Fourteen-day Administration of Carbutamide, Tolbutamide and Cortisone

Effects on Metabolism of Rat Liver and Diaphragm

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Much of the recent work on the effects of oral hypoglycemic agents has involved measurements in animals after a brief period of treatment with the drug. In general, it has been concluded that there is no direct effect of these drugs in enhancing glucose utilization of the peripheral tissues. Studies on the isolated rat diaphragm have confirmed this conclusion, since added sulfonylurea did not enhance the glucose uptake or glycogen synthesis of that muscle in vitro.[1-3] Studies in vitro of liver slices also have suggested that the sulfonylureas exert an inhibiting effect upon glycogenolysis[1,4] though under certain conditions tolbutamide seemed to affect only the potentiating effect upon glycogenolysis of epinephrine or of glucagon.[5] Some workers have felt that the levels of drug required to give an in vitro effect are so high that this effect cannot be considered of physiological significance.[6]

We have studied certain changes resulting from a more prolonged administration of carbutamide or of tolbutamide in the rat. A previous report by Root[7] indicated that if tolbutamide were given for a period of time, rather than as a single dose, there was a reduction in the extractable insulin of the pancreas, a reduction in liver glycogen, and in some cases a fall in muscle glycogen. Root interpreted these results with caution, since but few animals were used, and some of the treated animals were in poor condition. Candela and Candela have reported that carbutamide, given orally to rats four hours before sacrifice, caused an increased glucose uptake of the diaphragm[8] but that the stimulating effect of added insulin was unaffected.[9] Miller, Krake and Van der Brook also have shown that six hours after the administration of tolbutamide, there is an increased incorporation of glucose in muscle glycogen.[10]

We have found that glycogen levels in the rat diaphragm were significantly increased by carbutamide administration over a period of time. This may conceivably be a consequence of increased plasma insulin activity. Liver glycogen values were not significantly changed as a result of carbutamide administration. Liver fat was increased by cortisone. Tolbutamide, in general, did not seem to exert any effect on glycogen levels in liver or diaphragm. This may be a consequence of not giving enough tolbutamide, though the doses of tolbutamide and of carbutamide were the same on a weight basis, and very nearly the same on the basis of molecular weight.

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MATERIALS AND METHODS

A group of fifty-four male Wistar rats, weighing 200-225 gm., was divided into six equal subgroups. Each group was given a daily subcutaneous injection for fourteen to fifteen days, as shown below:

Group 1—1.0 ml. saline (control group).
Group 2—50 mg. carbutamide (approx. 250 mg./kilo body weight).
Group 3—50 mg. carbutamide plus 10 mg. cortisone.
Group 4—50 mg. tolbutamide (approx. 250 mg./kilo body weight).
Group 5—50 mg. tolbutamide plus 10 mg. cortisone.
Group 6—10 mg. cortisone (approx. 50 mg./kilo body weight).

The cortisone was given as 0.4 ml. of Merck's "Cortone," containing 25 mg. cortisone acetate per ml. Carbutamide and tolbutamide were dissolved in alkaline solution to give a 4 per cent solution. When solution was completed, the *p*H was adjusted to 7.5 with hydrochloric acid. For one experiment, one animal in each group was used, so that a total of nine replicate experiments were performed on nine different days.

At the end of the injection period, the animals were fasted for twenty hours. Fasting increases the glucose uptake and the glycogen synthesis in the isolated diaphragm, in the absence of insulin, and seems to yield results with a somewhat smaller spread of values.[11] The animals were then decapitated and tissues were removed for the various studies. In some of the animals, a portion of the liver was weighed and used for a determination of the nitrogen by the micro-Kjeldahl method. Two other

portions were rapidly weighed, and dropped into hot KOH for a determination of liver glycogen according to the method of Good, Kramer and Somogyi.[12] The remainder of the liver was used for a determination of the liver fat, according to the method of Best, Lucas, Patterson and Ridout.[13] The diaphragm was removed and divided into three portions. One portion, which usually weighed between 70 and 90 mg., was used for a determination of the initial glycogen content. The other two, which usually weighed between 100 and 120 mg., were incubated in a Gey-Gey medium,[14] with 200 mg. per cent added glucose. (One portion acted as a control, the other was incubated with added insulin, 1 unit/ml.). Tissues were placed in 10 ml. of medium in 25 ml. flasks, and were shaken in air, at 37° C., in a Dubnoff shaker for one hour. The tissues were then removed, blotted and put into hot KOH for a determination of glycogen. One ml. aliquots were removed from the incubation media for the determination of the glucose concentration, according to the method of Somogyi.[15]

From the "final glycogen" values obtained in the tissues after incubation, the "initial glycogen" values were subtracted, and these are the values shown as "glycogen difference" in table 2. A positive value indicates that there was net glycogen synthesis, a negative value indicates that the process of glycogen breakdown predominated over those processes leading to glycogen synthesis. The "insulin effect" on either glycogen synthesis or glucose uptake is the difference between values obtained with hemidiaphragm incubated in a medium with added insulin, and values obtained with a diaphragm incubated in a medium without added insulin.

RESULTS

The results of the liver analyses are seen in table 1. Cortisone increased the liver fat, but there was no significant effect of either carbutamide or of tolbutamide on liver fat. The percentage of nitrogen in the liver was unaltered by any of the other treatments. Cortisone increased liver glycogen, but the other drugs did not have any significant effect.

The results from the analyses of the diaphragms are

TABLE 1
Results of liver analyses

	Control	Carbutamide	Tolbutamide	Cortisone	Carbut. + Cortisone	Tolbut. + Cortisone
Glycogen (mg./gm.)	1.78± 0.50	1.74 ±.11	1.75 ±.26	16.90 ±2.51	15.20 ±1.40	18.89 ±1.93
Nitrogen (mg./gm.)	38.4 ±.84	37.1 ±.29	37.1 ±.39	38.2 ±1.00	37.5 ±.51	37.1 ±1.43
Fat (per cent dried fat-free residue)	32.1 ±1.18	34.6 ±1.54	35.0 ±1.39	42.1 ±3.74	49.2 ±6.40	42.5 ±2.38

TABLE 2
Results of diaphragm analysis

	Control	Carbutamide	Tolbutamide	Cortisone	Carbut. + Cortisone	Tolbut. + Cortisone
1. Initial glycogen (mg./gm.)	0.88	1.15	1.12	1.87	2.33	1.93
2. Glycogen difference (mg./gm.)	0.25	—0.01	0.10	—0.53	—1.10	—0.67
3. Insulin effect on glycogen synthesis (mg./gm.)	0.47	0.72	0.68	0.61	0.93	0.86
4. Glucose uptake without insulin (mg./gm.)	5.23	5.44	4.49	5.24	5.52	5.10
5. Insulin effect on glucose uptake (mg./gm.)	1.72	1.37	2.14	2.01	1.32	1.68

shown in tables 2 and 3. Average results are shown in table 2, and table 3 gives a summary of the results of the statistical procedures which were applied to the experimentally determined values.

The design and analysis of the experiment is in the form of a simple split-plot experiment.[16] The significance of the effect of the cortisone treatment may be obtained directly from the table of the analysis of variance by dividing the mean square for cortisone treatment by the mean square for error (a). The significance of the effect of tolbutamide is obtained by finding the difference between the mean values obtained from all animals treated with tolbutamide and the mean values from animals whose treatment was similar, except that they were not treated with tolbutamide. This difference is compared with an estimate of the error of the determination, as in a "t" test. The significance of the effect of carbutamide is similarly calculated.

In line 1 of table 2 there are shown the amounts of glycogen in portions of diaphragm which were removed immediately after sacrifice of the rat. The statistical analysis of the results (see table 3) shows that pretreatment with carbutamide or with cortisone significantly increased the initial concentration of muscle glycogen. No such effect was seen from the pretreatment with tolbutamide.

In line 2 of table 2 are seen the "Glycogen Difference" values. In general, when there has been a high initial glycogen, the "Glycogen Difference" values are small and may even be negative. Figure 1 shows the relationship between glycogen differences and initial glycogen levels in the diaphragm.

The statistical analysis shows that pretreatment with cortisone or carbutamide significantly decreased the "Glycogen Difference." Tolbutamide had no significant effect. It is felt that the changes are probably reflections of the different initial glycogen levels, as indicated above.

In line 3 of table 2 the amounts of additional glycogen synthesized as a result of the presence of insulin in the medium are recorded. Cortisone, tolbutamide and carbutamide all significantly increased the insulin effect upon glycogen synthesis.

TABLE 3

Summary of results of statistical analysis according to split plot design

		Initial glycogen		Glycogen difference		Insulin effect on glycogen difference		Glucose uptake		Insulin effect on glucose uptake	
	d.f.	Mean square	F.	M.S.	F.	M.S.	F.	M.S.	F.	M.S.	F.
Replications	8	1.16	3.14	1.04	3.38	0.27	16.9*	14.1	12.6*	1.33	2.77
Cortisone treatment	1	13.25	35.8*	10.48	34.0*	0.42	26.1*	0.73	<1	0.08	<1
Error (a)	8	0.37		0.31		0.02		1.12		0.48	
Hypoglycemic agent treatment	2	0.63	3.30*	0.80	4.7*	0.40	3.92*	2.18	<2	1.78	<1
Interaction (agent × cortisone)	2	0.14	<1	0.13	<1	0.01	<1	0.49	<1	1.49	<1
Error (b)	32	0.19		0.17		0.10		1.16		1.76	
Standard error of difference between two treatment means for hypoglycemic agents		0.146		0.138		0.106					
Mean value (no hypoglycemic agent)		1.37		—0.14		0.54		Detailed analysis not performed since results above show there was no significant effect of hypoglycemic agents.			
Mean value (with tolbutamide)		1.52		—0.29		0.82					
t		0.15/0.15=1.0		0.15/0.14=1.08		0.28/0.106=2.64*					
Mean value (with carbutamide)		1.75		—0.55		0.77					
t		0.38/0.15=2.53*		0.41/0.14=2.97*		0.23/0.106=2.15					

*Results are significant at 5 per cent level or better.
Error (a) is used to test significance of "Replications" and of "Cortisone Treatment."
Error (b) is used to test significance of "Hypoglycemic Agent Treatment" and of "Interaction (Agent × Cortisone)."
"F" values less than 2 are not significant.

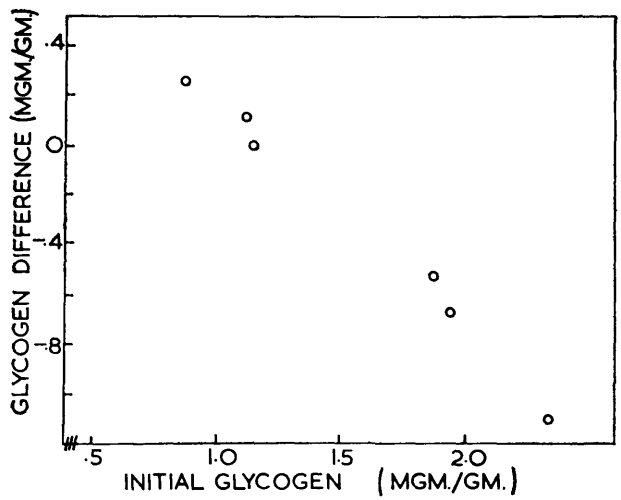

FIGURE 1

The glucose uptake of the diaphragm, as shown in line 4 of table 2 was not significantly altered by any of the treatments. Similar conclusions were reached regarding the insulin effect upon glucose uptake, as seen in the bottom line of table 2.

DISCUSSION

The increase in glycogen in the diaphragm caused by the pretreatment with carbutamide is consistent with the hypothesis of an increased effective insulin concentration in the plasma. This increase might come about through an increased secretion by the pancreas, or through a decreased activity of a plasma insulinase[17] or a decreased activity of a plasma insulin inhibitor. It would be expected that there might be an increased glucose uptake, but the experimental values do not show any such effect. There is no significant effect of pretreatment upon the insulin effect on glucose uptake, though the figures may suggest a reduction. Such a reduction would agree with the results reported by Field[3,18] for somewhat different experimental conditions. It may be, therefore, that carbutamide has no effect on a "basic" glucose uptake of the diaphragm, untreated by insulin, but that there is an inhibitory action of this compound upon the stimulating effect of insulin. This possible inhibition of the effect of insulin upon glucose uptake is difficult to reconcile with the significant increase in the insulin effect upon glycogen synthesis following carbutamide pretreatment, unless this agent favors those reactions leading to glycogen synthesis, relative to those which lead to glycolysis.

There is no significant effect of the hypoglycemic agents upon liver fat, though there does seem to be a suggestive trend in the figures. The direction of the trend is such as to be compatible with increased insulin activity. The effect of cortisone is highly significant, and is probably a consequence of the fat mobilizing action of the steroid. The amounts of nitrogen and of glycogen in the liver are unaffected by carbutamide or by tolbutamide, though cortisone gives the expected increase in glycogen. The lack of effect of hypoglycemic agents upon liver glycogen is in contrast to the decrease reported by Root[7] after administration of carbutamide to dogs, and the increase reported by Miller and Dulin[17] after administration of tolbutamide to rats.

In no cases did the cortisone seem to affect the action of the hypoglycemic agents, i.e., they seemed to act quite independently.

SUMMARY

1. The effects of subcutaneous administration of carbutamide and tolbutamide on certain aspects of carbohydrate metabolism in the rat have been studied.

2. The effect of cortisone upon the action of the above agents has been investigated.

3. Administration of carbutamide causes an increase in muscle glycogen. Net glycogen synthesis in the muscle after incubation in vitro in a glucose containing medium is reduced after treatment with carbutamide.

4. Liver glycogen, liver nitrogen and liver fat are not significantly altered by carbutamide treatment.

5. Cortisone caused an increased liver glycogen and increased liver fat.

6. Tolbutamide, in the dose levels given, only affected the insulin effect upon glycogen synthesis.

7. These results are consistent with the hypothesis that one of the effects of carbutamide is to cause an increased insulin activity in the plasma. It may be that there is an additional effect which tends to reduce the magnitude of the effect of this additional insulin upon the glucose uptake of the diaphragm.

SUMMARIO IN INTERLINGUA

Le Administration, Durante Dece-Quatro Dies, De Carbutamido, Tolbutamido, E Cortisona: Effectos Super Le Metabolismo Del Hepate E Diaphragma De Rattos

1. Esseva studiate le effectos del administration subcutanee de carbutamido e tolbutamido super certe aspectos del metabolismo de hydratos de carbon in le ratto.

2. Le effecto de cortisona super le action del supramentionate agentes esseva investigate.

3. Le administration de carbutamido causa un augmento de glycogeno muscular. Le nette synthese de glycogeno que occurre in le musculo post incubation in vitro in un medio que contine glucosa es reducite post

le tractamento con carbutamido.

4. Le glycogeno del hepate, le nitrogeno del hepate, e le grassia del hepate non es alterate significativemente per le tractamento con carbutamido.

5. Cortisona causava un augmento del glycogeno del hepate e del grassia del hepate.

6. In le doses usate, tolbutamido afficeva solmente le effecto de insulina super le synthese de glycogeno.

7. Iste resultatos concorda con le hypothese que un del effectos de carbutamido es le causation de un augmentate activitate de insulina in le plasma. Il es possibile que il existe un effecto additional que tende a reducer le magnitude del effecto de iste insulina additional super le acceptation de glucosa per le diaphragma.

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Human Experimentation

Biologic and medical investigation is accepted as essential to progress in health. Recent advances in the prevention, cure and treatment of diseases attest to the power and value of research. Today, the public and the professions alike agree that medical research must precede and then succeed practice to provide a basis for action and an evaluation of its worth. Such research, frequently in the most critical areas, takes the form of experimentation on human beings.

Development of standard diagnosis and treatment calls first for trial of novel methods. Ultimately, every innovation intended to benefit mankind must be tried on a human being—perhaps on thousands, as witness the trials with the Salk and the announced Sabin poliomyelitis vaccine—before it may be considered acceptable practice or procedure. Properly conducted experimentation by qualified scientists must therefore be considered an integral branch of biologic and medical science, but it does not thereby become customary medical practice. Nor does its essentiality and acceptance establish clearly its character or place the methods employed beyond scrutiny. The responsible professions have a duty to delineate for their own members and for a critically vigilant public the nature of medical research and the limits within which it may be properly undertaken.

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