hibitor of the enzyme by PLP-GABA, the inhibitor (PLP-GABA)-enzyme dissociation constant (K_i) was calculated as 1.4 μM,

$$\frac{1}{v} = \frac{K_m}{V_m}\left(1 + \frac{[I]}{K_i}\right)\frac{1}{[S]} + \frac{1}{V_m} \quad (1)$$

where v: initial rate of the enzymic reaction, Vm: maximum rate of the enzymic reaction, Km: Michael's constant, [I]: concentration of PLP-GABA, K_i: PLP-GABA and enzyme dissociation constant, [S]: concentration of GABA.

The magnitude of the K_i indicates that PLP-GABA is a powerful GABA-T inhibitor. Such an inhibitor might be a useful tool in the in vivo study of brain GABA metabolism if it transpires that it can cross the blood-brain barrier. As well, it could serve as a potential ligand in affinity chromatography for the purification of GABA-T.

Effect of chronic alloxan diabetes and insulin treatment on adipose tissue lipid composition of rats

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Summary. Decreased content of the adipose tissue lipids was observed in chronic alloxan diabetic rats and was restored to normal with insulin treatment. Prolonged insulin treatment in normal rats also resulted in increase of the lipid content of the adipose tissue.

Adipose tissue is characterized by its high content of fat which comprizes mostly triglycerides. The composition of yellow and brown adipose tissue lipids and its variations with different periods of starvation have been reported[1]. BENJAMIN et al.[2] and PECKHOLM et al.[3] studied the effects of age and diet on adipose tissue lipids of rats. The adipose tissue lipids are also under the influence of the hormonal status of the animal[4]. However, information regarding the effect of diabetes on the adipose tissue lipids and on their changes with insulin treatment are scanty. Therefore a study was undertaken to examine the detailed lipid composition of the adipose tissue of chronic alloxan diabetic rats and the effect of insulin treatment on the composition.

Materials and methods. Male rats of Institute colony (Wistar strain) weighing 160 ± 25 g were kept on commercial rat pellets (Hind Lever, Bombay) and water ad libitum. Diabetes was produced in 36 h fasted rats by i.p. injections of freshly prepared aqueous solution of alloxan monohydrate (150 mg/kg body wt.). After the production of diabetes, half of the animals were treated with s.c. injections of 3 units of protamine zinc insulin (Boots) daily, and the other half were given the same amount of 0.15 *M* saline. In non-diabetic animals, the dose of insulin was started from 0.3 units which was increased every 3rd day by 0.2 units depending on the tolerance of the animals, till a final level of 3 units was reached, and this dose was maintained throughout the rest of the experimental period. The animals were observed for 120 days. Then they were sacrificed after overnight fast under light ether anaesthesia. Insulin injections were stopped 24 h before sacrifice.

Epididymal fat pads were quickly removed and the lipids were extracted[5]. Neutral lipids were separated by thin layer chromatography on silica gel G plates using solvent system petroleum ether-ether-acetic acid, 90:10:1 (V/V). Neutral lipids from silica gel G plates were eluted successively with chloroform and chloroform-methanol 2:1 (V/V). Cholesterol[6], triglycerides[7], free fatty acids[8] and phospholipid phosphorus[9] were measured in the lipid extracts. Blood sugar[10] estimations were also done in the animals before sacrifice.

Results and discussion. The initial and final body weights and the blood sugar levels of rats at the time of sacrifice have been presented in Table I. There was increase of body weights in all the groups except in the saline-treated diabetic group. Blood sugar levels of the diabetic groups (Groups C and D) were higher as compared to those of the non-diabetic groups (groups A and B). However, insulin-treated diabetic group (group D) showed much lower values as compared to that of the saline-treated diabetic group (group D).

Table I. Body weight and blood sugar values of rats in different groups

Groups	Body weight (g)		Blood sugar at the time of sacrifice (mg/100 ml)
	Initial	Final	
A) Saline treated control	156±8	222±11	80±7
B) Insulin treated control	171±7	266±5	68±6
C) Saline treated diabetic	152±3	144±4	393±12
D) Insulin treated diabetic	174±9	231±18	276±6

Values were Mean ± SE of 5 animals in each group

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The lipid composition of epididymal fat pad in different groups are shown in Table II. There was significant reduction of total lipids in the saline-treated diabetic group which was mainly due to reduction in triglyceride fraction. Significant decrease in esterified cholesterol and increase in phospholipid and free fatty acid has been observed in this group. With insulin treatment, total lipid, neutral lipid and triglyceride were restored to the values observed in saline treated control group. There was no effect of insulin on esterified cholesterol, while free fatty acid increased further. In the non-diabetic animals, continued insulin treatment caused an increase in the total lipid which was mainly due to increase in triglyceride fraction. Slight though significant decrease was also seen in free fatty acid level in insulin treated non-diabetic animals.

Table II. Adipose tissue lipids in various groups

Lipids (mg/g)	Saline treated control	Insulin treated control	Saline treated diabetic	Insulin treated diabetic
Total lipid	715±26.5	812±7.6 $p<0.005$[a]	595±4.6 $p<0.005$[a]	718±31 $p<0.001$[b]
Neutral lipid	713±24	810±7.6 $p<0.005$[a]	592±5.7 $p<0.005$[a]	714±31.4 $p<0.001$[b]
Phospholipid	2.49±0.21	2.58±0.04	3.66±0.15 $p<0.001$[a]	3.92±0.39 $p<0.01$[a]
Triglyceride	452±8.6	518±9.2 $p<0.001$[a]	345±9.4 $p<0.001$[a]	471±9.2 $p<0.001$[b]
Total cholesterol	6.38±0.69	7.05±0.1	4.28±0.48 $p<0.02$[a]	4.44±0.48 $p<0.05$[a]
Free cholesterol	3.29±0.35	3.85±0.12	2.88±0.39	2.94±0.38
Esterified cholesterol	3.09±0.38	3.14±0.07	1.35±0.05 $p<0.001$[a]	1.35±0.13 $p<0.01$[a]
Free fatty acid	3±0.15	2.3±0.1 $p<0.005$[a]	4.8±0.2 $p<0.001$[a]	7.6±0.25 $p<0.001$[a] $p<0.001$[b]

Values were Mean ± SE of 5 observations in each group
[a] As compared to saline treated controls
[b] As compared to saline treated diabetics

It has been reported that adipose tissue clearing lipase is low in alloxan diabetic rats[11,12]. At the same time, there is increased mobilization of free fatty acids from the adipose tissue in diabetes[13]. These factors might have contributed to the decreased lipid content of the adipose tissue in chronic diabetic rats. Insulin administration restored the lipid content of the adipose tissue of the diabetic animal, possibly due to enhanced lipogenic effect and decreased lipolytic effect of the hormone[14]. Hyperinsulinism has been involved with such risks as obesity[15], with or without glucose intolerance[16] and hypertriglyceridemia[17]. The long-term in vivo effect of hyperinsulinism on adipose tissue lipid metabolism in normal non-diabetic animals has received relatively little attention in the past. From the results of the present investigation, it is clearly evident that long-term insulin administration leads to increased lipid accumulation in the adipose tissue.

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MAO inhibition, an unlikely mode of action for chlordimeform

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Summary. Inhibition constants of several formamidines, their corresponding formanilides and other representatives of compounds derived from aniline, such as phenylureas, N-phenyl-carbamates and acylanilides, were determined for rat liver monoamine oxidase. The reversability of the inhibition and the lack of correlation between inhibition potencies and toxicities of the compounds tested add to the opinion that MAO inhibition is not a prominent factor in chlordimeform poisoning.

The insecticide/acaricide/ovicide chlordimeform, N'-(4-chloro-o-tolyl)-N,N-dimethylformamidine, and its N-demethyl derivative are known to inhibit monoamine oxidase (MAO) in homogenates of rat liver[1,2], cattle ticks[3,4] and cockroach heads[5]. Whereas the first publications[1,2] attempted to illustrate the importance of this inhibition with regard to the biochemical mode of action of chlordimeform, subsequent papers did only express some[3,5] or no primary involvement[4] of MAO inhibition in the lethal action of formamidines.

All inhibition data published so far are I_{50}-values obtained from pre-incubation experiments. The type of inhibition, however, as compared to that of known inhibitors of pharmacological importance has not yet been evaluated and, with exception of the N-demethyl derivative, no other metabolite of chlordimeform was tested for its inhibition potency. In addition, the I_{50}-determinations did not consider further types of pesticides con-

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