crease the glutaminase activity of the kidney, it is possible that prolonged treatment with either acid or alkali could diminish both blood and renal glutamine stores and increase the glutamate available to the synthesizing enzyme. This in turn would stimulate the synthesis of glutamine by the kidney and lead to increased activity of the glutamine synthesizing enzyme.

Summary. Increased activity of the enzymes involved in ammonia metabolism in the kidneys of guinea pigs was produced by prolonged treatment with acid or alkali. When the results were expressed on a per cell basis, Glutaminase I was increased in acidosis while in alkalosis Glutaminases I and II and the Glutamine synthesizing enzyme were increased. These increases in activity are suggestive of enzyme induction though all the criteria for this process have not yet been fulfilled.

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Lipide Analysis of Cellular Fractions in Liver Necrosis.* (22734)

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Within the past few years considerable information has been obtained concerning the pathogenesis of necrotic liver degeneration. It may be produced by dietary(1), chemical (2), or virus(3) means. The biochemical changes occurring in the liver cell during the production of necrosis are probably different for each agent. The necrotic livers of rats fed a *Torula* yeast diet are characterized by a progressive failure of oxygen consumption, ketogenesis, lipogenesis and oxidation of labeled acetate to CO_2(4). Popper *et al.*(5,6) have observed that necrosis caused by bromobenzene administration produces a decrease in the concentration of liver esterase, succinic dehydrogenase and total lipides. However, no analyses were made on the phospholipide content of the liver. Biochemical detoxification of bromobenzene causes depletion of cysteine and its precursors such as methionine from the liver(5). Koch-Weser *et al.*(7) have suggested that the condition produced in the bromobenzene treated rats is an example of a chemical conditional amino acid deficiency. In view of the above findings, experiments were undertaken to determine the lipide content of the various cellular fractions of the liver following bromobenzene administration to ascertain whether the lecithins and cephalins are involved in this process.

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Experimental. Male albino rats of the

Wistar strain weighing 100-110 g were maintained on a Purina stock diet. Acute liver necrosis was produced within 48 hours by the injection of bromobenzene, utilizing the method of Koch-Weser *et al.*(8). Both control and experimental animals were fasted 24 hours. Following the fasting period 11 control rats received an intraperitoneal injection of 1 ml of corn oil. One ml of corn oil containing 0.2 ml of bromobenzene was administered to 15 experimental animals. Forty-eight hours later the rats were sacrificed by decapitation, livers were quickly removed, weighed on a Sartorius Balance and divided into 2 samples, one for histological examination and the other for lipide analysis.

The sample for lipide analysis from each liver was minced in an iced beaker and diluted 10-fold with cold 0.88 M sucrose. The mince was homogenized in a Potter-Elvehjem type tissue grinder with Teflon pestle for approximately 4 minutes in an ice bath, forced through a No. 20 gage hypodermic needle to remove stroma, and centrifuged in a Servall angle centrifuge (Type RR-1), according to a modification of the procedures of Hogeboom *et al.*(9) and Griffiths and Pace(10) for the separation of the cellular fractions by differential centrifugation. Mitochondria Fraction: An aliquot of the homogenate was centrifuged at 1,000 x g for 15 minutes to remove nuclei. The supernatant was then centrifuged at 10,000 x g for 30 minutes and the mitochondria precipitate was washed twice with cold 0.15 M NaCl and finally recentrifuged at 10,000 x g for 20 minutes. The mitochondria fraction was then treated with 10% trichloroacetic acid (TCA) containing 0.4 M $MgCl_2$(11) to remove the acid-soluble phosphorus and was then recentrifuged at 10,000 x g for 10 minutes. The precipitate was washed with 5% TCA containing 0.4 M $MgCl_2$ and recentrifuged at 10,000 x g for 15 minutes. The mitochondria precipitate was then covered with ethanol for dehydration for 6 hours and then washed quantitatively into Soxhlet extracting thimbles with ethanol. Nuclear Fraction: An aliquot of the homogenate was recentrifuged at 10,000 x g for 30 minutes. The precipitate was washed with cold 0.15 M NaCl and 10% and 5% TCA solutions and recentrifuged. The precipitate represents nuclei and mitochondria. The value of the nuclei component was obtained by difference. Homogenate Fraction: The homogenate fraction was precipitated by the addition of 10% TCA, centrifuged at 1000 x g for 15 minutes, washed with 5% TCA and recentrifuged. The lipides from the cellular fractions were extracted with 95% ethanol for 6 hours in Soxhlet continuous extractors. The lipides were purified with chloroform (12). Lipide P(13), cholesterol(14), lecithin and cephalin(15) were determined on aliquots of the chloroform solution. Histological examination showed that the mitochondria and nuclear fractions obtained corresponded in appearance and staining properties with those described by Claude(16) and Hogeboom(9).

Results. The lipide content of cellular fractions from the necrotic and control livers is reported in Table I. The *t* test of significance(17) was applied to the differences between the means for the control and experimental values to evaluate the statistical significance of the results. Histological examination of the liver section showed that necrosis was acute, central, fairly extensive and often confluent, extending between adjacent liver lobules in all specimens. The data in Table I show that bromobenzene necrosis produces a decrease in total lipides, lecithin and cephalin phosphorus in the homogenate fraction, the *t* test being 6.58, 3.90, 3.08 respectively. The probability, P, for chance occurrence of these differences was <0.01. There was a decrease in the lecithin phosphorus of the nuclei of the necrotic livers with a *t* value of 2.15 and $P < 0.05$. The mean values of the total lipide, lecithin and cephalin phosphorus in the mitochondria fraction of the necrotic liver are lower. However, the difference is not significant. The data show that the cholesterol content was significantly greater in the necrotic liver nuclei and homogenate fractions. The mitochondria, nuclei and homogenate *t* values were 1.0, 5.30, 3.61 respectively. The probability, P, for chance occurrence of these differences was <0.01

TABLE I. Lipide Analysis of Cellular Fractions of Liver 48 Hours following Bromobenzene Administration.*

	mg of P or cholesterol per g of wet tissue											
	Mitochondria				Nuclei				Homogenates			
Exp.	Total lipide P	Lecithin P	Cephalin P	Cholesterol	Total lipide P	Lecithin P	Cephalin P	Cholesterol	Total lipide P	Lecithin P	Cephalin P	Cholesterol
Controls	.25 ± .04	.12 ± .02	.12 ± .02	.39 ± .09	.28 ± .05	.13 ± .05	.14 ± .03	.61 ± .11	1.07 ± .06	.53 ± .06	.46 ± .06	2.92 ± .26
Necrosis	.22 ± .03	.11 ± .02	.10 ± .02	.43 ± .09	.23 ± .04	.09 ± .02	.13 ± .03	1.26 ± .31	.85 ± .07	.42 ± .04	.35 ± .08	3.60 ± .48

* Figures preceded by ± sign indicate stand. dev.

for the nuclear and homogenate fractions. The rise of cholesterol content in the liver may be the result of increased synthesis, decreased catabolism or excretion. It is of interest that in necrotic liver of animals fed *Torula* yeast there has been observed a decreased conversion of labeled acetate to lipides(4). The pathogenesis of liver necrosis produced by *Torula* yeast is probably different from that of bromobenzene. However, the biochemical changes of lipides in liver necrosis produced by bromobenzene may reflect cellular activity and indicate that there is a lipide disturbance in this process.

Summary. (1) Lipide analyses of cellular fractions in liver necrosis produced by the administration of bromobenzene was studied in the rat. (2) A statistically significant decrease occurred in the total lipide, lecithin and cephalin phosphorus in the homogenate fraction of the necrotic livers as compared to the controls. However, the total cholesterol content was significantly increased. (3) In the nuclei of the necrotic livers a decrease in the concentration of the lecithin phosphorus and an increase of total cholesterol were noted.

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Oxidation of Unsaturated Fatty Acids in Embryonic and Adult Tissues of Golden Hamster.* (22735)

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Kohn and Liversedge(1) gave evidence that rat tissue homogenates as well as slices incubated aerobically produced a compound which condensed with either p-amino benzoic acid (PAB) or thiobarbituric acid (TBA) and gave a pink color. Bernheim and co-workers showed that the color obtained by Kohn and Liversedge upon addition of TBA to incubated tissues was due to a product of the oxidation of certain unsaturated fatty acids (2). Crystalline hemoglobin and ascorbic acid were found to catalyze the reaction involved. Analysis of the TBA compound showed that a 3-carbon fragment, probably a peroxide, had combined with TBA. Such a reaction, namely, the oxidation of a double bond and the breaking of a fragment containing 3-carbon atoms from the end of the chain could occur in linolenic acid. Wilbur's group suggested the use of TBA as a test for oxidation of highly unsaturated fatty acids, principally linolenic(3). Donnan, working with rat tissues, noted that the results obtained in using the TBA test to estimate the amount of oxidized highly unsaturated fatty acid present in tissue preparations paralleled those obtained by the usual procedure for fat estimation(4). Holman, Lundberg and Malkin(5) reemphasized the TBA test as a means of qualitative and indirect measure of the oxidative products of highly unsaturated fatty acid (*i.e.*, linolenic, linoleic and oleic) content of tissues. Increased concentration of highly unsaturated fatty acids has been associated with increased rate of cell division(6). The following investigation was carried out to compare the oxidation of highly unsaturated fatty acids (HUFA) in terms of their oxidative products (HUFA-p) in brain, testes and liver, tissues of the embryo and young animal, with those of the adult. Experimental procedures were designed also to give evidence for *in vivo* oxidation of highly unsaturated fatty acids. Correlation between HUFA content and development of the tissues, as well as evidence of *in vivo* HUFA-p formation are presented.

Materials and methods. All pregnant females and weaned (15 days) animals derived HUFA's from a diet of Purina rabbit chow checkers supplemented with lettuce twice each week. The analysis of this diet, supplied by the Ralston Purina Co., is shown in Table I. About 50% of the 2.5% fat is made up of unsaturated fatty acids. Entire organs (with exception of adult livers, where only the left lobe was used) were removed immediately from decapitated animals, weighed wet, homogenized at 0-4°C and diluted with phosphate buffer, pH 6.0 to final concentration of 50 mg/ml. One 0.5 ml aliquot of this stock homogenate was added to 1.5 ml of buffer or to a mixture of 1 ml of buffer and 1.5 ml of ascorbic acid (concentration 0.2 mg/ml.) These mixtures contained in 50 ml Erlenmeyer flasks were either incubated in a con-

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