Effect of Diabetes and Diet on the Distribution of Tracer Doses of Chromium in Rats[1] (36248)

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There is increasing evidence that chromium as a trace element is required for normal carbohydrate metabolism in animals and man (1). A chromium containing complex called glucose tolerance factor (GTF) was isolated by Schwarz and Mertz (2) in 1959; and when the GTF complex was administered to chromium deficient rats, previously impaired glucose disposal rates were restored to normal.

Earlier workers have studied the distribution of ^{51}Cr in animals (3–6). We have extended these studies and examined the intracellular distribution of ^{51}Cr in various tissues of normal rats. The effect of diabetes on the organ distribution of ^{51}Cr also was observed. In addition, the results of feeding a high carbohydrate and a high fat diet on the distribution of ^{51}Cr was investigated. The results of these experiments are reported below.

Materials and Methods. All animals were obtained from Holtzman and Company and were maintained on a commercial chow diet, unless otherwise indicated. The high carbohydrate diet contained 21% casein, 74% glucose, and 5% salts; the high fat diet contained 21% casein, 37% corn oil, 37% cellulose, and 5% salts. Both diets had adequate vitamins added.[2]

Diabetes was produced by administration of streptozotocin (10 mg/rat in citrate buffer, pH 4.5) as described by Dulin.[3] Sixteen days after administration of the streptozotocin, 5 μCi of ^{51}Cr were administered intravenously; and the animals were sacrificed on day 21.

Chromic chloride, $^{51}CrCl_3$, was obtained from International Chemicals and Nuclear Corporation, Irvine, CA. All radioactive samples were counted in an automatic well scintillation spectrometer (Packard 500 B). Samples and an aliquot of the injected dose were counted at the same time, such that decay corrections were unnecessary.

Tissues were fractionated in the cold by the method of Hogeboom (7). For the distribution study given in Table I, animals were given a tracer dose of 0.022 μ of ^{51}Cr (5 μCi) intravenously and sacrificed after 96 hr. Tissues were homogenized in 0.25 *M* sucrose; and after fractionation, the amount of radioactivity present was determined.

Results. The distribution of ^{51}Cr in normal rat tissue fractions is shown in Table I.

Since chromium appears to be involved in both carbohydrate and lipid metabolism, the organ distribution of chromium in normal and diabetic rats was compared. It was of interest to ascertain if the diabetic animals would have an altered distribution of chromium. These results are shown in Table II.

It is well recognized that, in the diabetic state, lipogenesis is impaired (8). Similarly, it is known that fasting and refeeding a high carbohydrate, low-fat diet leads to an in-

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[2] The rat-chow diet was obtained from Country Best Agway, Inc., Syracuse, NY, and contained 24% protein, 5% fat, and 5% fiber. The casein, glucose, and Phillips and Hart salt mix were obtained from Nutritional Biochemicals, Cleveland OH.

[3] Streptozotocin was kindly furnished by Dr. W. E. Dulin, The Upjohn Company, Kalamazoo, MI.

TABLE I. Distribution of Chromium in Normal Rat Tissue Fractions, 96 hr After an Intravenous Injection of 5 μCi of $^{51}CrCl_3$.

Tissue	Data (% of reconstituted total)[a]			
	Nuclear	Mitochondrial	Microsomal	Supernatant
Liver	25	26	36	13
Heart	31	12	14	43
Spleen	24	11	38	27
Kidney	24	15	13	47
Pancreas	11	18	22	48
Adipose	12	16	25	46

[a] Mean value of 4 animals.

creased rate of lipogenesis in the liver, while feeding a high-fat diet depresses lipogenesis (8). The effects of diabetes and diet on the distribution of ^{51}Cr in liver fractions are shown in Tables III and IV.

Discussion. In experiments not shown, rats were given 50 and 500 μCi of ^{51}Cr with no apparent change in the pattern of distribution due to dosage. The distribution of ^{51}Cr in the subcellular fractions of various normal tissues is given in Table I. It suggests that the heart, pancreas, and adipose tissue have the bulk of the ^{51}Cr in the supernatant fraction in contrast to the liver. Kidney also has a high level of chromium in the supernatant

TABLE II. Distribution of Chromium 51 in Normal and Diabetic Rats After an Intravenous Injection of 5 μCi of $^{51}CrCl_3$ Containing 4.2×10^{-2} μg of Chromium(III).

Tissue	% of inj. dose/g of tissue ± SE		
	Normal,[a] av body wt, 212.5 (6)	Diabetic,[b] av body wt, 141.0 (5)	p
Kidney	1.03 ± 0.04	0.74 ± 0.08	<.01
Spleen	0.50 ± 0.05	0.78 ± 0.17	NS
Liver	0.45 ± 0.05	0.67 ± 0.11	NS
Heart	0.16 ± 0.005	0.15 ± 0.022	NS
Pancreas	0.20 ± 0.008	0.14 ± 0.019	NS
Adipose	0.12 ± 0.009	—[c]	—
Muscle	0.09 ± 0.003	0.07 ± 0.006	NS
Serum (1 ml)	0.07 ± 0.003	0.24 ± 0.038	<.005

[a] 96 hr after the injection.
[b] 120 hr after the injection.
[c] Adipose tissue was not present in sufficient quantity due to the diabetic state.

TABLE III. Distribution of Chromium 51 in Liver Fractions of Normal and Diabetic Rats After an Intravenous Injection of 5 μCi of $^{51}CrCl_3$.

Fraction	Data (% of reconstituted total ± SE)		p value
	Normal (12)[a]	Diabetic (11)[b]	
Nuclear	25.2 ± 1.20	36.1 ± 1.14	<.001
Mitochondrial	26.1 ± 1.49	19.8 ± 0.75	<.005
Microsomal	35.7 ± 2.50	25.5 ± 0.77	<.001
Supernatant	12.7 ± 0.34	18.3 ± 0.63	<.001

[a] 96 hr after the injection.
[b] 120 hr after the injection.

fraction, but this may reflect the urinary excretion of chromium. It is of interest that muscle and adipose tissue are the major sites of action of insulin; and Mertz (1) has suggested that chromium in the form of GTF is required for a normal metabolic response to insulin. GTF (1) has been shown to be a low molecular weight, dialyzable substance, and thus might be expected to occur in the supernatant fraction of tissues.

A comparison of the distribution of ^{51}Cr in normal and diabetic rat tissues did not show any major alterations in terms of the percent of the dose retained. Muscle, which constitutes the major portion of body mass, retained Cr at about the same level in the normal and diabetic animals. The serum of the diabetic animals appear to retain a somewhat higher percentage of the dose than was retained in the normal animals. The diabetic animals lost considerable body weight due to the diabetic state. There was no visible adipose tissue remaining when the animals were sacrificed. Since an equal tracer dose was given to both groups, the diabetics might have been expected to have higher chromium levels due to the lower body weights, but the percentage of the dose retained is similar in both groups. It was shown previously that human diabetics excrete more of a tracer dose of Cr than do normal subjects (9).

Because of the known alterations in hepatic metabolism in diabetic animals, it was decided to fractionate the livers and examine the distribution of ^{51}Cr. Table III shows that the diabetic rats retain more ^{51}Cr in the nuclear and supernatant fractions and lesser

TABLE IV. Distribution of Chromium 51 in Liver Cell Fractions of Rats[a] on High Carbohydrate and High Fat Diets.

Cell fraction	(cpm/whole liver ± SE) High carbohydrate (6)	High fat (6)	*p* value
Nuclear	7186 ± 161.6 (30.1)[b]	11,165 ± 831.3 (44.8)	<.001
Mitochondrial	4057 ± 162.9 (17.0)	4675 ± 237.2 (18.8)	NS
Microsomal	7201 ± 351.1 (30.2)	4663 ± 146.9 (18.7)	<.001
Supernatant	5428 ± 239.6 (22.7)	4415 ± 273.5 (17.7)	<.02
Whole liver (av)	23,872	24,918	
Av wt of livers (g)	10.78	7.62	

[a] Given an intravenous dose of 5 μCi of $^{51}CrCl_3$, fasted 72 hr, and refed 96 hr prior to sacrifice.
[b] % of reconstituted total is given in parentheses.

amounts in the mitochondrial and microsomal fractions than did normal animals. Table IV shows that fasting and refeeding either a high-carbohydrate or a high-fat diet gives a pattern of Cr distribution similar to that shown in Table III. That is, refeeding a high-fat diet results in a hepatic ^{51}Cr distribution much like that observed in the diabetic animals, while refeeding a high carbohydrate diet gives a distribution similar to the normal animals, as shown in Table III. There is an increased amount of chromium in the nuclear fraction of the cell, and a decreased amount in the microsomal fraction after ingestion of a high fat diet. The data are given as counts per minute per whole liver and percentage of reconstituted total. The total liver weight increased in the high-carbohydrate-diet animals in part due to increased glycogen deposition as shown by others. The total counts per liver remain essentially unchanged, only the distribution is altered by the two different diets.

Wacker and Vallee (10) found that chromium was associated with the RNA fraction of cells and suggested it might play a role in nucleic acid metabolism. Curran (11) demonstrated that Cr may be involved in fatty acid and cholesterol biosynthesis. In the livers where lipogenesis is depressed (*i.e.*, diabetic and high-fat diet), the amount of Cr in the microsomes is decreased compared to livers where lipogenesis is normal or elevated.

These data suggest that when lipogenesis is increased, there is a movement of chromium from the nuclear fraction to the microsomal fraction of liver cells. The biological role(s) of chromium are as yet unknown, but these data suggest that the distribution of chromium within the liver cell is influenced by the hormonal and nutritional state of the animal.

Summary. The distribution of ^{51}Cr in tissue fractions of various organs in normal rats has been ascertained. Induced diabetes in rats caused no major alterations in the organ distribution of ^{51}Cr. However, the distribution of ^{51}Cr in liver fractions was altered by induced diabetes or by the feeding of high-fat diets. When the rate of hepatic lipogenesis was normal or elevated, chromium appeared to move from the nuclear to the microsomal fraction of liver.

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