control), the pH did not change more than 0.1 unit as a result of incubation. This pH stability may have resulted from the insolubility of the fatty acids produced allowing little or no effect on pH. The inhibition of enzyme activity by the materials tested, therefore, could not be accounted for by an effect on pH of the incubating mixture.

Discussion. It appeared, therefore, that protein components most likely accounted for the lipase inhibition seen with plasma and red blood cells. Tauber(1) in fact, reported presence of serum antilipase which inhibited lipolytic activity normally found in some sera; he also found that laked rabbit red blood cells inhibited serum lipase activity and concluded that hemoglobin was the inhibitor. The ineffectiveness of ashed plasma and red blood cells in the present study indicated that inorganic electrolytes were not responsible for the inhibition.

The mechanism of an inhibitory activity of plasma proteins and hemoglobin on this enzyme is unknown. If the enzyme were autocatalytic, as reported for a lecithinase (6), the protein could prevent activation of the enzyme by binding the fatty acid products. The lack of albumin inhibition on triacetin hydrolysis by this enzyme, however, is evidence against this possibility, and suggests that the inhibitory effect of albumin and the other protein fractions on enzyme hydrolysis of the olive oil emulsion might have been the result of coating of the lipid droplets by these proteins, thereby preventing access of the substrate to the enzyme.

Summary. Human plasma, red blood cells, plasma protein fractions and a hemoglobin preparation were found to inhibit lipolytic activity of a microbiological lipase preparation when an olive oil emulsion was used as substrate. Human serum albumin, which was inhibitory in proportion to its concentration, was not inhibitory when triacetin was used as substrate. Hydrolysis of the olive oil emulsion by pork pancreatic extract was not inhibited by human plasma, red blood cells or albumin.

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Influence of Age on Liver Phospholipide Metabolism of Mice.* (26283)

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Phospholipides are involved in many basic structures of the cell, constituting 16-32% dry weight of nuclei, mitochondria and microsomes(1), and are chemically part of certain enzymes(2). To our knowledge, the influence of age upon radioactive turnover and chromatographic separation of the individual phospholipides has not been previously reported.

Methods. Alteration of phospholipide metabolism and distribution with increasing age was studied in inbred strains of female mice (AxZb). Six hours before sacrifice 25 μc of $NaH_2P^{32}O_4$ was administered intraperitoneally. This time interval corresponds to the ascending part of the specific activity

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TABLE I. Influence of Age on Liver Phospholipide Composition and Relative Specific Activities of Mice.*

Age (days)	No. of animals	Total lipide phosphorus, μg/mg liver N	% total lipide phosphorus				
			Phosphatidyl serine	Phosphatidyl ethanolamine	Phosphatidyl inositol	Lecithin	Sphingomyelin
8	8	16.93	7.94 (.20)	24.39 (.94)	20.10 (.81)	40.00 (.88)	7.92 (.46)
17	5	29.08	5.00 (.06)	30.10 (.31)	12.04 (.21)	45.44 (.42)	7.42 (.22)
24	4	26.40	3.17 (.05)	32.48 (.26)	13.74 (.15)	40.06	10.55 (.21)
60	3	36.44	2.52 (.11)	29.54 (.36)	10.00 (.21)	52.81 (.36)	5.12 (.26)
145	2	34.99	17.18 (.22)	15.41 (.26)	9.37 (.12)	51.05 (.28)	6.99 (.25)
365	3	40.41	16.06 (.19)	16.21 (.22)	8.74 (.12)	51.41 (.21)	7.59 (.16)
840	2	35.45	2.94 (.06)	30.78 (.24)	10.30 (.17)	49.14 (.20)	6.84 (.14)

* Values in parentheses are relative specific activities.

time curves, where synthesis of phospholipides occurs to a degree greater than breakdown(3). The livers were removed, pooled for each age group, and homogenized in the cold for 2 minutes in 95% ethanol in a Potter-Elvehjem homogenizer. An aliquot was taken of the homogenate for extraction of the inorganic soluble phosphorus with 10% trichloroacetic acid, and radioactivity(4) and phosphorus(5) were determined. Nitrogen was determined by the semimicro-Kjeldahl method on another aliquot. The lipides were extracted from the remainder of the homogenate, chromatographed on silicic acid columns, and eluted with chloroform-methanol by the method of Hanahan(6) using a Gilson Medical Electronics fraction collector. Radioactivity(4) and phosphorus(5) were determined on aliquots of the chloroform-methanol fractions. The composition of each of the individual phospholipide fractions was further substantiated by utilizing the paper chromatographic technic of Marinetti *et al.* (7). As a measure of phospholipide synthesis the relative specific activity has been calculated(8). This is the ratio of specific activities of phospholipide phosphorus to specific activities of acid soluble phosphorus. Under the conditions of our experiments an increase in formation of phospholipides may reasonably be assumed when higher values of relative specific activities are found. Radioactivity was determined with a ultra-thin window, gas flow geiger tube (Tracerlab TGC-14). Specific activity is defined as counts per minute per aliquot/mg of phosphorus per aliquot.

Results. Table I shows phospholipide concentration and chromatographic separation of liver phospholipides (phosphatidyl serine, phosphatidyl ethanolamine, phosphatidyl inositol, lecithins and sphingomyelins) and relative specific activity of each fraction in 8, 17, 24, 60, 145, 365 and 840 day old mice. There was an increase in concentration of μg phospholipide P/mg liver N for the 8 to 60 day old mice after which the concentration remained unchanged with age. This constancy of phospholipide phosphorus with ageing in mouse liver was observed by Schulz (9) in mice 21 to 390 days old. Williams *et al.*(10) have demonstrated a similar increase in concentration in liver of young rats 15 to 70 days old. The ratio of phospholipide P/liver N is constant in adult rats maintained on diets of different protein concentration (11) and is unaltered in animals maintained on low protein diets whether or not the liver is fatty, fibrosed, or normal(12). Concentration of phospholipides in the liver cell apparently increased only during growth. There was little change in % of total lipide phosphorus for the various phospholipide fractions with respect to age. However, the relative specific activities of each phospholipide fraction were greater in the younger mice. This observation of an increase in relative specific activities of the phospholipides is similar to that observed in the total phospholipides of the liver of 4 week old rats as compared to that of 10 weeks(13) and in 1.5 day old cultures of ascites tumor cells as compared to 6 days(14). The evidence presented supports the idea that tissue phospho-

lipides are involved in cellular growth.

Summary. 1) The influence of age upon radioactive turnover and chromatographic separation of the individual phospholipides was studied in female mice. 2) There was an increase in concentration of μg phospholipide P/mg liver N for the 8 to 60 day old mice after which the concentration remained unchanged with age. There was little change in % of total lipide phosphorus for the various phospholipide fractions with respect to age. However, the relative specific activities of each phospholipide fraction were greater in the younger mice.

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Ionic Alterations Associated with Resuscitation of the Isolated Rabbit Heart.* (26284)

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Resuscitation of the heart after chemically-induced arrest produced by potassium chloride, potassium citrate or acetylcholine has been accomplished experimentally(1,4,8), and also clinically in cardiac surgery. The isolated perfused rabbit heart may be subjected to as many as 10 successive periods of potassium ion-induced arrest with complete resuscitation in all cases(1). Furthermore, a close correlation has been found between resuscitability of the heart and temperature at which arrest was induced as well as duration of arrest. The importance of the electrolyte composition of the perfusion media employed in resuscitation procedures was stressed by all investigators. However, specific requirements for the optimum compositions are unknown. Hence the effects of cardiac arrest *per se* on electrolyte content of myocardium was of interest since it might shed some light on the significance of electrolyte distribution in reinitiation of the heart beat following arrest. Therefore, the alterations in heart muscle electrolytes incident to prolonged periods of cardiac arrest were determined.

Methods. Isolated rabbit hearts were perfused by the Langendorff technic in air at an ambient room temperature of 22-24°C. Three groups of hearts were studied: 1. *Control Group* consisted of 18 isolated hearts perfused with aerated Ringer-Locke solution at 36°C for a period of 25 minutes. After this period of stabilization, 30 ml of 5% dextrose in demineralized water were perfused through the hearts to flush excess electrolytes from the vascular system. Determinations on these tissues served to establish electrolyte and water content of normal isolated heart

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