Rat Strain Differences in the Utilization of Glucose-U-^{14}C and Acetate-1-^{14}C for Fat Synthesis (35980)

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(Introduced by D. L. Trout)

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In our laboratories the fat metabolism of BHE and Wistar rats has been extensively investigated (1–3). It has been shown that the BHE rats tend to accumulate more fat in the liver than do Wistar rats under comparable dietary treatment. Recently efforts have been made to study the mechanisms controlling lipogenesis in these two strains of rats. The activity of liver glucose-6-phosphate dehydrogenase (G6PD) was found to be significantly higher in BHE than Wistar (4, 5). A higher level of G6PD could be taken as a sign of a higher hexosemonophosphate (HMP) shunt activity, and it was associated with higher liver lipids in BHE rats (3, 4). A more direct indication of shunt activity was obtained by measuring the respiratory ratio of expired $^{14}CO_2$ from glucose-1-^{14}C vs glucose-6-^{14}C. This ratio proved to be higher for the Wistar strain than for BHE rats (4). It appeared that, in spite of low G6PD activity in liver, Wistar rats utilized the shunt pathway in some tissues to a greater extent than did BHE animals.

To further clarify strain differences in fat metabolism, the present study involving the metabolic fates of glucose-1-^{14}C (G-1-^{14}C), glucose-6-^{14}C (G-6-^{14}C), uniformly labeled glucose (G-U-^{14}C) and acetate-1-^{14}C was undertaken. The incorporation of label from G-U-^{14}C and acetate-1-^{14}C in the lipid of various portions of the body and lipid classes of plasma and liver was investigated. The respiratory ratio of $^{14}CO_2$ from G-1-^{14}C vs G-6-^{14}C was reexamined, since the diet used in the present study did not contain cholesterol, as had the previous diet (4).

Materials and Methods. Three groups of male weanling BHE and Wistar rats were housed individually in wire bottom cages and fed a diet consisting of the following (g/100 g): casein, 27; methionine, 0.2; beef tallow, 14; corn oil, 2; salt mix, 4; celluflour, 2; vitamin mix, 1; and glucose, 49.8. The detailed composition of salt mix and vitamin mix and the care of rats were as previously reported (4). All rats were fed this diet for 70 days (to an age of 3 months), after which they were fasted overnight. At 8 a.m. each rat was then refed 4 g of diet. One hour after the start of refeeding, five BHE and five Wistar each were given intraperitoneally, 2 μCi of G-1-^{14}C[1] in 0.5 ml of isotonic saline solution containing 5 mg of nonlabeled dextrose and each rat was immediately placed in an individual all-glass metabolic cage. The respiratory $^{14}CO_2$ was trapped during each of the first four 1-hr periods in a 3:7 mixture of ethanolamine and ethylene glycol monoethyl ether. Radioactive $^{14}CO_2$ was assayed by liquid scintillation counting (4). After the last sample was collected, all rats were transferred to their regular cages and were maintained on the diet for 3 days. The same rats were then fasted overnight, refed 4 g of diet, and injected with 2 μCi of G-6-^{14}C.[1] The ratio between respiratory 1-$^{14}CO_2$ and 6-$^{14}CO_2$ was determined for each rat.

The second group of BHE and Wistar rats was given 4 μCi of G-U-^{14}C[1] each. After 4 hr each rat was killed by decapitation. Blood was collected in heparinized tubes and plasma was separated by centrifugation. Liver, abdominal fat pads, and carcass[2] were kept frozen for chemical analysis later.

[1] Specific activity of glucose-1-^{14}C, 15.9 μCi/mg; glucose-6-^{14}C, 21.6 μCi/mg; glucose-U-^{14}C, 21.6 μCi/mg; acetate-1-^{14}C, 354 μCi/mg. All radioactive compounds were purchased from Nuclear Chicago.

[2] Eviscerated carcass.

Each of the third group of rats was given 2 μCi of acetate-1-^{14}C[1] in 0.5 ml of isotonic saline. After 4 hr, each rat was also sacrificed in the same manner as was in the previous group. Blood was collected and liver, abdominal fat pads, and carcass[2] were kept frozen for chemical analysis later.

Analysis of tissue lipids. Liver. Total liver lipid was extracted with 2:1 (v/v) chloroform:methanol and purified by washing 5–7 times with 0.04% $CaCl_2$ (6). The lipid extract was evaporated to dryness and weighed. A known portion of lipid was used for measuring total radioactivity by liquid scintillation counting.

Fractionation of liver lipids. From another known portion of liver lipid, phospholipids were separated from the neutral lipids by a silicic acid column (7, 8). After drying the separated fractions, neutral lipids and phospholipids were weighed. A portion of phospholipids was used for measuring radioactivity by liquid scintillation counting, and the neutral fat was further separated into fatty acid, glyceride–glycerol, and cholesterol after saponification with alcoholic KOH (9). Cholesterol was determined by the method of Kabara (9) and glycerol by the Carlson procedure (10). The radioactivity in a known amount of cholesterol and glycerol was determined (9). Fatty acids were extracted with *N*-hexane after acidifying the saponified mixture to pH 2–3 following digitonide precipitation of cholesterol (11). The total radioactivity of fatty acids was counted although their quantity was not determined.

Lipids in plasma. An equal volume (usually 1.5 ml) of plasma from each rat was pooled according to strain and isotopic treatment. After a thorough mixing, duplicate 0.05 ml aliquots were taken for determination of total cholesterol. Four samples of 1.0 ml aliquot were used for extraction of total lipids (12). The extract was washed 5–7 times with 0.04% $CaCl_2$. Two lipid samples were used for determining total radioactivity, and the other two samples were used for further fractionation. The separation of phospholipids from neutral lipids was also accomplished by silicic acid column chromatography. The quantity of phospholipids was determined by a colorimetric method (13) and radioactivity was determined by liquid scintillation counting. The neutral lipid fraction was saponified with 2.5 *N* alcoholic KOH. The same method as for the liver lipids was used for quantitative measurement of cholesterol and glycerol. Cholesterol and fatty acid were also separated by thin-layer chromatography with the running solvent 80:20:1 (v/v) *N*-hexane:ethyl ether:acetic acid. A mixture of known reference compounds was applied on the same plate as a guide. Radioactivity was then determined in the isolated cholesterol and fatty acids.

Abdominal fat pads. Epididymal and perirenal fat pads were quickly excised and weighed. The respective fat pads were minced and duplicate samples (200–300 mg) were used for fat extraction (14) and measurement of radioactivity.

Carcass fat. Carcass fat was extracted as previously described (15). A known amount of fat was used for counting the radioactivity.

Results. Figure 1 shows quantity of $^{14}CO_2$ collected over each of four successive 1-hr periods from BHE and Wistar rats given G-1-^{14}C and G-6-^{14}C. The 4-hr collections of CO_2 showed a ratio of $^{14}CO_2$ from injected glucose-1-^{14}C vs glucose-6-^{14}C, which was 0.93 in BHE animals and 1.14 in Wistar rats. The difference in this ratio was consistent but small and may not have been meaningful.

Lipid distribution within the bodies of BHE vs Wistar rats is presented in Table I. Present data, indicating that BHE rats had larger perirenal fat pads and more fat in liver and plasma than did Wistar rats, confirmed previous findings (2, 5).

The higher content of fat in the livers and plasma of BHE rats was accompanied by a greater incorporation in lipids of radioactivity from acetate-1-^{14}C. However, label from G-U-^{14}C was higher only in the liver fat of BHE rats. The BHE animals showed a greater incorporation of acetate-1-^{14}C but not of G-U-^{14}C in the perirenal fat than did the Wistar rats (Table II).

The level of fractionated lipids of liver and plasma of BHE vs Wistar rats is shown in

TABLE I. Body Lipid Distribution in BHE and Wistar Rats.

Strain	Carcass		Fat pads (g)		Liver		Plasma (mg/100 ml)
	Total	(%)[a]	Epididymal	Perirenal	Total (mg)	(%)	
BHE (9)	40.8 ± 2.8[b]	13.8 ± 0.96	7.4 ± 0.54	9.2 ± 0.76	775 ± 50[c]	7.0 ± 0.24[c]	525
Wistar (9)	40.4 ± 1.7	13.3 ± 0.64	8.5 ± 0.55	8.7 ± 0.63	569 ± 38.9	5.3 ± 0.34	477

[a] One gram of fat per 100 g of wet weight of carcass.
[b] Mean ± SE.
[c] $p \leqslant .01$.

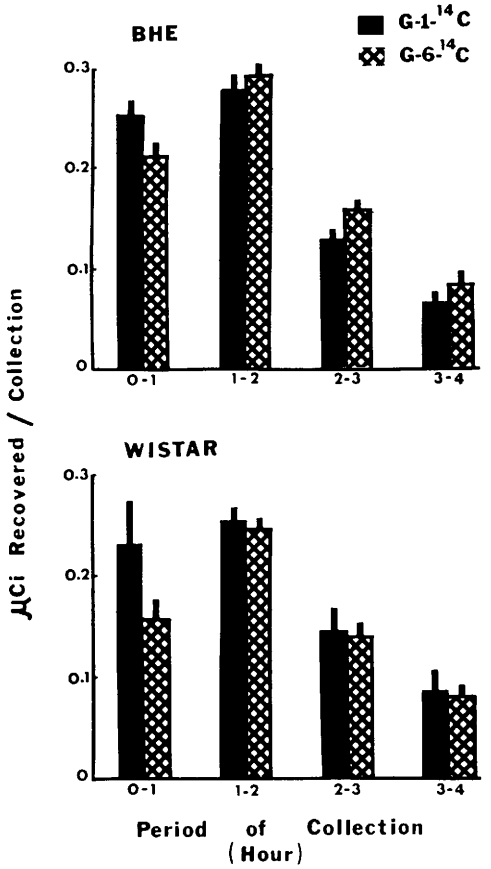

FIG. 1. Recovery of $^{14}CO_2$ from rats after the administration of glucose-1-^{14}C and glucose-6-^{14}C: five rats per group.

Table III. The amount of radioactivity and its relative percentage distribution in different fractions from the total is shown in Table IV. The level of liver phospholipids, glycerides, and cholesterol were significantly higher in BHE than in Wistar rats, and the amount of radioactivity incorporated into these fractions from acetate-1-^{14}C was also markedly higher in BHE than in Wistar rats. The level of fatty acids in Plasma neutral lipids was not determined but was found to contain a higher amount of radioactivity from acetate-1-^{14}C in BHE than in Wistar rats.

The relative percentage distribution of total incorporated radioactivity to lipid classes in liver and plasma lipids was different between strains. The pattern of distribution be-

TABLE II. Incorporation of Label from Glucose-U-^{14}C and Acetate-1-^{14}C into Body Lipids of BHE and Wistar Rats.

Strain	Recovery of radioactivity (dpm/μCi injected)				
	Carcass	Epididymal	Perirenal	Liver	Plasma[a] per ml
	After injection of glucose-U-^{14}C				
BHE (5)[b]	62,151[c]	1705	2576	8568[c]	30.85
	±3521	±252	±129	±867	
Wistar (5)	63,239	3925	3409	4815	44.61
	±7242	±965	±549	±180	
	After injection of acetate-1-^{14}C				
BHE (4)	133,449	17,876	18,383[e]	31,936[d]	171.7
	±14,522	±8337	±6345	±7861	
Wistar (4)	207,497	3672	1250	8016	50.5
	±69,534	±3260	±218	±883	

[a] Pooled plasma sample used.
[b] Number of rats is given in parentheses.
[c] Mean ± SE.
[d] $p \leqq .05$.
[e] $p \leqq .01$.

tween liver and plasma was not similar. This discrepancy might be due to the transport of lipids in plasma from various origins rather than from liver alone. Percentage of radioactivity in phospholipids from total compared with that in neutral lipids in liver was higher in Wistar than in BHE rats, but BHE had higher percentage in glyceride–glycerol from G-U-^{14}C and higher percentage in fatty acids and cholesterol from acetate-1-^{14}C.

Discussion. The ratio of 1-^{14}C/6-^{14}C of $^{14}CO_2$ from administered G-1-^{14}C and G-6-^{14}C may serve as an indicator for the body as a whole but does not indicate the contribution of tissues or organs to the total HMP activity. Lee and Lucia (16) reported that animals restricted in caloric intake showed little whole-body HMP activity, as indicated by a 1-CO_2/6-CO_2 value close to 1.0, yet showed an elevated activity of hepatic G6PD. They interpreted that an increased utilization of the HMP shunt in the liver may be masked by the activity of Embden-Meyerhof pathway in other tissues. The present study presents a similar situation. Data from whole-body radiorespirometry indicates that BHE animals show less HMP activity than animals of the Wistar strain;

TABLE III. Phospholipid, Glyceride, and Cholesterol Levels in Liver and Plasma[a] of BHE and Wistar Rats.

Strain	Phospholipids (mg)	Glyceride (μmoles)	Cholesterol (mg)
	Liver		
BHE (9)[b]	366 ± 30[c,d]	157 ± 26[e]	28 ± 2[d]
Wistar (9)	297 ± 17	82 ± 10	20 ± 2
	Plasma (per 100 ml)		
BHE	154	5.0	70.2
Wistar	150	5.8	64.7

[a] Pool of plasma sample was used.
[b] Number in parentheses represents number of rats.
[c] Mean ± SE.
[d] $p \leqq .05$.
[e] $p \leqq .01$.

TABLE IV. Distribution of Radioactivity in Phospholipids, and Glycerol, Fatty Acids, and Cholesterol of Neutral Lipids of Liver and Plasma in BHE and Wistar Rats.

Strain	Phospholipids		Neutral lipids					
			Glycerol		Fatty acids		Cholesterol	
	Total (dpm)	(%)[a]	Total (dpm)	(%)	Total (dpm)	(%)	Total (dpm)	(%)
	After injection of glucose-U-^{14}C (per μCi injected)							
	Liver							
BHE (5)[b]	4739		3408[c]		163		Nil	0
	±509[c]	57	±581	41	±69	2		
Wistar (5)	3460		880		119		Nil	0
	±259	77.6	±165	19.7	±16	2.7		
	Plasma (per ml)							
BHE	14	39	20	56	1.7	5	Nil	0
Wistar	13	29	30	66	2.4	5	Nil	0
	After injection of acetate-1-^{14}C (per μCi injected)							
	Liver							
BHE (4)	14,211[d]		361		12,716[d]		4810[d]	
	±3196	44.3	±149	1.1	±3540	39.6	±1478	15
Wistar (4)	4810				2374		358	
	±771	63.8	Nil	0	±274	31.5	±59	4.7
	Plasma (per ml)							
BHE	26	22	21	18	55	47	15	13
Wistar	7	15	19	40	13	28	8	17

[a] Relative percentage of total radioactivity recovered from liver phospholipids and neutral lipids.
[b] Number of rats is given in parentheses.
[c] Mean ± SE.
[d] $p \leqq .05$.
[e] $p \leqq .01$.

yet the BHE have been shown to have higher levels of hepatic G6PD under a variety of dietary conditions (4, 5), including the feeding of the same diet used in this study (5).

Results from a time course study suggested that labeled fat in adipose tissue and liver up to 6 hr after administered labeled acetate to fed animals represented the synthesis in these tissues (17). Since the present results were obtained from fed animals 4 hr after the administration of labeled compounds, isotope incorporation into fat in the tissues has been assumed to represent synthesis in these tissues. The markedly higher amount of radioactivity from G-U-^{14}C acetate-1-^{14}C was found in liver fat of BHE than that of Wistar rats (Table II) and indicates the BHE rats had a higher rate of hepatic synthesis of fat. This may explain the higher level of liver fat in the present study (Table I), as well as the previous finding (5) for BHE rats over Wistar rats. Because of wide variations between individuals, the differences in amount of radioactivity in lipids of other tissues (indicated as high SE), were not significant except for a greater incorporation of label from acetate-1-^{14}C into lipids of perirenal fat of BHE rats. Thus, the rate of fat synthesis in tissues, especially in liver, shows strain differences at this age.

Most of the glucose label incorporated into the neutral lipids of liver was found in the glycerol moiety. Apparently, an appreciable portion of glucose in the liver, on being converted to dihydroxyacetone phosphate during glycolysis, was reduced to α-glycerophosphate, the chief glycerol precursor of triglyceride in the liver. A greater portion of the glucose label appeared in the glycerol portion of neutral lipids in BHE than Wistar animals. A possible explanation is a higher activity of α-glycerophosphate dehydrogenase in

the livers of BHE than Wistar rats.[3]

In the present studies, BHE rats had a higher rate of synthesis of glyceride–glycerol in liver but also of fatty acids and cholesterol (Table IV). Livers of BHE animals synthesized phospholipid from acetate-1-^{14}C faster than did the Wistar animals. However, the strain difference in lipogenesis was most prominent in the neutral lipid fraction, which was larger and incorporated more acetate label in BHE than Wistar animals. Since it was known that hepatic glucose-6-phosphate dehydrogenase (4, 5) and malic enzyme (18) were markedly higher in BHE than in Wistar, the higher rate of fatty acid synthesis from acetate could be the result of the activity of the enzyme systems which produced NADPH, a factor required for fatty acid synthesis.

The forms of lipogenesis observed here included synthesis of fatty acids, glyceride–glycerol, phospholipids, and cholesterol. The results suggest that the major difference in fat metabolism between these two strains occurred in liver, and BHE had a higher rate of lipogenesis in this organ.

Summary. The difference in utilization of glucose and acetate for fat synthesis between rats of the BHE and Wistar strains was studied after the administering of acetate-1-^{14}C or ^{14}C-glucose labeled either uniformly or at the C-1 or C-6 position. Wistar rats were found to show a higher 1-$^{14}CO_2$/6-$^{14}CO_2$ ratio than BHE animals; but the difference was too small to demonstrate a real difference in whole-body utilization of the HMP shunt. The BHE animals incorporated a larger fraction of glucose and acetate label into the liver lipids than did Wistar animals; but incorporation of these labels into total carcass lipid was similar in the two strains at this age. Results indicated that hereditary differences in lipogenesis between these strains occur chiefly in the liver, where BHE animals synthesize fatty acids, glyceride–glycerol, phospholipids, and cholesterol at a higher rate than Wistar animals.

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[3] Unpublished data.

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