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The Effect of Oxytetracycline on the Response to Insulin of Diaphragm Muscle and on Lipid Synthesis *in Vivo* and *in Vitro* in the ob/ob Mouse

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Summary. The insulin sensitivity of the peripheral tissues in ob/ob mice treated with oxytetracycline was assessed both *in vivo* and *in vitro* by measuring the effect of insulin on the incorporation of glucose into glycogen in diaphragm muscle and on the incorporation of glucose and ^3H_2O into lipids in epididymal adipose tissue. The results indicated that OTC treatment improved the insulin sensitivity of muscle but not that of adipose tissue. The results presented also indicated that oxytetracycline treatment led to a decrease in the basal incorporation of lipids into the liver *in vivo* and the adipose tissue *in vivo* and *in vitro*. The results are discussed in view of the relative importance of liver and adipose tissue in the lipid metabolism of ob/ob and lean mice and in view of results obtained by other workers on the effects of B-cell cytotoxic agents.

Key words: Obese hyperglycemic mice, insulin resistance, oxytetracycline, skeletal muscle, epididymal adipose tissue.

It is well established that the obese-hyperglycemic syndrome (ob/ob) in mice is characterized by a high degree of insulin resistance [1, 2, 3]. The *in vivo* studies carried out by Stauffacher and Renold [4] indicated that the muscle is more resistant to insulin than the adipose tissue, which may explain the simultaneous occurrence of hyperglycemia, hyperinsulinaemia and obesity. We have found in previous studies [5] that the antibiotic oxytetracycline (OTC) afforded some improvement in the insulin response of the ob/ob mouse, indicated by normalized plasma glucose and insulin levels, normalized glucose and insulin tolerance tests and partial restoration of the ability of liver membranes to bind insulin.

The normalization of glucose tolerance by oxytetracycline would be consistent with improvement of the insulin responsiveness of the peripheral tissues, in particular the muscle [4, 5]. Experiments were therefore designed to test the response to insulin of the diaphragm and epididymal adipose tissue of oxytetracycline – treated mice and their respective controls and also to assess the differences observed in the relative degree of incorporation of labelled precursors in the lipids of liver and adipose tissue of obese and lean mice.

Materials and Methods

Animals

Male $C_{57}Bl/6J$ ob/ob mice and their lean controls were obtained from the Jackson Laboratories, Bar Harbor, Maine, U.S.A. The mice were 9–11 weeks of age at the beginning of the experiments. They were maintained on Purina chow and water *ad libitum* except as otherwise stated.

Treatment

The oxytetracycline treatments and the food restriction schedule were described in detail previously [5].

Incorporation of Labelled Compounds in Vivo and in Vitro

a. Measurements of the Incorporation of Tritiated Water into Lipids in Vivo. The following method for studying the accumulation of newly synthesized lipids in the tissues is based on the work of Fain and Scow [6]. The theoretical aspects of the method (isotope effect, exchange reactions and the metabolic pathways which lead to incorporation) have been discussed in detail by Jungas [7]. Each animal was injected in a tail

vein with 0.2 ml of a solution containing about 1 mCi of tritiated water per ml. The solution was made isotonic by the addition of NaCl and its pH was adjusted to 7.4 with 1 N NaOH. In some of the experiments, half of the mice each received 6 mU of crystalline porcine insulin (a gift of Eli Lilly & Co.) in the injection fluid. The mice were sacrificed by decapitation at the stated times. The blood was collected and the liver and epididymal fat pads were quickly removed, immediately frozen on solid CO_2 and placed in weighed vials. The tissues were kept at – 20°C until the lipid extractions were done. A barium-zinc filtrate of the blood was prepared (1 : 20 final dilution) and a 1 ml portion was frozen until the samples could be counted. Unless otherwise specified in the text, the animals were killed 120 minutes after the injection.

b. Incorporation of [^{14}C-U] – Glucose into the Glycogen of Diaphragm and into the Lipids of Liver and Adipose Tissue and in Vitro. The animals were injected intravenously with 2 μ-Ci of [^{14}C-U] – glucose in 0.2 ml of 0.9% NaCl adjusted to pH 7.4 with 1 N NaOH. Half of the animals were also given 6 mU each of crystalline porcine insulin. After 120 min, the animals were sacrificed by decapitation and the diaphragm, liver and epididymal adipose tissue were removed. The removal of the tissues was done within 60 seconds of sacrifice. The tissues were quickly frozen on solid CO_2 and kept as described above. This procedure is similar to that employed by Stauffacher and Renold [4].

c. Incorporation of [^{14}C-U] – Glucose into Glycogen, of Diaphragm in Vitro. The incorporation of glucose into glycogen of diaphragm was measured as follows: The intact diaphragms were removed from the animals and placed into ice cold Krebs-Ringer Phosphate buffer (118 mM NaCl; 1.2 mM $MgSO_4$; 5 mM KCl and 100 mM sodium phosphate buffer at pH 7.4). The muscle was dissected from the rib cage and divided into approximately equal parts weighing 45–60 mg. The tissues were weighed to the nearest mg on a torsion balance and they were incubated in a medium containing 3 ml of the Krebs-Ringer phosphate medium containing 1 mM $CaCl_2$ and 2% bovine serum albumin. The medium also contained 10 mM [^{14}C-U] – glucose at a specific activity of 0.01 mCi per mMole. Half of the incubation vessels contained also 2.5×10^{-6} M insulin (6.25×10^{-5} units/ml of incubation medium), so that one half of each diaphragm was incubated in the presence of insulin, while the other half was incubated in the absence of insulin. After 60 min of incubation, the tissues were removed from the incubation medium, placed into tubes containing 0.5 ml of 30% KOH and processed as described below.

d. The Production of $^{14}CO_2$ from [^{14}C]-Glucose by Epididymal Fat Pads. The fat pads were removed from the mice and placed in Krebs-Ringer bicarbonate buffer kept at room temperature. In the case of the lean animals, the whole fat pad was used; in the case of the obese animals, pieces (150–200 mg) were cut from the thinnest portion of the fat pads. The tissues were incubated in the Krebs-Ringer bicarbonate medium which contained in addition 2% bovine serum albumin and 5.5 mM [^{14}C-U]-glucose at a specific activity of 0.01 mCi/mMole. The insulin concentration, where applicable, was 2.5×10^{-7} M (6.3×10^{-6} units per ml of incubation medium). The media were gassed with 95%/5% CO_2 and tightly capped. The $^{14}CO_2$ produced was trapped in a removable centre well containing 0.2 ml of Hyamine hydroxide. After a 60 min incubation period, 0.5 ml of 5 N H_2SO_4 were injected through the rubber cap of each flask and the CO_2 was allowed to diffuse into the hyamine for a further 60 min. The centre wells were removed, placed in a toluene-base scintillation fluid (3 gm 2,5-diphenyloxazole and 0.1 gm p-bis (2-(5-phenyloxazolyl))-benzene per liter of toluene) and counted in a Nuclear Chicago Isocap Liquid Scintillation spectrometer (Searle Instruments, Toronto, Canada).

e. The Incorporation of Glucose into Total Lipids of Adipose Tissue. The tissue preparation and the incubation conditions were as described above. At the end of 60 min incubation at 37°, the tissues were removed from the incubation flasks and homogenized in 1.5 ml of 2 : 1 chloroform-methanol. The total lipids were then extracted and counted as described below.

Lipid Extraction and Counting

Lipid extraction of the tissues was done by a modification of the method of Folch *et al* [cf 8]. Essentially, the tissues were homogenized in chloroform-methanol 2 : 1 (v/v). The homogenates were filtered to remove the solids. The extract was then partitioned into two phases by the addition of 0.2 vol of 0.29% NaCl. The tubes were shaken thoroughly and the phases were separated by centrifugation. The upper phase was removed and the chloroform phase was washed three times with 0.5 vol of methanol: H_2O: chloroform (48 : 47 : 3). The lipid extracts were placed in counting vials and the solvent was evaporated at 60° under a stream of N_2. In the cases where the label was from ^3H_2O, care was taken to remove any residual water by several cycles of evaporation with benzene. After the addition of scintillation fluid, the samples were counted in a liquid scintillation spectrometer. When ^3H_2O was used, the specific radioactivity of the body water was determined for each animal by counting 1 ml of a barium-zinc filtrate of the blood (1 : 20 dilution). In this case, both the lipid extract and the blood filtrate were counted using Bray's scintillation fluid [9]

Table 1. The ratio of fat free dry weight to wet weight in the liver and epididymal fat pad of lean and obese mice

Group	Liver			Epididymal fat pad	
	g	g ff dry wt/g wet wt.	mg lipid/g	wet wt.	g dry wt/g wet wt.
Lean					
control	1.4±0.06	0.21 ±0.04	75±14	0.49±0.05	0.137+0.01
OTC-treated	1.2±0.05	0.225±0.03	41± 9	0.32±0.02	0.192±0.01
Obese					
control	3.4±0.16	0.145±0.07	184± 9	3.67±0.06	0.044±0.01
Food-restricted control					
a) 4.5 g/day	2.5±0.15	0.167±0.04	135±17		
b) 2.5 g/day	2.1±0.15	0.186±0.02	110±15	3.03±0.08	0.067±0.03
OTC-treated	1.6±0.08	0.192±0.04	76± 5	3.35±0.08	0.071±0.01

The tissues were extracted and weighed as described in the Methods section. The results given are means ± SEM for at least 6 observations

and the results were expressed as μmoles H_2O incorporate per g fat free dry weight. When [^{14}C]-glucose was the isotope, the lipid extracts were counted in the toluene scintillation fluid described above and the results are expressed as dpm per g fat free dry weight. In some cases, the saponifiable fatty acids were separated [10]. After extraction, the samples were evaporated to dryness and counted after the addition of 10 ml Bray's scintillation mixture.

Isolation of Glycogen and Counting Procedure

Glycogen was isolated by placing the tissues in 0.5 ml of 30% KOH in a boiling water bath for 10 min. After digestion of the tissues, the tubes were cooled and 0.2 ml of 2% Na_2SO_4 and 1.6 ml of absolute ethanol were added and the glycogen was allowed to precipitate at −20° for one hour. The samples were then centrifuged at 1500 × g for five minutes and the precipitate was washed with 5 ml of 66% ethanol. The precipitates were dissolved in 0.5 ml of 4N HCl and the glycogen was hydrolyzed in a boiling water bath for one hour. The samples were then neutralized with 2N NaOH and the volume was adjusted to 2.5 ml with distilled water. The samples were transferred to scintillation vials to which 7.5 ml Aquasol (New England Nuclear Corp. of Canada, Montreal) were added and the samples were counted. In cases where the glycogen content of the diaphragm was measured, a portion of the hydrolyzed glycogen was removed after neutralization and the glucose content of the solution was determined by a enzymatic method (Glucostat, Worthington Biochemical Corp.).

Calculation of Results

Because of the large amount of lipid in the liver and adipose tissue from the obese mice and because of the changes in the tissue lipid content in the oxytetracycline treated mice, the results presented below were calculated on the basis of the fat free dry weight of the tissues. For comparison, the average wet weights of the tissues and the corresponding fat free dry weights are given in Table 1. Values for the food-restricted controls were obtained from mice given 2.5 g of food per day, which is slightly less than the intake of the *lean* mice [5] and mice given 4.5 g of food per day which is equivalent to the average intake of the OTC − treated obese animals. Unless otherwise indicated the results presented below for food restricted controls were for mice given 2.5 g of food/day.

Abbreviations: OTC: oxytetracycline
IRI: immunoreactive insulin

Results

The effect of OTC treatment was first assessed by testing the effect of insulin on the incorporation of radioactive glucose into glycogen by the diaphragm muscle *in vivo*. The results of these experiments are given in Table 2. In both groups of lean animals, there was a 3 fold stimulation by insulin of the incorporation of glucose into glycogen. Neither the diaphragms from the obese control or food-restricted animals responded to insulin but the diaphragms from the obese OTC-treated animals responded as well as those from the lean animals.

Isolated hemidiaphragms were then incubated in the presence and in the absence of insulin to test their response in an isolated system. The results of these experiments are given in Table 3. The experiments *in vitro* confirmed the findings *in vivo* that OTC-treatment was capable of rendering the diaphragm of the obese mice sensitive to insulin.

It should also be noted that the diaphragms of the OTC-treated lean mice were significantly more responsive than those of the lean controls.

Incorporation of [^{14}C]-Glucose and $^{3}H_2O$ into Lipids in Vivo

Liver. The *in vivo* incorporation of labelled glucose and tritiated water into the lipids of the liver were measured in the control and OTC-treated lean animals as well as in the control, OTC-treated and food-restricted obese animals (Table 4). As would be expected with the experimental protocol used [11], there was no stimulation by insulin of the incorporation of label into the lipids of the liver. The results presented indicate, however, that a) the incorporation of radioactive glucose was several fold greater in the liver of the obese control mice than in the liver of the lean controls; b) the incorporation of radioactive water was twice as great in the livers of the obese control animals than in the livers of the lean mice; and c) OTC-treatment and food restriction decreased the incorporation of both labels in the liver of the obese treated animals whereas OTC-treatment did not appreciably affect the incorporation in the lean animals.

Epididymal Fat Pads. The incorporation of radioactivity in the epididymal fat pad *in vivo* was measured in the absence and in the presence of insulin (Table 5). In both the control and OTC-treated lean animals, there was a significant ($P < 0.01$) effect of insulin on the incorporation of both radioactive glucose and tritiated water. It should also be noted that

Table 2. The incorporation of [^{14}C-U]-glucose into glycogen in diaphragm *in vivo*

Group	− Insulin	+ Insulin	*P*
	dpm × 10^{-2}/mg glycogen		
Lean			
control	41±16	120±20	<0.05
OTC-treated	46± 8	145± 8	<0.001
Obese			
control	53± 9	50± 6	ns
Food-restricted control	30± 5	38± 8	ns
OTC-treated	51±10	148±20	<0.005

The experimental procedure was as reported in the Materials & Methods section. Each value represents the average ± SEM for at least 7 samples. The *P* values are based on t-statistics for unpaired samples

Table 3. The incorporation of [^{14}C-U]-glucose into glycogen of diaphragm *in vitro*

	− Insulin	+ Insulin	P^a	△ due to insulin	P^b
	nmoles/100 mg wet weight/hour				
Lean					
Control	23±2 (4)	30±2 (4)	<0.001	7.1±4.2	
OTC-treated	20±3 (3)	43±1 (3)	<0.01	22.4±5.3	<0.001
Obese					
Control	20±4 (4)	21±4 (4)	ns	1.4±0.3	<0.001
Food-restricted control	24±2 (4)	29±3 (4)	ns	5.9±2.9	<0.005
OTC-treated	19±3 (4)	31±5 (4)	<0.005	11.8±2.9	<0.005

The experimental procedure was as described in the Method section. Each value represents the mean ± SEM for the number of observations given in brackets. P^a was based on t-statistics for paired samples and P^b was based on chi-square analysis

Table 4. The incorporation of [^{14}C-U]-glucose and $^{3}H_2O$ into liver total lipids *in vivo*

Group	Insulin	[^{14}C-U]-glucose	$^{3}H_2O$
Lean		dpm × 10^{-3}/g fat free dry wt	μmoles/g fat free dry wt
Control	−	73± 23	282± 47
	+	88± 19	422± 77
OTC-treated	−	83± 20	264± 39
	+	96± 23	315± 49
Obese			
control	−	869±148	867±114
	+	1055±231	1031±190
Food-restricted	−	325± 78	294± 23
control	+	305± 39	322± 20
OTC-treated	−	156± 23	292± 10
	+	151± 21	342± 26

The experimental procedure was as described in the Methods section. Each value represents the mean + SEM for 7–11 observations. The values were corrected for the differences in the specific radioactivity of the blood glucose. The average blood glucose levels for each group were lean control: 125 ± 6; lean OTC-treated, 110 ± 8; obese control, 275 ± 25; obese OTC-treated, 132 ± 16; obese food restricted, 280±30

Table 5. The incorporation of [^{14}C-U]-glucose and ^3H_2O into total lipids of adipose tissue *in vivo*

Group	Insulin	[^{14}C-U]-glucose	^3H_2O
		dpm × 10^{-3}/g fat free dry wt.	μmoles/g fat free dry wt.
Lean			
control	–	304± 98	56± 8
	+	591± 72[a]	160±22[a]
OTC-treated	–	167± 44	51± 5
	+	501± 53[a]	98±33[a]
Obese			
control	–	376± 42	207± 5
	+	408± 52	202± 5
Food-restricted control	–	502±112	136±15
	+	706±184	120±29
OTC-treated	–	198± 54	71± 8
	+	299± 86	78±12

The experimental procedures and calculations were as described in the note to Table 4.
[a] denotes a statistically significant effect of insulin ($P < 0.01$, based on t-statistics for unpaired samples)

Table 6. The incorporation of glucose into total lipids by epididymal fat pads *in vitro*

Group	μmoles glucose incorporated/g ffdw		
	– Insulin	+ Insulin	*P*
Lean			
control	109.7±15	179.1±22	<0.001
OTC-treated	89.5± 9.8	138.1± 9.7	<<0.001
Obese			
control	70.5± 8.1	79.5± 5.7	ns
Food-restricted control	34.3± 3.8	61.4± 6.5	<0.005
OTC-treated	35.7± 2.8	42.8± 3.1	<0.01

The experimental procedures were as described in the Methods section. Each value represents mean ± SEM for 7 determinations. *P* values are based on t-statistics for paired samples. In addition all values obtained for the obese animal are significantly lower than the values obtained for the lean control animals ($P < 0.01$) and OTC-treatment and food restriction of the obese animals significantly reduced incorporation relative to the obese controls ($P < 0.01$)

Table 7. The production of $^{14}CO_2$ from glucose by epididymal fat pads

Group	μmoles $^{14}CO_2$ produced/g ffdw		
	– Insulin	+ Insulin	*P*
Lean			
Control	479±57	660±90	<0.001
OTC-treated	250±27	458±63	<0.01
Obese			
control	218±34	368±46	<0.01
Food-restricted control	128±27	231±47	<0.005
OTC-treated	111± 6.9	128± 8.6	<0.025

The experimental procedure was as described in the Methods section. Each value represents the mean ± SEM for 7 determinations. *P* values are based on t-statistics for paired samples

OTC-treatment diminished the incorporation of label in the lean animals. None of the obese groups showed a response to insulin, however, OTC-treatment greatly decreased the incorporation of label in the epididymal adipose tissue.

The in Vitro Metabolism of [^{14}C-U]-Glucose by Epididymal Adipose Tissue

The incorporation of [^{14}C]-glucose into lipids was studied with isolated adipose tissue. The results obtained *in vitro* (Table 6) are in harmony with the observations made *in vivo* in the case of the lean animals. There was a significant stimulation ($P < 0.001$) of the incorporation of glucose by the addition of insulin to the incubation medium. In the *in vitro* system, there was significantly less incorporation of glucose into the tissues of the obese control. This has generally been observed when pieces of adipose tissue were used rather than adipocytes [12, 13]. The incorporation was even lower in the tissues from the obese-OTC-treated and food-restricted animals. There was no stimulation of the incorporation by insulin in the tissues of the obese controls and there was a significant but very small stimulation in the tissues of the OTC-treated obese. The response to insulin of the foodrestricted obese animal was of the same order as that of the lean animals, although the tissue incorporated less glucose per unit wet weight or, as expressed, per unit of fat free dry weight.

The production of $^{14}CO_2$ from glucose was also measured in the tissues obtained from the same groups of animals (Table 2). Insulin stimulated $^{14}CO_2$ production in all groups, although at different levels. The basal $^{14}CO_2$ production was less in the obese than in the lean animals; further, OTC-treatment and food-restriction decreased the basal levels in both the

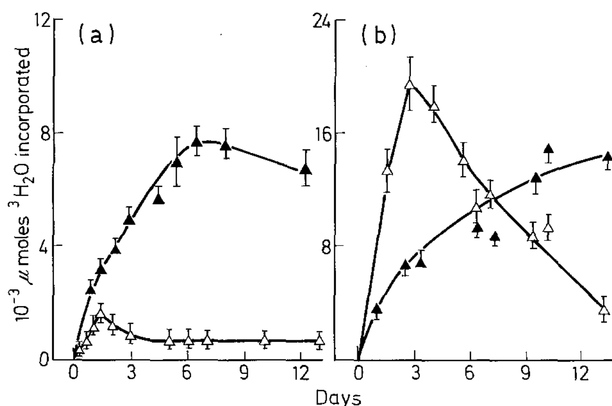

Fig. 1. The time of incorporation of label from ^3H_2O in the liver and epididymal fat pads of lean (*a*) and obese (*b*) mice. The mice were injected with tritiated water at Day O. At various interval some of the animals were killed and their livers and epididymal fat pads were removed and analyzed as described in the Methods section. Each point on the graph represents mean ± SEM for 3–6 observations △ – △ liver, ▲ – ▲ fat pad

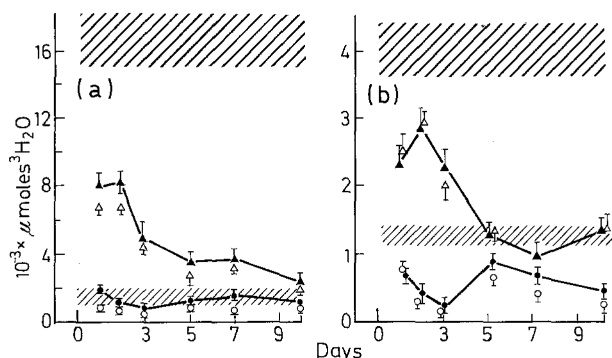

Fig. 2. The effect of the time course of oxytetracycline treatment on the incorporation of ^3H_2O into the lipids of liver (*a*) and epididymal fat pads (*b*) of lean and obese mice. The mice were subjected to their respective treatments for the times indicated. 24 hours before sacrifice, they were injected with ^3H_2O and the tissues were processed as described in the Methods. Some of the control animals were included at each time. Each point represents the mean ± SEM for 3 observations except in the case of the controls where there were 18 observations. o: lean OTC-treated: △: obese OTC-treated. The shaded areas in the upper portion of the graphs represent values obtained with the tissues of the control obese mice and those in the lower portion, the values for the control lean mice. Closed symbols represent the radioactivity incorporated into the total lipids, whereas the open symbols represent the radioactivity incorporated into the glyceride fatty acids

lean and the obese animals. The proportion of $^{14}CO_2$ produced (Table 7) to glucose carbon incorporated (Table 6) is consistent with the view that, in the obese mice, most of the glucose carbon utilized was incorporated into fatty acids.

Since the *in vivo* results obtained above indicated differences in the relative labelling of the liver and adipose tissue of the lean and obese mice, experiments were carried out to help assess the relative contribution of the liver to the deposition of triglycerides in adipose tissue. Control lean and obese mice were injected with ^3H_2O as described in the Materials and Methods section. Groups of untreated lean & obese mice were killed at various intervals ranging from 6 hours to 13 days after the beginning of the experiment. The livers and epididymal fat pads were removed and the lipids were extracted and counted. The results of these experiments are given in Fig. 1. Since the procursor utilized was ^3H_2O radioactivity was incorporated into the fatty acids via the reduced pyridine nucleotides [6] irrespective of the source of the acetyl units. Thus, there is no isotope dilution caused by different pool sizes of precursors.

Apart from the obvious difference in the magnitude of incorporation of label in the liver of the obese mice as compared with the lean mice, which was 6 fold at day 2, there was a striking difference in relative levels of incorporation between liver and adipose tissue in both groups. In the lean mice, the adipose tissue became labelled more rapidly than the liver and the total amount of radiocactivity incorporated at peak time was also greater in adipose tissue than in the liver. This relationship held whether the calculations were made on the basis of fat free dry weight, total weight of tissue (as calculated from Table 1) or total body adipose tissue estimated from [14]. In the obese mouse on the other hand, the maximum amount of radioactivity incorporated in the liver at the peak of incorporation was always equal to or greater than the amount incorporated into adipose tissue at its peak of incorporation. These results indicate that in the obese mice the liver might play a relatively greater role in total lipogenesis than it would in the lean mice and that it might furnish a greater proportion of the adipose tissue triglycerides in the obese than in the lean mice. Such long term experiments could not be

Table 8. The effect of discontinuing OTC treatment on the weight of the liver and the incorporation of radioactivity in liver lipids

Days after last injection	Weight of liver		^3H_2O incorporated in triglycerides	
	Control	OTC	Control	OTC
1	3.9±0.1	1.6±0.08	1423±130	740±110
6	3.7±0.1	2.7±0.13	1570±250	1312±150
P	ns	<0.005	ns	<0.05

carried out on the treated mice, since their metabolic state was changing [5]. Another type of experiment was therefore designed to provide further information on this point. Groups of lean and obese animals were submitted to the usual treatment. Some of each group were killed at day 1, 2, 3, 5, 7 and 10, and the adipose tissue and the liver were removed for analysis of the triglycerides. Twenty-four hours prior to sacrifice tritiated water was injected. The results of these experiments are given in Fig. 2. The following observations were made: Although OTC-treatment did not noticeably influence the incorporation of radioactive water into the liver triglycerides of the lean mice, it markedly reduced its incorporation into adipose tissue. On the other hand OTC-treatment markedly reduced the incorporation of radioactivity in the liver triglycerides of the obese mice and this reduction was very closely paralleled in the adipose tissue. The results shown in Fig. 2 also indicated that under these conditions the bulk of the incorporation of ^3H_2O was in the fatty acid fraction.

OTC-treatment did not cause permanent changes in the lipid metabolism of the obese mouse. Within 6 days after discontinuation of the treatment, the liver was already returning to pretreatment weight and the amount of ^3H_2O incorporated into the lipid fraction of the liver was nearly the same as in non-treated animals (Table 8).

Discussion

In attempts to delineate the role played by B-cell hyperplasia and hyperinsulinemia in the development of the obese hyperglycemic syndrome in mice, the B-cell dytotoxic agents alloxan and streptozotocin have previously been utilized [15–18]. Single injections of these agents produce long term toxic effects on the pancreatic B-cell [19] thus decreasing the release and the availability of pancreatic IRI [14, 16] leading to lower concentrations of IRI in the blood. In long term experiments with both streptozotocin and alloxan there was a decrease in the blood sugar levels in the ob/ob mouse [17, 18], whereas in experiments of shorter duration the blood sugar was increased as a result of treatment [14, 16]. In short term experiments, streptozotocin was shown to decrease markedly the incorporation of [^{14}C]-glucose into fatty acids in all tissues of lean and ob/ob mice [19]. In long term experiments, however, streptozotocin and alloxan were shown to have little effect on the adiposity of the ob/ob mouse [16, 17]. Streptozotocin treatment had little effect on the basal glucose metabolism in adipose tissue and its sensitivity to insulin as measured in *in vitro* experiments [18]. Whereas alloxan treatment was shown to increase the insulin sensitivity of the diaphragm muscle of the ob/ob mouse [16] streptozotocin treatment did not have such effects [18].

The present study differs from those mentioned above in many respects. Oxytetracycline was chosen as a potential agent to alter the metabolism of insulin in the obese mice because of its previously reported insulin potentiating effects [20, 21]. OTC did not have long lasting effects: in order to alter the metabolism of the obese mouse, chronic treatment with the drug was necessary [5] and within a few days after discontinuing OTC treatment, there were indications that the effects of the drug were no longer present (Table 8).

It was shown previously [5] that OTC-treatment produced the following sequence of events following the onset of treatment; 1) a decrease in the levels of serum IRI levels to within the range seen in lean animals (5 days); 2) an increase in the binding of insulin by the liver membranes (5 days) and 3) a normalization of the blood glucose levels (7 days). In the present study, it was also shown that the incorporation of radioactive precursors into liver triglycerides and the time course observed for this parameter was very similar to that observed for the effect of OTC on serum IRI levels and insulin binding to the liver membranes, and occurred before the blood sugar returned to normal levels. This may be a further indication that hyperinsulinaemia, but not hyperglycaemia, is a causative factor in the excessive lipogenesis seen in the obese animals [14].

OTC treatment also restored the sensitivity to insulin of the diaphragm muscle of the ob/ob mouse both *in vivo* and *in vitro*. In this respect, OTC had effects similar to those reported for alloxan [16], and it is possible that the greater sensitivity of muscle to insulin would account at least partially for the normalization of glucose tolerance seen in both OTC-treated and alloxan-treated obese mice.

OTC treatment did not render the adipose tissue of obese mice more sensitive to insulin. The most notable effect of OTC-treatment on the adipose tissue of the obese mouse was a large decrease in the incorporation of radiocactive precursors, observed both *in vivo* and *in vitro*, relative to the values found in the control obese mouse. This decrease was even greater than that observed for the food-restricted controls, although the food intake of the latter was far less than that of the OTC-treated obese mice.

It appears that the time course of incorporation of ^3H_2O was different in the obese and the lean mice. The *relative* differences observed between liver and adipose tissue may indicate that the liver contributes a greater proportion of the adipose tissue fatty acids in the obese than in the lean mice. This is strengthened by the finding that OTC treatment decreased fatty acid incorporation into both the liver and the adipose tissue of the obese mice while, in the lean animals,

only the adipose tissue was affected. The use of ^3H_2O to label lipids *in vivo* does not allow definite conclusions as to the exact site of synthesis of the fatty acids which are eventually incorporated into triglycerides. However, the results described above are in harmony with the observation that secretion of newly synthesized triglycerides was abnormally high in the liver of the ob/ob mice and that streptozotocin treatment corrected this anomaly [22]. They are also in harmony with the results of *in vivo* experiments on the incorporation of [^{14}C]-glucose in these animals [14].

Because OTC treatment somewhat decreased the food intake of the obese animals, OTC treatments were always done in parallel with a food restricted obese group. In the majority of the experiments the food restricted obese animals were given slightly less food than the *lean* controls. It is therefore not surprising that the metabolism of triglycerides was also decreased in these animals. Some experiments (cf Table 1) were carried out with groups of animals receiving 4.5 g of food per day which was the average intake for the OTC-treated obese. From comparison with the latter group, it is evident that OTC treatment had effects greater than could be accounted for simply by diminished food intake. It should be noted also that, unlike OTC treatment, even the more severe food restriction (2.5 g/day) did not lead to improved sensitivity of the diaphragm muscle to insulin.

Oxytetracycline does not seem to act as a B-cell cytotoxic agent, sinde the lean mice did not become diabetic. However, a close analog of OTC, chlortetracycline, has been shown to be concentrated in pancreatic islets and to be capable of forming complexes of definite stoichiometry with insulin in the presence of Zn ions *in vitro* [23]. It is possible, therefore, that oxytetracycline exerted its effects on the metabolism of the obese mouse by complexing with insulin *in vivo*. Investigations on the histology of the islet cells and on the kinetics of release of insulin from the pancreas of OTC-treated mice are now in progress.

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