Alterations in the metabolism of lipids in ischemia of the liver and kidney

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Abstract The metabolism of lipids in the ischemic liver has been examined in the attempt to define the cause of the previously described loss of phospholipid and to determine whether additional alterations occur that may be related to the disturbances in membrane function. With 3 hr of ischemia, 30% of the cellular phospholipid was lost when measured either as phosphate in a lipid extract of the whole liver or as fatty acyl esters after separation by thin-layer chromatography of the major lipid classes in the same extracts. All phospholipid species were equally affected, and there was no accumulation of lysophospholipids. The loss of phospholipid acyl chains was not accompanied by an increased number of acyl esters as mono-, di-, or triglycerides. There was no increase in the size of the free fatty acid pool, and the content of long chain acyl CoA esters decreased by 50%. The acyl chain composition of the free fatty acid and neutral lipid pools changed, however, to resemble more closely that of the phospholipids. There was no change in the fatty acid composition of the phospholipids. The incorporation of intraportally injected [^{3}H]arachidonic acid into total phospholipids was decreased upon reperfusion of liver that had been ischemic for only 20 min. These data are consistent with a loss of fatty acyl chains from the phospholipids into the free fatty acid pool. A few of these chains are incorporated into neutral lipids, but most are lost from the liver. A reduced rate of reacylation may produce an imbalance in the normally very rapid rate of deacylation and reacylation of glycerophospholipids resulting in this net loss of phospholipid. Renal cortical ischemia was accompanied by similar changes in the metabolism of lipids with one exception. The loss of fatty acyl chains from phospholipids was accompanied by their quantitative accumulation as free fatty acids. The acyl chain composition of the enlarged free fatty acid pool in the ischemic renal cortex came to resemble more closely the fatty acid composition of the phospholipids. The loss of phospholipid and the accumulation of free fatty acids was accompanied by a progressive increase in tissue calcium. The changes in lipid metabolism, however, could be dissociated from this influx of calcium ions. This suggested that the changes in calcium homeostasis are a consequence rather than a cause of the alterations in lipid metabolism. —**Finkelstein, S. D., D. Gilfor, and J. L. Farber.** Alterations in the metabolism of lipids in ischemia of the liver and kidney. *J. Lipid Res.* 1985. **26:** 726–734.

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Disturbances in membrane function in general and in the plasma membrane in particular characterize the irreversible cell injury in ischemia (1, 2). An accelerated degradation of phospholipid has been previously reported to be a potential cause of the membrane dysfunction in both hepatic (3–6) and myocardial ischemia (7, 8). With 3 hr of liver ischemia, there is loss of 25–35% of the total phospholipid content (3). Alterations in the structure and function of both the plasma membrane and the membranes of the endoplasmic reticulum accompany such a disordered phospholipid metabolism (2, 9).

In the present report, we have examined in more detail the effect of ischemia on hepatic lipid metabolism in the effort to define further the cause of the phospholipid loss and to determine whether additional alterations in lipid metabolism occur that may also relate to the altered membrane function. In addition, we have examined the effect of renal ischemia on the metabolism of lipids in the attempt to determine the generality of the alterations in lipid metabolism in ischemia.

MATERIALS AND METHODS

Induction of hepatic and renal ischemia

Female Sprague-Dawley rats (180–200 g, Charles River Breeding Laboratories) were fasted overnight prior to use. Liver ischemia was induced by ligation of the portal venous and hepatic arterial blood supply to the left lateral and median lobes of the liver as previously described (3). In order to determine the extent of hepatic swelling with ischemia, whole livers were transplanted to the abdominal cavity of another rat. After 3 hr, the liver had increased in weight by 6.5 ± 0.5%. The changes in total protein, calcium, phospholipid, free fatty acids, or cholesterol were identical in livers made ischemic in situ or by transplantation (data not shown). All the data reported below with respect to hepatic ischemia are expressed on a per gram wet weight basis obtained by dividing the postischemic wet weight by 1.065. Renal ischemia was induced by modification of the total pedicle clamping procedure of

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Sheehan and Davis (10–12). Under light ether anesthesia, a midline abdominal incision was made. The left kidney was elevated and a ligature was passed around the pedicle to completely occlude the renal artery and vein. The kidney was then quickly removed, weighed, and replaced in the left posterior abdominal cavity. The laparotomy incision was closed, and the animal was allowed water ad libitum. At the times indicated in the text, the animal was killed by decapitation, the ischemic kidney was retrieved, and the cortex was separated from the medulla by dissection. Preischemic wet weight for the renal cortex was calculated with the following formula: cortical preischemic wt = (whole kidney preischemic wt/whole kidney postischemic wt) × cortical postischemic wt.

Liver and renal cortical tissues were homogenized in 20 volumes (w/v) of deionized water by 15 strokes in a Teflon-glass homogenizer. Protein was determined by the method of Lowry et al. (13). The total calcium content of the homogenates was assayed according to Macdonald et al. (14). Readings were taken on a Perkin-Elmer Model 460 atomic absorption spectrometer.

Lipid analyses

Total lipids were extracted from aliquots of the homogenates by the method of Bligh and Dyer (15). Lipid phosphate in the range 0.1–1 μmol was assayed according to Harris and Popat (16). When greater sensitivity was required (0.01–0.15 μmol), a modification of the method of Bartlett (17) was used. Cholesterol was measured using the *o*-phthalaldehyde reagent (18). Acyl esters were quantitated by the ferric hydroxymate method of Snyder and Stephens (19). Free fatty acids were measured by three separate methods: *1*) the copper–triethanolamine assay of Itaya (20) performed on the total lipid extract freed of phospholipid by means of $MgCl_2$ precipitation (21); *2*) potassium dichromate reduction (22) after isolation of free fatty acids from the total lipid extract by thin-layer chromatography (17); and *3*) the difference between total esterified fatty acids before and after methylation (23) of the total lipid extract. Thin-layer chromatography was performed on 20 × 20 cm silica gel G plates, 0.25 mm thick (Supelco). Total lipids were separated with a solvent system consisting of hexane–diethyl ether–acetic acid 70:50:4 (17). Phospholipids were resolved with a solvent system consisting of chloroform–methanol–water–acetic acid 25:15:4:2 (17). Fatty acid compositional analysis was performed by gas–liquid chromatography after methylation with boron trifluoride–methanol (Sigma) according to Morrison (23). Methylation was performed using 2 ml of 14% BF_3–methanol–hexane 0.7:0.7:0.6 in sealed glass vials placed in boiling water for 45 min. The samples were cooled and 0.5 ml of 5 N NaOH was added. Methyl esters were extracted four times with 2 ml of hexane, dried under N_2, and resuspended in hexane. Fatty acid methyl esters were separated on a Perkin-Elmer 5% DEGS-PS column (6 ft, 1/4 in o.d., 120 mesh Chromasorb WHP) isothermally at 175°F at 40 ml/min helium flow with a Perkin-Elmer Model 3920 gas chromatograph equipped with a flame ionization detector. Peaks were integrated with a Perkin-Elmer Model 1 computing integrator. The long chain acyl CoA pool was measured fluorometrically as free CoA with the use of α-ketoglutarate oxidase after alkaline hydrolysis of a perchloric acid precipitate of the whole liver homogenate (24).

Radiochemical studies

Measurement of the turnover of the glycerol moiety of hepatic triglycerides and glycerophospholipids was performed on animals fasted overnight (18 hr) after receiving by intraperitoneal injection 10 μCi of [2-^{3}H]glycerol (New England Nuclear, 9.52 Ci/mmol). Ischemia was induced as described above. Immediately after placing the ligature around the hepatic artery and portal vein, a portion of the ischemic lobe was removed to serve as the time "0" control. Bleeding was not encountered in keeping with the absence of blood flow in the ischemic lobe. After 3 hr tissue samples were obtained from the ischemic and residual nonischemic liver. Lipids were extracted, separated by thin-layer chromatography, and counted in Omnifluor (New England Nuclear). The turnover of fatty acids in ischemic liver was measured 18 hr after an intragastric administration of 10 μCi of [(5,6,8,9,11,12,14)5-^{3}H]-arachidonic acid (New England Nuclear, 95.4 Ci/mmol) in corn oil. Tissue samples were obtained from the ischemic lobe immediately after placing the ligature, as well as from both the ischemic and nonischemic lobes 3 hr later. The radioactivity in aliquots of the whole liver homogenates as well as in total lipid extracts was determined. The rate of reacylation of liver phospholipids was measured by the intraportal injection of 1 μCi of [^{3}H]-arachidonic acid. The solvent was evaporated under nitrogen and the residue was resuspended in 2% fatty acid-free albumin in 0.15% NaCl. Tissue samples were removed 30 sec after administration of the isotope and immersed immediately in liquid N_2. The samples were homogenized and an aliquot was removed for determination of total radioactivity. From another aliquot, total lipids were extracted. Free fatty acids, neutral lipids, and phospholipids were separated by thin-layer chromatography and the radioactivity in each lipid fraction was measured. The data are expressed as the percentage of the total radioactivity in free fatty acids or neutral lipids and phospholipids.

RESULTS

Lipid metabolism in hepatic ischemia

With 3 hr ischemia, there was a loss of 30% of the total liver phospholipid from the initial level of 52 ± 4 to 33

± 3 μmol of lipid phosphate/g preischemic wet weight. Most of the major phospholipid species were lost in parallel (**Table 1**). There was no accumulation of lysophospholipids. These data are similar to those reported previously (3) and are the primary event to which the further changes in cellular lipids detailed below are to be related.

The effect of ischemia on the metabolism of lipids other than the phospholipids was examined by quantitation of fatty acyl chains. The number of esterified fatty acids in total lipid extracts also decreased by close to 30% with 3 hr of liver ischemia (**Table 2**). The same extracts were then fractionated by thin-layer chromatography to isolate phospholipids, mono-, di-, and triglycerides. The number of esterified fatty acids in each of these lipid classes was then measured and the data are also shown in Table 2. The decrease in phospholipid acyl chains could account for the entire loss of esterified fatty acids in the total lipid extract. Acyl ester content of the total lipid extract decreased by 32 μmol/g preischemic wet weight. The decrement in phospholipid acyl chains was 30 μmol. The neutral glyceride fractions showed no significant change in acyl ester content.

This specific change in phospholipid content was confirmed by following the fate of hepatic lipids prelabeled with [^{3}H]glycerol. Eighteen hours after an intraperitoneal administration of 10 μCi of [^{3}H]glycerol, liver ischemia was induced. After 3 hr the livers were removed, and the total lipids were extracted and fractionated by thin-layer chromatography. A 25% loss of radioactivity from the phospholipid fraction accounted for the entire loss of radiolabel from a total lipid extract (data not shown). Again, there were no changes in the neutral glycerides.

The loss of 30 μmol of phospholipid acyl chains/g preischemic wet weight was not accompanied by an accumulation of free fatty acids. Free fatty acids were measured in control and ischemic livers by three independent methods (**Table 3**). In no case was there a detectable increase in the content of free fatty acids. This was not, however, a consequence of the insensitivity of the assays. As shown below (Table 7), renal ischemia was also accompanied by phospholipid depletion. In this case, an equimolar accumulation of free fatty acids was readily detected by the same assays used in Table 3.

Fatty acids lost from phospholipids did not accumulate in a form that escaped extraction into hydrophobic solvents. Rats were given 10 μCi of [^{3}H]arachidonic acid in corn oil via intragastric instillation 24 hr prior to induction of liver ischemia for 3 hr. The radioactivity in the whole liver homogenate was identical to that in the total lipid extracts derived from the same homogenate with both the nonischemic and ischemic lobes (data not shown). Homogenates of ischemic livers and their total lipid extracts, however, had a reduced total radioactivity reflecting the loss of phospholipid acyl chains. There was also no increase in ketone bodies in the ischemic tissue (measured as β-hydroxybutyrate; data not shown).

Further clues to the fate of the fatty acyl chains lost from ischemic liver phospholipids were sought by examination of the fatty acid composition of the various lipid classes (**Table 4**). The fatty acid composition of the phospholipids prior to induction of ischemia was quite different from that of the other lipid classes. After 3 hr ischemia, the fatty acid composition of the phospholipids had not changed despite the loss of 1/4 to 1/3 of the total phospholipid. Each of the other lipid classes, however, showed changes in fatty acid composition. This was best seen in the case of the free fatty acids. The relative content of stearic (18:0) and arachidonic acid (20:4) increased, while palmitic (16:0) and linoleic acid (18:2) decreased. Stearic and arachidonic acids also increased in the neutral lipid fractions. The net result of these changes tended to make the fatty acid composition of the free fatty acid pool in the ischemic liver more closely resemble that of the phospholipids. These changes in fatty acid composition were a sensitive indicator of the effects of ischemia. Significant changes in the fatty acid composition of the free fatty acid fraction were detected with as little as 45 min of ischemia (data not shown). There was no change in the composi-

TABLE 1. Phospholipid content and composition in ischemic liver

	Control	% of Control	3 Hr Ischemic	% of Control
Total phospholipid	51.9 ± 4.4[a]		33.1 ± 3.0	
Phosphatidylcholine	31.6 ± 3.5	61	19.2 ± 2.2	58
Phosphatidylethanolamine	12.8 ± 1.9	25	9.6 ± 1.3	28
Phosphatidylinositol and phosphatidylserine	4.3 ± 1.1	8	2.8 ± 0.9	10
Sphingomyelin	2.2 ± 2.0	4	1.4 ± 1.4	4
Lysophospholipids	0.6 ± 0.5	1	0.5 ± 0.5	2
Total	51.5	99	33.5	102

[a] Results are μmol of lipid phosphate/g preischemic wet weight and represent the mean ± SD of the determinations on at least three separate livers. Total phospholipid content was measured on an extract of whole liver. Contents of individual phospholipid species were determined after thin-layer chromatography of this same extract.

TABLE 2. Acyl ester content and distribution in ischemic liver

	Control	% of Control	3 Hr Ischemic	% of Control
Total acyl esters	122.6 ± 3.4[a]		90.0 ± 8.2	
Phospholipid acyl esters	95.9 ± 6.3	78	65.6 ± 5.0	70
Glyceride acyl esters				
Mono- and diglycerides	3.8 ± 1.8	3	4.3 ± 2.1	5
Triglycerides	23.5 ± 0.9	19	23.4 ± 1.8	25

[a]Results are μmol of acyl esters/g preischemic wet weight and represent the mean ± SD of the determinations on at least three separate livers. Total acyl ester content was measured on an extract of whole liver. Contents of phospholipid and neutral lipid acyl esters were measured after thin-layer chromatography of this same extract.

tion of the serum free fatty acids and triglycerides after 3 hr of liver ischemia. In addition, there were no changes in the fatty acid composition of the free fatty acids or neutral lipids in the nonischemic lobe. There was also no change in the amount of total liver cholesterol after 3 hr ischemia (data not shown). Because of the absolute fall in phospholipid, however, the cholesterol/phospholipid molar ratio increased significantly in extracts of the whole liver homogenate.

The above data suggested that fatty acyl chains released from a shrinking phospholipid pool were appearing in the free fatty acid pool and, at least in part, in neutral glycerides. Their ultimate fate remained unclear, although the data indicate that they are lost from the liver itself. In the final studies, an attempt was made to assess the rate of flow of fatty acids into phospholipid in control and ischemic liver, since a decline in this rate could lead to a net loss of phospholipid given a constant or increased rate of phospholipid deacylation.

In control rats, over 90% of an intraportal injection of [^{3}H]arachidonic acid was incorporated into phospholipids and triglycerides within 30 sec (**Table 5**). In contrast, less than 2% of a similar injection of either [^{14}C]choline or [^{14}C]ethanolamine was incorporated into phospholipids after 10 min (data not shown). Such a measurement of the extent of esterification in the first 30 sec after injection of a tracer pulse of radiolabeled fatty acid was used as an index of the rate of reacylation. Since the amount of isotope injected relative to the total number of esterified acyl chains is quite small and the pool of phospholipid acyl chains is over 100 times greater than that of the free fatty acid pool, the return of radioactivity to free fatty acids is negligible over such a short interval and could, therefore, be discounted.

After periods of ischemia of increasing duration, the vascular occlusion was removed and [^{3}H]arachidonic acid was immediately injected into the portal vein. Thirty seconds later the extent of incorporation of the isotope into phospholipids was measured (Table 5). With as little as 20 min of ischemia, a reduction in the rate of esterification of radiolabel and an accompanying increase in the radioactivity in free fatty acids was seen. With ischemia of longer duration, there was a further decline in the extent of esterification of the tracer pulse. After 1, 2, and 3 hr, esterified counts represented only 60, 50, and then only 30% of the total radioactivity in the liver. There were no changes in the size of the free fatty acid pool (Table 3), and the size of the pool of long chain acyl CoA esters declined from 37.7 ± 7.9 to 11.6 ± 1.9 μmol/g with 3 hr ischemia. While the relative content of arachidonic acid in the free fatty acid pool increased with 3 hr ischemia (Table 4), a similar decline in the rate of phospholipid acylation was seen after injection of [^{14}C]palmitic acid (data not shown), a fatty acid that decreased in concentration in the free fatty acid pool.

Lipid metabolism in renal ischemia

In order to achieve ischemia without interstitial congestion or hemorrhage, it was necessary to sever the kidney not only from the renal artery and vein but also from the vessels reaching the cortex through the capsule. This was accomplished by completely removing the kidney following total pedicle ligation and then immediately replacing it in the posterior abdominal cavity. This procedure for

TABLE 3. Free fatty acids in ischemic liver[a]

	Control	3 Hr Ischemic
1. Method A		
Copper triethanolamine assay on whole lipid extract freed of phospholipid by $MgCl_2$ precipitation	4.9 ± 2.1	3.9 ± 1.9
2. Method B		
Dichromate oxidation assay after thin-layer chromatography of whole lipid extract	1.9 ± 1.0	1.7 ± 0.8
3. Method C		
Ferric hydroxymate assay with and without BF_3 methylation	2.0 ± 1.0	2.1 ± 1.9

[a]Results are μmol of free fatty acid/g preischemic wet weight and represent the mean ± SD of the determinations on three separate livers.

TABLE 4. Fatty acid composition of control (C) and ischemic (I) liver lipids[a]

	Phospholipids		FFA		Mono- and Diglycerides		Triglycerides	
	C	I	C	I	C	I	C	I
16:0	11	13	33	24	26	23	34	31
18:0	31	33	15	30	16	21	8	13
18:1	5	5	14	10	16	9	20	18
18:2	10	11	20	12	9	18	24	25
20:4	28	23	9	18	13	17	4	8
22:6	8	11	1	4	11	9	5	5
	93%	96%	92%	98%	91%	97%	95%	100%

[a]Results are the mean % peak area of the fatty acid methyl esters separated by gas-liquid chromatography and derived from the respective lipid classes isolated by thin-layer chromatography from an extract of the whole liver homogenate.

producing renal ischemia also allowed determination of the pre- and postischemic weights with each experiment.

Fig. 1 shows the time course of the increase in weight of an ischemic kidney. By 12 hr kidney weight had increased by 40%. In contrast, the maximum hepatic swelling was never more than 7% and was achieved by 3 hr. This relatively large increase in the weight of the kidney, affecting cortex as well as medulla (data not shown), emphasizes the need to normalize subsequent measurements to the preischemic wet weight.

The time course of accumulation of Ca^{2+} ions in the ischemic renal cortex (**Fig. 2**) differed significantly from that of the increase in weight. Calcium accumulation generally accompanies the histological transformations that denote cell death (1), and as such it was a convenient and quantitative method for documenting the extent of renal cortical necrosis. The Ca^{2+} content of ischemic renal cortex was significantly increased only after 6 hr. Between 6 and 24 hr, the Ca^{2+} content continued to increase, reaching a level some 8 times the control. In contrast, the increase in wet weight started earlier and was essentially complete after 8 hr (Fig. 1).

Fig. 3 illustrates the time course of the decrease in the phospholipid content of ischemic renal cortex. Compared to the liver, the renal cortex reacted more slowly to ischemia with significant changes in phospholipid content evident only after 6–8 hr. By 18 hr there was a loss of 30% of the total phospholipid. As with liver ischemia, most major classes of phospholipids were affected, and there was no accumulation of lysophospholipids (**Table 6**).

The number of acyl chains in total lipid extracts of ischemic renal cortex decreased in parallel with the loss of phospholipid (**Table 7**). The distribution of acyl chains among the major lipid classes is also shown in Table 7. The phospholipid fraction was again the only lipid class that changed, and the decrement in phospholipid acyl chains accounted entirely for the change in acyl ester content of the unfractionated extracts.

The major difference between the effects of ischemia on lipid metabolism in the kidney and the liver was observed in the fate of the acyl chains lost from phospholipids. In the kidney, the decrement in phospholipid acyl chains could be entirely accounted for in an accumulation of free fatty acids (Table 7). Free fatty acids accumulated by 20 μmol/g preischemic wet weight. This represented a 10-fold increase over the control and closely corresponded to the fall in total acyl chains of 16–20 μmol/g preischemic wet weight measured in lipid extracts of the whole cortex.

Changes in the fatty acid composition of the various lipid classes supported a conclusion that the elevated free fatty acids were the product of the degradation of phospholipids. In the normal renal cortex, the fatty acid composition of the small free fatty acid pool was different from that of the much larger phospholipid pool. While similar to that of the renal triglycerides, the fatty acid composition of the free fatty acid pool had a higher content of 18:0 and 20:4. Ischemia did not change the fatty acid composition of the phospholipids (data not shown). There were, however, changes in the other lipid classes. The accumulation of free fatty acids was accompanied by changes in fatty acid composition. Most striking was the loss of 16:0 and the rise in 20:4. The content of these two fatty acids represented the major differences between the phospholipids and free fatty acids prior to ischemia. With ischemia the changes in these fatty acids tended to reduce the differences between the two lipid classes. The fatty acid composition of renal neutral glycerides changed in a manner similar to that of the free fatty acids. As with liver ischemia, the total cholesterol content remained unchanged, resulting in an increased cholesterol/phospholipid ratio (data not shown).

Reperfusion of ischemic liver is accompanied by a large accumulation of Ca^{2+} ions (24). In this case, it can be reasonably presumed that the disordered calcium homeostasis is a consequence of the disordered phospholipid metabolism. The relationship between phospholipid degradation and the accumulation of Ca^{2+} ions in the ischemic renal cortex was explored in the final experiment. Whole kidneys were removed from the animal and incubated for 24 hr in vitro in a tissue culture medium either

TABLE 5. Inhibition of phospholipid acylation in ischemic liver

Duration of Ischemia	% CPM in FFA	% CPM Esterified	Total CPM/g Preischemic Wet Weight
0	7.2 ± 0.5	92.8[a]	3350 ± 1447
5 min	6.9 ± 0.4	93.1	4988 ± 1033
15 min	7.9 ± 0.8	92.1	4110 ± 425
20 min	15.4 ± 1.4	84.6	3250 ± 1090
1 hr	39.8 ± 40	60.2	3113 ± 1331
2 hr	51.7 ± 2.9	48.3	4883 ± 1928
3 hr	68.5 ± 5.4	31.5	2875 ± 1265

[a]Phospholipids accounted for ≥60% of esterified CPM at all times.

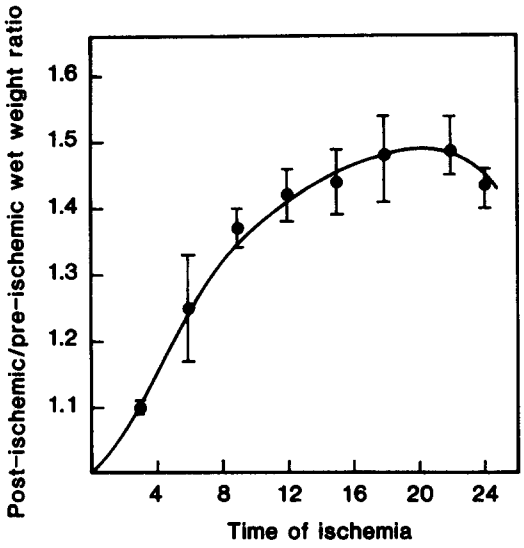

Fig. 1. Increase in the wet weight of ischemic renal cortex. Results are the mean ± SD of the determinations on three separate animals.

free of total Ca^{2+} ions (less than 20 μM) or containing 3 mM total calcium. **Table 8** indicates that phospholipid loss was seen in either case. Renal cortical calcium ion accumulation, however, occurred only in those kidneys incubated in calcium-containing medium. Free fatty acids accumulated in both cases. It would seem, therefore, that the phospholipid degradation in renal ischemia is independent of calcium influx, and the increased calcium content most likely represents an influx across cellular permeability barriers destroyed by the disordered lipid metabolism.

DISCUSSION

Lipid metabolism in ischemic liver cells is characterized by a loss of phospholipid quantitated by measurement of lipid phosphate (Table 1), long chain acyl esters (Table 2), or glycerol. Loss of as much as 1/3 of the total liver phospholipid with 3 hr ischemia occurred without any change in the proportion of the different phospholipid species (Table 1) or in the fatty acid composition of the total phospholipids (Table 4). Whatever the mechanism responsible for this striking alteration in lipid metabolism, it clearly does not discriminate among the very heterogeneous phospholipids either on the basis of polar head group or fatty acid composition.

An additional feature of the disordered lipid metabolism in hepatic ischemia is that the changes in phospholipid content are not accompanied by corresponding changes in the quantities of other lipid species. The loss of 30 μmol of fatty acyl chains from the total phospholipid pool was not accompanied by increases in the content of either free fatty acids (Table 3) or neutral glycerides including mono-, di-, and triglycerides (Table 2). While the data presented do not exclude increases in free fatty acids at the level of nmol/g wet weight, they certainly indicate that a 3-hr decrement of 30 μmol of fatty acyl chains is not accompanied by significant increases in free fatty acids in the range of μmol/g wet weight (Table 3).

Such a quantitative analysis of the changes in lipid metabolism in liver ischemia would suggest that there are no manifestations of the disordered phospholipid homeostasis in the other lipid species present in an hepatocyte. Qualitative analysis, however, of the fatty acid composition of the various lipid classes revealed that phospholipids are not the only lipids affected (Table 4). Normally free fatty acids released from fat cells into the blood are taken up by the liver. The acyl chain composition of the free fatty acid pool in the liver generally closely resembles that of the blood. In the liver, these fatty acids may be oxidized or incorporated into neutral glycerides, mainly triglycerides for storage or export, or into phospholipids. Normally the acyl chain composition of the hepatic neutral lipids closely resembles that of the free fatty acid pool. Liver cells do, however, regulate the selection among the different acyl chains in the free fatty acid pool for incorporation into phospholipids, since the fatty acid composition of these membrane lipids is different from the free fatty acids and neutral lipids (Table 4). The fatty acyl chains lost from the phospholipids with liver ischemia must have moved, at least in part, through the free fatty acid pool. This is implied by the change in the acyl chain composition of the free fatty acid pool to more closely resemble that of the phospholipids (Table 4). Some of these fatty acyl chains entering the free fatty acid pool can also be presumed to have passed into the neutral lipids,

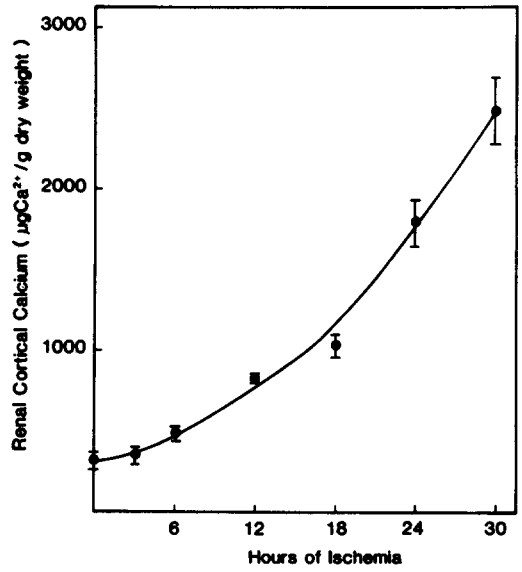

Fig. 2. Total calcium content of the ischemic renal cortex. Results are the mean ± SD of the determinations on five separate animals.

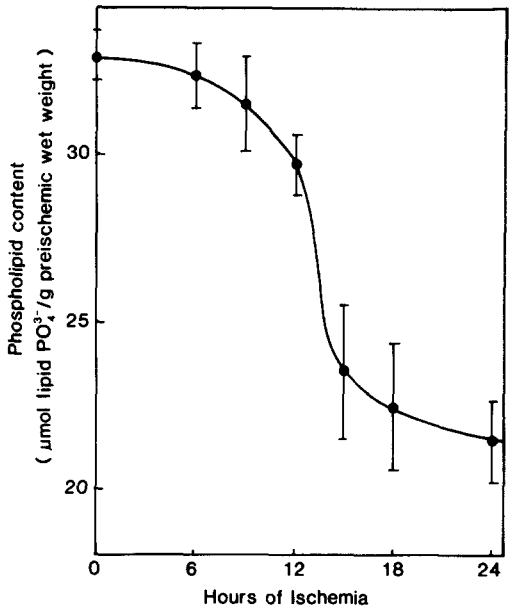

Fig. 3. Phospholipid content of ischemic renal cortex. Results are the mean ± SD of the determinations on three to five separate animals.

as evidenced by changes in the fatty acid composition of the neutral glycerides (Table 4).

The size of the free fatty acid pool (2–5 μmol of acyl chains/g wet weight) is an order of magnitude smaller than the 30 μmol of acyl chains lost from the phospholipids. Only a fraction of the acyl chains lost from phospholipids would have to pass through the free fatty acid pool in order to significantly change its fatty acid composition. The triglyceride pool has 20–25 μmol of acyl chains/g wet weight. The data in Table 4 limit the total change in fatty acid composition of the triglycerides to not more than 15%. To produce such a change would require only 3–4 μmol of acyl chains/g wet weight being incorporated into triglycerides. The changes in fatty acid composition, then, of the free fatty acid and neutral lipid pool would need to involve, at most, only 10–15% of the fatty acyl chains lost from phospholipids. The changes attributable to only a fraction of the acyl chains lost from the phospholipids would suggest, nevertheless, that the entire pool was initially present as free fatty acids prior to its loss from the liver.

The acyl chains of liver cell phospholipids are normally in a very dynamic state of flux with continual deacylation and reacylation. In principle, an alteration in either arm of this cycle could lead to disequilibrium with net loss of phospholipid as occurs in ischemia. Either an accelerated rate of deacylation, as a result of phospholipase A activation, or an inhibited rate of reacylation, as a result of the inhibition of the acylation of lysophospholipids, could disrupt the normal balance between the rates of fatty acid reacylation and deacylation. In either case, the end result would be a decrease in the number of phospholipid acyl chains and a net loss of phospholipids. Somewhat against phospholipase activation is the absence of any evidence of specificity in the mechanism causing loss of phospholipid for either polar head group or fatty acid composition. In vitro, at least, the endogenous phospholipase A activities of liver cell membranes do seem to have such specificity, the best example being the preferential hydrolysis of phosphatidylethanolamine by the calcium-activated phospholipase of rat liver microsomal membranes (25).

That there is decreased reacylation of lysophospholipids in ischemic liver cells is suggested by the decreased rate of incorporation of [^{3}H]arachidonic acid into phospholipids (Table 5). Interpretation of this experiment is dependent upon a knowledge of the specific activities of both precursor pools, the free fatty acid and long chain acyl CoA

TABLE 6. Phospholipid content and composition in ischemic renal cortex[a]

	Control	% of Control	20 Hr Ischemic	% of Control
Total phospholipid	33.5 ± 0.8		24.9 ± 1.0	
Phosphatidylcholine	15.1 ± 1.1	45	9.9 ± 0.1	40
Phosphatidylethanolamine	8.4 ± 0.5	25	6.9 ± 0.4	28
Phosphatidylinositol and phosphatidylserine	3.0 ± 0.8	9	1.7 ± 0.2	7
Phosphatidylglycerol, cardiolipin, and phosphatidic acid	3.0 ± 0.8	9	2.3 ± 0.1	9
Sphingomyelin	3.4 ± 0.8	10	2.3 ± 0.3	9
Lysophospholipids	1.3 ± 0.5	4	1.9 ± 0.1	8
Total	36.9	102	27.3	100

[a] Results are μmol of lipid phosphate/g preischemic cortical wet weight and represent the mean ± SD of the determinations on at least three separate kidneys. Total phospholipid content was measured on an extract of the whole renal cortex. Contents of individual phospholipid species were determined after thin-layer chromatography of this same extract.

TABLE 7. Acyl ester and free fatty acid contents of ischemic renal cortex[a]

	Control	20 Hr Ischemic
Acyl esters[a]		
Total	71.5 ± 3.9	55.6 ± 3.6
Phospholipids	55.1 ± 3.1	40.4 ± 2.7
Mono- and diglycerides	4.4 ± 0.7	3.7 ± 0.3
Triglycerides	10.7 ± 2.4	11.4 ± 2.3
Free fatty acids[b]		
Method A		
Copper triethanolamine assay on whole lipid freed of phospholipid by $MgCl_2$ precipitation	2.2 ± 1.0	21.7 ± 1.8
Method B		
Ferric hydroxymate assay with and without BF_3 methylation	2.3 ± 2.0	23.9 ± 2.2

[a] Results are μmol of acyl esters/g preischemic cortical wet weight and represent the mean ± SD of the determinations on at least three separate kidneys. Total acyl ester content was measured on an extract of whole cortex. Contents of phospholipid and neutral lipid acyl esters were measured after thin-layer chromatography of this extract.

[b] Results are μmol of free fatty acid/g preischemic cortical wet weight and represent the mean ± SD of the determinations on at least three separate kidneys.

pools. As noted above, there was no increase in the total free fatty acid pool (Table 3). There was also no change in the total radioactivity in the liver during the time that incorporation into phospholipids was inhibited (Table 5). It was possible that the increase in the proportion of free fatty acids represented by arachidonic acid, 50% increase after 3 hr (Table 4), could have contributed to the decreased rate of incorporation of the labeled arachidonic acid. The rate of incorporation, however, of palmitic acid into phospholipids also decreased, despite the decrease in its concentration in the free fatty acid pool. After 3 hr ischemia, the long chain acyl CoA pool was reduced by 50%, possibly reflecting some decrease in the rate of fatty acid activation in the face of reduced ATP levels. It is not known to what extent such a change in the rate of incorporation of the [^{3}H]arachidonic acid into the arachidonyl CoA pool contributed to the rate of labeling of phospholipids, since the specific acitivity of arachidonyl CoA is not known. The decreased rate of incorporation of [^{3}H]-arachidonate into phospholipids in ischemic liver could, therefore, reflect a decreased rate of entry of the label into the acyl CoA pool or a decreased rate of the reacylation of lysophospholipids. In either case, a declining ability of the liver to keep up the reacylation of lysophospholipids in the face of continuing deacylation of phospholipids would seem to be a significant factor in the loss of phospholipid in ischemia.

Ischemic renal cortex showed a similar yet slower loss of phospholipid (Fig. 3). In this case, however, the loss of fatty acids from phospholipids was accompanied by their equimolar accumulation as free fatty acids (Table 7). The conclusion that the rise in free fatty acids represents the accumulation of the products of the degradation of phospholipids is supported by the change in chain composition of the free fatty acid pool to more closely resemble that of the phospholipids. The reason the free fatty acids accumulate in the ischemic renal cortex and not in the liver is not known. The difference cannot be attributed to the mechanism of inducing ischemia, as either method gave the same result in the liver.

It would seem, therefore, that ischemia induces in both the kidney and liver a similar disorder in lipid metabolism. Whether the loss of phospholipid from the ischemic renal cortex is related to the genesis of irreversible cell injury in this model is dependent upon the time course of the former correlating with that of the latter. It might appear that the time course of the loss of phospholipid is too slow to account for the presumably much more rapid loss of viability of the renal cortex in ischemia. Loss of viability, however, of the ischemic kidney is generally assessed as the loss of reversibility upon reperfusion. Because of the so-called "no-reflow" phenomenon (26, 27), where blood flow does not return despite reperfusion, loss of reversibility may relate more to loss of prompt reperfusion than to actual changes in the parenchymal cells themselves. The time course of the loss of phospholipid from ischemic renal cortex reported here may actually represent the rate of change of cellular constituents, presumably membranes, that critically determine the viability of the cells independent of considerations of the ability to adequately resupply the cells with oxygen upon reperfusion. In addition, the lipid loss reported here represents that from whole cells rather than specifically from a particular cellular membrane, presumably the plasma membrane, alterations

TABLE 8. In vitro incubation of the kidney with and without calcium ions[a]

	Control	+ Ca^{2+}	- Ca^{2+}
Post/preischemic wet weight	1	1.5 ± 0.3	1.6 ± 0.2
Calcium content (μg/g dry wt)	332 ± 28	1793 ± 201	231 ± 26
Protein content (mg/g preischemic wet weight)	177 ± 4	151 ± 7	148 ± 6
Phospholipid content (μmol lipid PO_4/g preischemic wet wt)	33 ± 1	25 ± 2	24 ± 4
Free fatty acids (μmol/g preischemic wet wt)	2 ± 2	37 ± 9	35 ± 2

[a] Results are the mean ± SD of the determinations on three separate kidneys.

in whose structure and function may relate more directly to the loss of viability of the ischemic cells. Consideration of the effect of ischemia on the phospholipids of the plasma membranes of renal cortical epithelial cells may increase the sensitivity for detection of alterations critically related to irreversible cell injury in ischemia.

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