son's disease is due to formation of abnormal proteins in tissues which have a high avidity for copper(10,11). Such a mechanism does not appear to be necessary for excessive deposition of copper in the liver and kidneys of animals with bile ducts ligated or in animals fed a high copper diet(12).

Summary. Ligation of the bile ducts of adult male rats was followed by hypercupremia and deposition of copper in liver and kidneys, but not in the brain.

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Relation Between Structure of Sulfonylurea Compounds and their Effect on Frog Muscle Metabolism.* (24250)

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Two sulfonylurea compounds, carbutamide and tolbutamide,† have been studied extensively as hypoglycemic agents. Although in normal dogs, rats and toads the 2 compounds are equally effective in reducing concentration of glucose in blood(1), they differ markedly from each other and from insulin in their effect on *in vitro* metabolism of intact frog muscle(2). Carbutamide (R = NH_2 in Fig. 1) inhibits oxygen consumption of frog muscle to a small but significant degree, as does the same concentration of sulfanilamide. Tolbutamide (R = CH_3 in Fig. 1), on the other hand, greatly stimulates oxygen consumption of muscle due to increased production of lactate from muscle glycogen. The fact that these compounds differ only in part of molecule labeled R in Fig. 1, suggested that the difference in effect on muscle was due to the type of substitution on the R position of the molecule. To investigate this possibility, similar experiments were done with a series of sulfonylbutylurea compounds which differ only in the R position in Fig. 1.

Methods. Oxygen consumption of intact

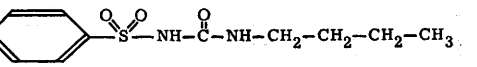

FIG. 1. Structural formula of the *para*-substituted sulfonylbutylurea compounds.

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TABLE I. Effect of Sulfonylbutylurea Compounds with Different Substitutions in the *para* Position of the Ring on Oxygen Consumption of Frog Muscle *in Vitro*.

Substitution	Conc., mM	No. of muscle pairs	O_2 consumption, μM/g/hr Control	Exp.	Difference, %
NH_2-	7.5	12	1.18 ± .03	1.08 ± .04	− 8*
Nothing	7.5	6	1.18 ± .05	1.35 ± .08	+ 14*
CH_3-O-	7.5	6	1.01 ± .08	1.22 ± .07	+ 21*
COOH-	7.5	9	1.39 ± .11	1.42 ± .05	+ 2
CH_3-	7.5	12	1.33 ± .06	3.75 ± .34	+182†
	10	15	1.31 ± .04	6.65 ± .32	+408†
CH_3-CH_2-	2.25	6	1.15 ± .06	5.15 ± .69	+348†
$(CH_3)_2$-CH-	.90	6	1.19 ± .08	3.97 ± .34	+234†
$(CH_3)_3$-C-	.54	6	1.26 ± .07	4.56 ± .20	+262†

* Significant at 5% level. † Significant at 0.1% level.

muscles dissected from legs of the Leopard frog, *Rana pipiens* Schreber, was measured in a Warburg respirometer at 20.0 ± 0.02°C over 6 hours as described previously(3). The iliofibularis and tibialis anticus longus muscles from one leg were placed together in Ringer's solution to serve as controls while the 2 corresponding muscles from the opposite leg of same animal were placed in Ringer's solution in which the compound under study was dissolved as sodium salt.

Results. Increased rate of oxygen consumption caused by tolbutamide is considered from previous work(2) to be due to oxidation of excess lactate. The lactate appearing in muscle and later in medium results from increased breakdown of muscle glycogen. Muscle glycogen and concentration of lactate in the medium at end of incubation period were also determined routinely, and increased oxygen consumption coincided with decreased glycogen and increased lactate in the muscle. Since measurement of oxygen consumption rate provides an accurate and sensitive measurement of the primary effect of the compounds, only these data are presented.

The results are summarized in Table I in which average hourly rate of oxygen consumption is given in μmoles/g wet weight of tissue. The molecular group substituted in the R position of the sulfonylbutylurea molecule in Fig. 1 is listed in the first column. The data obtained previously(2) with carbutamide (R = NH_2) and tolbutamide (R = CH_3) are included for comparison. At a concentration of 7.5 mM tolbutamide rate of oxygen consumption rose within 3 hours to an average of 182% of control level, and this new rate was maintained for the remainder of the 6-hour incubation period. In 10 mM tolbutamide oxygen consumption stimulation was much greater but concentrations of tolbutamide higher than 10 mM were deleterious to muscles, and the increased rates of oxygen consumption were not maintained throughout period of observation. Therefore, comparison of effects of different compounds on oxygen consumption was made at concentrations of 7.5 mM or less.

The sulfonylbutylurea molecule with no substitution at position R increased oxygen consumption rate only slightly compared with tolbutamide at the same concentration. Similarly, when a methyl group is connected to the molecule through oxygen (R = CH_3-O) instead of directly as in tolbutamide, or when a carboxyl group is connected to the molecule (R = COOH), the effect on oxygen consumption is relatively small or absent. The negative result with the carboxyl analog (1-butyl-3-*p*-carboxyphenylsulfonylurea) is of interest because Louis, Fajan, Conn, Struck, Wright and Johnson(4) found that this compound is the principal metabolic product formed when tolbutamide is administered to humans. The carboxyl analog does not produce hypoglycemia in dogs, rats or humans. However it cannot be concluded that the effect of tolbutamide on frog muscle metabolism is related to its hypoglycemic effect *in vivo,* since carbutamide had no effect on frog muscle glycogen (by direct analysis) although it, too, is an effective hypoglycemic agent.

The compound with an ethyl group in the

R position of Fig. 1 is very effective in stimulating oxygen consumption. In fact, to avoid deleterious effects to the muscle, the concentration of this compound had to be lowered to 2.25 mM. The effectiveness of the molecule increased when the substituted group was changed to either isopropyl or tertiary butyl and the concentrations of these compounds also had to be decreased to avoid deleterious effects to the muscle. By making rough approximations from the data in Table I the relative potencies of the 4 effective substitutions on the sulfonylbutylurea molecule are estimated to be: methyl, 1; ethyl, 4; isopropyl, 8; tertiary butyl, 16. The relative effect of these compounds on blood glucose level of frogs is not known.

It has been known for many years that caffeine, like tolbutamide, stimulates glycolysis in frog muscle with consequent lactate accumulation and extra oxygen consumption(5, 6). Fenn(7) concluded that oxygen consumption stimulation was correlated with contracture of the muscle caused by caffeine. To determine whether tolbutamide is a contracture-producing agent like caffeine, experiments were done in which a tibialis anticus muscle was suspended in 7.5 mM tolbutamide-Ringer's solution from a carefully balanced heart lever to magnify any change in length of muscle. The opposite muscle from the same frog was similarly suspended in Ringer's solution. Muscle length was recorded at beginning of immersion and at intervals for 6 hours and no change in length was observed in either solution. This simple experiment strongly suggests that the metabolic activity initiated by the effective sulfonylbutylurea compounds occurs without mechanical change in muscle, and in this respect their action differs from that of caffeine. There is evidence that increased metabolic activity of frog muscle in a concentration of 10 mM potassium also occurs without accompanying contracture(8).

It is not known whether the effect of the caffeine molecule on oxygen consumption of frog muscle is related to addition of the methyl groups to the xanthine nucleus. However, recalculation of Gemmill's data(9) on the effect of l-substituted theobromine derivatives on oxygen consumption of rat diaphragm muscle indicates that, in general, as the carbon chain attached to theobromine increased, the molar concentration required to stimulate the oxygen consumption by about 30% decreased. Thus it appears that addition of methyl groups in a chain to theobromine also increases the effectiveness of this compound in stimulating muscle metabolism.

Summary. The effect of sulfonylbutylurea derivatives on frog muscle metabolism depends upon the molecular group substituted on the benzene ring *para* to the main chain of the molecule. Substitution of an amino group produces an inhibitory compound; absence of any group or substitution of either a methoxy or a carboxyl radical produces a compound with little or no effect. Substitution of a methyl, ethyl, isopropyl or tertiary butyl radical produces compounds that are increasingly effective in stimulating glycolysis and oxygen consumption. These effects are similar to those caused in frog muscle by caffeine although, unlike caffeine, the sulfonylbutylurea derivatives apparently exert their effects without causing contracture.

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