fication of DNA which might cause loss of desirable qualities of the extracts. With the aid of such procedures attempts are now being made to study genetic recombination at ABO locus in human amniotic cells cultured *in vitro*.

Summary. Evidence is presented that epithelial cells of human amnion and kidney are capable of binding heterologous blood group substances in human sera and in animal tissue extracts, thereby displaying altered blood group phenotypes following short exposures to such sera and extracts. The antigen-binding property of such cells disappears following treatment with trypsin, but reappears gradually upon appropriate recultivation of the cells. The property is apparently lacking in fibroblasts, erythrocytes and HeLa cells.

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Glutamic Pyruvic Transaminase in Rat Liver after Single Injection of Carbon Tetrachloride. (27171)

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It is known that carbon tetrachloride intoxication decreases the following enzymes of the liver: pyridine nucleotides(1), adenylnucleotides(2), cytochrome C(3), cytochrome oxidase(4), succinic oxidase(4,5), acid and alkaline phosphomonoesterase and liver esterase(4). Koch-Weser *et al.*(6) have found an increase of alkaline phosphomonoesterase. According to Rahman *et al.*(5) xanthine, choline and d-amino acid oxidases are unaffected.

The transaminases in the liver are also known to decrease in various conditions of liver damage. The release of these enzymes from the hepatic tissue is easily determined by the increase in their serum levels. It is common practice to estimate the serum level of glutamic pyruvic transaminase (GPT) in liver diseases in man, as GPT activity in hepatic tissue is much greater than in any other tissue of the body, whereas glutamic oxaloacetic transaminase (GOT) activity is high in many other organs. The enzyme in the hepatic tissue, however, has not been studied as intensively as have the serum levels of the enzyme. Sommerville *et al.*(7) and Zelman *et al.*(8) have found a significant decrease of hepatic GPT in various liver diseases in man, using different methods. Molander and Friedman(9), studying the transaminase tissue level in experimental carbon tetrachloride injury in rats, could find no decrease in activity. There is, however, no information as to which of the transaminases was determined. In a dog which died 4 days after gastric instillation of 2 ml of carbon tetra-

chloride per kilo of body weight, Fleisher *et al.*(10) found that hepatic GPT activity had been reduced to about half the normal value. In a personal communication to Wróblewski (11), Asada reports that GPT located primarily in the mitochondrial and supernatant fractions after homogenization decreases within 24 hours of CCl_4 administration, reaches its lowest activity at 72 hours and returns to normal values within a week. In a study of liver healing after carbon tetrachloride injury, we used determination of hepatic GPT as an index of regeneration and obtained results that differed from those of Asada. We therefore investigated further the effects of carbon tetrachloride.

Methods. Male rats (Sprague Dawley) weighing 300 ± 25 g, reared on the laboratory diet, were injected intraperitoneally with chemically pure carbon tetrachloride in an amount of 0.1 ml/100 g body weight. The animals were sacrificed 1, 2, 3, 4, 5, 6 and 10 days thereafter. All samples were taken from the left lobe, where the injury is much more pronounced than in other lobes(12).

Liver homogenate was prepared for determination of glutamic pyruvic transaminase (GPT) activity in hepatic tissue. This preparation was in accordance with the method described by Schmidt, Schmidt and Wildhirt (13). The liver sample was immediately dried with filter paper to free it from blood. Half was used for determination of dry weight; the other half weighing 10 to 50 mg, was weighed in a previously chilled Potter-Elvehjem homogenizer, then homogenized for exactly 2 minutes in 0.4 ml of 0.9% NaCl per mg wet liver. The time of these procedures never exceeded 5 minutes.

The following steps in the determination were in accordance with Ordell's modification (15) of Karmen's method(14). The homogenate was centrifuged for 15 minutes at ± 1.5°C at 4300 rpm. To 0.025 ml of the supernatant clear fluid (which was pipetted to avoid the supernatant cloudy layer of lipids) was added 3.0 ml of a commercial reagent (manufactured by AB Kabi, Sweden) containing l-alanine and the enzyme system Lactic Dehydrogenase-DPNH in TRIS-EDTA buffer (pH 8.0 ± 0.1).* The sample was then incubated for exactly 45 minutes at 26°C. (We found it important to adhere strictly to this incubation time since the reduction of activity increased with time.) 0.1 ml of another commercial reagent (Kabi), containing alpha-keto-glutaric acid in the same buffer as the previous one,† was added, and the increase in activity was measured in a Beckman DU spectrophotometer at 340 mμ wave length. One unit of enzyme activity is equivalent to a change in optical density of 0.001 per ml per minute at this wave length.

TABLE I. GPT in Units per mg Dry Liver Tissue.

Days after administration of CCl_4	No. of animals	Mean	95% confidence interval	$\delta_{\bar{x}}$
0 Controls	11	201	159-243	21.3
1	11	182	159-205	11.8
2	11	150	128-173	11.5
3	12	138	112-164	13.2
4	12	125	111-139	7.1
5	11	115	99-131	8.1
6	17	130	111-149	9.6
10	15	159	136-182	11.9

Results. The results summarized in Table I show that hepatic GPT decreases as a result of a single administration of carbon tetrachloride. This decrease is not significant two days after the poisoning ($0.1>P>0.05$). After 3 days, however, the reduction is significant ($0.05>P>0.01$) and reaches its maximum after 5 days. From the fifth day transaminase activity begins to increase. 10 days after the trauma the activity is not significantly lower than normal values ($0.9>P>0.8$).

Discussion. It is known that carbon tetrachloride poisoning is associated with an enormous rise of GPT in serum(10,16). As Wróblewski and LaDue report a rise of up to

* *Reagent A.* Lyophilized content of a vial of the reagent is reconstituted with distilled water to a volume of 30 ml, giving the following concentrations of active constituents:

dl-Alanine	25 mg/ml
DPN (Reduced diphosphopyridine-nucleotide)	0.1 mg/ml
Lactic dehydrogenase	6.000 units/ml

† *Reagent B.* Consists of a solution of alpha-keto-glutaric acid at a concentration of 40 mg/ml in a buffer at pH 8.0.

1000 times normal(17), our finding of only about 40% decrease of GPT activity in the hepatic tissue seems astonishing. We have not been able to correlate our findings with those of Asada, as Wróblewski does not report on the extent of reduction. Molander and Friedman(9) in spite of high serum transaminase levels, could find no significant decrease of liver transaminase activity after repeated doses of carbon tetrachloride to white rats, but there is no report of which transaminase was determined. Our results, however, are in good correlation with the observation of Fleisher and Khalil(10), who found a reduction of about 50%. The fact that an elevation in serum GPT up to 1000 times normal corresponds to a reduction in liver GPT of only half normal, can however be explained, since the liver contains such enormous quantities of the enzyme compared with serum. In a normal rat weighing 300 g, the liver weighs about 9 g and contains 200 units per mg making, roughly estimated, a total of $18 \cdot 10^5$ units. Under the same circumstances there will be about 9 ml of serum (18), which contains about 20 units per milliliter, a total of approximately 180 units. It will be seen that a loss of about 30% of the liver enzyme, as we found 3 days after poisoning when serum concentration is reported to be at its maximum(10), will give rise to an increase of as much as roughly 4000 times the normal serum level. The fact that our calculated figure is still higher than the reported one might be because the damage is not quite as pronounced in the other lobes as it is in the left one, to which we have restricted our calculation. This might also depend upon the fact that there is an excretion and/or breakdown of the enzyme.

As mentioned above, the serum GPT after an acute damage rises to its maximum after 48 to 72 hours. As shown in Table I enzyme levels of hepatic tissue, in contrast to the report of Asada, reach their minimum between the third and the fifth day after poisoning. Although there is no absolute significance between the third and the fifth day ($0.1 > P > 0.05$), it appears that the values continue to decline up to the fifth day. This time difference between maximal serum GPT and minimal hepatic GPT might depend upon a concomitant damage to the kidneys induced by carbon tetrachloride, as proposed by Kato and Murakami(19). Sommerville *et al.*(7) made observations in jaundiced patients which indicated that GPT was probably not excreted in appreciable quantities thru the urinary tract. These observations, however, were random, without controls, and little is known about the further fate of the transaminases.

From these results, it seems that for practical purposes in the study of liver damage, it is preferable to estimate the GPT in serum, where the alterations are much more pronounced, and because the normal values in hepatic tissue vary within wide limits.

Summary. Glutamic-pyruvic transaminase in hepatic tissue decreases from the second day after a single injection of carbon tetrachloride in rats. The enzyme activity does reach normal values by the tenth day. The finding of a relatively small reduction of the enzyme in hepatic tissue as compared with the known enormous increase in the serum, and the finding of a time difference in maximum response to injury between serum and hepatic tissue are discussed.

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Effect of Cortisone and Growth Hormones on Cellular Proliferation During Early Embryogenesis.* (27172)

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A number of studies have dealt with the effects of cortisone acetate and pituitary growth hormone on embryonal tissue(1-7). In chick embryos the growth-retarding effect of cortisone is well known(1,5,6,7). It has been reported that this effect is demonstrable as early as the fourth day of embryogenesis (5). Such an early retardation of growth is manifested in an alteration of the average length of the embryos and in a suppression of mitotic activity. Growth hormone administered to chick embryos during the last half of embryogenesis results in a striking increase in size and in mitotic activity of the tibia, increase in body weight, elevation in the blood sugar of newly hatched chicks and in increased nucleic acid synthesis(2,3,4). Effects of growth hormone during early embryogenesis appear not to have been reported heretofore.

The present report compares the effects of cortisone and growth hormone during an even earlier period of embryogenesis (18-72 hours). While it has already been shown that cortisone can exert an effect on the chick embryo before development of its own functional endocrine system, it is not yet known if growth hormone can also be effective during this period.

Materials and method. Fertile white New Hampshire chick eggs were incubated at a temperature between 98° and 98.5°F and 80% relative humidity. Suspensions of cortisone acetate (Merck Sharp and Dohme) and beef growth hormone (Armour) were prepared in sterile normal saline. Twenty dozen eggs were divided into 4 equal groups, which were injected chorioallantoically on initial day of incubation. One group received 1 mg of cortisone in 0.2 ml of saline and a second group 20 μg of growth hormone in 0.2 ml of saline. One control group received 20 μg of albumin in 0.2 ml of saline and a second 0.2 ml of saline only. Because of the high mortality in the younger embryos receiving albumin, it was used only in the 72-hour experiment.

Embryos were removed after 18, 30, 60 and 72 hours of incubation, fixed in Bouin's fluid, stained *in toto* with hematoxylin, embedded in paraffin and serially sectioned at 12 μ. All mitotic figures were counted in 10 random sections on each slide under oil immersion. This was done on ectodermal, mesodermal and endodermal tissues, or organs of these origins. In the case of 18-hour embryos only ectodermal and mesodermal tissues could be counted, whereas in 60- and 72-hour embryos dividing cells were counted in neural tube (ectodermal), mesenchyme and mesodermal segments (mesodermal), and the lining of the gut (endodermal). A cell was counted as a dividing one when it was in any stage between early metaphase and late telephase. Late telephases were counted as two cells, and late prophases were not counted. The number of mitoses in a field was first determined, then the total number of cells in that field was calculated by inserting a disc divided into 36 equal squares into one of the

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