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An Effect of Tolbutamide on Nucleotides of Incubated Whole Human Blood.* (26888)

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Tolbutamide is an oral hypoglycemic agent whose mode of action is still in dispute. If this class of arylsulfonamides acts only in the animal containing some islet tissue(1), then these compounds should have no effect *in vitro* on animal tissue since no insulin-producing tissue would be present to be acted upon. Lundbaek *et al.*(2) noted that tolbutamide added to the isolated rat diaphragm would increase glucose uptake, and Renold *et al.* (3) reported that tolbutamide inhibited ketogenesis by rat liver slices and affected glucose oxidation and lipogenesis from glucose by surviving rat adipose tissue. The present work shows that tolbutamide affects the nucleotide pattern of incubated whole human blood and since this pattern is closely related to glycolysis in the red cell, suggests, but does not explain, an influence on carbohydrate metabolism.

Materials and methods. Blood was collected from 2 donors in such a way that the blood samples contained about 2,000 units of heparin and 350 mg of glucose per 100 ml of blood. Aliquots were placed in 2 tissue culture spinner flasks specially water jacketed to maintain 37° and to one flask in each experiment was added 250 mg tolbutamide (Orinase) suspended in 2.5 ml of saline. Samples were withdrawn from all flasks aseptically at several subsequent time periods and trichloracetic acid extracts were made for subsequent nucleotide fractionation(4).

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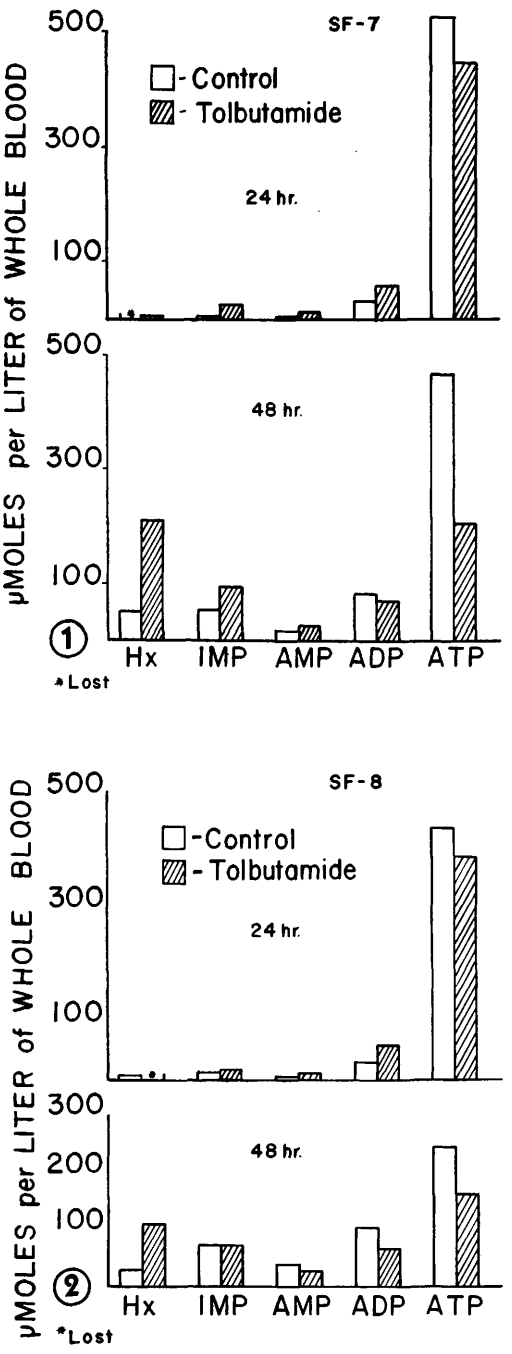
Results. In Fig. 1 and 2 are shown the concentrations of hypoxanthine (Hx), inosine monophosphate (IMP), and adenosine mono-, di-, and tri-phosphates, (AMP, ADP, ATP) in the control and tolbutamide flasks for the 2 subjects. Since ATP is the key energy compound and since on breakdown it follows the pattern(5):

$$ATP \rightarrow ADP \rightarrow AMP \rightarrow IMP \rightarrow\rightarrow Hx$$

it is apparent that in both experiments the presence of tolbutamide was associated with some breakdown in ATP and buildup of degradation products. At 24 hours the changes have started and by 48 hours they are easily seen.

Other measurements such as glucose, pH, and plasma hemoglobin were made in both experiments. The pH in the tolbutamide samples was as much as .1 pH unit below the corresponding control pH at intermediate time intervals but both control and tolbutamide samples came to the same pH by 48 hours. Glucose utilization under the two conditions was not different.

Discussion. Since in the mammalian red cell, glycolysis is the sole source of energy, ATP buildup must be directly coupled with glycolysis. The effect of tolbutamide in reducing blood ATP levels *in vitro* means either that tolbutamide slows ATP synthesis or accelerates its breakdown. The latter might suggest activation of "latent ATPase" while the former could be explained as analogous to "uncoupling" in respiratory phosphorylation. Either process could be compatible with essentially no change in glucose utilization in this *in vitro* system but would



not necessarily imply no effect on carbohydrate metabolism in the whole animal. In a sense then, the effect of tolbutamide on blood nucleotides indicates more subtle changes in energy metabolism than can be measured by more conventional determinations such as glucose utilization. The effects shown here have been demonstrated in several different experiments and hence are verifiable if not explicable.

Summary. Fresh human blood collected in heparin and glucose was incubated without and with tolbutamide for up to 48 hours. ATP levels in the tolbutamide samples were less than in the control samples although glucose utilization appeared to be the same. It was concluded that the changes in the blood nucleotide pattern were more subtle indicators of effects of tolbutamide on energy metabolism than more conventional measures such as glucose utilization, but the implications of the findings are not entirely clear.

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FIG. 1. Nucleotide and hypoxanthine levels at 24 and 48 hr in human blood *in vitro* without and with added tolbutamide.

FIG. 2. Same as Fig. 1 except blood from another subject.