CLIII. THE RELATIONSHIP OF PHOSPHATES TO CARBOHYDRATE METABOLISM.

I. TIME RELATIONSHIP OF THE CHANGES IN PHOS- PHATE EXCRETION CAUSED BY INSULIN AND SUGAR.

By SAHIB SINGH SOKHEY

(Fellow International Health Board of Rockefeller Foundation)

AND FRANK NATHANIEL ALLAN.

From the Department of Physiology, University of Toronto.

(Received July 29th, 1924.)

ACCOMPANYING changes in the metabolism of sugar, variations in the inorganic phosphate content of the blood, and in the excretion of phosphate in the urine, have been observed by several investigators. Fiske [1920] found that ingestion of sugar resulted in a temporary diminution in the urinary output of phosphorus followed by a compensatory increase, and more recently Blatherwick, Bell and Hill [1923] have reported that the inorganic phosphates of the blood and urine become diminished after giving glucose for tolerance tests. It has also been shown that the administration of insulin is followed by a decrease in the inorganic phosphate of the blood. This was first observed in rabbits by Wigglesworth, Woodrow, Smith and Winter [1923], Winter and Smith [1924]. The same change in the blood of normal and diabetic individuals, accompanied by a decrease in excretion of phosphate in the urine, has been found by Blatherwick, Bell and Hill [1923], Perlyzweig, Lathan and Keefer [1923] and Harrop and Benedict [1923, 1924]. Another significant fact has been reported by Elias and Weiss [1922], to the effect that the intravenous injection of phosphate solutions is followed by a reduction of the blood sugar, in cases in which this is above the normal level as in diabetes or in alimentary hyperglycaemia.

The present paper presents further evidence to show the existence of a close relationship between phosphorus and the metabolism of carbohydrate. It is the outcome of an investigation which was started to test the possibility of the occurrence of a disturbance of the acid base equilibrium during insulin hypoglycaemia. Such striking changes in phosphate excretion were met with, that attention was directed to this feature. In order to determine the nature of the metabolic changes which might be responsible, analysis of the urine was also made for total nitrogen, ammonia and creatinine.

METHODS.

Observations were made on normal and depancreatized animals, which were starved throughout the experiment, so that the effect of food might be eliminated. The animals were catheterised at intervals of 3 hours during the day, to obtain the urine in fractional quantities and the night urine was collected as a whole, in metabolism cages. 50 cc. of water were given by stomach tube at the beginning of each of the day periods, and 150 cc. were given at night. The bladder was irrigated with 30 cc. water at the end of each catheterisation, and no evidence of infection or bladder irritation developed during the experiments. Chloroform was added to the samples of urine and these were kept at low temperature. The analytical methods used were: for phosphate, that of Briggs; for sugar, that of Shaffer and Hartmann; for nitrogen, ammonia and creatinine, those of Folin; and for titratable acidity, that of Fiske. The results are expressed graphically, those of one experiment being also given in tabular form. In the graphs the vertical lines mark equal periods of 3 hours, beginning with 10 a.m. on each day. The horizontal lines represent the amounts of the various substances excreted per hour during each of the periods. The figures on the curves give the total amount of the corresponding substance excreted for each day. The total excretion is calculated to the 24-hour value when the period is not exactly of this duration.

RESULTS.

I. *Normal animals.*

Dog. B. Female, weighing 12.0 kilos. at the commencement of the experiment, was observed from March 4th until March 12th, following a preliminary

Fig. 1. Normal dog B. Phosphorus ———. Nitrogen - - - -. Ammonia - · - · - ·. Titratable acidity · · · · ·

period of 3 days' fasting. The total nitrogen, ammonia, acid and phosphorus were determined at the usual periods, insulin or sugar being given on certain days.

On the normal days, the excretion of nitrogen per hour was somewhat

higher during the day than during the night, the highest values being usually reached in the early afternoon. The total daily excretion did not change significantly during the period of observation. The phosphorus excretion was at its lowest in the forenoon hours, increased during the remainder of the day, and reached its highest in the evening, the output for the night being considerably higher than that for the day. This diurnal variation has been observed in man by Fiske [1921], Campbell and Webster [1921] having also noted a higher excretion of phosphate during the night than during the day. The ammonia and the acidity varied in the same direction as the phosphorus.

Insulin was given at 12 noon on the second day, March 5th, the dose being insufficient to cause severe symptoms of hypoglycaemia although the dog became lethargic and inactive for a few hours after the injection. The excretion of phosphorus fell from 4.54 to 1.42 mg. per hour in the 1st period of $2\frac{1}{4}$ hours after the injection and to 0.18 mg. per hour in the 2nd period of $1\frac{3}{4}$ hours. This was accompanied by a corresponding reduction in the acidity and ammonia, but by no immediate change in total nitrogen. During the 2nd period of $1\frac{3}{4}$ hours the nitrogen rose markedly, accompanied by a lesser rise in ammonia, the acidity, however, continuing to fall with the phosphate. In the 3rd period, between the 4th and 7th hours following the injection of insulin, a most remarkable increase occurred in the excretion of phosphorus, and the acidity and ammonia rose in sympathy. In the next period (7th to 10th hours following insulin) the phosphorus rose still higher but the total nitrogen began to decline, with the ammonia following the latter, and the acidity the former. In the night urine the phosphorus remained above the normal, so that in the total 24-hours' urine it amounted to 337 mg., which, when compared with that observed on the normal days for this animal (4th, 7th and 10th), is approximately 45 % above the average. The total nitrogen fell to about its normal level during the night, but was increased for the 24 hours. The ammonia and the acidity for the 24 hours were decidedly greater than the normal.

Insulin was again given to this animal on March 8th with the same general results. The decrease in phosphorus was, however, more marked, and traces only could be detected in the samples of urine collected in the first 6 hours after insulin, the post-hypoglycaemia peak being reached in about 10 hours. The nitrogen excretion rose in the 2nd period after insulin, the peak being reached in the 3rd period, i.e. about 8 hours after the injection. The ammonia fell in the 1st period, when the phosphate was low, then rose in the 2nd, when the nitrogen was rising, reaching its peak, later, when the phosphate was at its highest. The acidity ran parallel with the phosphate throughout. The total quantities of phosphorus, nitrogen and ammonia for the 24 hours were greater than normal. The volume of urine was found to be diminished during the hypoglycaemic period on both insulin days, this being followed by a compensatory increase occurring in from 7 to 12 hours after the injection. On March 15th the urine was reduced from 9 cc. per hour before insulin to

5 cc. per hour in the first 4 hours following it, and it rose to 20 in the next 3 hours. On March 8th the excretion was reduced from 13 to 8 and 5 cc. per hour in the first 4 hours, rising to 32 between the 6th and 8th hours.

On March 6th the dog was given 50 g. of glucose (approximately 5 g. per kilo.) by stomach tube. The phosphate excretion which continued its usual afternoon increase in the 1st period ($2\frac{1}{4}$ hours) fell to 0.14 mg. per hour in the 2nd period. Then a large increase occurred, lasting about 5 hours. The total 24-hour output was not greater than the average normal output. The nitrogen excretion gradually diminished, reaching its lowest level during the night, and on the following day it was considerably below the average. The ammonia and titratable acidity followed the changes of the phosphate.

On March 11th, at 12.55 p.m., 50 g. glucose were given along with 30 units of insulin. Owing to vomiting it is not known how much sugar was absorbed. The phosphate excretion fell to 1.84 mg. then rose to 22.30 mg. per hour, the total for the day being somewhat increased. The nitrogen showed a temporary increase but doubt exists as to whether this may not have been due to some vomitus having become mixed with the urine; later, a decrease occurred so that the total for the day was not changed. The ammonia was little changed and the acidity varied with the phosphate.

On March 9th, 100 cc. of $N/10$ H_2SO_4 were given by stomach tube. The phosphate excretion did not deviate from the normal and there was no increase in excretion for the day. The ammonia increased immediately, but the total for the day was very little more than the normal. The titratable acidity of the urine followed the usual course during the day, and was unaltered for the 24 hours.

Dog A. A small terrier was starved from March 6th to March 25th, during which period its weight declined from 7.0 kilos. to 5.5 kilos. Phosphorus and ammonia were determined in the usual fractions of the urine, but the nitrogen, only for the whole 24 hours, except for one day, March 20th.

On March 10th and 11th, which were control days, the excretion of phosphate per hour was a little lower in the morning than the average for the preceding night, but rose decidedly during the afternoon and evening. The same diurnal variation occurred on March 14th and March 21st, the days following insulin. The ammonia curve paralleled that of phosphate.

Insulin (10 units) was given at the beginning of the 1st morning period on March 13th. This resulted in almost complete disappearance of phosphate from the urine collected during the succeeding 6 hours; in the next 6 hours the excretion reached a very high level. The total for the day though 40 % above that of the following day (14th) was no greater than that of the first two control days.

A smaller dose of insulin (7 units), given on March 15th, and again on March 17th, was followed by the same changes in phosphates, except that the secondary increase was earlier in appearing. The rise had begun in the 2nd period, 3 to 6 hours after insulin, reaching its maximum in the 3rd period, 6 to 9 hours. It should be noted that on March 15th, although the morning value was quite low before the insulin was given, it was reduced still further

after the injection. A smaller dose (5 units), given on March 23rd, caused the same definite changes. On each of these three insulin days there was a small increase in the phosphate output for the 24 hours, but that of the following day was also high especially on the 16th and 24th.

The nitrogen output was decidedly increased on the days when insulin was given. Thus, on March 13th, 3.587 g. was excreted, as compared with 2.108 g. on the following day and 2.278 g. and 2.046 g. on the first two normal days. On March 15th the nitrogen was 2.829 g. On March 17th the increase in nitrogen was less evident, the total for the day amounting to only 2.335 g. compared with 2.008 g. and 1.980 g. on the preceding and following days, respectively. On March 23rd, when the small dose of insulin was given, the output was only slightly increased, amounting to 2.443 g. with 2.311 g. on the day following.

Fig. 2. Normal dog A. Phosphorus ———. Nitrogen - - - -. Ammonia -.-.-.-.

On March 20th, insulin was given three times (at 10 a.m., 8 units; at 4 p.m., 5 units; at 10 p.m., 3 units) without producing any symptoms, except lethargy. The first three fractions of urine collected during the day, did not contain phosphorus in measurable quantities. In the period from 7 to 10 p.m. the excretion had risen to 8.94 mg. per hour and there can be little doubt that a marked increase in phosphate occurred during the night. It is interesting to note, however, that the output of phosphorus for the 24 hours (156.93 mg.) was slightly below the normal (about 180 mg.); neither was there an increase on the following day. The nitrogen rose promptly to a very high level and during the 24 hours 4.064 g. were excreted. This represents an increase of

nearly 100 % over the normal output of 2.0 to 2.5 g. per day. The ammonia followed the total nitrogen.

On March 12th, 35 g. of glucose were given at 12.50 p.m. The phosphate excretion fell from 7.86 mg. per hour to 3.22 mg. in the next 3 hours, returning to 7.34 mg. in the following period. In the 3rd period, 6 to 9 hours after the ingestion of the sugar, the phosphate had risen to 26.60 mg., but the total for the day was not increased. The nitrogen output was markedly reduced, the decrease amounting to more than 20 %.

In two out of the four days in which a single dose of insulin was given a marked diminution was observed to occur in the volume of urine excreted. On March 15th and 23rd, the volume per hour fell to 4 and 2 cc. respectively during the hypoglycaemic period. Compensatory increases occurred several hours later. On one other day, March 19th, there was a smaller decrease, to 8 cc. from the normal rate of 12 or 13 cc.

This dog, on two or three occasions, passed a dark green viscous material by the bowel during the hypoglycaemic period. This was probably bile and intestinal secretion evacuated on account of the increased activity of the intestine, which is known to occur after insulin.

Dog F. A small fat terrier weighing 7.3 kilos. was subjected to a somewhat longer fast, from March 18th to April 10th. It stood the fasting well, though its weight fell to 4.0 kilos.

Fig. 3. Normal dog F. Phosphorus ———. Nitrogen — — —. Ammonia ·····.

The excretion of nitrogen and phosphorus was fairly constant from day to day though a gradual reduction became evident towards the end. The usual

Dog F.

No.	Period	Time	Duration hrr.	Urine cc.	Total N. g.		Ammonia N. mg.			Creatinine mg.		Phosphorus mg.	
					Total	Per hour	Total	Per hour	Per cent.	Total	Per hour	Total	Per hour
1	Mar. 20,	9.50 a.m.											
	-Mar. 21,	10.00 "	24-17	295	2.340	0.097	65.00	2.70	2.78	165.0	6.8	210.0	8.6
2	Mar. 21,	10.00 a.m.											
	-Mar. 22,	10.15 "	24-25	300	2.448	0.101	49.80	2.05	2.02	155.0	6.4	209.0	8.6
3	-Mar. 22,	12.45 p.m.	2.50	29	0.281	0.112	4.97	1.99	1.77	18.0	7.2	12.8	5.1
4		-3.50 "	3.17	25	0.315	0.099	7.33	2.31	2.32	21.0	6.6	30.5	9.6
5		-6.45 "	2.83	45	0.279	0.099	7.07	2.50	2.54	19.5	6.9	28.1	9.9
6		-9.45 "	3.00	32	0.302	0.100	6.53	2.18	2.16	20.0	6.7	32.0	10.7
7	-Mar. 23,	9.45 a.m.	12.00	130	1.059	0.088	25.20	2.20	2.38	72.8	6.1	83.7	7.0
8	-Mar. 23,	12.45 p.m.	3.00	25	0.263	0.088	6.25	2.08	2.38	18.5	6.2	27.1	9.0
* 9		-3.45 "	3.00	—	0.330	0.110	5.80	1.93	1.76	21.0	7.0	5.9	1.9
10		-7.10 "	3.42	64	0.444	0.130	8.91	2.61	2.02	22.6	6.6	29.0	8.4
11		-10.00 "	2.83	60	0.204	0.070	3.96	1.40	1.94	15.5	5.5	60.0	21.2
12	-Mar. 24,	9.50 a.m.	11-83	120	0.884	0.075	19.65	1.66	2.22	67.9	5.7	100.1	11.6
13	-Mar. 24,	12.50 p.m.	3.00	35	0.244	0.081	5.40	1.80	2.21	21.1	7.0	12.9	4.3
14		-4.10 "	3.33	38	0.283	0.085	5.75	1.93	2.04	20.7	6.2	12.6	3.8
15		-6.50 "	2.67	25	0.189	0.071	2.84	1.06	1.50	16.7	6.3	4.8	1.8
16		-9.50 "	3.00	30	0.282	0.094	4.64	1.55	1.64	21.2	7.1	20.0	6.7
17	-Mar. 25,	9.50 a.m.	12.00	150	0.954	0.080	29.30	2.44	3.08	74.5	6.2	112.0	9.3
†18	-Mar. 25,	12.50 p.m.	3.00	45	—	—	—	—	—	(21.0)	—	—	—
19		-4.10 "	3.33	25	0.337	0.101	7.78	2.34	2.31	23.4	7.0	2.4	0.7
20		-6.55 "	2.75	70	0.467	0.170	10.37	3.79	2.22	16.7	6.1	0.6	0.2
21		-9.50 "	2.92	50	0.337	0.115	6.76	2.32	2.00	18.2	6.2	82.5	28.2
22	-Mar. 26,	9.55 a.m.	12.08	120	0.977	0.081	24.20	2.00	2.50	69.8	5.8	111.5	9.5
23	-Mar. 26,	12.55 p.m.	3.00	27	0.227	0.079	3.45	1.15	1.52	19.5	6.5	2.5	0.8
24		-3.50 "	2.92	29	0.232	0.080	5.60	1.92	2.41	18.6	6.4	14.4	4.9
25		-6.55 "	3.08	53	0.249	0.081	6.15	2.00	2.47	19.5	6.3	21.3	6.9
26		-10.00 "	3.08	38	0.262	0.085	7.08	2.30	2.70	20.0	6.5	30.6	9.9
27	-Mar. 27,	9.55 a.m.	11-92	140	0.910	0.076	25.60	2.15	2.82	72.8	6.1	83.5	7.0
‡28	-Mar. 27,	12.45 p.m.	2.83	20	0.220	0.078	4.85	1.71	2.20	17.6	6.2	1.6	0.6
§29		-3.45 "	3.00	80	0.366	0.122	7.27	2.42	1.98	17.7	5.9	trace	trace
30		-6.50 "	3.08	50	0.273	0.089	5.10	1.66	1.87	13.3	4.3	"	"
¶31		-9.50 "	3.00	25	0.278	0.093	9.36	3.12	3.37	18.5	6.2	75.0	25.0
32		-11.55 "	2.08	20	0.213	0.102	4.89	2.35	2.30	10.5	5.0	56.7	27.3
33	-Mar. 28,	9.55 a.m.	10.00	85	0.870	0.087	22.00	2.20	2.53	57.9	5.8	67.2	6.7
34	-Mar. 28,	12.50 p.m.	2.92	40	0.280	0.091	5.86	2.00	2.10	22.0	7.5	4.1	1.4
35		-3.50 "	3.00	47	0.263	0.088	7.26	2.42	2.76	18.0	6.0	15.0	5.0
36		-6.50 "	3.00	32	0.254	0.085	5.40	1.80	2.12	18.2	6.1	10.5	3.2
37		-9.50 "	3.00	30	0.248	0.083	6.56	2.19	2.65	17.9	6.0	23.2	7.8
38	-Mar. 29,	9.50 a.m.	12.00	140	0.988	0.082	23.70	1.98	2.40	61.7	5.1	100.1	8.4
39	-Mar. 29,	12.50 p.m.	3.00	30	0.249	0.083	5.34	1.78	2.15	18.2	6.1	6.9	2.3
40		-4.00 "	3.17	42	0.286	0.090	6.39	2.01	2.24	18.2	5.7	19.5	6.2
41		-6.45 "	2.75	36	0.218	0.079	3.08	1.12	1.41	15.6	5.7	8.0	3.0
42	-Mar. 30,	9.55 a.m.	15-17	170	1.120	0.074	21.55	1.42	1.92	83.8	5.5	127.2	8.4

* Insulin 6 units at 12.45 p.m. (weight 5.5 kg.).

† Insulin 9 units (weight 5.3 kg.).

‡ Insulin 9 units at 9.55 a.m. (weight 5.0 kg.).

§ Partial collapse from 12.45-3.0 p.m.

|| Insulin 3 units at 3.45 p.m. Convulsions and coma at 6.45 p.m.

¶ Dog better at 9.45 p.m.

Dog F (continued)

No.	Period	Time	Duration hrs.	Urine cc.	Total N. g.		Ammonia N. mg.			Creatinine mg.		Phosphorus mg.	
					Total	Per hour	Total	Per hour	Per cent.	Total	Per hour	Total	Per hour
43	-Mar. 31,	9.55 a.m.	24-00	170	—	—	43.25	—	—	127.0	5.3	151.1	—
44	-Apr. 1,	9.55 a.m.	24-00	170	1.904	0.079	44.94	1.87	2.36	—	—	144.5	6.0
45	-Apr. 2,	9.50 a.m.	23-92	230	1.750	0.073	43.70	1.83	2.50	—	—	108.0	4.5
*46	-Apr. 2,	12.50 p.m.	3-00	40	0.242	0.081	5.93	1.97	2.45	—	—	20.9	7.0
47		-3.50 „	3-00	25	0.202	0.067	6.15	2.05	3.04	—	—	11.2	3.7
48		-6.50 „	3-00	35	0.206	0.069	6.77	2.26	3.29	—	—	trace	—
49		-9.55 „	3-08	115	0.207	0.069	—	—	—	—	—	12.2	4.0
50	-Apr. 3,	9.50 a.m.	11-92	155	0.479	0.040	18.08	1.51	3.77	—	—	100.0	8.4
51	-Apr. 3,	12.50 p.m.	3-00	14	0.095	0.032	3.02	1.01	3.18	—	—	4.9	1.6
52		-4.30 „	3-67	37	0.169	0.046	4.80	1.31	2.84	—	—	14.0	3.8
53		-6.50 „	2-33	28	0.102	0.043	3.09	1.33	3.03	—	—	5.0	2.1
54		-9.50 „	3-00	38	0.166	0.055	5.48	1.83	3.30	—	—	15.8	5.3
55	-Apr. 4,	9.50 a.m.	12-00	142	0.558	0.047	24.00	2.00	4.30	—	—	43.5	3.6
56	-Apr. 4,	12.55 p.m.	3-08	28	0.140	0.045	4.89	1.58	3.50	—	—	23.0	7.5
57	-Apr. 5,	9.50 a.m.	20-92	70	1.341	0.064	24.33	1.16	1.81	—	—	69.0	3.3
58	-Apr. 6,	9.50 a.m.	24-00	285	1.315	0.055	39.10	1.63	2.97	—	—	86.8	3.6
59	-Apr. 7,	9.50 a.m.	24-00	250	1.312	0.055	39.20	1.63	2.98	—	—	90.9	3.8
†60	-Apr. 7,	12.50 p.m.	3-00	28	0.209	0.070	5.57	1.86	2.66	—	—	11.3	3.8
61		-3.50 „	3-00	40	0.190	0.063	7.28	2.43	3.83	—	—	18.5	6.2
62		-7.00 „	3-17	70	0.210	0.066	6.15	1.94	2.93	—	—	4.9	1.5
63		-9.50 „	2-83	78	0.139	0.049	3.33	1.18	2.40	—	—	18.9	6.7
64	-Apr. 8,	9.50 a.m.	12-00	110	0.400	0.033	15.93	1.33	3.98	—	—	74.6	6.2
65	-Apr. 8,	12.50 p.m.	3-00	15	0.163	0.054	3.91	1.30	2.40	—	—	5.2	1.7
66		-3.55 „	3-08	35	0.138	0.045	3.05	0.99	2.21	—	—	4.9	1.6
67		-6.50 „	2-92	40	0.126	0.043	3.57	1.22	2.84	—	—	5.8	2.0
68		-9.55 „	3-08	40	0.136	0.044	5.16	1.68	3.80	—	—	19.2	6.2
69	-Apr. 9,	9.50 a.m.	11-92	145	0.504	0.042	16.51	1.39	3.28	—	—	33.5	2.8
70	-Apr. 10,	9.50 a.m.	24-00	325	1.119	0.047	43.30	1.80	3.88	—	—	6.9	2.9

* 25 g. sugar, insulin 10 units.

† 25 g. glucose.

daily rhythm in the excretion of phosphorus was noted on the normal days. Single doses of insulin were injected on two occasions, 6 units on March 23rd and 9 units on March 25th, but no symptoms developed on either date. The decrease and increase in phosphorus excretion occurred as before, the greater change following the larger dose. In the latter case the phosphorus almost disappeared from the urine. The increase for the day amounted to about 20 and 30 % respectively.

The nitrogen output was increased in the first 3 hours in each case, reaching the maximum in the second 3 hours, then falling. There was little if any increase for the whole day in the first case and a small increase, amounting to about 10 %, in the second case.

On March 27th, 9 units of insulin, given at 9.55 a.m., were followed by moderately severe symptoms—salivation, tremors, ataxia and partial collapse.

The animal recovered and a second dose of 3 units was administered at 3.45 p.m. Convulsions and coma occurred between 6 and 7 p.m., but the dog recovered without requiring sugar. The first injection was followed by almost complete disappearance of phosphorus from the urine and the second prolonged this condition for another three hours. Then the inevitable rise in phosphate occurred, probably lasting for several hours during the night. The total for the day was increased about 30 %.

The nitrogen rose immediately after the first injection, falling again after 6 hours. The second injection caused another increase, though smaller, reaching its peak in 6 hours. The total for the day was 2.220 g. and for the following day 2.033 g., compared with 1.880 g. the day before and 1.873 g. the second day after. The ammonia followed the total nitrogen excretion very closely in these experiments.

The creatinine excretion measured for the first 8 days showed very little change from day to day, or for the different periods during the day although there appeared to be a slightly higher output in the morning than in the afternoon and night. This difference, though apparently very constant, is probably not great enough to be of any significance. Administration of insulin did not affect the creatinine excretion on the days in which single doses were given. On March 27th, however, when the animal was in a state of collapse as the result of a second injection of insulin, the creatinine output was 4.3 mg., the normal being from 5.5 to 6.0 mg.

On April 7th, 25 g. of glucose (5 g. per kilo.) were given at 12.50 p.m. The phosphate excretion showed the usual changes but the effect was delayed, as it will be recalled was also the case when sugar was given to Dog *B* on March 6th. The usual afternoon rise continued in the first period after sugar was given but a decided fall took place in the next period, 3 to 6 hours later. In the subsequent period the increase had developed but the maximum was probably reached during the night. The total for the day was increased over 30 %.

The nitrogen output showed a gradual diminution, the lowest rate of excretion being reached during the night. The total for the day was decreased from 1.312 g. to 1.148 g., that of the day following being decreased still more—to 1.067 g.

On April 2nd, at 12.50 p.m., 25 g. of sugar and 10 units of insulin were given together. The phosphate excretion dropped at once, becoming a trace only in the second period. The increase which followed reached its maximum during the night. The total increase for the day amounted to over 40 %, and on the following morning the phosphate excretion was decidedly low. A further depression of the usual low morning phosphate seems to occur regularly on the days following large changes in phosphate excretion whether brought about by insulin, or sugar, or both together. This feature was very prominent on March 26th and 28th.

The volume of urine was diminished on March 25th in the first period after insulin from 15 to 7 cc. per hour. On March 27th, immediately after the first

dose of insulin, the excretion was low—8 cc. per hour—but in the next two periods, when the animal was in collapse, it was high, 26 and 16 cc. per hour.

II. Diabetic animals.

Depancreatized dogs were investigated in the same way as normal animals except that, as a rule, 100 cc. of water, instead of 50 cc., were given before each day period, and 250 cc. at night.

Dog 23 had been depancreatized several months previously, being meanwhile fed on a diet of meat and cane sugar, with insulin twice daily most of the time. It was in good condition and had maintained its weight of about 7 kilos.

Fig. 4. Diabetic dogs 23 and E. Phosphorus ———. Sugar ○○○○. Ammonia - - - - -. Titratable acidity

On March 5th, it was given no food or insulin and on this day the excretion of phosphate was extremely high, the total output for the day, nearly 600 mg., being more than double the output of a normal dog of similar weight. The high rate of excretion in the morning became still higher in the afternoon, this change being similar to the normal diurnal variation. The urinary sugar was high in the morning but fell during the day, probably on account of loss of glycogen deposited as a result of the sugar feeding and insulin treatment on the preceding days.

On March 6th insulin (15 units) was given at 12.10 p.m. The phosphate excretion, which had been extremely high in the morning (35-30 mg. per hour) fell immediately, almost reaching zero in the period from 5 to 7 hours after insulin. This was followed by an increase to 16.78 mg. per hour during the night. The total for the day was 333.86 mg., which is about 50 % less than the excretion on the preceding and following days. The sugar and acetone bodies disappeared immediately after the injection, returning in about 8 hours.

On March 8th, a dose of 8 units of insulin was given with similar results. The fall in phosphate was not quite as great, though a decrease from 26 mg.

per hour to 1.41 mg. took place in 7 hours. An increase to 14.42 mg. followed. The experiment had to be terminated, on account of symptoms, at 9.30 p.m., before the phosphate had risen to its highest level.

The excretion of ammonia paralleled fairly closely that of the phosphate. Changes in the titratable acidity corresponded almost exactly with the changes in phosphate. When the phosphate was low after insulin the urine became almost neutral on both occasions.

Dog D, also depancreatized several months previously, had been fed meat and sugar and received insulin before each feeding. On March 11th, the day before observations were begun, an overdose of insulin was injected and severe hypoglycaemic symptoms developed. Administration of glucose was necessary to restore the animal.

Fig. 5. Diabetic dog *D*. Phosphorus ———. Sugar ○ ○ ○ ○. Ammonia - - - - -. Nitrogen - - - - -.

On March 12th the dog was apparently in good condition, its weight being 7.0 kilos. It was given 15 units of insulin at 1 p.m. and again hypoglycaemic symptoms with mild convulsions developed. Temporary recovery took place after giving about 7 g. of cane sugar by stomach tube at 4 p.m. At 7 p.m. the dog was found to be in a comatose state with slow irregular heart and respiration. It was given 10 g. of glucose subcutaneously. It recovered consciousness but was still very weak at 10.20 p.m.

The phosphate excretion before insulin was given amounted to 19 mg. per hour which is two or three times the normal value. After insulin a decrease occurred but in the 3rd period (9 to 12 hours) the excretion (22.6 mg. per hour) had returned to the original level.

The excretion of nitrogen showed a steady decrease, reaching the lowest level during the night.

On March 13th and 14th the dog appeared normal; no food or insulin was given though the animal seemed to be still under the influence of insulin to some extent, since the amount of sugar excreted was small and acetone bodies were absent from the urine.

On March 15th, 20 g. of glucose were given by stomach tube; most of it,

but not all, was excreted within the next $7\frac{1}{2}$ hours, the total output for this period being 15.5 g. The phosphate excretion showed a marked increase in the 3rd period, 7 hours after the glucose, the nitrogen and ammonia being also increased in this period.

During the night the excretion of all three had again returned to normal. The usual preliminary decrease found after giving sugar to normal animals was not observed, perhaps because it was too small and of too short duration to be apparent in the periods over which urine was collected. Otherwise the result resembles that produced by giving sugar to a normal animal and, as more than 25 % of the sugar given was retained, may differ from the result of sugar ingestion on a diabetic where none is utilised.

On March 17th a smaller dose of insulin, 10 units, was given and no symptoms resulted. The phosphate fell immediately, becoming only a trace in the urine for the 2nd period. The increase which followed was not great and the original level was reached in the night.

The nitrogen diminished immediately, the decrease lasting for 6 hours, but a small increase took place during the night.

Dog E was depancreatized on February 14th. It was fed 200 g. meat and 50 g. sugar twice daily and received insulin before each feeding until March 19th, when no food was given. (Its weight was 7.5 kilos.) The phosphate excretion in the morning was high, as in the other diabetic dogs (17.30 mg. per hour rising to 18.18 mg. between 1 and 3 p.m.). 10 units of insulin were given at 3 p.m. with the result that there was an immediate decrease in phosphates followed after 5 hours by an increase which, however, did not bring the excretion to its original level. The average for the night was 13.10 mg. per hour.

SUMMARY.

1. *Effect of Insulin on Normal Animals.* The administration of insulin to normal animals resulted in an immediate reduction in the excretion of inorganic phosphate in the urine. After a large dose, not sufficient, however, to cause symptoms of hypoglycaemia, the amount of phosphate became too small to measure. The period of reduced excretion lasted for about 6 hours, and was followed by a period of increased excretion, the maximum being reached in from 9 to 12 hours after the injection. The total output of phosphorus for the day was usually increased from 25 to 35 %, though in some cases the increase in phosphate excretion occurred partly or mainly on the following day.

The effect of repeating the dose was to prolong for a time the phase of diminished excretion. This was, however, followed by the inevitable increase. The cause of this cannot be that there is lack of insulin, or that the effect of insulin has passed off, since it was observed when the animals were obviously under the influence of insulin; for example, the increase occurred in *Dog F* when severe hypoglycaemic symptoms were still present. On the other hand, when *Dog A* was given three injections of insulin in one day the increase did

not balance the decrease which had lasted for about 9 hours, so that the total output for the day was diminished instead of being increased.

The nitrogen excretion showed an increase which was evident in the first 6 hours after insulin. On the average the total output for the day was increased 20 % and on March 20th, when Dog *A* was given three injections of insulin, the nitrogen output was approximately doubled.

The excretion of ammonia and the titratable acidity of the urine paralleled, in general, the excretion of phosphorus. On some occasions, however, the ammonia became greater while the phosphorus was low, but this increase corresponded with an increase in the total nitrogen output. The titratable acidity was always low after insulin, when the phosphate output was low, and on some occasions it became zero.

The volume of urine was usually diminished in the first 3- or 6-hour periods after insulin. The initial decrease was followed by a compensatory increase, usually appearing in the post-hypoglycaemic period. The increase occurred, however, in the case of Dog *F*, on March 27th, when severe hypoglycaemic symptoms were still present.

The excretion of creatinine does not appear to be affected by administration of insulin. It did not increase when there was an increased output of the total nitrogen.

2. *Effect of Sugar.* The ingestion of sugar caused changes in excretion similar to those caused by insulin. The decrease in phosphate was almost as great, but its onset appeared to be slower. On two occasions the usual afternoon increase continued for the 1st period after sugar ingestion; it always decreased during the 2nd period however. This was followed after 6 hours by a large increase but in only one case out of three was the phosphorus output for the 24 hours greater than normal.

The excretion of nitrogen gradually declined so that the total for the day was diminished as well as that for the following day. The ammonia fell in proportion to the fall in total nitrogen. The titratable acidity paralleled the excretion of phosphate.

3. *Effect of Insulin and Sugar.* Insulin and glucose given at the same time brought about the same changes in phosphate excretion as were observed after giving insulin or glucose separately. The nitrogen (Dog *F*, April 2nd) gradually diminished, reaching the lowest level during the night. The output for the day was decreased, but the greatest decrease was shown on the following day.

4. *Effect of Acid.* The administration of acid did not alter the excretion of phosphate.

5. *Effect of Insulin and Sugar on Diabetic Dogs.* The phosphorus excretion of diabetic dogs without insulin was very high, being more than twice that of the normal. Administration of insulin caused a decrease in the excretion, then an increase, with return to the level for a normal dog. The output for the 24 hours was reduced to a value approximating to the normal.

The one experiment in which sugar was given did not yield a clear cut result. Further experiments now in progress suggest that sugar will not affect the phosphate excretion if there is complete absence of insulin.

DISCUSSION.

The striking changes in the excretion of phosphate following the administration of insulin together with the fact that the same changes occur after the ingestion of sugar establish beyond question the existence of an intimate relationship between the metabolism of carbohydrate and phosphoric acid. It would appear that after the ingestion of carbohydrate, or after administration of insulin when change in glucose is taking place, there is a demand for phosphate to form some compound with a carbohydrate product. This compound exists only temporarily and when it breaks up, the phosphoric acid is released, and the excretion therefore increased.

The rôle of phosphate in the fermentation of glucose demonstrated by Harden and Young and by Lebedev [see Harden, 1923], and the relationship of lactacidogen to muscular contraction established by Embden and others [1914], suggest that the hypothetical compound of phosphorus is hexosephosphate. Study of the lactacidogen and inorganic phosphate content of rabbit's muscle after insulin administration and after insulin along with sugar, made in this laboratory, has not, however, yielded results which could be considered to afford direct proof for this hypothesis. This is contrary to the results reported by Audova and Wagner [1924], and Harrop and Benedict who claim to have found an increase in lactacidogen in muscle after insulin. The technical difficulties of these experiments are very great, so that further work will be necessary to settle this point.

The increase in nitrogen excretion when insulin is given to a normal fasting animal, may indicate that when there is not sufficient carbohydrate present for the insulin to act upon, there is increased catabolism of protein to provide the necessary sugar. When, however, there is sufficient sugar available, as is the case in a normal well-fed animal, or in a fasting animal when sugar is given at the same time as insulin, there is a decrease in nitrogen excretion. Here the increased carbohydrate combustion exerts a protein-sparing action. It may be noted that previous work [Macleod and Allan, 1923] showed that when insulin was given to a well-fed animal, the nitrogen excretion was diminished, the greatest decrease frequently occurring on the day following the insulin administration. This result has been confirmed in the present observations when insulin and sugar were given together. The fact that the administration of insulin diminished the nitrogen loss of a fasting diabetic probably indicates not only that there is abundant sugar present in the body for it to act upon, but also that the previous rapid breakdown of protein giving rise to sugar, is retarded. The other explanation of protein-sparing action, namely that there is re-synthesis of amino acids from decomposition products of protein and carbohydrate metabolism must also be kept in mind.

The increase in the phosphorus output for the 24 hours did not occur except where there was also an increased nitrogen output (with one exception). In view of the continuous high phosphorus excretion in diabetic animals in which the nitrogen excretion is also high this observation suggests a common origin of the phosphorus and nitrogen. It may be that substances of the nature of nucleoprotein or inosinic acid furnish the material from which the sugar and phosphorus are derived. Another possibility which must be considered is that insulin is concerned with fat synthesis and that the phosphorus becomes incorporated in a phospholipin. These suggestions are under investigation.

The possibility that the changes in phosphate excretion are the result of alteration in the acid-base equilibrium can be dismissed. In an investigation of the effects of acidosis in cats (Sokhey, unpublished work) no change in urinary phosphate was found to occur, an observation which is confirmed in the present investigation. There is, however, a change in the reaction of the urine after insulin dependent no doubt on the change in phosphates. The parallelism between the titratable acidity of the urine and the phosphate content has shown itself to be remarkably close.

CONCLUSION.

The changes in excretion of inorganic phosphate in the urine, after ingestion of sugar and administration of insulin to normal and diabetic animals, are described in detail and the time relationships studied. The intimate association of the metabolism of carbohydrate and phosphoric acid is demonstrated. Coincident metabolic changes are discussed and their significance suggested.

The authors wish to acknowledge their indebtedness to Professor J. J. R. Macleod for his advice and interest in this investigation.

REFERENCES.

- Audova and Wagner (1924). *Klin. Wochensch.* Feb. 5, No. 6.
Blatherwick, Bell and Hill (1923). *Proc. Amer. Soc. Biol. Chem.* **6**, 35.
Campbell and Webster (1921). *Biochem. J.* **15**, 660.
Elias and Weiss (1922). *Wien. Arch. inn. Med.* **4**, 29.
Emlden, Griesbach and Laquer (1914). *Z. physiol. Chem.* **93**, 1.
Fiske (1920). *J. Biol. Chem.* **41**, lix.
—— (1921). *J. Biol. Chem.* **49**, 171.
Harnden (1923). *Alcoholic Fermentation*. (Longmans, Green & Co.)
Harpen and Benedict (1923). *Proc. Soc. Exp. Biol. Med.* **29**, 430.
—— (1924). *J. Biol. Chem.* **59**, 683.
Macleod and Allan (1923). *Trans. Roy. Soc. Canada*, Section v, 47.
Perlyzweig, Lathan and Keefer (1923). *Proc. Soc. Exp. Biol. Med.* **21**, 33.
Wigglesworth, Woodrow, Smith and Winter (1923). *J. Physiol.* **57**, 447.
Winter and Smith (1924). *J. Physiol.* **58**, 327.