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Validity of Chemical Balance Studies in Eviscerated Animals, as Index of Carbohydrate Utilization.

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We have recently employed the chemical balance method in the abdominally eviscerated animal, to study the utilization of carbohydrate by the peripheral tissues of dogs under the following conditions (a) normal and depancreatized;[1] (b) normals treated with insulin;[2,3] (c) normals treated with an anterior pituitary extract;[4] (d) hypophysectomized;[4,5] (e) phlorhidzinized.[6] The chemical balance was struck from the blood sugar, blood lactic acid and the muscle glycogen values at the beginning and end of the experiments; and from the amount of sugar administered during the experiment. The calculations from these data were based upon distribution ratios reported by others, namely, 1/6 of the body weight for sugar and lactic acid[7,8] and 1/2 of the body weight for muscle glycogen.[9,10]

There are 2 possible objections to the manner in which we calculated the utilization of carbohydrate by the muscles: (1) The possible presence of significant amounts of free sugar or of higher carbohydrate intermediates in the muscles, which would not be taken into account by glycogen estimations alone. (2) The possible presence of amounts of lactic acid in the muscles, which are not in equilibrium with the blood values. We have therefore repeated utilization experiments on eviscerated normal and depancreatized

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NORMAL DOG, EXP. 1.

Wt 11.4 kg. Exp. period 4 hr. Avg blood sugar throughout exp. was 92 mg%. Muscle mass calculated as 50% of body wt[9] equals 5700 g. In calculation (A) the blood and extracellular fluid is taken as 1/6 of the body wt,[7,8] equal to 1900 cc. In calculation (B), the blood and extracellular fluid is considered equal to 30% of body wt.[13,14] Blood volume equals 7%. Extracellular fluid of muscle (30 — 7)/2 equals 11.5%. Blood plus non-muscle extracellular fluid equals 18.5%, or 2109 cc.

Calculation (A)		Calculation (B)
	Initial total CHO of muscle 449.9 mg%	
	Final " " " " 290.9 "	
	Difference 159.0 "	
$\frac{159 \times 5700}{100} = 9063$ mg	Total CHO decrease	$\frac{159 \times 5700}{100} = 9063$ mg
	Initial blood sugar 84.0 "	
	Final " " 122.0 "	
	Diff. 38.0 "	
$\frac{38 \times 1900}{100} = 722$ mg	Increase in extracellular sugar	$\frac{38 \times 2109}{100} = 802$ "
	Initial blood lactic acid 32.4 "	
	Final " " " 129.0 "	
	Diff. 96.6 "	
$\frac{96.6 \times 1900}{100} = 1835$ mg	Increase in extracellular lactic acid	$\frac{96.6 \times 2109}{100} = 2038$ "
	Initial muscle lactic acid 37.4 "	
	Final " " " 72.6 "	
	Diff. 35.2 "	
	Increase in muscle lactic acid	$\frac{35.2 \times 5700}{100} = 2006$ "
5455 mg	Dextrose injected during experiment	5455 "
(9063 + 5455) = 14518 "	Total sugar disposed of	(9063 + 5455) = 14518 "
722 + 1835) = 2557 "	Total retained sugar	(802 + 2038 + 2006) = 4846 "
(14518 — 2557) = 11961 "	Sugar utilized	(14518 — 4846) = 9672 "
$\frac{11961}{11.4 \times 4} = 262$ mg	Sugar utilized per kg original body wt per hr	$\frac{9672}{11.4 \times 4} = 212$ "

TABLE I.

Summary of Data for Alternative Calculations of Carbohydrate Utilization by Eviscerated Normal and Depancreatized Dogs Respectively.

Dog	No.	Wt, kg	Duration of exp.		Total sugar injected, g	Blood sugar			Blood lactic acid		Total CHO in muscle		Lactic acid in muscle	
			Hr	Min		Initial, mg%	Final, mg%	Avg, mg%	Initial, mg%	Final, mg%	Initial, mg%	Final, mg%	Initial, mg%	Final, mg%
Norm.	1	11.4	4	0	5.46	84	122	92	32.4	129.0	449.9	290.3	37.4	72.6
”	2	9.7	4	0	6.97	236	277	262	47.2	125.0	461.7	264.0	12.9	56.3
”	3	9.4	3	0	9.50	474	675	574	32.4	61.0	581.5	399.5	25.0	34.5
Dep.	4	8.4	3	0	1.60	205	175	193	51.2	111.6	470.0	392.0	97.0	98.0
”	5	12.5	3	0	14.90	337	646	506	132.8	160.0	431.6	387.3	154.8	102.2
”	6	7.4	2	40	1.59	390	392	403	77.0	204.0	443.0	207.0	54.2	148.6

dogs respectively, in which we have determined the total carbohydrate content of the muscle instead of muscle glycogen, and the lactic acid content of the muscle as well as blood lactic acid. Our results by these methods are presented, and are compared with our previous results.

Methods and Results. The details regarding the technic of these experiments and the chemical methods employed were described in a previous publication.[1] The total carbohydrate estimations in the present work were made by the method of Tsai, as modified by Benoy and Elliott.[11] The blood and muscle lactic acids were determined by a slight modification of the method of Miller and Muntz.[12]

The data are summarized in Table I. Calculations of utilization from these data were made in 2 ways: (A) depending on the distribution ratios used in our previous work, (B) treating blood and muscle separately. These 2 types of calculation are exemplified in parallel, for animal No. 1.

It may be seen that the results of the above calculations, although they show some difference, are of the same order of magnitude. Fig. 1 graphically represents the results of both calculations for all our present animals, and offers a comparison with our previously published results and calculations. The present methods of calculating introduce only a slight and consistent difference in the data as a whole. It is understood, of course, that none of the above methods can be held to yield absolute utilization values. But it is equally evident that any of them give good comparative results.

Discussion and Summary. It is clear that neither of the possible objections to our previous method of calculating carbohydrate utilization is sufficiently valid to materially affect the end results. That is, the amounts of free sugar, higher carbohydrate intermediates, or lactic acid present in the muscles are not such as to invalidate calculations based on blood sugar and lactic acid values, and distribution ratios. This agrees with the earlier, basic work of Best, Dale, Hoet and Marks,[9] who demonstrated that the sugar which disappeared from the blood of eviscerated spinal cats was equal to the sum of the glycogen deposited in the muscles and the glucose equivalent of the oxygen consumed.

The close correspondence of utilization rates, as determined by

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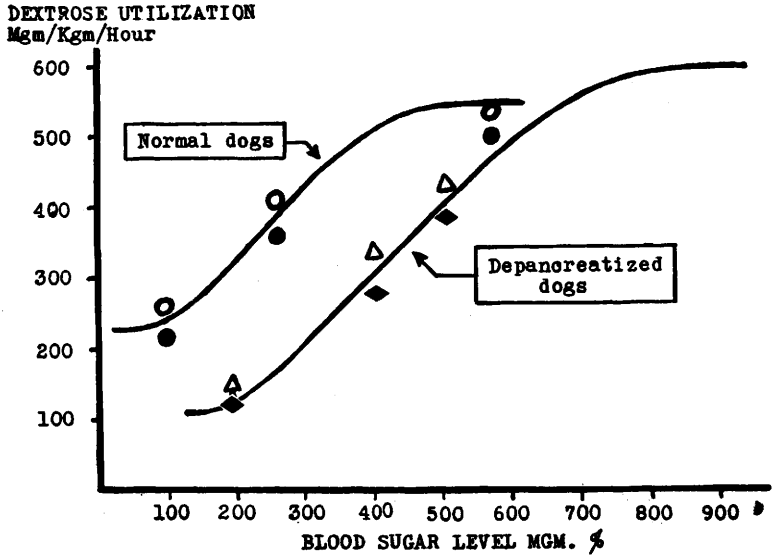

FIG. 1.

Comparison of Carbohydrate Utilization by Normal and Depancreatized Dogs by Different Calculations.

The plotted points represent the results of the present work and calculations, and are *not* the points through which the smooth curves are drawn. The curves are included for comparison, and are derived from previous work[1] on the rates of sugar utilization by the extra-hepatic tissues of normal and depancreatized dogs, respectively.

○ Normal dogs, calculation (A).
● Normal dogs, calculation (B).
△ Depancreatized dogs, calculation (A).
◆ Depancreatized dogs, calculation (B).

different methods and by different calculations, strongly supports the validity of the chemical balance method as a means of determining carbohydrate utilization in liverless animals.