INFLUENCE OF CALCIUM ON CARBOHYDRATE METABOLISM.

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Extensive studies on the calcium metabolism of body, particularly from the point of view of explaining the cause of ricket, tetany, and other diseases have been made. But so far as the author knows very little study has been made on the influence of calcium on carbohydrate metabolism regarding diabetes. The present investigation is undertaken on this point with a connection with diabetes.

It is known that a normal adult needs more than 0.5 g. of calcium per day. Pregnant women and nursing mothers require a greater amount. It is believed that an average daily intake of calcium per capita exceeds

0.5 g. However only a small amount of calcium thus taken in is absorbed through the upper intestine while the rest is excreted mostly in the feces and partly in the urine as its salts.[1] The absorption depends upon its form in the food, or the degree of its solubility of the state.

Acid-base Balance. The balance of calcium content in the blood serum is ordinarily disturbed little in mild diabetes, but in the severe case with ketosis as well as in severe malnutrition, the amount of calcium in the serum is slightly lowered.[2] In these conditions the calcium deficiencies in the blood serum may be associated with low serum proteins. The most interesting symptom in diabetes is the acidosis which is commonly developed as a result of retention of ketonic acid, and is formed by the incomplete combustion of fats. Accordingly the CO_2 content in the plasma is lowered from 45–55 to somewhere 35–45 volume per cent.[3] with the fall of the *p*H before the symptoms appear (Table 1). In severe cases the whole electrolyte in the blood is strongly affected,[3][4] for example in the terminal condition of the diabetes the *p*H in the plasma falls below 7.1 and, the CO_2 content likewise becomes somewhere below 15 volume per cent. In addition to the fall of the *p*H and CO_2 content in the plasma there may also be a decrease in the content of Cl while the glycemia and glycosuria increase with an increase of the blood diastatic activity (Table 1).

Effect of Insulin Injection. After the injection of insulin, the ketosis and glycemia may be reduced with a transitory increase of the serum calcium[5] and the blood lactic acid,[6] while inorganic phosphorus in the serum decreases[7] either directly or indirectly. Accordingly the blood bicarbonate may likewise rise in this instance. This opposite effect is observed after adrenalin injection.[8]

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Insulin Substitutes and their Actions. If insulin is taken by mouth there is little influence on the fall of glucose in the blood and urine. For this reason, recently various substitutes for insulin, which can be taken by mouth, have been proposed and tried. Among these an extract of huckleberry leaves[9] and guanidine derivatives[10][11] may be mentioned. Allen claims that the extract of huckleberry leaves reduces both hyperglycemia and glycosuria without any toxic action. Guanidine derivatives also have a distinct reducing effect on the hyperglycemia and glycosuria with an increase of the calcium in the serum,[12] but they have deleterious effect upon the livers and kidneys.[10] Both hydrazine derivatives[13] and sodium selenite[14] have actions similar to guanidine compounds, but the former is known to be injurious to the livers.

It is presumed that all these substitutes which are claimed by some to have therapeutic actions on diabetes mellitus, have no specific actions upon the disease, but they elevate the calcium content of the blood serum, and thereby both hyperglycemia and glycosuria may be reduced, either by the stimulation of calcium metabolism or by the decalcification of calcium stored in the body. If so, the parathyroid gland might play an important rôle on diabetes, since it controls the calcium metabolism of the body. The extract of parathyroid gland is effective on the metabolism, either when given by mouth or by intravenous injection, but an over-dose of the extract causes a negative calcium balance[15]. Therefore, the calcium for the metabolism must be supplied by intake of sufficient calcium in the form of food or of calcium compound. The commonest food to supply calcium is milk which contains about 1.2 g. per liter.

Food as a Cause of Diabetes. It is doubtful that all diabetes is due to disease of pancreas (the Islands of Langerhan) since there are no definite evidences given by either chemical or pathologic studies of the

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(14) V. E. Levine and R. A. Flaherty, *Proc. Soc. Exp. Biol. Med.*, **24** (1926), 251.

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gland. It seems that dietary in-balance is one of the most important factors causing diabetic acidosis. Whether the food has an acidifying or alkalinizing effect depends upon the amount of protein and unoxidizable acid or upon the amount of alkaline elements in the food.[16] Meat and other foods consisting largely of protein have acidifying effects. It is shown by Hasselbalch[17] that a change from a high carbohydrate to a high protein diet causes the fall of CO_2 capacity of blood about three per cent. by volume. Therefore, it seems that the high protein diet rather than high carbohydrate is responsible for a cause of diabetes.[18] If so, there might exist an important relation between the calcium, protein, and the carbohydrate in food, which prevents the causing of diabetes.

The author is interested only in the calcium compounds, which could increase the serum calcium by oral administration without accompanying acidosis or other toxic action, in order to carry out the present experiment.

Effect of Calcium Salts on the Serum. Inorganic calcium compounds such as $CaCl_2$[19] and $CaSO_4$ cause acidosis by selective excretion but not selective absorption. However, oral administration of calcium lactate does not cause acidosis because of its organic radical, and when absorbed, it is burned in the organism. After an oral administration of 5 g. of calcium lactate there is a rise of 5–14 per cent. of calcium in the serum[20] and keeps the elevation above normal level long as for 12 hours. However the increase of the amount of calcium appears to lengthen the duration rather than to elevate the calcium content.[21] Calcium lactate is a neutral drug and has no toxic action. The influence of orally administered calcium salts on the serum calcium content have been studied by Hjört.[22] The following order is given according to the degree of its absorption power or of its solubility:

$$CaCl_2 > CaFeC_9H_9O_{10}{}^{[23]}\,(C_6H_5O_7Fe + Ca(C_3H_5O_3)_2 = C_6H_5O_7Fe\cdot Ca\,C_3H_4O_3 + C_3H_6O_3) > Ca(C_3H_5O_3)_2 > Ca\,PO_4C_3H_5(OH)_2 > CaCO_3\,.$$

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(22) A. M. Hjört, *J. Biol. Chem.*, **65** (1925), 783.
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Animal Experiment. Experiment 1. The influence of calcium lactate on the glucose tolerance in rabbits: 200 c.c. of 37.5% glucose aqueous solution was made by aid of heat, boiled for a few minutes, and the solution was divided into two equal parts. To one part 2.5 g. of calcium lactate was dissolved while it was hot. Both solutions were boiled again for a couple of minutes under same condition and cooled down to room temperature; then the solutions were made up to 100 c.c. with sterilized distilled water.

The urine in the bladder of rabbits were first evacuated by means of a catheter, and the rabbits were allowed to fast for 24 hours and at the end of that period the urine was collected by the same means as before for the control.

Then 20 c.c. of the glucose solution was injected to one rabbit in the peritonial cavity and the glucose-calcium-lactate solution to the other. While they were kept in this condition, the blood was taken out first one half hour then one hour intervals for four hours and also at the end of twenty four hours from the veins of each rabbit.

On the urine collected at the end of twenty four hours the sugar content was determined by the method of Benedict on one hand, and the sugar content in the blood was determined by Jeghers-Myer's modification of Folin's micro-method[24] on the other. The results are tabulated in Table 2 and 3.

Experiment 2. The influence of calcium lactate on blood sugar which should be elevated by the injection of adrenalin in rabbits was also studied in same manner as Experiment 1. The injection was made as follows: One with 0.2 c.c. of adrenalin (1:1000 dilution) only as a control, and the other with the same amount of adrenalin which was followed immediately after the injection of 20 c.c. of 2.5 per cent. calcium lactate solution. The results are given in Table 4 and 5.

Experiment 3. The influence of adrenalin-glucose (Table 6-A and 7-C) of adrenalin-glucose-calcium-lactate (Table 6-B and 7-B), and of adrenalin-calcium-lactate (Table 7-A) on the glycemia and the glycosuria were studied and compared as described in Experiment 1 and 2. However in this experiment the concentration of glucose-calcium-lactate, and calcium-lactate were reduced to one half with sterilized distilled water.

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Table 1.

Condition	Volume % plasma with CO_2 reduced to 0°., 760 mm.[25]	Diastatic units[25]	24 hr. excretion of 0.1 N acid NH_3 for 60 kg.[26] (c.c.)	*p*H of plasma corresponding to CO_2 combing power[27]	Blood sugar[25] (%)	$NaHCO_3$ required to turn urine alkaline for 60 kg.[26] (g.)
Normal resting adult	75—55	15—20	0—1600	7.51—7.40	0.09—0.12	0—30
Mild diabetic acidosis	55—45	20—40	1600—4000	7.40—7.34	0.15—0.30	30—50
Moderate diabetic acidosis	45—35		4000—6000	7.34—7.28		50—65
Severe diabetic acidosis	below 35	35—75	over 6000	below 7.28	0.30—1.20	over 65

Table 2. A.

♂ Wt.: 2015 g. Glucose (15 g.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.1063	0.1617	0.1894	0.2188	0.1457	0.1169	0.1111
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	37 c.c., 0.250%, 0.093 g.			33 c.c., 1.736%, 0.573 g.			

B.

♂ Wt.: 2125 g. Glucose (15 g.) and calcium lactate (0.5 g.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.0968	0.1530	0.1546	0.1578	0.1457	0.1071	0.1111
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	50 c.c., 0.250%, 0.125 g.			37 c.c., 0.592%, 0.219 g.			

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Table 3. A.

♂ Wt.: 2125 g. Glucose (15 g.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.1095	0.1578	0.2205	0.2.05	0.1704	0.1119	0.0920
Urine sugar	for 24 h. before treament			24 h. after treatment			
	40 c.c., 0.250%, 0.100 g.			60 c.c., 2.000%, 1.200 g.			

B.

♂ Wt.: 1800 g. Glucose (15 g.) and calcium lactate (0.5 g.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.1034	0.1471	0.1578	0.1578	0.1500	0.1234	0.1127
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	23 c.c., 0.266%, 0.061 g.			42 c.c., 1.407%, 0.591 g.			

Table 4. A.

♂ Wt.: 2040 g. Adrenalin (0.2 c.c.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.1127	0.1428	0.1851	0.1327	0.1250	0.1136	0.1127
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	115 c.c., 0.182%, 0.209 g.			60 c.c., 0.388%, 0.233 g.			

B.

♂ Wt.: 1990 g. Adrenalin (0.2 c.c.) and calcium lactate (0.5 g.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.0938	0.1250	0.1281	0.1851	0.1472	0.1270	0.1000
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	95 c.c., 0.216%, 0.206 g.			45 c.c., 0.529%, 0.238 g.			

Table 5. A.

♂ Wt.: 2050 g. Adrenalin (0.2 c.c.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.0904	0.1327	0.1744	0.1530	0.1875	0.1630	0.1127
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	115 c.c., 0.182%, 0.209 g.			30 c.c., 1.071%, 0.321 g.			

B.

♂ Wt.: 2000 g. Adrenalin (0.2 c.c.) and calcium lactate (0.5 g.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.1027	0.1250	0.1500	0.1744	0.1401	0.1292	0.0938
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	95 c c., 0.216%, 0.206 g.			66 c.c., 0.341%, 0.225 g.			

Table 6. A.

♂ Wt.: 2280 g. Adrenalin (0.2 c.c.) and glucose (15 g.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.1095	0.3333	0.3750	0.4545	0.5000	0.4687	0.0980
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	35 c c., 0.074%, 0.026 g.			13 c.c., 3.934%, 0.511 g.			

B.

♂ Wt.: 2300 g. Adrenalin (0.2 c.c.), glucose (15 g.) and calcium lactate (0.5 g.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.0987	0.2586	0.2727	0.2500	0.2830	0.2885	0.0820
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	24 c.c., 0.121%, 0.029 g.			15 c.c., 2.522%, 0.378 g.			

Table 7. A.

♂ Wt.: 2240 g.: Adrenalin (0.2 c.c.) and calcium lactate (0.25 g.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.1154	0.1667	0.1724	0.2239	0.2235	0.1875	0.1292
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	30 c.c., 0.04%, 0.012 g.			17 c.c., 4.638%, 0.789 g.			

B.

♂ Wt.: 2100 g. Adrenalin (0.2 c.c.), glucose (7.5 g.) and calcium lactate (0.25 g.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.1154	0.3125	0.2778	0.2778	0.2459	0.2143	0.1119
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	95 c.c., 0.190%, 0.181 g.			28 c.c., 1.875%, 0.525 g.			

C.

♂ Wt.: 2135 g. Adrenalin (0.2 c.c.) and glucose (7.5 g.)

	Control	1/2 h.	1	2	3	4	24
Blood sugar in %	0.1200	0.3846	0.4167	0.4167	0.3750	0.3571	0.1281
Urine sugar	for 24 h. before treatment			24 h. after treatment			
	95 c.c., 0.130%, 0.124 g.			28 c.c., 7.200%, 2.016 g.			

Conclusion from the Results. When calcium in the form of organic salts as described above was injected with glucose (Table 2 and 3) especially with glucose-adrenalin (Table 6 and 7) into rabbits it prevented considerably the elevation of the sugar contents in both the blood and urine, which would occur when glucose alone or glucose-adrenalin without calcium was administered. However the calcium had little influence on the activity of adrenalin on the blood sugar (Table 4 and 5). Why calcium assists the combustion of the carbohydrate in the body is not clear. It is presumed that the disease of diabetes is probably attended by an imperfect calcium metabolism, thereby the combustion of carbohydrate being disturbed either directly or indirectly.

In other words the production or the activity of insulin will probably depend upon the calcium content in the blood.

Summary.

(1) The glucose tolerance on albino-rabbits was studied.

(2) The influence of calcium upon the activity of adrenalin on the blood sugar in the rabbit was experimented. It gave little change.

(3) An interesting relation between calcium and the carbohydrate metabolism of the body was observed: that is, calcium prevents considerably the elevation in both hyperglycemia and glycosuria which would occur when glucose alone or glucose-adrenalin without calcium was administered.

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