedure but in addition underwent left partial hepatectomy.

No significant change in SGO-T activity was found after the sham operation, but partial hepatectomy resulted in an appreciable rise in SGO-T activity 24 hours postoperatively with a return toward normal activity in the ensuing 6 days (Table III). A group of 50 unoperated mice had an average SGO-T activity of 115 units.

TABLE III

The Effect of Partial Hepatectomy and Sham Operation on SGO-T Activity

	Time after operation	SGO-T activity*
		Serum
		units
Partial hepatectomy	24 hrs.	1722
	48 "	271
	72 "	254
	4 days	273
	5 "	195
	7 "	107
Sham operation	24 hrs.	120
	48 "	70
	72 "	80
Control		115‡

* Average of three individual determinations.

‡ Average of determinations on fifty mice.

DISCUSSION

Glutamic-oxaloacetic transaminase is widely distributed in animal tissues. Although most concentrated in cardiac muscle, the enzyme is present in considerable quantities in the liver (6). The sera of human beings and of all animals tested showed glutamic-oxaloacetic transaminase activity. The range of normal values appeared to be species specific, varying from 8 to 40 units in the human being, 100 to 375 units in the rat, and 50 to 125 units in the mouse per ml. of serum.

Injury to cardiac muscle is followed by an increase in SGO-T activity which is proportional to the degree of heart cell damage (7). Simultaneously a decrease in glutamic-oxaloacetic transaminase activity was found in the infarcted muscle (8). This suggests that the rise of SGO-T activity results from the

release of intracellular glutamic-oxaloacetic transaminase into the blood stream from the damaged heart muscle. Injury to liver tissue in rats by the instillation of carbon tetrachloride into the gastrointestinal tract has been shown to cause a rise in SGO-T that is directly proportional to the amount of carbon tetrachloride given. Similarly, the degree of microscopic evidence of liver cell injury was roughly proportional to the height of the SGO-T (9).

In view of the observation that a marked rise in SGO-T occurred in patients with acute infectious hepatitis (1), the present study was undertaken to determine whether the same relationship existed in mice with virus hepatitis. Although mouse hepatitis differs from human infectious hepatitis, especially in that the incubation period and course of the disease are appreciably shorter, the qualitative and quantitative changes in SGO-T activity appear to be comparable.

The observations on mouse hepatitis herein reported suggest that liver cell injury due to virus hepatitis likewise results in an increase in SGO-T activity which is roughly proportional to the amount of the inoculum given, to the amount of the virus in the blood, and to the extent of histological necrosis of the liver. Increased SGO-T activity following partial hepatectomy in mice suggests that the SGO-T rises as a result of release of intracellular glutamic-oxaloacetic transaminase from damaged cells. Elevation of SGO-T following partial hepatectomy in human beings has been reported (1). It would appear, therefore, that in the experimental animals studied, toxic, infectious, or physical trauma to hepatic tissue results in increased SGO-T activity which is roughly proportional to the amount of liver cell injury.

SUMMARY

Experimental infectious mouse hepatitis is associated with an increase in glutamic-oxaloacetic transaminase activity of the serum (SGO-T).

A relationship appears to exist between the rise in SGO-T activity and (*a*) the size of the virus inoculum, (*b*) the blood virus titer, and (*c*) the degree of liver necrosis.

Trauma to the liver following partial hepatectomy results in a rise in SGO-T activity in mice.

Although mouse hepatitis differs from human hepatitis in the incubation period, histological changes, and natural course, both infections bring about comparable changes in SGO-T activity.

BIBLIOGRAPHY

1. Wróblewski, F., and La Due, J. S., *Ann. Int. Med.*, 1955, **43**, 345.
2. Braunsteiner, H., and Friend, C., *J. Exp. Med.*, 1954, **100**, 665.
3. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, **27**, 493.

4. Karmen, A., Wróblewski, F., and La Due, J. S., *J. Clin. Inv.*, 1955, **34,** 126.
5. Friend, C., unpublished data.
6. Cammarata, P. S., and Cohen, P. P., *J. Biol. Chem.*, 1950, **187,** 439.
7. La Due, J. S., Wróblewski, F., and Karmen, A., *Science*, 1954, **120,** 497.
8. Nydick, I., Wróblewski, F., and La Due, J. S., *Circulation*, 1955, **12,** 161.
9. Molander, D., Wróblewski, F., and La Due, J. S., *J. Clin. and Lab. Med.*, 1955, in press.