A Comparative Study on the Effects of Three Oral Hypoglycemic Agents on Amino Acid Decarboxylation (35028)

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It has been verified in the past few years that the oral hypoglycemic agents which have wide clinical use today have a definite effect both on *in vitro* and *in vivo* amino acid metabolism. These agents inhibit both the *in vitro* incorporation of radioactive amino acids into protein and the *in vitro* evolution of $^{14}CO_2$ from tissue preparations. The *in vitro* effects have been shown for tolbutamide in rat hemidiaphragm (1) and for tolbutamide and phenethylbiguanide in rat liver homogenates (2–5). The *in vivo* studies have also shown a metabolic effect on protein biosynthesis while also utilizing a rat liver system (6).

Until now, all of the work involving the effects of the oral hypoglycemic agents on amino acid metabolism has been done using rat tissues. The purpose of the present study was to determine the relative effects of three oral hypoglycemic agents on the decarboxylation of isotopically labeled amino acids by liver homogenates prepared from the tissue of three different mammalian species.

Materials. The source of the $^{14}CO_2$ was a mixture of 13 labeled amino acids. The amino acids were all labeled in the carboxyl position so that a measurement of any evolved $^{14}CO_2$ was a measurement of the catabolic decarboxylation processes.

The incubation mixture contained, in addition to tissue homogenate, isotope, and hypoglycemic agent, a premix solution as described by DeChatelet and McDonald (2). This premix solution contained co-factors necessary for the maintenance and optimal metabolic activity of the tissue preparation. The buffer solution was 0.08 *M* phosphate buffer at pH 7.6.

Methods. Liver tissue from anesthetized or decapitated animals was removed, rinsed in ice-cold buffer solution, weighed, and ground gently with 3 vol of ice-cold buffer in a Potter-Elvehjem homogenizer. This preparation was centrifuged at 0° for 10 min at 700*g* to remove unbroken cells, cell debris, and nuclei. All experiments used 0.5 ml/flask of this supernatant as the homogenate.

The homogenate, along with the premix solution, isotope, and the hypoglycemic agents of varying concentrations, were incubated in flasks at 37° for 1 hr in an Elmac-type incubator-shaker. The labeled amino acids had an average specific activity of 140 mCi/mmole (range 75–225) and a relative concentration range of 50–125 μCi/mCi of mixture. In the experiments involving dog and cat tissue, 1 mCi of isotope was used whereas 2 mCi were used in the rabbit experiment. The incubation flasks had disposable center wells which contained 10% KOH to trap any CO_2. After 1 hr, 10% TCA was added to terminate the incubation and the flasks were shaken until the absorption of CO_2 by KOH was complete. The center wells were then removed and washed with distilled water into 5 ml of 10% $BaCl_2$. The resulting $BaCO_3$ precipitate was filtered, washed, dried, and counted. All sample results were corrected for self absorption.

Results and Discussion. The data are presented in Figs. 1–3. These data represent the effect of concentration of the various drugs on dog, cat, and rabbit liver tissue,

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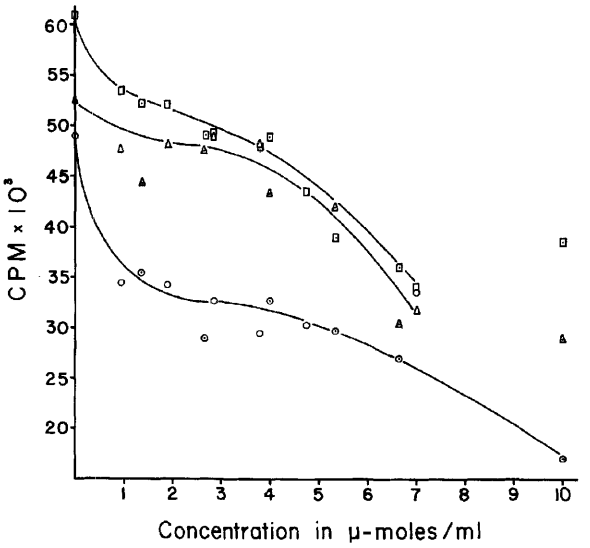

FIG. 1. The effect of concentration of hypoglycemic agents on the evolution of $^{14}CO_2$ from dog liver homogenate: concentration (μmoles/ml of incubation mixture); The drugs are: Phenethylbiguanide (○); Chlorpropamide (□), and Tolbutamide (△); a dot in the center of a symbol differentiates the first experiment from the second. Points represent the mean of two determinations.

respectively. From the results, it is possible to draw the following conclusions.

First, all three agents definitely show a marked inhibitory action on amino acid catabolism. The inhibition was approximately 35–45% of control using 5 μmole of agent/ml of incubation mixture (homogenate protein approximately 25 mg/flask as determined spectrophotometrically by using a biuret reaction). In all experiments, the effect of concentration on $^{14}CO_2$ evolution was nonlinear throughout the entire concentration range.

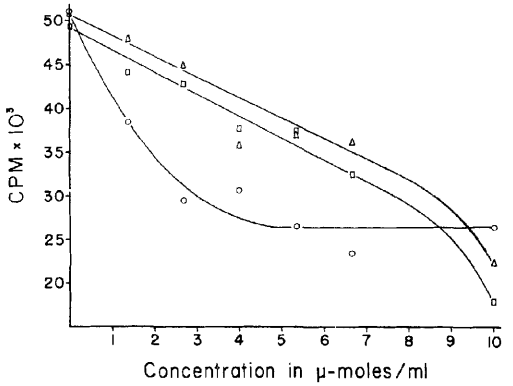

FIG. 2. The effect of concentration of hypoglycemic agents on the evolution of $^{14}CO_2$ from cat liver homogenate. Symbols used similarly as in Fig. 1. Points represent the mean of two determinations.

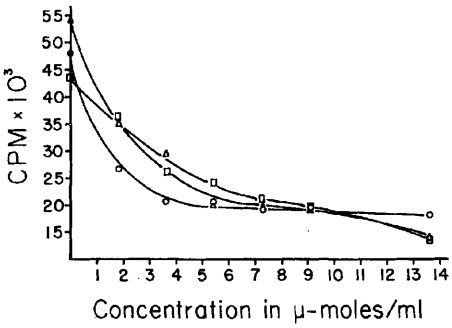

FIG. 3. The effect of concentration of hypoglycemic agents on the evolution of $^{14}CO_2$ from rabbit liver homogenate. Symbols used similarly as in Fig. 1. Points represent the mean of two determinations.

The seond point to be noted is the relative effects of the drugs with respect to one another and with respect to the tissues used. Tolbutamide and chlorpropamide, relative to each other, seem to have approximately the same effect on catabolism in all three cases. However, there seems to be a major difference in their action depending upon usage of cat or dog tissue and rabbit tissue.

The seemingly different action in rabbit tissue cannot be explained at the present time. The different effects of tolbutamide and chlorpropamide, on the one hand, and phenethylbiguanide (at least in Figs. 1 and 2), on the other, could be expected since they are known to act in different ways as far as their hypoglycemic effects are concerned. No adequate explanation can be presented to explain similar results obtained when rabbit tissue was used.

The third point involves the effect of phenethylbiguanide on dog tissue. It has been reported that dogs do not respond to phenethylbiguanide as an oral hypoglycemic agent (7). Since there is a definite inhibition of amino acid metabolism and protein biosynthesis (unpublished results of the authors), it may be concluded that the two activities of phenethylbiguanide, the hypoglycemic activity and its inhibitory effect on amino acid metabolism, are independent of each other. The action of phenethylbiguanide on amino acid metabolism may, however, be determined by its action on more than one metabolic mechanism. This has been shown by its different effects on rat whole-liver homogenate and microsomal systems (4, 8).

Summary. An investigation was made as to the comparative effects of three oral hypoglycemic agents on the *in vitro* evolution of $^{14}CO_2$ from dog, cat and rabbit liver whole-homogenate systems. All three drugs, tolbutamide, chlorpropamide, and phenethylbiguanide, show strong inhibitory effects over the concentration ranges studied, regardless of tissue species. The mode of action of tolbutamide and chlorpropamide was the same, but different from that of phenethylbiguanide in the dog and cat systems. In the rabbit system, the effects of the two classes of drugs could not be differentiated. This might be explained on the basis that the metabolic effects of the two classes of drugs normally follow different pathways.

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