amino acids which might be bound in this fashion. A filtrate prepared in this manner did not contain an increased amount of any of the amino acids in human milk or of arginine in cow's milk. Histidine, however, must be present in a bound form in cow's milk, since only a trace could be detected in the ultrafiltrate, but an amount comparable to that of many of the other amino acids was present in the sulfosalicylic acid filtrate.

No unexpected components could be detected among the ninhydrin-positive substances of human milk. The samples of cow's milk, however, contained 3 unidentified materials: with the system of Spackman, Moore and Stein(4) all were detected in the eluate from the 50 cm column. One is a minor component which emerges 7 ml before phenylalanine and tyrosine, the major substance emerges 18 ml before ornithine, and the remaining one is eluted with lysine. This last material is detectable because it alters the usual ratio of 570 mμ to 440 mμ absorption given by lysine, and is not present in the sulfosalicylic acid filtrate.

*Summary.* The concentrations of the free amino acids in human and cow's milk are reported.

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## Hypoglycemic Activity of 3,5 Dimethylisoxazole. (28461)

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Although numerous chemicals and extracts of biological materials have been reported to possess oral hypoglycemic activity(1,2,3), the discovery of an orally effective agent with an insulin-like mechanism has remained an elusive goal. Sulfonylureas and biguanides have offered partial success but are not effective in all diabetics. Therefore, 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.

This report describes the hypoglycemic action of 3,5 dimethylisoxazole* (U-21221, Fig. 1) which is a highly potent orally active compound with a mechanism of action different from that of available antidiabetic drugs.

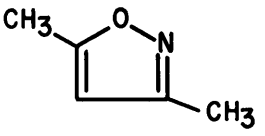



FIG. 1. Structure of 3,5 Dimethylisoxazole (U-21221).

*Methods. Blood sugar of intact rats.* This method has been described(5) and consists of measuring ability of compounds to depress blood sugar of glucose-primed fasted intact rats 2 hours after treatment. Potency ratio was calculated by standard USP method(4).

*Oxidation of glucose-U-$C^{14}$ by intact rats.* This method(6) consists of intraperitoneal injection of glucose U-$C^{14}$ (1.4 μc) one-half hour after oral administration of compound. Animals were placed in glass metabolic units and expired $CO_2$ trapped and sampled periodically, precipitated as $BaCO_3$ and counted

* Compound synthesized by Dr. J. B. Wright, Chemistry Dept., The Upjohn Co.

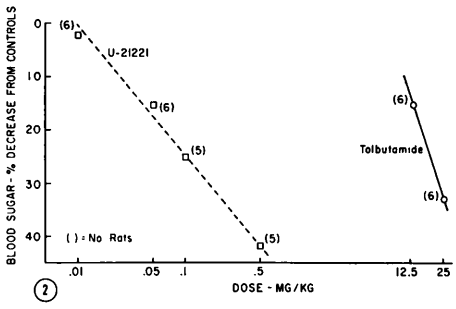
in the gas flow counter (Baird Atomic).

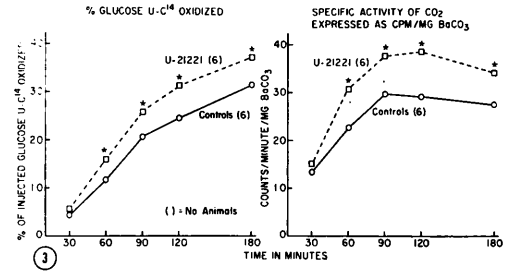
*Blood sugar of alloxan diabetics.* Alloxan diabetes was induced in fasting (18 hours) intact male rats (Sprague-Dawley) by intravenous injection of 40 mg/kg of alloxan monohydrate (Eastman). Animals had been diabetic for approximately 9 months prior to this study and were maintained on 1-3 units of regular insulin (Iletin). They were kept in regular cages and allowed food (Purina Lab Chow) and water *ad libitum*. Last dose of insulin was 24 hours prior to treatment with oral compound and food withdrawn for 18 hours. Immediately before treatment a 0-hour blood sample was taken from the tail and again at 2, 4 and 6 hours after treatment. Experimental design was a crossover as previously described(5). Blood sugar was determined by the glucostat method(7). Statistical examination of data was done by analysis of co-variance(8).

*Blood sugar of eviscerate rats.* Male rats (Charles River) weighing 190-200 g were prepared by spinal transection between the 5th and 6th cervical vertebrae the afternoon before evisceration or laparotomy. At time of transectomy, adrenals were removed, bladder cannulated and each animal injected with 1 mg of hydrocortisone after which the animals were placed in a constant temperature room at 33°C. Transectomy was required since it was found that several anesthetics blocked blood sugar response to the isoxazole. Evisceration(9) or laparotomy was done about 18 hours after transectomy and withdrawal of food. Eviscerated rats were connected to constant infusion pump *via* posterior vena cava and received glucose at a rate of 40 mg/100 g/hour in .9% saline. Laparotomized rats received 160 mg glucose subcutaneously. Animals were kept in a constant temperature box (33°C) during the experiment. Compound was given subcutaneously in the neck 30 minutes after evisceration and initiation of glucose infusion or at time of glucose injection in the laparotomized animals. Blood samples were taken from orbital sinus at time of compound injection and from the femoral vein 2 hours later in the eviscerates. In laparotomized animals a single blood sample was taken 2 hours after treatment. Blood glucose was determined by micro AutoAnalyzer method.

*Results.* U-21221 was approximately 188 times (95% confidence limits 97-344) more potent than tolbutamide in depressing blood sugar of glucose-primed fasted intact rats (Fig. 2).

Oxidation of glucose U-$C^{14}$ was significantly increased when expressed either as % of injected dose oxidized or as specific activity of expired $CO_2$ as measured by counts/



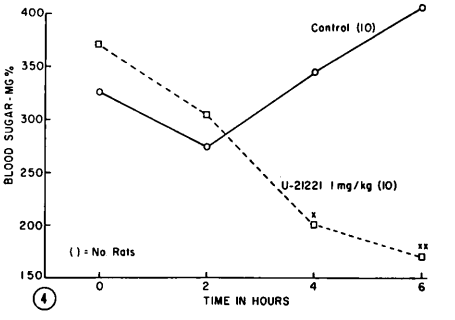




FIG. 2. Comparative hypoglycemic activity of U-21221 and tolbutamide in glucose-primed fasted intact rats 2 hr after oral administration.

FIG. 3. Effects of U-21221 on glucose U-$C^{14}$ oxidation by fasted intact rats.

Footnote to Fig. 3:

* P = <.01.

FIG. 4. Effect of U-21221 on blood sugar of fasted alloxan diabetic rats.

Footnotes to Fig. 4:

* F value between 4-hr control and treated=20.4.

† F value between 6-hr control and treated=77.8.

TABLE I. Effects of U-21221 on Blood Sugar of Eviscerated or Laparotomized Spinal Transectomized Rats.

| No. rats | Operation | Treatment | Dose (mg/kg) | Blood sugar (mg%) ± S.E.* 0 hr | 2 hr |
|---|---|---|---|---|---|
| 10 | Laparotomized | Control | — | — | 87 ± 3.4 |
| 10 | " | U-21221 | 5 | — | 60 ± 2.7† |
| 8 | Eviscerated | Control | — | 95 ± 7.7 | 155 ± 10.6 |
| 10 | " | U-21221 | 5 | 102 ± 4.8 | 164 ± 12.3 |

* Standard error.

† Significant at 1% level by "t" test.

minute/mg $BaCO_3$ (Fig. 3). Blood sugar of alloxan diabetic rats which had been found to be unresponsive to tolbutamide (unpublished) was significantly depressed 4 and 6 hours after treatment with 3,5 dimethylisoxazole (Fig. 4). Blood sugar of eviscerated spinal transectomized rats was not altered by U-21221 (Table I). Laparotomized spinal transectomized rats responded to 3,5 dimethylisoxazole (Table I).

*Discussion.* 3,5 dimethylisoxazole is the most potent orally active hypoglycemic agent reported. It lowered blood sugar of glucose-primed fasted intact rats at an oral dose of approximately 10γ per 150 g rat.

Mechanism of action of 3,5 dimethylisoxazole is not like insulin, tolbutamide or biguanides. It is different from insulin in lack of action in eviscerates in which insulin is effective(10). It is unlike tolbutamide since it was active in alloxan diabetic rats which were unresponsive to the sulfonylurea. Biguanides differ from the isoxazole in lack of ability to increase glucose oxidation(11).

Although the mechanism is not fully understood, lack of effect in eviscerates with activity in intact and diabetic rats supports the hypothesis that its action depends on the presence of the liver and/or intestinal tract. Since the isoxazole induced increased oxidation of glucose, it is suggested that it stimulated the oxidation by the gut and/or liver.

*Summary.* 3, 5 dimethylisoxazole (U-21221) was found to be 188 times more potent orally than tolbutamide in glucose-primed fasted intact rats. U-21221 also increased glucose oxidation by intact rats. It did not lower blood sugar of eviscerate rats but was active in alloxan diabetic animals which were unresponsive to tolbutamide. It was suggested that the mechanism of action of 3,5 dimethylisoxazole was by stimulation of glucose oxidation by the gut and/or liver.

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