Incorporation of acetate-1-^{14}C into lipids by the perfused liver of normal, X-irradiated, or partially hepatectomized rats

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ABSTRACT In order to study lipid metabolism in the liver without interference due to transport from and to the liver the isolated livers of normal, X-irradiated, and partially hepatectomized rats were perfused with acetate-1-^{14}C and the distribution of radioactivity in total lipids, total fatty acids, individual lipids, and fatty acids of individual lipids was determined. In X-irradiated animals, an increased incorporation of acetate into many lipids, particularly into cholesterol, was observed. Lipids in the liver of the partially hepatectomized rats exhibited a marked increase in triglyceride content together with a decreased rate of incorporation into all but the phospholipid fractions.

It is concluded that the increase usually observed in lipid pontent of the regenerating liver is due to the changes in transcort rather than to changes in synthesis. The changes observed in irradiated liver could be the result of alterations in the metabolism of precursors common to most lipids.

KEY WORDS rat · normal · X-irradiated · partially hepatectomized · liver perfusion · incorporation · acetate-1-^{14}C · total lipids · lipid fractions · fatty acids

Changes in the concentration of lipids in the liver, such as are found during regeneration after partial hepatectomy (1, 2), can result not only from a hepatic alteration in synthesis or catabolism of these substances but also from a change in the transport of lipids to and from the liver. It is difficult to distinguish between contributions from the liver and those from other organs. The isolated perfused liver has been shown to be a useful system for the study of many metabolic pathways, including those of lipid metabolism (3, 4), and permits an approximation of in vivo conditions without interference from other organs. We have, therefore, studied the synthesis from acetate of various lipid fractions in perfused normal, regenerating, and X-irradiated livers.

Abbreviations: GLC, gas–liquid chromatography; TLC, thin-layer chromatography.

METHODS

Rats

Male Wistar rats, 3 months old, were used. The technique of perfusing the isolated rat liver has been described elsewhere (5, 6). The liver is perfused outside the body in an incubator held at 37°C. Perfusate enters the liver under a hydrostatic pressure of 13 cm via the portal vein and returns to the oxygenator reservoir via a cannula in the superior vena cava. The bile duct was also cannulated during this operation, and bile was collected during perfusion. Thirty milliliters of rat blood, diluted with one-third of Ringer's solution, to which 150 mg of glucose was added, served as perfusate.

All animals were starved for 18 hr prior to perfusion. Partial hepatectomy of the donors of blood and liver was performed 24 hr before perfusion (7). Total body X-irradiation (1000 r) of blood and liver donors was also carried out 24 hr prior to perfusion. The conditions of X-irradiation were 300 kv 100 r/min, filter 2 mm Cu (8). Livers from eight normal, eight partially hepatectomized, and nine X-irradiated animals were perfused.

Perfusion

Acetate-1-^{14}C (33 μc, specific activity 2–3 mc/mmole) was added to the perfusate after the flow had become stable (about 30 min after the start of perfusion). Samples

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of the perfusate as well as of the liver were taken at various intervals during the perfusion. CO_2 was collected in ethanolamine–methyl Cellosolve 1:2 (v/v) and its radioactivity as well as that from all other samples was determined by liquid scintillation counting.

Analysis of Liver Lipids

The liver samples were homogenized in 20 volumes of chloroform–methanol 2:1 (9) and the homogenates were filtered. After the radioactivity in an aliquot had been determined, the crude extracts containing the total lipids were washed repeatedly with water until the washings were free from radioactivity. The extracts were then taken to near dryness under vacuum and finally dried over anhydrous Na_2SO_4. Weight and radioactivity of total lipids were determined.

At this point two different procedures were used for further processing. Either the lipids were separated into lipid classes or the methyl esters of the fatty acids from total lipids were prepared.

In the latter case, the lipids were methanolyzed in 5% methanolic HCl under reflux for 5 hr. The crude methyl esters were extracted with petroleum ether and saponified in 0.5 N methanolic NaOH under reflux for 3 hr. Unsaponifiable material was removed with petroleum ether and the free fatty acids were esterified with 5% methanolic HCl.

For the fractionation of the methyl esters into saturated, monounsaturated, and polyunsaturated esters, they were dissolved in methanol containing an excess of mercuric acetate (as calculated from the iodine number of the methyl esters) and kept at room temperature in the dark in stoppered flasks under nitrogen for 3 days (10). The samples were dried, dissolved in benzene, and heated to 50°C, and the solution was filtered in order to remove excess mercuric acetate.

The saturated methyl esters and the mercuric adducts of mono- and polyunsaturated esters were separated on a silicic acid column by successive elution with benzene, ether, and methanol–acetic acid 9:1. Monounsaturated esters were regenerated from their adducts by dissolving the residues (1 g) in 10 ml of methanol, followed by dropwise addition of 4 ml of 15% HCl. Petroleum ether (50 ml) was then added, the mixture was shaken for 1 hr at room temperature, and the methyl esters were extracted with petroleum ether. Polyunsaturated methyl esters were regenerated by dissolving the adducts (50 mg) in 2 ml of methanol, adding 4 ml of concd HCl–methanol 1:1 dropwise, and extracting with 10 ml of petroleum ether as described above. The purity of saturated, monounsaturated, and polyunsaturated fractions was checked by determining the iodine number and by GLC.

For fractionation of lipid classes, the crude total lipid extracts were first separated into phospholipid and neutral lipid fractions by dialysis against petroleum ether through a rubber membrane (11). Separation was complete after 48 hr of dialysis, as ascertained by TLC and determination of the phosphorus content. The neutral lipids of the dialysate were separated further into triglycerides, cholesterol, and cholesterol esters by column chromatography on Florisil, 60–100 mesh, with a stepwise gradient of ethyl ether in petroleum ether (12). Aliquots were taken and analyzed by TLC on Silica Gel G (Merck). Chloroform was used as developer for the neutral lipids and chloroform–methanol–water 65:25:4 for the phospholipids.

Methyl esters of the triglycerides and phospholipids were prepared as mentioned above and analyzed by GLC on a Pye gas chromatograph with argon as carrier gas and an ionization detector. Separations were carried out on 4 ft × 4 mm I.D. columns packed with 15% ethylene glycol succinate polyester on Gas Chrom P, 80–100 mesh (Applied Science Laboratories, Inc., State College, Pa.). The columns were operated at 175°C and at 10.6 psi inlet pressure. The percentage of each methyl ester in a sample was calculated from area measurements obtained by triangulation. The analysis of standards for GLC showed reliable linearity of response to the detector.

Analysis of Lipids in the Perfusate

Total lipids in the perfusate were extracted with chloroform–methanol 2:1 and the extract was washed repeatedly with a 0.04% aqueous solution of $CaCl_2$. The total lipids were further partitioned by dialysis through a rubber membrane. Thin-layer chromatography of the resulting fractions revealed no differences among the perfusates of X-irradiated, normal, and regenerating liver with respect to the amount of individual fractions present. Therefore, further separation of the lipid fractions from the perfusate was not undertaken.

Incorporation of Label into Bile

Bile was collected over a 60 min period and separated by TLC in butyl acetate–acetic acid–ethanol–water 6:4:2:1. Radioactivity of conjugated bile acids, phospholipids, and cholesterol as well as that of total bile was determined.

RESULTS

Data concerning the radioactivity in the perfusate and the formation of radioactive CO_2 are shown in Fig. 1. The rates of disappearance of radioactivity and the rates of formation of CO_2 were slightly increased in X-irradiated liver compared with normal liver, but were markedly decreased in regenerating liver. However, when the difference in weight of the livers is taken into

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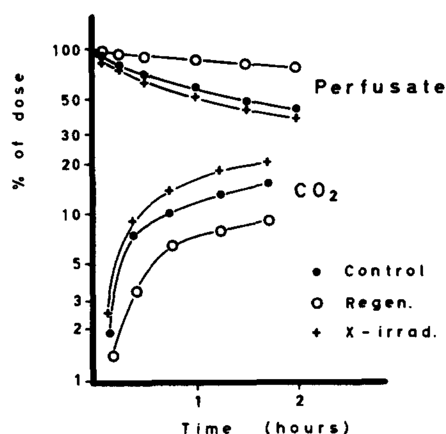

Fig. 1. Disappearance of radioactivity from perfusate containing acetate-1-^{14}C and formation of $^{14}CO_2$ by perfused normal, X-irradiated, and regenerating liver (after partial hepatectomy). The liver weights were: normal 7.0 g, X-irradiated 8.5 g, regenerating 3.4 g.

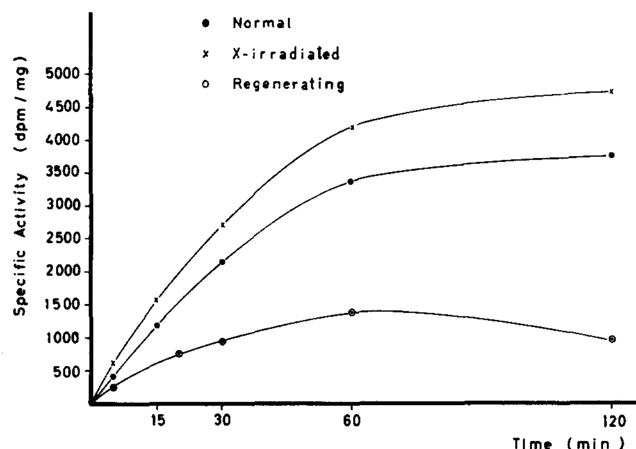

Fig. 2. Incorporation of acetate-1-^{14}C into fatty acids of total lipids of perfused liver. There were four animals per group.

account, the differences between regenerating and normal liver with respect to these rates disappear almost completely.

The graphs of specific activities of methyl esters from total lipids against time demonstrate (Fig. 2) that incorporation proceeded over a period of 1–2 hr in the case of normal and X-irradiated liver, but was already complete after 1 hr in the case of regenerating liver, where a slight decrease in activity occurred after 1 hr. A perfusion time of 1 hr was, therefore, selected for most experiments. Fig. 2 also shows that radioactivity was highest in the X-irradiated liver, followed by normal and regenerating liver.

Data (Table 1) on total, saturated, and unsaturated methyl esters from the experiment referred to in Fig. 2 confirm that the specific activity of lipids in the X-irradiated liver was generally higher than that in normal liver, whereas the specific activities in regenerating liver were markedly lower. It is also noteworthy that the specific activity of monounsaturated methyl esters increased markedly from 1 to 2 hr of perfusion, in contrast to the behavior of saturated and polyunsaturated methyl esters, where only a slight increase or even a decrease in specific activity was seen.

Total content of lipid in the liver was about the same in normal and regenerating liver (Table 1), but since the weight of the regenerating liver was only 3.4 g vs. 7.8 g for the normal one, the concentrations of these fractions in regenerating liver were much higher. If incorporation of acetate is expressed in terms of radioactivity incorporated per gram of liver, differences between normal and regenerating liver with respect to total saturated and polyunsaturated fatty acids become much smaller for the 1 hr period of perfusion. On the other hand, much less acetate was incorporated into monounsaturated fatty acids of regenerating liver than into those of normal liver after 1 hr of perfusion and the difference becomes even more marked if the 2 hr values are compared, since no further increase in activity takes place after 1 hr in monounsaturated fatty acids of regenerating liver. On the other hand, radioactivity in saturated fatty acids of regenerating liver decreases whereas it remains constant in normal liver.

Data on several lipid fractions in the liver after an hour's perfusion are presented in Table 2. Again it can be seen that the concentration of triglycerides is markedly increased in regenerating liver, but that normal and X-irradiated livers are similar with respect to the concentration of the various lipid fractions.

The specific activities of triglycerides, cholesterol, and particularly of cholesterol esters increased after irradiation (Table 2), but no difference was found in phospholipids. Most lipid fractions in regenerating liver showed a marked decrease in specific activity, but if incorporation is corrected for the amount of lipid present and for liver weight, i.e. is expressed as activity per gram of liver, it appears that synthesis of triglycerides is even slightly increased during regeneration. Synthesis of cholesterol is decreased, whereas that of phospholipids remains unaltered.

The gas chromatographic separation of the fatty acid methyl esters (Table 3) indicates that the composition of fatty acids remained the same during regeneration, but that slightly more arachidonic acid was present in phospholipids after irradiation.

The radioactivity in various lipids of the perfusate followed the same pattern as in liver, i.e., specific activity increased (or remained constant) after irradiation and decreased during regeneration (Table 4).

In bile, higher activities occurred in conjugated bile acids, phospholipids, and cholesterol from X-irradiated liver than in the same substances derived from bile of normal and regenerating liver (Table 5).

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TABLE 1 Specific Activities and Amounts of Fatty Acids (Measured as Methyl Esters) of Total Liver Lipids after Perfusion with Acetate-1-^{14}C

Fatty Acids	Time	Quantity N*	Quantity X*	Quantity R*	Spec. Activity N	Spec. Activity X	Spec. Activity R	Total Activity × 10³ N	Total Activity × 10³ X	Total Activity × 10³ R	Activity/g Liver × 10³ N	Activity/g Liver × 10³ X	Activity/g Liver × 10³ R
	min		*mg/liver*			*dpm/mg*			*dpm*			*dpm*	
Total	60	232† ±43	285 ±34	248 ±32	3350 ±695	4200 ±615	1350 ±296	782	1190	345	105	140	103
	120	296 ±26	312 ±31	244 ±23	3760 ±532	4700 ±705	955 ±117	1100	1560	232	147	184	68
Saturated	60	79 ±23	97 ±16	78 ±14	2870 ±440	5530 ±783	1950 ±330	222	536	189	24	63	55
	120	97.1 ±8.9	107 ±9.4	86 ±8.7	4200 ±794	5790 ±783	975 ±96	408	619	84	54	73	25
Monounsaturated	60	32 ±6.4	40 ±13	45 ±16	1183 ±289	2060 ±296	289 ±56	38	82.5	13.1	5.15	9.8	3.8
	120	44 ±4.5	44 ±6.3	40 ±3.8	5340 ±735	5478 ±806	342 ±59	235	300	13.8	31	35	4.1
Polyunsaturated	60	115 ±17	128 ±19	110 ±35	3489 ±510	3740 ±349	1092 ±186	461	480	121	53	56	35
	120	142 ±11.6	147 ±15.1	115 ±11.3	2700 ±420	3514 ±454	1076 ±119	383	517	123	50	61	36

* N, normal; X, X-irradiated (1000 r); R, regenerating.
† Means of four animals per group ± SD.

TABLE 2 Amount and Radioactivity of Lipid Fractions after 60 Min Perfusion of the Liver with Acetate-1-^{14}C

	Experimental Group	Amount		Radioactivity Spec. Activ.	Total Activ. × 10³	Activ./g Liver × 10³
		mg/liver	*% wet wt*	*dpm/mg*	*dpm*	*dpm*
Total lipid*	Normal	335 ± 21	4.26 ± 0.46	9120 ± 840	3050	385
	Regenerating	204 ± 13	7.9 ± 0.4	2800 ± 400	572	222
	X-irradiated	334 ± 16	4.14 ± 0.29	11,640 ± 1070	4000	490
Phospholipids	Normal	251	3.19	2830	710	90
	Regenerating	74	2.86	2415	180	70
	X-irradiated	255	3.16	3180	810	99
Phospholipid fatty acids	Normal	88	—	2690	237	30.2
	Regenerating	40	—	1970	79	30.4
	X-irradiated	93	—	2840	264	32.3
Triglycerides	Normal	44	0.56	6560	288	36.4
	Regenerating	94	3.63	1425	134	52.0
	X-irradiated	42	0.52	9920	417	51.0
Triglyceride fatty acids	Normal	31		5275	163	20.4
	Regenerating	76	—	998	76	29.5
	X-irradiated	34	—	7750	263	32.3
Cholesterol	Normal	19	0.24	95,500	1820	230
	Regenerating	6.5	0.25	29,700	193	75
	X-irradiated	15	0.21	152,000	2290	281
Cholesterol esters	Normal	11	0.14	3650	40.0	5.05
	Regenerating	7.0	0.27	875	6.15	2.38
	X-irradiated	11	0.13	7314	80.5	9.75

* Values for total lipids are the means of three animals per group ± SD. Values for the individual lipid fractions were obtained from the pooled lipid extract from each experimental group.

DISCUSSION

Effects of X-Irradiation

Microscopic sections suggest an increase in lipids of the liver of irradiated animals (13), but the biochemical evidence for such an increase is questionable. Some authors have observed an increase in fatty acids and changes in the amount of unsaturated fatty acids after total body exposure (14–16), whereas others have found such an increase only after local irradiation of the liver (17).

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TABLE 3 Fatty Acid Composition of Triglycerides and Phospholipids of Liver after 60 Min of Perfusion

Fatty Acid	Triglycerides			Phospholipids		
	Normal	X-irradiated	Regenerating	Normal	X-irradiated	Regenerating
	% of total fatty acids					
14:0	0.71	0.5	0.5			
16:0	23.0	21.6	19.0	19.6	16.1	19.2
16:1	1.96	1.1	1.9	1.35	0.9	0.75
18:0	3.1	2.6	2.5	25.7	16.1	18.7
18:1	25.7	25.2	26.2	7.2	6.1	9.2
18:2	36.5	37.4	39.6	17.2	18.9	22.7
18:3	5.9	6.8	7.1			
20:4	2.8	3.5	2.8	28.8	43.0	27.7

Studies on incorporation of acetate into lipids also have not provided unequivocal answers. An increase in incorporation of acetate into fatty acids (18, 19) and especially into cholesterol (19, 20) is reported, but it has been claimed that forced feeding with glucose abolishes this effect (21).

Our experiments do not show a significant difference in the concentration of hepatic lipids, but incorporation into triglycerides and particularly into cholesterol and its metabolites, i.e. bile acids, was increased after irradiation. The effect is not as great as has been described for the intact animal (18, 19), but it is possible that dietary conditions were controlled more efficiently in our experiments since all animals were starved before perfusion and glucose was added to the perfusate. Indeed, starvation has been found to play an important role in the development of other metabolic disturbances hitherto ascribed to irradiation (22, 23).

Our data do not allow conclusions as to the mechanism which might be responsible for the increased incorporation of acetate after irradiation. However, it seems likely that since incorporation into most lipids of the liver as well as breakdown of acetate is affected, the metabolism of a low molecular weight precursor of fatty acids is altered.

Effects of Partial Hepatectomy

The lipid content in the liver increases soon after partial hepatectomy (2) and this increase precedes the increase in mitotic activity (24–26). Such a rapid accumulation of lipids in the liver, which is also seen after administration of toxic substances, has been interpreted as an expression of an impairment of liver function (27). However, it seems likely that changes in the transport of lipids rather than changes in the synthesis of lipids are responsible for the accumulation of lipids during regeneration (1) and after intoxication (4, 28).

The incorporation of acetate into lipids of regenerating liver in the intact rat is not increased as markedly as would be expected from the increase in lipid content (29, 30). Our results, obtained on the isolated perfused liver, confirm that synthesis of total lipids in the regenerating liver does not increase but rather decreases. The increase in concentration of lipids during regeneration appears to be the result of an altered transport of lipids rather than of an increased synthesis. However, other changes occur during regeneration which are the result of alterations in the hepatic metabolism of lipids. The utilization of acetate per gram of liver is not altered markedly, yet marked differences exist in the incorporation of acetate into the various lipid fractions. This observation indicates that changes in the pool of acetate or in the precursors common to all lipids are not responsible

TABLE 4 Amounts and Specific Activities of Lipids in the Perfusate* after 60 Min of Perfusion

Experimental Group	Total Lipids			Phospholipids			Neutral Lipids		
	Quantity	Spec. Activ.	Total Activ. × 10^3	Quantity	Spec. Activ.	Total Activ. × 10^3	Quantity	Spec. Activ.	Total Activ. × 10^3
	mg	*dpm/mg*	*dpm*	*mg*	*dpm/mg*	*dpm*	*mg*	*dpm/mg*	*dpm*
Normal†	37 ±4.2	3150 ±480	115	19 ±1.6	870 ±95	16.5	16 ±1.9	5950 ±875	95
X-irradiated	21 ±3.8	5190 ±534	108	9 ±1.2	870 ±68	7.8	10 ±2.3	9911 ±965	99
Regenerating	36 ±4.9	750 ±140	26.5	14 ±2.1	615 ±83	8.6	20 ±3.4	825 ±56	16.5

* The perfusate consisted of a mixture of 2 parts heparinized rat blood and 1 part Ringer's solution.
† Mean of three animals per group ± SD.

TABLE 5 Excretion into the Bile of Various Radioactive Lipid Fractions during 60 Min Liver Perfusion with Acetate-1-^{14}C

Experimental Group	Total Activity Excreted	Conjugated Bile Acids	Phospholipids	Cholesterol
	dpm × 10^{-3}			
Normal	80	54	8	6.0
X-irradiated	105	70	10	8.0
Regenerating	34	18	4.2	3.0

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for the changes in lipid synthesis. Most marked is the difference in turnover during regeneration in monounsaturated fatty acids which not only appear to be synthesized at a considerably lower rate but also are converted or degraded more rapidly.

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