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LIVER GLUCOSE, GLYCOGEN AND LIPID SYNTHESIS IN FED AND 24-HOUR FASTED RATS SOON AFTER A PULSE OF [3-^{14}C]PYRUVATE THROUGH THE PORTAL VEIN

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Abstract—1. A pulse of [3-^{14}C]pyruvate was given to rats through the portal vein and blood was collected at brief intervals from the inferior cava vein at the level of the suprahepatic veins.

2. In 24 hr fasted rats, the appearance of [^{14}C]glucose in blood and blood glucose specific radioactivity were higher than in fed animals from the first minute after delivery of the tracer. At this time total radioactivity did not differ between the two groups.

3. After 5 and 20 min, liver radioactivity present in glycogen and glyceride glycerol was enhanced while in fatty acids it was reduced in fasted as compared with fed animals.

4. It is proposed that, in the fasted state, both glycogen and glyceride glycerol synthesis are predominantly gluconeogenic processes.

INTRODUCTION

Although liver glycogen is generally considered the major product in the disposal of increased blood glucose concentrations (Stalmans, 1976), loading the perfused liver with high amounts of glucose yields very little formation of glycogen from glucose, whereas the addition of gluconeogenic substrates results in substantial glycogen synthesis (Hems *et al.*, 1972; Whitton & Hems, 1977). After the "*in vivo*" injection of [3-^{14}C]pyruvate in the fasted rat, we found the appearance of [^{14}C]glycogen in liver greatly enhanced (Herrera *et al.*, 1969). More recently, Katz *et al.* (1979) reported that the contribution of gluconeogenic precursors to liver glycogen synthesis becomes quantitatively important when liver glycogen is depleted. The problem of liver glycogen synthesis remains unclear as no correlation has been found between liver glycogen enzymes and actual glycogen concentrations and/or synthesis (Den Otter, 1974; Remesar & Alemany, 1980; Shikama *et al.*, 1980). Determination of the role of gluconeogenic precursors in glycogen and glucose synthesis is complicated by the substrate recycling which varies with the animals' dietary status (Freidmann *et al.*, 1971; Okajima *et al.*, 1981). In the present study, the conversion of [3-^{14}C]pyruvate into glucose and glycogen in the liver of fed and 24 hr fasted rats was investigated with a technique previously used for assessing the role of the liver in the hypoglycemic effects of insulin (Chieri *et al.*, 1966). This technique allows sampling directly from the liver at very short intervals after administration of the tracer, avoiding artefacts produced by its recycling. In view of the recent interest in the role of glycerol-3-phosphate availability in glyceride glycerol synthesis in the liver of fasted animals (Debeer *et al.*, 1981), our study was extended to determine the conversion of the administered [3-^{14}C]pyruvate into liver fatty acids and glyceride glycerol.

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MATERIALS AND METHODS

Male Wistar rats weighing 200 ± 5 g were studied when fed a commercial purina chow diet "*ad libitum*" or after a 24 hr fast. Animals were anesthetized by an i.v. injection of sodium pentobarbital (60 mg/kg b.w.) and a pulse of 1 μCi of [3-^{14}C]pyruvate (from the Radiochemical Center, Amersham; specific activity 15.8 mCi/mmol) containing 100 μmol of sodium pyruvate/100 g b.w., was given through the portal vein. Blood aliquots were collected at the level of the suprahepatic veins at different intervals thereafter. The surgical procedure used was similar to that previously described by Chieri *et al.* (1966) for other purposes and may be summarized as follows: After laparotomy, a cannula (PE-10 Intramedic, Clay Adams, NJ) is introduced through the ileocholic vein up to the level of the portal vein. After the suture of the abdominal wall, another cannula is introduced (PE-50, Intramedic, Clay Adams, NJ) through the right jugular vein passing by the superior cava vein up to the level of the suprahepatic veins. Once cannulation is completed, a saline–albumin solution (0.5 g bovine albumin/l of 0.9% NaCl) is perfused through the cannula in the portal vein, at the rate of 5 ml/hr for 5 min, after which the pulse of [3-^{14}C]pyruvate is given, followed by perfusion with saline–albumin solution until the end of the experiment. Blood aliquots of about 0.2 ml are collected into heparinized syringes from the cannula placed at the level of the suprahepatic veins, at 1, 2, 5, 10 and 20 min after the tracer. At the last blood collection, a piece of liver is placed into liquid N_2. In other animals, blood was collected for only the first 5 min when the experiment was ended by placing a piece of liver into liquid N_2. The correct disposition of the cannulas was always checked by animal autopsy and values were not used unless an adequate cannula location was observed. Blood aliquots were deproteinized (Somogyi, 1945) and supernatants were used to measure glucose (Huggett & Nixon, 1957) and placed in

Duolite A-561 (generously supplied by Diamond Shamrock, CA)–Dowex 1 × 2–400 (Sigma, St Louis, MO) (Herrera *et al.*, 1969) that were eluted with double distilled water until 3 ml of eluate was produced. Aliquots of the eluates were used to determine the radioactivity in [^{14}C]glucose. Recovery experiments were performed by adding ^{14}C-standards to normal rat plasma. In the 3 ml eluates, recovery of the label was 99.6% when the standard was [U-^{14}C]glucose, while it was 0.59% with [U-^{14}C]alanine, 0.17% with [1-^{14}C]lactate and 0.24% with [3-^{14}C]pyruvate. Aliquots of frozen livers were placed in 30% KOH at 100°C for 10 min and glycogen was precipitated three times with ethanol in the cold (Good *et al.*, 1933). Glycogen precipitates were hydrolyzed with 5 N H_2SO_4 at 100°C for 2 hr and after neutralization, aliquots were used for counting the radioactivity and for determination of glucose with glucose oxidase. This method has previously been validated by one of us (Herrera *et al.*, 1969). Another aliquot of frozen liver was used for lipid extraction and purification (Folch *et al.*, 1957) and fractionation, following a modified version of the method of Kerpel *et al.* (1961), as described previously (Carmaniu & Herrera, 1980), to determine the radioactivity in both fatty acids and glyceride glycerol. Radioactive measurements were performed in a PPO/POPOP based scintillation cocktail dissolved in xylene and Trition-X-100 and samples were counted in a Nuclear Chicago (Isocap 300) counter provided with an external standard device. Quenched standards (NEN chemicals GmbH) were always counted with samples to determine the channels-ratio to convert CPM into DPM. Tests for significant differences between means were done with the Student's *t*-test.

RESULTS

Fed and 24 hr fasted rats were given a pulse of [3-^{14}C]pyruvate through the portal vein and blood was subsequently collected from the inferior cava vein at the level of the suprahepatic veins at different times. As shown in Table 1, total blood radioactivity did not differ between either group of animals at the 1 min time, while it decreased more slowly in fasted than in fed animals, the difference becoming significant from the 2nd min on. A certain proportion of this circulating radioactivity corresponded to [^{14}C]glucose and, as shown also in Table 1, this parameter augmented with time after tracer administration in both groups of animals, although it was significantly greater in fasted than in fed rats from the 1st min on. Blood glucose concentration did not change in either group throughout the experiment (Table 1) and it was lower in the fasted than in the fed animals. Consequently, blood glucose-specific activity was much greater in the fasted than in the fed animals from the 1st until the 20th min after tracer administration (Table 1). To determine the incorporation of radioactivity into liver glycogen and lipids, some animals in each group were sacrificed at 5 and 20 min. As seen in Table 2, the liver content of [^{14}C]glycogen changed very little after tracer administration and at the two times studied, it was significantly higher in the fasted than in the fed animals. Liver glycogen concentration was much lower in fasted than in fed animals (Table 2) and in both groups it decreased progressively. Liver glycogen-specific activity was much greater in fasted than in fed animals (Table 2) and it rose in the former group after tracer administration. As shown in Table 3, the appearance of radioactivity in total lipids was slightly but not significantly higher in fasted than in fed animals, and in both groups, values at the 20 min sample were higher than at 5 min. The distribution of this radioactivity among the lipidic fractions differed markedly between the two groups. In the fasted animals, radioactivity in the fatty acids was much lower than in the fed subjects while radioactivity in the glyceride glycerol fraction was much greater in the fasted rat (Table 3). These relations did not differ between the groups when studied at 5 or 20 min after the [^{14}C]pyruvate pulse although the amount of label found in liver lipids augmented with time in both groups.

DISCUSSION

The administration of a pulse of [3-^{14}C]pyruvate to the rat at the level of the portal vein and collection of blood from the supraheptic veins permitted deter-

Table 1. Effect of 24 hr fast in the rat on liver production of [^{14}C]glucose after a pulse of [3-^{14}C]pyruvate through the portal vein

Minutes after [3-^{14}C]pyruvate:		1	2	5	10	20
Total blood radioactivity (dpm/ml)						
	Fed	40,640 ± 2549	26,103 ± 902	16,180 ± 619	10,927 ± 468	8411 ± 215
	Fasted	42,933 ± 2323	32,390 ± 857	24,330 ± 779	20,064 ± 497	18,456 ± 689
	P	NS	<0.001	<0.001	<0.001	<0.001
[^{14}C]Glucose in blood (dpm/ml)						
	Fed	585 ± 73	1042 ± 113	1018 ± 140	1154 ± 235	1378 ± 164
	Fasted	3067 ± 332	4580 ± 380	5350 ± 464	4959 ± 655	5134 ± 531
	P	<0.001	<0.001	<0.001	<0.001	<0.001
Blood glucose (mg/dl)						
	Fed	119 ± 4	106 ± 8	120 ± 11	116 ± 8	116 ± 8
	Fasted	71 ± 4	79 ± 4	74 ± 4	74 ± 6	75 ± 6
	P	<0.001	<0.01	<0.001	<0.001	<0.001
Blood glucose specific radioactivity (dpm/mg)						
	Fed	501 ± 84	1162 ± 116	911 ± 149	924 ± 218	991 ± 90
	Fasted	4224 ± 531	5407 ± 442	7718 ± 894	6925 ± 1020	6909 ± 620
	P	<0.001	<0.001	<0.001	<0.001	<0.001

Blood samples were collected from the inferior cava vein at the level of the suprahepatic veins, at different times after trace administration. Means ± SEM of 6–14 rats/group. *P* values correspond to the comparison between fed and fasted animals (NS = not significant, $P > 0.05$).

Table 2. Effect of 24 hr fast in the rat on liver glycogen parameters after a pulse of [3-^{14}C]pyruvate through the portal vein

	[^{14}C]Glycogen (dpm/g)		Glycogen conc. (0/00)		Glycogen specific radioactivity (dpm/mg)	
Minutes after [3-^{14}C]pyruvate	5	20	5	20	5	20
Fed	624 ± 79	488 ± 93	42 ± 3	20 ± 2	16 ± 3	24 ± 6
Fasted	1784 ± 257	2252 ± 439	0.8 ± 0.1	0.4 ± 0.0	1889 ± 274	5139 ± 994
P	<0.01	<0.01	<0.001	<0.001	<0.001	<0.001

Animals were sacrificed at 5 or 20 min after tracer administration and a piece of liver was immediately placed into liquid N_2 for glycogen purification. Means ± SEM of 5–10 rats/group. *P* corresponds to the comparison between fed and fasted animals.

mination of production of [^{14}C]glucose by the liver within minutes of tracer administration. This procedure minimizes the problem of glyconeogenetic substrate recycling which is known to be significant in other experimental conditions (Freidmann *et al.*, 1971; Freminet *et al.*, 1976; Okajima *et al.*, 1981) and is affected by the dietary status of animals (Friedmann *et al.*, 1971; Freminet *et al.*, 1976). An augmented production of [^{14}C]glucose by the liver was found in fasted compared with fed animals 1 min after tracer delivery. At this time, blood [^{14}C]glucose corresponded to a minor percentage (1.4–7.1%) of total blood radioactivity and this latter parameter did not differ between fed and fasted animals. Thus sampling soon after tracer delivery avoids the changes which may be produced by differences in peripheral utilization of the newly formed glucose in the two experimental groups. It also allows measurement of the [^{14}C]glucose formed when the availability of the labelled substrate does not differ between groups. These two factors (peripheral utilization of glucose and substrate availability) are known to be affected by dietary status (Blackshear *et al.*, 1974; Freminet *et al.*, 1976) and changes in circulating labelled glucose at later periods may indeed be influenced by them. Present results showing an intense activation of liver gluconeogenesis after 24 hr of fasting confirm other findings obtained with different methodologies (Herrera *et al.*, 1969; Schimmel & Knobil, 1970; McGarry *et al.*, 1973) but the greater sensitivity obtained with the technique used here offers the possibility of studying very rapid actions of effectors on this pathway.

Changes in the appearance of ^{14}C in liver glycogen and in liver glycogen-specific activity indicate that after 24 hr of food deprivation, there is increased conversion of gluconeogenic substrates into liver glycogen. The reduced concentration of liver glycogen after fasting indicates an augmented net degradation but release into circulation of newly synthesized glucose in the fasted liver may require the previous synthesis of glycogen from gluconeogenic substrates and its subsequent breakdown, resulting in the formation of free glucose. This "futile" cycle between glycogen synthesis and glucose formation would facilitate hepatic glycogen deposition on re-feeding after starvation by continued gluconeogenesis and reduced glycogen breakdown, as already proposed (Hems *et al.*, 1972). Our results also agree with those of Katz *et al.* (1979) who have emphasized the important role of glycogen synthesis from gluconeogenic precursors when liver glycogen is depleted, suggesting that in the fasted state, glycogen synthesis is predominantly a gluconeogenic process.

The conversion of [^{14}C]pyruvate into fatty acids appeared greatly reduced in the liver of fasted rats, in agreement with previous reports (Aranda & Herrera, 1974; Llobera *et al.*, 1979). In the fed animals, a similar amount of pyruvate was canalized to both lipogenesis and glyceride glycerol formation. In the fasted rats, however, the reduction in liver lipogenesis was counteracted by a marked increase in the formation of [^{14}C]glyceride glycerol, which was observed already at 5 min after tracer administration, suggesting that the esterification of fatty acids reaching the liver from peripheral fat depot mobilization is maintained in the fasted animal by the glycerol-3-phosphate formed from glucogeneogenic precursors. It is known that for a given load of fatty acids, "*in vitro*" preparations of liver from fed rats esterify a greater

Table 3. Effect of 24 hr fast in the rat on the formation of ^{14}C-lipids in liver after a pulse of [3-^{14}C]pyruvate through the portal vein

	Total lipids		Fatty acids		Glyceride glycerol	
Minutes after [3-^{14}C]pyruvate	5	20	5	20	5	20
Fed	2659 ± 585	3845 ± 415	1648 ± 481	1825 ± 372	1024 ± 260	1345 ± 111
Fasted	3658 ± 376	5390 ± 799	198 ± 18	200 ± 40	3192 ± 389	5010 ± 773
P	NS	NS	<0.01	<0.001	<0.01	<0.01

Animals were sacrificed at 5 or 20 mm after tracer administration and a piece of liver was immediately placed into liquid N_2 for lipids extraction and fractionation. Means ± SEM of 5–10 rats/group. *P* corresponds to the comparison between fed and fasted animals. Values are expressed as dpm/g. (NS = not significant, $P > 0.05$).

proportion of the incoming fatty acids than do similar preparations from starved rats. In the latter, fatty acid oxidation increases in relation with esterification (Mayes & Felts, 1967; McGarry & Foster, 1971; Ontko, 1972). It has also recently been reported that the rate of esterification in hepatocytes from starved rats is limited by their deficiency of glycerol-3-phosphate (Debeer *et al.*, 1981). In the "*in vivo*" situation, starvation is associated with an enhancement in fat deposit mobilization which produces an increase in circulating free fatty acids concentration (Schimmel & Knobil, 1970; Llobera *et al.*, 1978; Palou *et al.*, 1981) and their subsequently increased delivery to the liver. This action alone is not sufficient to provoke increased hepatic ketogenesis (McGarry *et al.*, 1973) and there is actually a considerable time lag between the increase in circulating free fatty acids and ketone body levels in the starved rat (McGarry *et al.*, 1973; Llobera *et al.*, 1978; Palou *et al.*, 1981). Thus, since the ability of the liver to store fatty acids in the free form is very limited (Göransson & Olivecroma, 1964; Kohout *et al.*, 1971), they are forced to be temporally esterified even in the fasted state (Palmer *et al.*, 1978). In this condition, the limited availability of glycerol in the liver (Berdanier *et al.*, 1979) as compared to the needs of glycerol-3-phosphate for esterification (Debeer *et al.*, 1981) necessitates the use of gluconeogenic precursors for glyceride glycerol formation. The increased cytosolic redox state in the liver of the starved animal (Williamson *et al.*, 1967 Berdanier *et al.*, 1979) facilitates this diversion of dihydroxyacetone phosphate to glycerol-3-phosphate as it does for the formation of glyceraldehyde phosphate from 1,3-diphosphoglycerate, producing a parallel enhancement of liver glycerolgenesis and gluconeogenesis from the same substrate.

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