bation of the blood of rats in which both intestinal and renal vessels had been ligated regardless of the time interval after injection of the antigen is evidence that the enzyme is released from one of these 2 areas. With one exception the animals with ligated renal vessels showed evidence of histaminase release when the blood was drawn 6 to 13 minutes after injection of the antigen. Hence, it must be concluded that little if any histaminase is released from the kidneys following anaphylactic shock.

With one exception the data in Table II strongly favor release of histaminase only from the intestinal tract. This confirms the *in vitro* findings of Waton(2) in a study carried out in the tissue of normal animals.

The source of histamine released during anaphylactic shock in the rat is still not clear. The perfusion studies of Brocklehurst (6) indicate that histamine is not released from lung, liver, or hindquarters. Our study shows that histamine is released despite ligation of both intestinal and renal vessels. This seems to eliminate the small intestine which has previously been considered the source(1, 7).

Conclusions. 1. Histaminase is released after anaphylactic shock in rats. Its release is consistently noted in blood drawn 6 to 13 minutes after injection of the antigen, less consistently in blood drawn prior to 6 minutes. 2. The site of histaminase release during anaphylactic shock in rats is the intestinal tract, probably the small intestine. 3. Histamine is released into the blood stream following anaphylactic shock in the rat despite ligation of intestinal and renal blood vessels.

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Hepatic Antigens in the Blood of Rats with Toxic Liver Damage.* (27737)

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Liver cell disintegration and the increase of enzymes in plasma during acute hepatic damage(1,2,3) suggest leakage of liver substances into the blood. Furthermore, release of hepatic proteins into blood perfusing isolated rat livers has been shown by Ouchterlony's method(4). We present here evidence obtained by the double diffusion technic(5) for the presence of hepatic antigens in the blood of rats with toxic liver injury, and description of the time course of the levels of these antigens and their immuno-electrophoretic(6) behavior.

Methods. Anti-rat liver serum. Antiserum was prepared in 10 rabbits according to Freund's technic(7), employing whole rat liver homogenate as the antigen. Sera showing the highest levels of antibodies were pooled and merthiolate was added to a final concentration of 1 × 10,000. Anti-rat serum protein antibodies were absorbed by successive addition of normal rat sera until no pre-

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cipitation was observed. Complete removal of anti-rat serum protein precipitins was confirmed by a gel diffusion method(5). Anti-rat liver serum was stored at 4°C in sealed vials. Organ specificity of the antiserum was studied by testing it against liver, spleen, pancreas, intestine, kidney, heart, skeletal muscle, lung and brain homogenates, according to Ouchterlony's technic(5). All of them revealed precipitin lines except those of brain, heart and skeletal muscle. No precipitin lines were obtained when these homogenates were tested against non-immunized rabbit serum. The relationship between the precipitin zones produced by individual organs and liver homogenate placed in adjacent reservoirs of Ouchterlony plates, when tested against the antiliver serum, indicated the presence of cross-reacting and identical antigens(5,8,9).

Serum liver antigen estimations. Liver antigens in sera were studied by double diffusion (5) and immunoelectrophoresis(6). The optimum incubation period of the agar plates at 4°C was studied by serial photographic records. Lines of precipitation began to develop at 36-48 hours, reached a peak at 5-6 days and remained there for a month. Thus a 10-day incubation period was selected. Liver antigen levels were studied in rat serum samples taken at different intervals after toxic injection by determining the highest dilutions showing lines of precipitation. A tentative estimation of the liver proteins contained in the highest titer sera was made by comparison with serial dilutions of known amounts of rat liver extract.

Toxic hepatonecrosis and carbon tetrachloride cirrhosis. Experiments were carried out using male albino adult rats weighing 300-350 g. Hepatonecrosis was induced by intrasplenic injection of CCl_4 (0.1 ml of 20 ml/100 ml solution in vaseline per 100 g body weight), intraperitoneal injections of $CHCl_3$ (0.3 ml of 33 ml/100 ml solution in vaseline per 100 g body weight) and phosphorus (0.2 ml of 1 g/100 ml solution in olive oil per 100 g body weight), and subcutaneous injection of tannic acid (2 ml of 5 g/100 ml solution in saline per 100 g body weight). Blood samples (0.3-0.5 ml) were taken from the tail at different intervals after injection. Liver damage was checked by histological examination of hepatic biopsies at 2 days and 15 days following injection. Carbon tetrachloride cirrhosis was produced by intraperitoneal injection of CCl_4 (0.1 ml of 10 ml/100 ml solution in vaseline per 100 g body weight) at 2-day intervals during 3 months followed by maintenance injections at weekly intervals for one month. Animals were bled at 2, 3 and 7 days after a maintenance dose and cirrhosis was confirmed by liver histology.

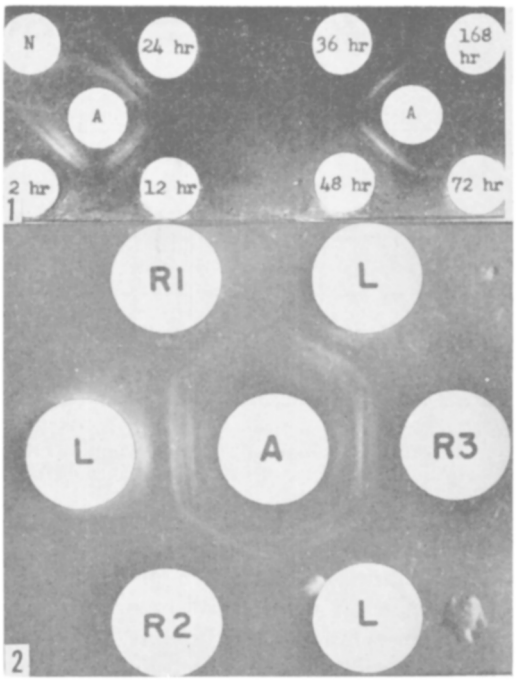

FIG. 1. Double diffusion analysis of liver antigens in serum samples of a CCl_4 poisoned rat. In peripheral wells time at which samples were collected is indicated. N: samples collected before inj. A: rabbit anti-rat liver serum.

FIG. 2. Double diffusion analysis of liver extract and serum samples from CCl_4 poisoned rats. A: rabbit anti-rat liver serum, L: liver proteins extract (5 mg/ml). Serum samples obtained from rats at 2 hr (R1), 24 hr (R2) and 36 hr (R3) after inj.

Results. Carbon tetrachloride hepatonecrosis. Fig. 1 shows a double diffusion analysis of liver antigens in time-spaced serum samples of a CCl_4 poisoned rat. The severe hepatonecrosis found 2 days after CCl_4 injection has disappeared at 15 days. Similar results were obtained in 10 other animals. In some rats receiving higher doses (0.2 ml of 20 ml/100 ml solution in vaseline per 100 g body

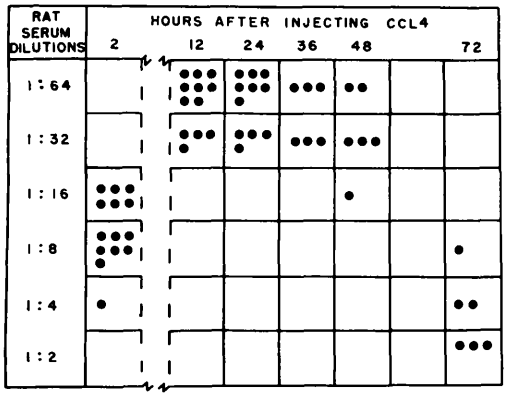

FIG. 3. Liver antigen titers in rat sera after inj. CCl_4 (14 rats). ●: individual serum samples. No precipitation was observed in samples taken before and a week after inj.

weight) up to 7 precipitin lines were recognized. No precipitin lines were produced with serum samples from a control group injected with saline solution. To confirm the specificity of precipitin zones the following tests were performed: a) normal rat serum plus CCl_4 against rat liver antiserum, and b) CCl_4 poisoned rat sera against non-immunized rabbit serum. Both experiments were negative.

To ascertain whether the antigens found in sera are of hepatic origin, a liver extract and serum samples were placed in adjacent reservoirs of Ouchterlony plates(5,8,9). Precipitin lines fused (Fig. 2).

Fluctuations of serum hepatic antigen titers in time-spaced serum samples from 14 rats injected with CCl_4 are shown in Fig. 3. Highest titers indicated that there were approximately 3 mg liver proteins per ml serum (see *Methods*). The minimum amount of hepatic protein detectable was 25 μg/ml serum.

Liver antigens in sera of 8 CCl_4 poisoned rats were further resolved by means of immunoelectrophoresis. Precipitin lines appeared only in the gamma, beta and alpha globulin regions as shown in Fig. 4 for 2 of these animals.

Carbon tetrachloride cirrhosis. The sera of 15 rats with CCl_4 liver cirrhosis were tested against the liver antiserum in Ouchterlony plates 2 days, 3 days and one week following a CCl_4 maintenance injection. Precipitin lines appeared only in the 2- and 3-day samples.

Hepatic damage induced by chloroform, phosphorus and tannic acid. Serum from chloroform, phosphorus and tannic acid poisoned rats (5 animals per group) also showed precipitin lines when tested with rabbit anti-rat liver serum. However, the hepatic damage, and the number and intensity of lines were not so pronounced as in CCl_4 intoxicated animals.

Discussion. Experiments reported here demonstrate that serum obtained from rats with acute liver damage contains antigens which react with rabbit anti-rat liver serum. The presence of such antigens in the serum of animals with CCl_4 cirrhosis during only 2 and 3 days after a maintenance injection indicates that this phenomenon is related to the superimposed acute liver injury. The antigens found in the sera of rats given a single intrasplenic injection of CCl_4 are probably of hepatic origin, as was shown by the fusion (Fig. 2) of their precipitin lines with those produced by liver extract in Ouchterlony tests. However, since the antiserum does not react exclusively with liver material, as demonstrated when tested with other organ homogenates, specific recognition of organ antigens in blood using whole organ antisera is difficult and requires further research into the antigenic patterns of different organs.

The exact nature of the liver antigens in the blood has not been determined. Probably they consist partly of enzymes which are known to be present and to follow a similar time course in the blood after CCl_4 intoxication(3).

Quantitative estimation of liver proteins in sera draws attention to the mechanisms of serum protein modifications following hepatonecrosis(10,11). The highest titers of liver

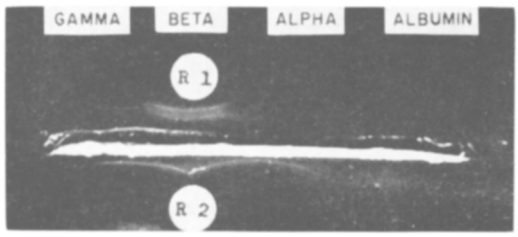

FIG. 4. Immunoelectrophoresis of liver antigens in sera of 2 CCl_4 poisoned rats. Rat serum obtained 24 hr after inj. 0.1 ml (R1) and 0.2 ml (R2) of 20 ml/100 ml solution CCl_4 in vaseline per 100 g body wt.

proteins found (approximately 3 mg/ml serum) constitute a small percentage of total serum proteins. Nevertheless, they may have significant quantitative importance if, as indicated by immunoelectrophoresis, their serum protein distribution is considered.

Summary. Liver antigens were demonstrated in the sera of rats with hepatic damage induced by single injections of carbon tetrachloride, chloroform, phosphorus and tannic acid, and with carbon tetrachloride cirrhosis by the Ouchterlony and immunoelectrophoresis methods, using rabbit anti-rat liver serum absorbed with normal rat serum proteins. In carbon tetrachloride poisoned rats, hepatic antigens in the sera began to rise within a few hours after injection, reached a peak at 12-24 hours, remained there for 2 days and then declined, to disappear in approximately one week. The highest titers indicated the presence of about 3 mg liver proteins per ml serum. The minimum amount of antigens detectable by the method employed was 25 μg hepatic proteins per ml serum. These liver antigens in the sera have electrophoretic mobilities of gamma, beta and alpha globulins, as shown by immunoelectrophoresis. Their influence on serum protein levels after acute liver injury is discussed. In rats with carbon tetrachloride cirrhosis, leakage of hepatic antigens into the blood was demonstrated only in the 2-3-day period following a maintenance injection, indicating that this phenomenon is related to the acute hepatic injury.

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Kinetics of Megacaryocyte Proliferation.* (27738)

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Studies on the proliferation of megacaryocytes in normal animals have, in the past, been based mainly on characteristics of the cells in various stages of differentiation(2,8, 12). Estimates of megacaryocyte proliferation have been made namely on the basis of the observed pattern of distribution of cells of different maturity following damage by means of physical or chemical agents, such as cytotoxic drugs or irradiation(5,7,10). The effects of total body irradiation (200 to 450 r) on the megacaryocytes and platelets in the rat manifest themselves as a decline of the number of the megacaryoblasts less than one day after irradiation, and as a fall in platelets observed starting within 5 days thereafter. In the recovery period, the rise in megacaryocytes precedes the rise in platelets by about 3 days. The fall in megacaryocytes after irradiation was ascribed to a block in cell division and cell death in the "stem"

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