Effect of a New Hypoglycemic Agent, 3,5-Dimethylpyrazole, on Carbohydrate and Free Fatty Acid Metabolism

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SUMMARY

As a hypoglycemic agent, 3,5-dimethylpyrazole (U-6245) was found to be fifty-four times more potent orally than tolbutamide in glucose-injected, fasted intact rats. U-6245 increased glucose oxidation by intact rats. It lowered plasma free fatty acids but not blood sugar of eviscerate rats and was effective in decreasing the fasting blood sugar levels of alloxan-diabetic rats which were unresponsive to tolbutamide. U-6245 markedly depressed plasma FFA fifteen minutes to three hours after its administration.

The mechanism of hypoglycemic activity of 3,5-dimethylpyrazole is not the same as insulin, sulfonylureas or biguanides in that: (1) It is ineffective in lowering the blood sugar of eviscerates while insulin is. (2) U-6245 is active in alloxan diabetic rats which are unresponsive to tolbutamide. (3) The pyrazole increases glucose oxidation and biguanides do not. Although the mechanism of action of 3,5-dimethylpyrazole is not understood, data are presented which support the hypothesis that its action may depend on its effect on plasma FFA and also on the presence of the liver and/or intestinal tract. Since the pyrazole increases glucose oxidation in intact rats, it appears that at least part of its action may be due to the stimulation of glucose oxidation by the intestinal tract and/or liver. DIABETES *14*:507-15, August 1965.

The extensive search for orally active hypoglycemic compounds is readily apparent from recent reviews.[1-3] The sulfonylureas and biguanides are the only classes of compounds which have been successfully developed and utilized extensively for the treatment of diabetes in man. Although these drugs are widely accepted as being efficacious in treating some diabetics, they are ineffective in many others.[4,5] Consequently, testing of many chemicals and plant extracts has continued. However, the need still remains for agents with oral activity via different mechanisms with the possibility of a broader spectrum of action than the presently available oral agents.

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Since insulin will depress plasma free fatty acids and blood sugar,[6] it was of interest to study effects of 3,5-dimethylpyrazole (U-6245) on both blood sugar and plasma free fatty acids. Recent observations by Shipp et al.[7] on isolated perfused rat hearts and by Randle[8] on the rat diaphragm in vitro suggest that free fatty acids can inhibit glucose uptake and utilization by the tissues. Diabetics have elevated free fatty acid levels which can be decreased by insulin administration,[6] and Randle et al.[9] have postulated that the control of plasma free fatty acids in the diabetic may be very important.

This paper describes studies on the mechanism of action of 3,5-dimethylpyrazole (U-6245) (figure 1) and its possible influence on interrelationships between carbohydrate and free fatty acid metabolism. U-6245 is an interesting compound since it has a marked influence on both carbohydrate and lipid metabolism and therefore, may be useful for studying the possible interrelationships between them and for contributing to the understanding of metabolic abnormalities associated with diabetes.

METHODS

Blood sugar and plasma free fatty acids.

Blood glucose was determined by the AutoAnalyzer, which utilizes a modification of a method described by Hoffman.[10] Manifold tubing sizes and/or reagent concentrations were modified in order to assure blood sample readings in the most sensitive portion of the curve.

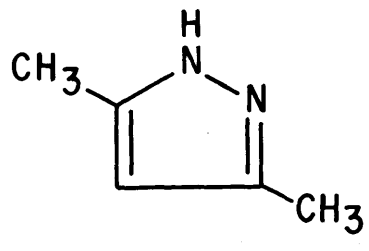

FIG. 1. Structure of 3,5-dimethylpyrazole (U-6245).

Blood glucose levels of alloxan-diabetic animals were measured by the glucostat procedure.[11]

Plasma free fatty acids (FFA) were determined in triplicate by the method of Dole.[6]

Animal preparations.

All animals used for these studies were male rats of the Charles River C.D. Strain. Potency estimates were determined in intact rats weighing 130-150 gm. following a fifteen-hour (overnight) fast and were calculated by a standard USP method.[12] The fasted animals were injected subcutaneously with 100 mg. of glucose immediately prior to oral administration of 3,5-dimethylpyrazole in 0.5 ml. of CMC Vehicle.[13] Blood was withdrawn two hours after treatment from the posterior vena cava while the rats were under Cyclopal* anesthesia.

The effects of U-6245 were also studied by the above procedure in rats injected intraperitoneally with 40 mg./kg. of a metabolic inhibitor of drug metabolism[14] (U-5446)† thirty minutes prior to oral treatment with the pyrazole.

Adrenalectomized rats were operated at a body weight of 130-150 gm. seven days prior to use and maintained on 1 per cent sodium chloride as the drinking fluid. The effects of U-6245 on blood sugar of adrenalectomized rats were measured as described above for intact rats, except potency estimates in adrenalectomized animals were obtained by a comparison of minimal effective doses.

The effect of U-6245 on epinephrine, lactic acid and hydrocortisone-induced hyperglycemia was measured in the adrenalectomized rat preparation described above. U-6245 was administered one-half hour prior to the hormones and blood sugar levels determined two and one-half hours after the pyrazole. U-6245 was given one hour prior to lactic acid and blood sugar measured two hours after U-6245.

Alloxan diabetes was produced in rats fasted overnight weighing 150-180 gm. by intravenous injection of alloxan monohydrate (Eastman) at a dose of 42 mg./kg. in 0.5 ml. of saline. The animals were alloxanized one month prior to use. They were housed in stainless steel metabolism cages and cup-fed 10 ml. of liquid diet[15] twice a day. The diabetes of some of the animals was of such severity that insulin (Regular Iletin) given twice daily at feeding times was required for survival. Those animals requiring insulin were given sufficient amounts to maintain their twenty-four-hour urinary glucose excretion between 3 to 4 gm. Diabetic animals which did not require insulin also had twenty-four-hour urinary glucose excretions between 3 to 4 gm. The last insulin dosage and feeding was twenty-four hours prior to treatment with U-6245, tolbutamide or vehicle. The experimental design was a crossover as previously described.[16] Immediately prior to treatment, a zero-hour blood sample was withdrawn from the orbital sinus and again at two, four and six hours after treatment.

Since anesthetics were found to block the hypoglycemic activity of 3,5-dimethylpyrazole (unpublished data), technics were developed to circumvent this problem to allow studies in animal preparations requiring surgery. Spinal transection and evisceration were conducted as previously described.[17]

Oxidation of glucose-U-C-14 and palmitate-1-C-14 in vivo

These experiments were conducted by a previously described method[18,19] and consisted essentially of an intraperitoneal injection of glucose-U-C-14 (.14 microcuries) or palmitate-1-C-14 in 1 cc. rat serum (.56 microcuries) one-half hour following oral administration of the compound. Animals were placed in glass metabolic units, and the expired air pulled out of the units into sodium hydroxide by a slight negative pressure. The sodium hydroxide was sampled periodically, and the CO_2 precipitated as $BaCO_2$ and counted in a gas flow counter (Baird-Atomic). All values were corrected for self-absorption by use of a calibration curve prepared for the instrument. The rats used had fasting weights from 160-180 gm. All rat weights were identical for any one experiment.

In vitro studies with rat epididymal adipose tissue.

The assay for insulin-like activity in vitro was a modification of Renold's procedure.[20] Epididymal fat pads were removed from rats weighing 160-165 gm. which were sacrificed by decapitation. The distal portion of one pad from each rat was placed in an incubation flask containing U-6245, and the segment from the other pad placed in the control flask. The experiment was designed so that half of the left pads and half of the right pads were treated. The incubation was carried out in 35 ml.-capacity glass bottles. They were stoppered with sleeve-type rubber stoppers with small glass cups suspended from the stopper; the incubation was carried out in a Dubnoff metabolic shaking incubator for ninety minutes at 37.5° C. in 3 ml. of

*5-(1-Cyclopenten-2-yl)[5]allylbarbituric acid, sodium.

†(SKF-525A) Valeric acid, 2,2-diphenyl-, 2-diethylaminoethyl ester, hydrochloride.

Krebs Ringer bicarbonate buffer at *p*H 7.4. The bottles were gassed for five minutes with 95 per cent O_2 and 5 per cent CO_2, after which the flasks were sealed for the duration of the experiment. Glucose-1-C-14 was added at 0.12 microcuries/flask. Unlabeled glucose from the National Bureau of Standards was added to the buffer at 200 mg./100 ml. At the end of the incubation period, 0.2 ml. of 0.3 N NaOH was placed in the center well and 0.2 ml. of 10 N H_2SO_4 was added to the incubation medium and the released CO_2 collected in the NaOH well for sixty minutes. The NaOH was diluted and a portion counted by liquid scintillation technics.

Effects of U-6245 on free fatty acid release from rat epididymal adipose tissue was measured in vitro by technics similar to those of Leboeuf et al.[21] Rats weighing 180 gm. were fasted overnight and killed by a blow on the head. The distal portion of one fat pad was removed immediately and placed in an incubation flask containing U-6245, and the segment from the other pad placed in the control flask. Experiments were designed so that half of the left pads and half of the right pads were treated. Incubations were carried out in 10 ml. Erlenmeyer flasks. Flasks containing 3.0 ml. of medium were preweighed and reweighed after the addition of tissue. Incubation was carried out in a Dubnoff metabolic shaking incubator for ninety minutes at 37.5° C. in a medium consisting of Krebs Ringer bicarbonate buffer at *p*H 7.4 containing 2 per cent crystallized bovine albumin (Armour, Lot No. T68412) and 100 mg. glucose per 100 ml. Free fatty acids released into the medium were measured by Dole's method.[6]

Specific activity of FFA.

Palmitate-1-C-14 (0.5 μc) in 0.5 ml. rat serum was injected intravenously into fasted rats weighing 170 gm. immediately after oral treatment with U-6245. The rats were bled one hour later from the posterior vena cava while under Cyclopal anesthesia. FFA were determined,[6] and an aliquot of the FFA extract counted by liquid scintillation technics. The specific activity is expressed as CPM/μE FFA/L.

RESULTS

Analyses of the blood sugar data obtained from intact, fasted, glucose-injected rats indicates that 3,5-dimethylpyrazole is fifty-four times more potent than tolbutamide (95 per cent confidence limits 37-73) (figure 2). In the fasted adrenalectomized rat, 0.0125 mg./kg. of 3,5-dimethylpyrazole depressed blood glucose more than 10 mg./kg. of tolbutamide (figure 3). A comparison of the minimal effective doses in fasted adrenalectomized rats indicates that U-6245 is approximately 800 times as potent as tolbutamide in this animal preparation. It is apparent that U-6245 completely blocks epinephrine or hydrocortisone-induced hyperglycemia (tables 1 and 2) but does not block lactic acid hyperglycemia (table 3) in the adrenalectomized rats fasted overnight.

The blood sugars of the U-6245-treated alloxan diabetic animals were depressed 40 to 50 per cent two to four hours after treatment (figure 4). Blood sugars of the same animals treated with tolbutamide did not decrease significantly from the fasting level.

In intact, fasted rats, U-6245 significantly increased the per cent of labeled glucose oxidized sixty to 180 minutes after the injection of C-14 glucose (figure 5). The specific activity of $BaCO_3$ obtained from expired C-14-O_2 was significantly increased during the entire

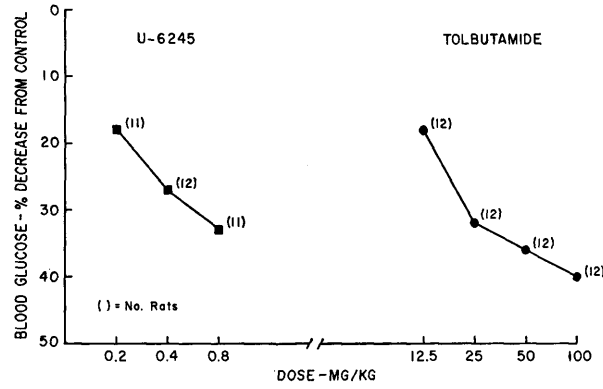

FIG. 2. The comparative hypoglycemic activity of U-6245 and tolbutamide in glucose-injected (100 mg.), fasted, intact rats—two hours after oral administration.

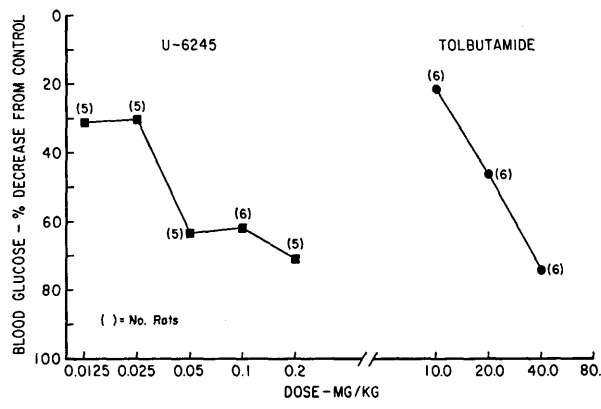

FIG. 3. The comparative hypoglycemic activity of U-6245 and tolbutamide in glucose-injected (100 mg.), adrenalectomized fasted rats—two hours after oral administration.

TABLE 1

Effect of U-6245 on epinephrine-induced hyperglycemia in adrenalectomized, fasted rats two hours after hormone treatment. U-6245 given one-half hour before epinephrine

Number of rats	Treatment	Dose	Blood sugar (mg./100 ml. ± S.E.)*
6	Controls	—	37 ± 1.2
6	U-6245	6.25 mg./kg.	10 ± 1.2†
6	Epinephrine	15 μg./rat	95 ± 2.6
6	U-6245 + Epinephrine	6.25 mg./kg. + 15 μg./rat	16 ± 1.1†

*Standard error
†P < 0.001; U-6245 + epinephrine treatment vs. epinephrine treatment. P < 0.001; U-6245 vs. control.

TABLE 2

Effect of U-6245 on hydrocortisone-induced hyperglycemia in adrenalectomized, fasted rats two hours after hormone treatment. U-6245 administered one-half hour before hydrocortisone

Number of rats	Treatment	Dose	Blood sugar (mg./100 ml. ± S.E.)*
6	Control	—	37 ± 1.2
6	U-6245	6.25 mg./kg.	10 ± 1.2†
6	Hydrocortisone	5 mg./rat	62 ± 2.1
6	Hydrocortisone + U-6245	5 mg./rat + 6.25 mg./kg.	19 ± 1.8†

*Standard error
†P < 0.001; U-6245 + hydrocortisone treatment vs. hydrocortisone treatment. P < 0.001; U-6245 vs. control.

TABLE 3

Effect of U-6245 on lactic acid-induced hyperglycemia in adrenalectomized, fasted rats two hours after U-6245 treatment. Lactic acid administered one hour after U-6245

Number of rats	Treatment	Dose	Blood sugar (mg./100 ml. ± S.E.)*
6	Control	—	48 ± 0.6
6	U-6245	6 mg./kg.	41 ± 1.5
6	Lactate	100 mg./rat	82 ± 2.5
6	Lactate + U-6245	100 mg./rat + 6 mg./kg.	72 ± 7.6

*Standard error

course of the experiment. This increase was maximal at sixty and ninety minutes after U-6245 treatment.

The results of studies on the effect of U-6245 on glucose oxidation by epididymal adipose tissue in vitro are summarized in table 4. It is seen that U-6245 at concentrations of 0.04, 0.4 and 4.0 mg./100 ml. of medium did not increase the oxidation of glucose-1-C-14 by epididymal adipose tissue in vitro while insulin did increase the oxidation of glucose-1-C-14.

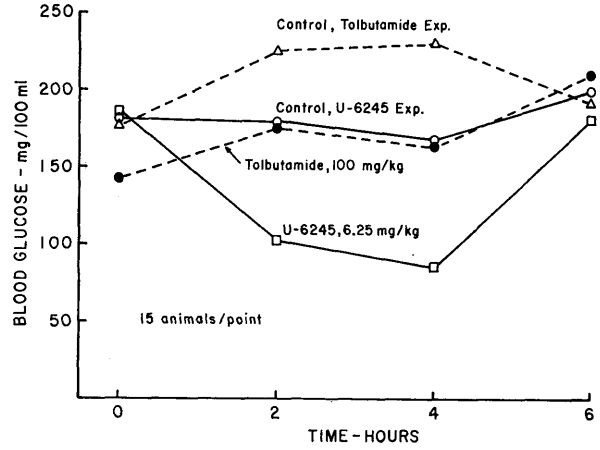

FIG. 4. Comparative effects of U-6245 and tolbutamide on blood glucose in the same group of fasted, alloxan-diabetic rats.

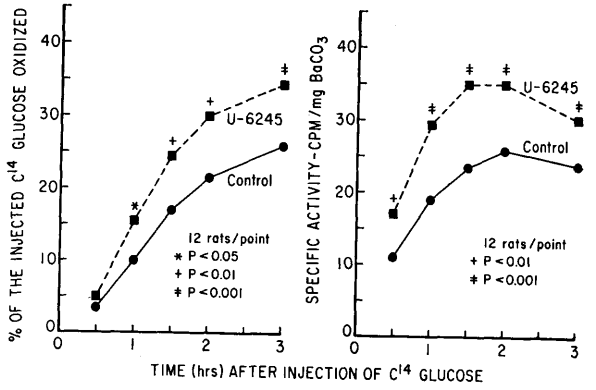

FIG. 5. Effect of orally administered U-6245 (6.25 mg./kg.) on the oxidation of glucose-U-C-14 to C-14-O_2 in intact fasted rats. Compound given one-half hour prior to glucose injection.

TABLE 4

Effect of U-6245 on the oxidation of glucose-1-C-14 by rat epididymal adipose tissue in vitro

Number assays	Treatment	Dose (mg./100 ml. medium)	(CPM/mg. tissue ± S.E.*)
5	Control	—	4.1 ± 0.3
5	U-6245	0.04	4.5 ± 0.4
5	Control	—	7.7 ± 0.6
5	U-6245	0.40	6.9 ± 0.7
5	Control	—	7.0 ± 0.5
5	U-6245	4.00	6.7 ± 0.6
3	Control	—	4.2 ± 0.2
3	Insulin	500 μU./ml.	28.8 ± 1.9†

*Standard error
†P < 0.001

U-6245, insulin and tolbutamide significantly depressed blood glucose in the spinal transected laparotomized animals (table 5). Only insulin was effective in the eviscerated rats.

The effect of U-6245 two hours after treatment on plasma FFA and blood sugar in rats with and without Cyclopal treatment is shown in table 6. U-6245 significantly depressed both blood sugar and FFA two hours after treatment in the unanesthetized rats. However, Cyclopal completely blocked the blood sugar response to U-6245 but did not affect the FFA depression two hours after treatment. The metabolic inhibitor of drug metabolism (U-5446) blocked the blood sugar response to the pyrazole but did not block plasma FFA depression (table 7).

Regular insulin at a dose of 0.125 U./rat significantly decreases both plasma FFA and blood glucose one hour after treatment while tolbutamide at 25 mg./kg. decreases only blood sugar (table 8). Tolbutamide is not effective in lowering plasma FFA at doses as high as 100 mg./kg. in intact rats (unpublished data). Figure 6 shows that plasma FFA depression occurs prior to a change in blood sugar after U-6245 treatment. The plasma FFA are significantly depressed fifteen minutes after oral treatment with the pyrazole and remain depressed for three hours. The blood sugar fall in the same rats is not significant until two hours after treatment.

Plasma FFA are significantly depressed in spinal transected eviscerate rats two hours after treatment with U-6245 (table 9). However, U-6245 did not lower blood sugar of the eviscerate rats.

At concentrations from 1.0 to 10 mg. U-6245/100 ml. of medium, no significant inhibition of FFA release into the medium was observed in vitro (table 10).

TABLE 6

Effect of U-6245 on plasma free fatty acids (FFA) and blood sugar of intact, fasted, glucose-injected (100 mg.) rats and on rats anesthetized with Cyclopal during the experiment. Blood obtained for analysis two hours after treatment.

Number of rats	Treatment	Dose (mg./kg.)	Blood sugar (mg./100 ml. ± S.E.*)	FFA (μE/L. ± S.E.*)
		Without Cyclopal		
5	Controls	—	75 ± 1.2	750 ± 52
6	U-6245	2.5	60 ± 1.1†	326 ± 16†
6	U-6245	5.0	54 ± 0.9†	287 ± 11†
		With Cyclopal		
6	Controls	—	79 ± 1.9	819 ± 64
6	U-6245	2.5	81 ± 2.8	354 ± 22†
6	U-6245	5.0	76 ± 3.1	308 ± 19†

*Standard error
†$P < 0.001$

TABLE 7

Effects of U-5446 on the blood sugar and FFA response to U-6245 in intact rats

Number of rats	Treatment	Dose (mg./kg.)	Blood sugar (mg./100 ml. ± S.E.*)	FFA (μE/L. ± S.E.*)
6	Control	—	68 ± 3.5	728 ± 43
6	U-6245	6.25	47 ± 4.0†	204 ± 12†
6	U-5446	40	68 ± 2.2	743 ± 54
6	U-6245 + U-5446	6.25 + 40	62 ± 1.8	194 ± 15†

*Standard error
†$P < 0.001$

Treatment of intact, fasted rats with U-6245 did not increase palmitate-1-C-14 oxidation in fasted rats (figure 7). It can be seen that the specific activity of C-14-O_2 expired by the U-6245 treated animals is similar to that of the controls.

TABLE 5

Effects of U-6245, insulin and tolbutamide on blood sugar of eviscerated, spinal transected rats

Number of rats	Operation	Treatment	Dose	Blood sugar (mg./100 ml. ± S.E.*) 0 Hrs.	2 Hrs.
15	Laparotomy	Control	—	—	86 ± 8.6
9	Laparotomy	U-6245	25 mg./kg.	—	64 ± 2.2†
14	Evisceration	Control	—	90 ± 4.1	158 ± 11.2
13	Evisceration	U-6245	25 mg./kg.	94 ± 3.7	170 ± 8.3
15	Laparotomy	Control	—	—	86 ± 8.6
10	Laparotomy	Tolbutamide	50 mg./kg.	—	70 ± 3.3†
8	Evisceration	Control	—	105 ± 14	150 ± 13.0
8	Evisceration	Tolbutamide	50 mg./kg.	99 ± 6.7	146 ± 13.0
15	Laparotomy	Control	—	—	86 ± 8.6
9	Laparotomy	Insulin	0.125 U./rat	—	43 ± 3.4†
9	Evisceration	Control	—	98 ± 7.8	157 ± 12.0
10	Evisceration	Insulin	0.125 U./rat	96 ± 3.7	46 ± 3.7†

*Standard error
†$P < 0.001$

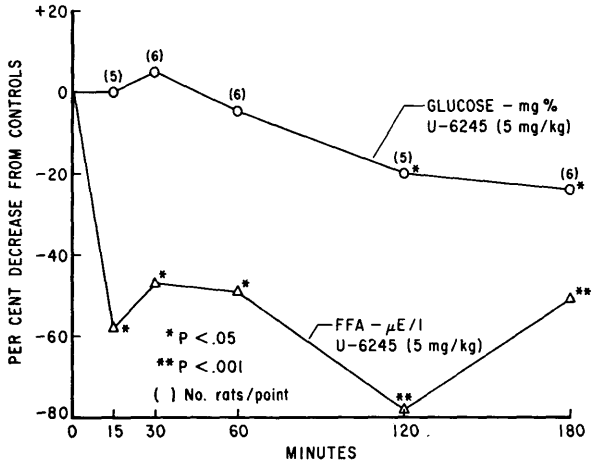

FIG. 6. Effect of U-6245 on blood sugar and plasma FFA in intact, fasted rats injected with glucose (125 mg.) at time of treatment.

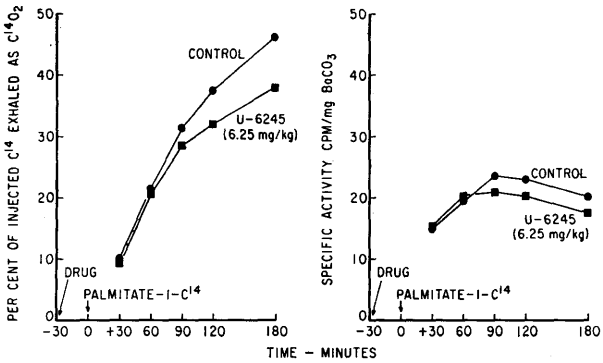

FIG. 7. Effect of U-6245 on oxidation of palmitate-1-C-14 by intact rats.

TABLE 8

Effect of insulin and tolbutamide on plasma free fatty acid (FFA) and blood sugar levels of intact, fasted, glucose-injected and nonglucose-injected rats. Blood obtained for analysis one hour after treatment

Number of rats	Treatment	Dose (mg./kg.)	FFA (μE/L.± S.E.*)	Blood sugar (mg./100 ml. ± S.E.*)
		Glucose injected (100 mg./rat SQ)		
5	Control	—	815 ± 32.0	89 ± 1.7
5	Insulin	0.125 U./rat	469 ± 88.3†	28 ± 1.5‡
5	Control	—	565 ± 36.0	86 ± 1.2
5	Tolbutamide	25 mg./kg.	656 ± 33.4	68 ± 2.5‡
		Nonglucose injected		
5	Control	—	793 ± 52.7	85 ± 1.4
5	Insulin	0.125 U./rat	360 ± 49.1‡	32 ± 2.2‡
5	Tolbutamide	25 mg./kg.	758 ± 9.7	56 ± 3.2‡

*Standard error
†P < 0.01
‡P < 0.001

TABLE 9

Effect of U-6245 on blood sugar and FFA of eviscerated, spinal-transected rats. Blood samples obtained from the posterior vena cava two hours after treatment

Number of rats	Treatment	Dose (mg./kg.)	Blood sugar (mg./100 ml. ± S.E.*)	FFA (μE/L.± S.E.*)
10	Controls	—	109 ± 15	769 ± 60
11	U-6245	25	106 ± 6	473 ± 47†

*Standard error
†P > 0.001

TABLE 10

Effect of U-6245 on release of free fatty acids by epididymal adipose tissue from fasted intact rats

Number of assays	Treatment	U-6245 (mg./100 ml.)	FFA release (μE/L./mg. tissue ± S.E.*)
6	Control	—	6.5 ± 0.5
6	U-6245	1.0	6.0 ± 0.7
12	Control	—	7.2 ± 0.8
12	U-6245	5.0	6.5 ± 0.5
6	Control	—	5.6 ± 0.4
6	U-6245	10.0	4.9 ± 0.5

*Standard error

U-6245 treatment caused a sixfold increase in the specific activity of plasma FFA one hour after simultaneous administration of pyrazole and tracer amount of palmitate-1-C-14 (table 11). The pyrazole also caused the expected decrease in the level of plasma free fatty acids.

DISCUSSION

The observation that 3,5-dimethylpyrazole is approximately fifty times more potent than tolbutamide as an oral hypoglycemic agent in intact, fasted rats injected with glucose makes this compound the most potent orally active hypoglycemic agent known with the exception of 3,5-dimethylisoxazole, which is approximately 200 times as potent as tolbutamide.[17] The minimal dose of U-6245 found capable of producing hypoglycemia in fasted, glucose-injected adrenalectomized rats (0.0125 mg./kg. or 2 γ/150 gm. rat) is in the same range as the amount of parenterally administered insulin required to lower blood sugar of this animal preparation.[22]

The mechanism of hypoglycemic activity of the pyrazole is not the same as insulin, sulfonylureas or biguanides. A comparison of the effects of these compounds is presented in table 12. Its action is similar to that of insulin in that: (a) It increases glucose oxidation and depresses plasma free fatty acids of intact rats

TABLE 11

Effect of U-6245 on the specific activity of plasma FFA

Number of rats	Treatment	Dose (mg./kg.)	Blood sugar (mg./100 ml. ± S.E.*)	FFA (μE/L.±S.E.*)	FFA (CPM/μE/L.±S.E.*)
6	Control	—	100 ± 2.2	839 ± 74	180 ± 20
6	U-6245	1.0	97 ± 5.1	104 ± 18†	1,300 ± 200†

*Standard error
†$P < 0.001$

TABLE 12

Comparison of the activities of U-6245, insulin and tolbutamide

Activity	U-6245	Insulin	Tolbutamide
Hypoglycemic potency (rats) (two hours)	50 × tolbutamide	1,000 × tolbutamide*	1
Oral hypoglycemic potency (rats) (two hours)	50 × tolbutamide	0	1
Alloxan-diabetic rats (fasted)	+	+	0
Glucose oxidation (intact rats)	+	+	+
FFA depression (intact rats)	+	+	0
Fat pad in vitro (glucose-1-C-14 oxidation)	0	+	0
Fat pad in vitro (FFA release)	0	+	0
Eviscerate rats (blood sugar)	0	+	0
Eviscerate rats (FFA)	+	+	0

*Insulin subcutaneous; U-6245 and tolbutamide oral

as reported for insulin[18,6]; and (b) lowers blood glucose. U-6245 is unlike insulin in that: (a) It is not effective in lowering blood sugar of eviscerated rats which respond to insulin; and (b) U-6245 does not stimulate the production of C-14-O_2 from glucose-1-C-14 by epididymal adipose tissue while insulin does.

3,5-Dimethylpyrazole is similar to tolbutamide in that it increases glucose oxidation in intact rats as tolbutamide does.[18] It is unlike tolbutamide in that: (a) U-6245 decreases plasma free fatty acids and tolbutamide does not; and (b) the pyrazole lowers the fasting blood sugar of alloxan-diabetic rats which are not responsive to tolbutamide. The mechanism of U-6245 is unlike the biguanides. This is apparent since 3,5-dimethylpyrazole increases glucose oxidation and the biguanides do not.[23]

Although the mechanism of action of 3,5-dimethylpyrazole is not understood, it is interesting to note that U-6245 markedly decreases plasma FFA fifteen minutes after administration. Recent observations by Shipp[7] and by Randle[8,9] have shown that a fatty acid, such as palmitate, can inhibit the utilization of glucose at high concentrations. These observations have been interpreted to suggest that the increase of free fatty acids occurring in diabetics can block the utilization of glucose. Thus, agents which will depress free fatty acids, should, secondarily, increase glucose utilization and may depress blood sugar. This concept is very interesting since 3,5-dimethylpyrazole depresses plasma FFA prior to changes in glucose oxidation rate and blood sugar depression. It can be visualized that if the free fatty acids in the fasted animal are decreased and if this source of energy is no longer available to the animal, a compensatory increase in glucose utilization could occur with a resultant increase in glucose oxidation and blood sugar depression. The observation that U-6245 administration results in a depression of blood sugar, which is secondary to a decrease in free fatty acids, supports the hypothesis of the glucose fatty-acid cycle proposed by Randle.[9]

It should be emphasized, however, that data are also presented suggesting that FFA depression and changes in glucose utilization are not necessarily interrelated. These are: (1) Tolbutamide increases glucose oxidation[18] and lowers blood sugar but does not depress plasma FFA in the intact rat; (2) cyclopal blocks the effects of U-6245 on glucose oxidation (unpublished data) and blood sugar, but the FFA response is still manifested and (3) U-6245 depresses FFA of eviscerate rats and rats treated with U-5446 without alteration in blood sugar. These observations suggest that the effects of FFA and activity on blood glucose by sulfonylureas and 3,5-dimethylpyrazole are not related. However, the possibility does exist that U-6245 can depress blood sugar by more than one mechanism. Also, it is possible that Cyclopal and U-5446 directly inhibit either the secondary or primary effect of the pyrazole on carbohydrate metabolism. Further, it is possible that

the gastrointestinal tract is necessary for part of the action of the pyrazole on blood sugar but not for lowering of FFA in the eviscerate rat.

U-6245 blocks epinephrine[24,25] and hydrocortisone-induced hyperglycemia[24] and is also very effective in depressing blood sugar of adrenalectomized rats, indicating that the pyrazole does not necessarily lower blood sugar in the intact rat by blocking the effects of epinephrine and hydrocortisone on blood sugar. Although epinephrine-induced hyperglycemia was completely blocked by U-6245, lactic acid-induced hyperglycemia was not. It can be concluded from these data that the blockage of epinephrine-induced hyperglycemia by U-6245 was not involved with the conversion of lactate to glucose by the liver[26] but does not eliminate the possibility that U-6245 inhibits the conversion of muscle glycogen to lactate by anaerobic glycolysis.[26] Since epinephrine inhibits hexokinase,[27] it is possible that U-6245 may antagonize epinephrine at this point.

The mechanism by which U-6245 depresses plasma free fatty acids appears to be an inhibition of FFA release from triglycerides in the fat depots. This hypothesis is supported by the observation that U-6245 did not significantly alter the oxidation of palmitate-1-C-14 in intact rats indicating that the decrease in plasma free fatty acids was not due to increased removal via oxidation. The level of labeled palmitate was not increased in muscle, liver or fat (unpublished data), thus further suggesting that the decrease in FFA was not due to increased disposal of plasma free fatty acids. The data showing a sixfold increase in the specific activity of plasma FFA are consistent with the hypothesis that U-6245 decreased free fatty acids by inhibiting release from the fat depots since an increase in specific activity can be interpreted as an indication of decreased release or unlabeled fatty acids.

Although U-6245 markedly depressed plasma FFA and stimulates glucose oxidation in vivo, it does not affect these parameters in vitro. These observations indicate that 3,5-dimethylpyrazole may be metabolized and that the metabolite per se exerts the hypoglycemic effect. The concept of an active hypoglycemic metabolite is supported by the lack of hypoglycemic activity of U-6245 in eviscerate rats which are functionally hepatectomized. The fact that U-5446, an inhibitor of drug metabolism,[14] blocks the blood sugar response to U-6245 further supports the hypothesis that U-6245 is metabolized to an active hypoglycemic compound.

The data presented indicate that U-6245 is a unique hypoglycemic compound which also has a marked effect on plasma free fatty acids. Although its mechanism of action is not understood, it is unlike that of insulin, sulfonylureas or biguanides. Further, 3,5-dimethylpyrazzole is a unique tool for studying possible interrelationships between carbohydrate and lipid metabolism which may be of considerable importance in the diabetic.

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Effects of Insulin on Carbohydrate and Fat Metabolism in Vitamin B_6 Deficiency

The need for vitamin B_6 coenzymes in the synthesis and catabolism of amino acids has explained many of the changes in nitrogen and amino acid metabolism in B_6 deficiency. This deficiency also leads to changes in carbohydrate and fat metabolism, including decreases in the levels of body fat, glycogen, and fasting blood sugar. These effects do not appear directly related to decreases in the levels of vitamin B_6 enzymes.

In an attempt to stimulate food intake of vitamin B_6 deficient rats, G. H. Beaton, A. M. Haufschild, and E. W. McHenry (J. Nutrition *60*:455, 1956) injected insulin, and found that the insulin increased food intake and restored body fat to a normal level. On the other hand, insulin did not prevent the fall in liver glutamic-pyruvic transaminase in the deficient animals. Sensitivity of the deficient animals to insulin also increased as the deficiency became more severe. Subsequently, M. Kuno (Vitamins *19*:140, 1959) observed changes in the pancreatic beta cells of B_6 deficient rats.

A detailed investigation of the effects of insulin on carbohydrate and fat metabolism in vitamin B_6 deficient rats has been made by A. M. Huber, S. N. Gershoff, and D. M. Hegsted (J. Nutrition *82*:371, 1964). This study was undertaken to test the possibility that alterations in the availability of insulin might be responsible for some of the changes observed in carbohydrate and fat metabolism in vitamin B_6 deficient animals. Young male rats were fed a purified diet of the following composition, grams per 100 gm.: casein, 15; sucrose, 75.7; cod liver oil, 1.0; corn oil, 4.0; mineral mixture, 4.0; choline chloride, 0.3; and a vitamin supplement lacking pyridoxine. Pyridoxine was added to the control diets (1 mg. pyridoxine hydrochloride per 100 gm. diet).

In the first experiment, deficient and control groups were fed the basal diets for four weeks. After the first week, the rats fed the deficient diet were divided into three groups. One group was injected intraperitoneally with one unit of Protamine Zinc Insulin per day. The second group was injected with four units of insulin, and the third group was not treated with insulin. These insulin levels were selected because the deficient rats could not tolerate more than six units per day, although the controls tolerated up to ten units per day when fed ad libitum.

The deficient rats injected with one unit of insulin grew somewhat more than the rats not given insulin, but growth and food intake of the group injected with four units were both significantly greater than corresponding values for the other deficient groups. The growth of this group, however, was still significantly less than that of the control group fed pyridoxine. The percentage of body fat in both of the groups injected with insulin increased to the level of the animals fed pyridoxine, but the level of fat in the group not given insulin was significantly lower. Percentages of body protein and ash were similar in all groups. The tail length of deficient groups injected with insulin was greater, an indication that insulin produced greater bone growth.

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