THE INFLUENCE OF CARBOHYDRATE AND FAT ON PROTEIN METABOLISM. III. THE EFFECT OF SODIUM SELENITE. BY E. P. CATHCART AND J. B. ORR.

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THE toxic effect of certain of the salts of selenium has long been known but so far no complete investigation of their influence on the animal metabolism has been attempted. A few years ago C. O. Jones[1] published a very interesting paper on the physiological effects of selenium compounds. He injected sodium selenite hypodermically and after the death of the animal extracted from the tissues the granular deposit observed by previous workers. This deposit he showed to be identical in every respect with the amorphous form of selenium obtained on reducing sodium selenite in the test tube. He found that the reduction took place if a solution of sodium selenite were allowed to act upon animal tissue in which enzyme action had been destroyed by heat and he concluded therefore that the reduction was a purely chemical action. He next examined the tissues for the particular constituent that effected the reduction and finally came to the conclusion that of substances of animal origin reduction could only be effected by glucose and a few allied sugars or bodies yielding these. This reduction could not be produced by organic matter from which these sugars were absent. To prove his point he carried out various forms of experiment; thus he administered hypodermically several sublethal doses of selenite of sodium to an animal which was then killed and its liver examined for glycogen. In a control animal the glycogen was abundant whereas in the injected animal it had almost completely disappeared. He inferred that the glycogen had been drawn upon to supply glucose for the reduction of the sodium selenite. As a result of these and other experiments Jones concluded, from apparently quite ample and well founded data, that sodium selenite injected hypodermically is carried by the blood stream to the tissues and is there reduced to selenium by glucose.

Assuming the validity of Jones's contention as to the intimate relationship between the reduction of sodium selenite and the glucose content of the tissues it was thought that a further study of the effect of these selenium salts might throw more light on intermediate carbohydrate metabolism and on the effect of disturbances of the same.

The animal employed in our experiments was an Airedale bitch of 12 kilos. weight. The bladder was emptied daily at 10 a.m. by catheter and the urine added to that collected in the receiver of the metabolism cage. The methods of estimation were total nitrogen by the Kjeldahl method, urea, ammonia, creatinine and creatine by Folin's methods, and amino-acids by the Henriques-Sörensen method. In all the experiments, with the exception of the first, the total amount of urine was made up to a constant volume before the estimations were made, as we have found in this laboratory that this procedure renders the analyses more accurate. The animal was always allowed to recover completely between the experiments. During each experiment the dog was kept on a constant fixed diet. After the diet had been given for from five to seven days, *i.e.* until the nitrogen output was steady for two or three days, a sublethal dose, viz. 2 c.c. of a 1 % solution of sodium selenite, was injected subcutaneously. The fixed diet was continued after the injection until examination of the urine showed that the output of the various nitrogenous constituents had returned to normal.

Throughout the series of experiments the protein content of the food was little altered but the amount of carbohydrate was varied from 33 to 204 grams per diem, while the fat content was varied inversely to maintain an approximately constant calorie value for the diet. Sodium chloride, 2 grams per diem, was added to each diet. The daily preparation of the food was carried out by J. B. O.

Exp. I. Diet. Oatmeal 40 grams, dry skimmed milk 30 grs., margarine 40 grs., tapioca 80 grs., containing approximately protein 28 grs., fat 44 grs. and carbohydrate 161 grs. with a total calorie intake of 1184 or about 99 Cal. per kilo.

Twenty minutes after the injection of the selenite the animal vomited, and continued to do so at intervals for about half an hour. The vomited matter consisted of saliva and mucus. Beyond this temporary sickness no ill effects were noted. The animal did not consume its full ration for the three following days but thereafter the appetite completely returned and the full amount was readily eaten.

TABLE I.

Day of exper.	Urine amount, c.c.	Total nitrogen, gr.	Urea nitrogen, gr.	NH₃ nitrogen, gr.	Amino acid nitrogen, gr.	Creatinine, gr.	Creatine, gr.	Remarks
3	970	2·63	1·96	·24	—	·362	·032	Full diet.
4	1099	2·71	2·00	·25	—	·367	·012	,,
5	1100	2·67	1·94	·24	—	·356	—	,,
7	Urine lost.							Injection.
8	240	4·02	3·29	·25	·03	·379	·153	⅓ diet.
9	349	2·93	2·29	·20	·01	·315	·148	½ diet.
10	560	2·57	2·03	·14	·02	·334	·045	½ diet.
11	460	2·12	1·65	·16	—	·343	—	Full diet.
12	700	1·94	?	·17	—	·357	—	,,

Exp. II. Diet. Oatmeal 70 grams, margarine 80 grs., and dry skimmed milk 30 grs., containing approximately protein 25 grs., fat 74 grs., and carbohydrate 64 grs., with a total calorie value of about 1053 Cal. or about 90 Cal. per kilo.

As in the previous experiment soon after the injection the animal vomited, but the vomiting persisted much longer. The animal appeared to be very lethargic and this condition of listlessness lasted during the following day and was still partially present on the second day after the injection. The animal refused its food on the day of the injection and on the two following days. On the fourth day it was quite normal and ate the full diet.

TABLE II.

Day of exper.	Urine amount, c.c.	Total nitrogen, gr.	Urea nitrogen, gr.	NH₃ nitrogen, gr.	Amino acid nitrogen, gr.	Creatinine, gr.	Creatine, gr.	Remarks
1	540	2·89	2·33	·19	—	·308	—	Full diet.
2	340	2·60	2·06	·18	·04	·318	·006	,,
3	602	2·98	2·27	·26	·01	·386	·028	,,
4	518	2·54	2·06	·20	·05	·346	·025	,,
5	390	2·24	—	·15	·06	·283	·061	,,
6	144	2·10	1·69	·13	·01	·368	·044	Fast.
7	520	2·55	2·06	·18	·05	·360	·053	Full diet.
8	525	2·55	1·74	·20	·04	·343	·054	,,
9	420	2·24	1·67	·20	·05	·291	·044	,,
10	340	4·27	3·59	·19	·06	·369	·157	Injection.
11	687	5·36	3·90	·38	·06	·302	·282	No food.
12	430	7·06	5·96	·41	·02	·352	·358	,,
13	200	3·37	2·67	·17	·09	·296	·036	Full diet.
14	330	2·32	1·84	·17	·06	·296	·016	,,

Exp. III. Diet. Oatmeal 100 grams, tapioca 80 grs., cane sugar 40 grs., dry skimmed milk 30 grs., containing approximately 25 grs. of protein, 27 grs. of fat and 189 grs. of carbohydrate with a total calorie value of 1128 or about 94 Cals. per kilo.

Twenty minutes after the injection the dog vomited mucus and saliva as in Exps. I and II but the vomiting was much less profuse. The animal was little upset and appeared quite lively and normal. No food was offered on the day of the injection. Next day it appeared quite normal but it only ate a portion of its food. On the following day the full diet was taken.

TABLE III.

Day of exper.	Urine amount, c.c.	Total nitrogen, gr.	Urea nitrogen, gr.	NH₃ nitrogen, gr.	Amino acid nitrogen, gr.	Creatinine, gr.	Creatine, gr.	Remarks
1	546	3·33	2·69	·32	·0003	·359	·029	Full diet.
2	650	2·63	2·18	·28	·09	·321	·029	,,
3	930	2·73	2·08	·31	—	·311	·027	,,
4	985	2·76	1·95	·29	—	·310	·027	,,
5	210	2·09	1·54	·13	·019	·288	·041	Fast.
6	840	2·45	1·98	·24	·027	·303	·015	Full diet.
7	690	2·08	1·55	·19	·029	·319	·011	,,
8	760	3·50	2·76	·23	·030	·339	·052	Injection.
9	420	4·65	3·48	·36	·105	·329	·121	Part food.
10	1000	3·67	2·52	·31	·036	·305	·011	,,
11	1000	2·86	2·15	·28	·020	·289	·005	Full diet.

Exp. IV. Diet. Oatmeal 30 grams, tapioca 100 grs., cane sugar 80 grs., dry skimmed milk 50 grs., and margarine 10 grs., containing about 23 grs. of protein, 12 grs. of fat and 204 grs. of carbohydrate with a calorie value of 1042 or about 87 Cals. per kilo.

Following the injection salivation was profuse but there was no vomiting. The dog appeared a little less lively than usual but was otherwise quite unaffected by the injection. No food was offered on the day of injection. Next day the animal appeared normal but ate only part of its food; on the day following it ate the full amount.

TABLE IV.

Day of exper.	Urine amount, c.c.	Total nitrogen, gr.	Urea nitrogen, gr.	NH₃ nitrogen, gr.	Amino acid nitrogen, gr.	Creatinine, gr.	Creatine, gr.	Remarks
1	698	2·84	1·85	·29	·02	·343	·026	Full diet.
2	690	2·71	1·79	·28	·05	·341	·004	,,
3	695	2·87	2·05	·29	—	·359	·016	,,
4	640	2·81	1·97	·30	—	·354	·012	,,
5	780	3·04	2·10	·29	—	·352	·011	,,
6	320	4·44	3·33	·26	—	·395	·055	Injection.
7	210	4·47	3·42	·28	—	·364	·035	⅓ food.
8	350	3·50	2·66	·29	—	·373	·032	Full diet.
9	630	3·22	?	·33	—	·357	—	,,

Exp. V. Diet. Oatmeal 30 grams, dry skimmed milk 30 grs., casein 9 grs., and margarine 100 grs., with about 25 grs. of protein, 85 grs. of fat and 34 grs. of carbohydrate, having a total calorie value of 1121, *i.e.* about 93 Cals. per kilo.

Within five minutes of the injection profuse salivation appeared and this was soon followed by vomiting of mucus, which was bile stained. The animal absolutely refused all food on the day of the injection and the following day but on the second day after it was induced with much coaxing to eat its full diet.

TABLE V.

Day of exper.	Urine amount, c.c.	Total nitrogen, gr.	Urea nitrogen, gr.	NH_3 nitrogen, gr.	Amino acid nitrogen, gr.	Creatinine, gr.	Creatine, gr.	Remarks
1	420	3·34	2·39	·20	·069	·384	—	Full diet.
2	375	3·23	2·33	·18	·075	·379	—	,,
3	410	3·03	2·10	·20	·052	·370	—	,,
4	255	2·94	2·12	·13	·063	·343	—	,,
5	Urine lost.							Injection.
6	275	4·42	3·32	·32	·081	·342	·298	No food.
7	220	5·24	3·91	·32	·098	·357	·189	Full diet.
8	510	4·68	3·52	·36	·125	·343	·053	,,
9	680	3·59	2·41	·33	·065	·331	·003	,,
10	340	3·31	?	·21	·069	·322	—	,,

Exp. VI. This was a short experiment to ascertain whether or no an acidosis resulted from the injection of the selenite.

Diet. Oatmeal 70 grams, margarine 60 grs., dry skimmed milk 30 grs., cane sugar 40 grs., containing approximately protein 25 grs., fat 52 grs. and carbohydrate 100 grs. with a total calorie content of 996 or about 83 Cals. per kilo.

Salivation began about three minutes after the injection. Vomiting of mucus and saliva occurred about half an hour later. Next day the dog ate about one third of its diet and on the following day two thirds.

TABLE VI.

Day of exper.	Urine amount, c.c.	Total nitrogen, gr.	NH_3 nitrogen, gr.	Amino acid nitrogen, gr.	Chlorides, gr.	Acetone+ diacetic acid, gr.	Creatinine, gr.	Creatine, gr.	Remarks
3	680	3·76	·21	·068	?	·0044	·388	·051	Diet.
4	480	3·86	·25	·051	1·790	·0041	·377	·060	,,
5	385	3·86	·20	·074	1·340	·0047	·377	·060	,,
6	305	6·00	·35	·060	·017	·0054	·411	·227	Injection.
7	300	6·76	·39	·067	·028	·0081	·397	·240	⅓ diet.
8	265	5·21	·25	·051	·061	·0056	·385	·073	⅔ diet.

The acetone and the acetoacetic acid were estimated by means of the Messinger-Huppert method. In estimating the creatinine the readings were taken after the acetone and acetoacetic acid had been driven off by the method advocated by Graham and Poulton[2].

In order to ascertain whether selenium itself produced any effect on the metabolism a subcutaneous injection of a colloidal solution of selenium containing about ·030 gram of the metal was given to a dog on a fixed diet comparatively poor in carbohydrate. The urinary analysis showed no evidence of a disturbed metabolism nor were any of the other toxic symptoms, *i.e.* salivation, vomiting, present. The colloidal selenium was kindly given to us by Dr C. Walker of the Cancer Research Laboratory.

DISCUSSION OF THE RESULTS.

The general effects of the injection of the sodium selenite may be summarised as salivation, vomiting, anorexia and depression of vitality. The salivation which has not been noted by previous observers was always quite distinct and was the earliest symptom of the intoxication. In Exp. V it appeared within three minutes of the injection. The actual salivation was always preceded by a curious twitching of the nostrils and lips which suggested that there may have been some irritation of the nasal passages. It is possible that salivation may be due, as Jones suggests, to the irritation of the nerve endings of taste and smell by the methyl-selenide which is excreted by the lung and which causes the garlic like odour so constantly noted and commented upon.

Vomiting occurred in all of the experiments except IV, where the carbohydrate intake was highest. It is interesting to note that although the vomiting was such a prominent feature of the intoxication it seemed to cause no pain or even discomfort. No sooner was the actual act of vomiting over, except in experiment II, where depression was a marked feature, than the animal would take an active interest in its surroundings until the next attack suddenly came on. The vomiting was curiously intermittent. Associated with the vomiting was the marked anorexia. It is difficult to offer an adequate explanation of these phenomena but the following hypothesis may be of interest. The organism as a whole finds its existence threatened by the presence within it of a substance whose toxic influence can be nullified by glucose. There is therefore a call for the mobilisation of the maximum amount of glucose in the blood. This can be effected by increasing the sodium chloride content of the blood (Fischer (3)). It therefore diverts all its available chlorides to this end and in consequence the chlorine is withdrawn from the oxyntic cells of the stomach. Absence of HCl is a recognised accompaniment of, if indeed not a cause of, gastric disturbances associated with anorexia. That chlorides are utilised to increase the carbohydrate content of the blood is suggested by Jones who found a decreased excretion of chlorides lasting four or five days after a sublethal dose of the salt. In an experiment which we carried out to confirm this observation we found that the average excretion of chlorides in the urine for the three days preceding the injection was 1·98 grams per diem and the average for the three days following the

injection was 0·035 gram. See also Exp. VI. This fall cannot possibly be accounted for by the abstention from food.

The severity of all these symptoms described above was in inverse proportion to the amount of carbohydrate previously ingested. Thus on the highest carbohydrate diet, viz. 204 grams per diem, there was no vomiting and the dog's health was but little disturbed, while on the lowest, viz. 34 grams, vomiting continued intermittently for 12 hours and for the two following days the animal was very lethargic.

Total nitrogen. As a result of the injection there is a marked rise in the output of nitrogen, indeed in every experiment the immediate increase is greater than fifty per cent. Whilst exact comparison is prevented owing to the varying amount of food taken on the days following the injection it will be noted that in general the increase is greater and continues longer where the preceding carbohydrate intake has been least.

As regards the source of this excreted nitrogen it can hardly be seriously maintained that the increased output is due to the drafting off of glucose to neutralise the toxic action of the twenty milligrams of sodium selenite injected and that as a result protein material is drawn upon to make up the deficiency. Of course part of the increased output on the days following the injection when no food was taken is probably accounted for on the grounds of energy requirement. In order to test this point in experiments II and III we interposed a day without food in the period preceding the injection but little or no rise in the output of nitrogen was observed. It is evident then that starvation does not play a very prominent part.

We believe that the increased excretion of nitrogen comes from a destructive disintegration of the protein molecule. It has been shown that the selenite is reduced to selenium within the cell, and that apparently the only substance capable of bringing about the reduction is carbohydrate. It is just possible therefore that the introduction of a small amount of selenite causes abstraction from the protoplasm of an equally small amount of carbohydrate, and that the loss of this carbo-hydrate results in a partial disintegration of the cell content with the subsequent excretion of the nitrogenous part.

Urea. The excretion of urea rises *pari passu* with the excretion of total nitrogen. Considered as percentage of the total nitrogen, taking the average of three days before injection and three days after, it will be seen from the following figures (Table VII) that, with the exception of experiment III, there is a rise in the percentage output of the urea

following the injection. It would seem as if the metabolic processes concerned in the synthesis of urea were stimulated rather than depressed.

TABLE VII. *Output of urea in per cent. of the total nitrogen.*

Experiment—	I	II	III	IV	V
Average of 3 pre-days	73·8	73·9	76·5	70·5	71·2
Average of 3 post-days	79·7	80·4	74·1	75·9	75·0

Ammonia. It may be said, generally speaking, that the effect of the injection on the course of the output of ammonia is to cause a rise in the absolute amount excreted but a fall in the percentage amount considered in per cent. of the total nitrogen. Table VIII brings out a very interesting fact for which we cannot at present offer an adequate explanation, viz. that the higher the intake of carbohydrate in the food the higher is the output of the ammonia in the days preceding the injection.

Turning to the percentage output it is seen that here again the high carbohydrate intake is associated with a high percentage output in the days preceding the injection whereas after the injection no regularity in the output is to be detected. The question of the production of an acidosis will be considered in the discussion of the creatine output.

TABLE VIII.

Experiment—		IV	III	I	VI	II	V
Carbohydrate intake in grms. per diem		204	189	161	100	64	34
Average output of 3 pre-days :							
Absolute (in grms.)	...	·29	·29	·24	·22	·20	·17
Per cent.	...	10·1	10·8	9·0	5·7	7·9	5·5
Average output of 3 post-days :							
Absolute (in grms.)	...	·28	·30	·20	·33	·32	·33
Per cent. ...	...	6·8	7·5	6·2	5·4	5·7	7·0

Amino acids. The output of these bodies is not very marked; the only experiment in which a definite rise in the output took place was V. Here on the three days before the injection the average excretion was 0·063 gram per diem and on the three days following the injection 0·101 gram per diem. In experiment III there was a very transitory rise.

Creatinine. The injection of the selenite causes an immediate rise in the output of creatinine either on the day of the injection or the day following in all our experiments with the possible exception of V. Following this rise there tends to be a fall in the output on the day or

two after this particularly noticeable in experiments II and V. The possible explanation of the fall below normal is that it is due to the presence of acetoacetic acid in these urines—the diets in both experiments were carbohydrate poor.

As our first five experiments were all carried out before the appearance of the observations on the influence of acetoacetic acid in the estimation of creatinine we controlled our results in experiment VI, in which the estimation of the creatinine was made *after* the removal of the acetone and acetoacetic acid. The only point which argues against such a conclusion is that in our observations (in another experiment, on a carbohydrate poor diet, the output of acetone and acetoacetic acid, as the result of the injection, rose from 7·7 mgrms. to 10·8 mgrms.) on the output of acetone and acetoacetic acid, after the injection of selenite, the rise, such as it was, only occurred on the day of the injection and the day after whereas the decrease in the creatinine output usually took place later.

When the output of creatinine is considered in percentage of the total nitrogen it is found, in every experiment, that the injection of the selenite produces a definite fall, as is clearly demonstrated in Table IX.

It will be noted that the fall is most marked in experiments II and V where the carbohydrate intake was lowest.

TABLE IX.

Experiment—	I	II	III	IV	V	VI
Average of 3 pre-days	5·05	5·38	4·31	4·54	4·40	3·70
Average of 3 post-days	4·09	2·35	3·10	3·43	2·70	2·48
Difference	·96	3·13	1·21	1·11	1·70	1·22

The results of these experiments certainly tend to subvert the view that creatinine is a direct derivative of creatine, formed from the latter substance by a method analogous to the formation of urea from ammonia. If the view, that some direct transformation occurred were correct, then one might expect a definite increase in the excretion of creatinine when a relatively enormous amount of creatine is being set free in the tissues and excreted in the urine (see also Table X) after each injection, unless the assumption also be made that some substance essential for this change is lacking as the result of the injections.

Creatine. Although we are inclined to agree with some recent workers who maintain that the available results are not sufficient to establish a direct causal relationship between carbohydrate and creatine

metabolism, we think that the present series of experiments shows at least that alterations in the amount of available carbohydrate in the body does affect the output of creatine in a very marked fashion. One of us (E. P. C.[4]) has already stated elsewhere his belief that the output of creatine is not so much evidence of the disturbance of the creatine metabolism *per se* as that " the appearance of creatine under these conditions (*i.e.* lack of carbohydrate) is merely to be regarded as an index of faulty metabolism in general and perhaps more particularly of the non-occurrence of resynthesis."

Before discussing our results it is necessary to refer to some recent work on the estimation of creatine. Greenwald[5] pointed out last year that the presence of acetoacetic acid in the urine produced an alteration in the estimation of creatinine. The red brown colour of the Jaffe reaction is bleached, the degree of bleaching being proportionate to the amount of acetoacetic acid present in the urine. This statement has been confirmed recently by Graham and Poulton (*l.c.*), who maintain that the recorded observations on the output of creatine during carbohydrate starvation are purely fictitious, and that the difference between the colorimeter readings for preformed creatinine and total creatinine, which has been interpreted by many recent workers (Cathcart[6], Mendel and Rose[7], Wolf[8], Meyer and Fine[9], Krause and Cramer[10] and others) as being due to the presence of creatine, is in reality due to the presence of acetoacetic acid. We have carried out a number of observations on this point and find that the original observation of Greenwald is correct but that the contention of Graham and Poulton, that the acidosis alone accounts for the difference in readings, is not true. There is no doubt about the fact that when acetoacetic acid is added to urine the actual creatinine reading on the colorimeter is raised, *i.e.* the colorimeter reading does not give the true creatinine content in the presence of the acetoacetic acid, the result is therefore too low. This observation is of very considerable importance when acidosis is a marked feature of the urine which is being examined as for example in severe diabetes.

The question arises as to whether these observations invalidate the conclusion reached by one of us (E. P. C. (*l.c.*)) and since confirmed and extended by many other workers, that the output of creatine is associated in some way with disturbances in the carbohydrate metabolism. As we [11] have shown elsewhere, in the case of starvation, the output of creatine, which was found, cannot be accounted for by the amount of acetoacetic acid present in the urine. These observations were checked

by the use of the method of Graham and Poulton (*l.c.*) for the removal of the acetoacetic acid, and still further controlled by the use of the diacetyl reagent of Walpole (12). We also found that creatine was actually excreted in the urine of a subject who was living solely on olive oil.

Still further evidence in support of the contention, that, if the course of carbohydrate be interfered with, creatine appears in the urine, is afforded by the output of creatine after the administration of phloridzin. In conjunction with Taylor one of us (E. P. C. (13)) found that, after the single injection of 0·75 grm. phloridzin, creatine appeared in the urine. It has been frequently demonstrated that it is extremely difficult to produce an acidosis in a dog, and further, Geelmuyden (14) and Baer (15) have both shown that a single small dose of phloridzin, such as we gave, does not produce an acidosis. This we have fully confirmed (*l.c.*). In the experiments of Wolf and Osterberg (16) large doses of phloridzin were repeatedly given and the output of acetoacetic acid was also estimated. They found that the creatine output preceded the appearance of the acetoacetic acid, and further, when the amount of the acid excreted is compared with the output of creatine, it is evident that the observed difference in readings between the preformed and the total creatinine could not be accounted for on the basis of an alteration due to the presence of the acetoacetic acid.

Then the recent experiments of McCollum and Hoagland (17) on carbohydrate starvation in the case of the pig yield the same result. They found that pigs fed on a carbohydrate diet excreted no creatine in the urine, but creatine was excreted on the last days of the experiment in which the diet consisted of fat and carbohydrate and was present throughout in the experiment in which fat alone was given as the foodstuff. At the same time they draw attention to the fact that it is difficult to produce an acidosis in the pig and they further state

TABLE X.

Experiment—	IV	III	I	VI	II	V
Amount of carbohydrate in grms. per diem	204	189	161	100	64	34
Amount of creatine excreted on the 3 days preceding the injection	·039	·083	·044	·171	·151	—
Amount of creatine excreted on the 2nd, 3rd and 4th days after the injection	·067	·137	·346	·540*	·676	·545
Increase in creatine output ...	·028	·054	·302	·369	·525	·545

* In Exp. VI the excretion of creatine is taken over the 1st, 2nd, and 3rd days following the injection as this experiment was ended on the third day after the injection.

definitely that "acetone and diacetic acid were found only during the last few days of fat feeding."

In the present series of experiments, as will be seen from the tables I–VI and more particularly from Table X and Fig. 1, the effect of the injection of the sodium selenite is to cause a very definite output of creatine which varies in amount inversely with the amount of carbo-hydrate present in the diet.

The effect of the amount of carbohydrate in the diet on the output of creatine is most clearly shown in Fig. 1.

Fig. I
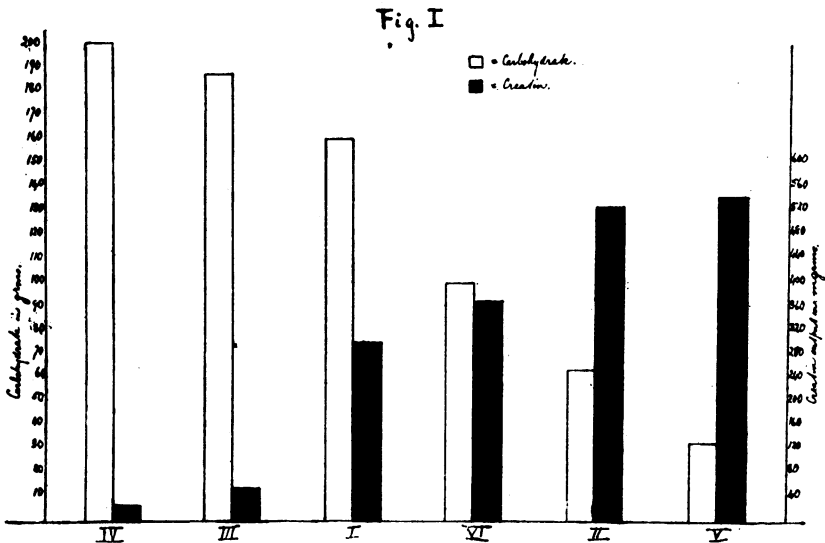
Incidentally it is interesting to note that in experiment V there was no excretion of creatine previous to the injection. The explanation of this may lie in the fact that the dog was allowed to get into first class condition before this experiment was carried out—it had a free open air life with abundant and varied diet. In order to test this the animal was again fed for some two months on an abundant mixed diet and allowed considerable freedom for exercise—the animal put on over a kilo. in weight—then given a carbohydrate low diet. No creatine was excreted for the first four days but on the fifth day a small amount appeared in the urine.

As was to be expected when the creatine output is considered in per cent. of the total nitrogen output it is found that following the injection there is always a very definite rise.

In view of the contention of Hawk[18] and his co-workers that crea-tine can be removed from muscle without of necessity causing complete disintegration of the protein molecule it is interesting to note the relation between the output of creatine and total nitrogen in the present series of experiments. We find that the ratio of creatine to total nitrogen in muscle tissue is approximately 1 : 10 and in practically all our experiments the creatine : total nitrogen ratio in the urine is greater than one to ten except in experiment V, where it is 1 : 9·4, and in experiment I. In this experiment the ratio for some unknown reason is only 1 : 5. This abnormal ratio results on the one hand from a very slight increase in the output of total nitrogen and on the other from an abnormally large output of creatine. Even experiment V, with its large output of creatine, does not in our opinion support Hawk. Here 0·545 gram of creatine was excreted, on the 2nd, 3rd and 4th days, which would represent the amount of creatine present in about 176 grams of muscle, as, according to the recent analyses of Beker[19], dog muscle contains about 0·31 °/₀ of creatine. If the output of total nitrogen is now considered it is found that the total increase (*i.e.* the total output of the three post-days less the total output of the three pre-days) as the result of the injection only amounts to some 5·14 grams. Assuming that the total nitrogen content of moist dog muscle tissue is about 3·3 °/₀ (the figure found in this laboratory by G. D. Cathcart), the output of nitrogen for the 176 grams of tissue should be about 5·8 grams. Thus in the experiment in which most creatine was excreted the output of creatine and the output of total nitrogen are practically in the proportion in which they exist in muscle tissue. Of course it is admitted that such a calculation as the above is only partially true, firstly because owing to the fact that the animal abstained from food on the 2nd day the actual extra output of total nitrogen must perhaps be regarded as 5·14 plus 4·42 = 9·56 grams, thus a ratio of 1 : 17·5, and secondly because it is highly improbable that muscle tissue is the only tissue attacked by the selenite and that therefore part of the nitrogen excreted will come from sources which are practically creatine free.

The question of a possible resynthesis having taken place does not in our opinion arise in the present series of experiments as the conditions for synthetic activity were far from favourable.

At present we are investigating the problem as to whether there may be some possible connection between the presence of creatine and the utilisation of carbohydrate or other source of energy in the muscle.

It is an interesting point that creatine is present in greatest amount about ·3 % in voluntary muscle where there are sudden demands for the liberation of energy, whereas in involuntary muscle it only amounts to about ·03 % ; in cardiac muscle it takes an intermediate place about ·2 %.

CONCLUSIONS.

1.   The injection of sodium selenite produces salivation, vomiting, anorexia, and lethargy.

2.   The total nitrogen and urea outputs are raised as the result of the injection; this is most marked in the experiments with carbohydrate poor diets.

3.   The ammonia output during the pre-days is highest with carbohydrate rich diets; on the days following the injection it is highest in the low carbohydrate experiments.

4.   There is no marked acidosis following the injection as estimated by the output of ammonia, acetone and acetoacetic acid.

5.   The injection of the selenite causes an immediate rise in the output of creatinine with possibly a subsequent fall below normal.

6.   As a result of the injection there is a very definite rise in the output of creatine which cannot be explained by the presence of acetoacetic acid. The output of creatine varies inversely with the amount of carbohydrate in the food.

7.   It may be said generally that the toxic effect of the selenite was most marked when the diet was poor in carbohydrate.

REFERENCES.

(1)   J o n e s.   Biochem. Jour. iv. 405. 1909.
(2)   G r a h a m and P o u l t o n.   Proc. Roy. Soc. B. lxxxvii. 205. 1914.
(3)   F i s c h e r.   Arch. f. ges. Physiol. cix. 1. 1905.
(4)   C a t h c a r t.   Physiol. of Protein Metabolism, p. 116. London, 1912.
(5)   G r e e n w a l d.   Jour. Biol. Chem. xiv. 87. 1913.
(6)   C a t h c a r t.   This Journal, xxxix. 311. 1909.
(7)   M e n d e l and R o s e.   Jour. Biol. Chem. x. 213. 1911.
(8)   W o l f.   Ibid. x. 473. 1911.   Biochem. Zeit. xxxv. 329. 1911.
(9)   M e y e r and F i n e.   Jour. Biol. Chem. xv. 305. 1913.
(10)   K r a u s e and C r a m e r.   Proc. Physiol. Soc. lxi. 1910 (This Journal, xl.).

(11) Cathcart and Orr. About to be Published in Proc. Physiol. Soc. for Mar. 14, 1914 (This Journal, xlviii.).

(12) Walpole. This Journal, xlii. 301. 1911.

(13) Cathcart and Taylor. Ibid. xli. 277. 1910.

(14) Geelmuyden. Zeit. f. physiol. Chem. xxvi. 381. 1898.

(15) Baer. Arch. f. exp. Path. u. Pharm. li. 271. 1904.

(16) Wolf and Osterberg. Amer. Jour. Physiol. xxvii. 71. 1911.

(17) McCollum and Hoagland. Jour. Biol. Chem. xvi. 317. 1913.

(18) Howe, Mattill and Hawk. Ibid. xi. 103. 1912.

(19) Beker. Zeit. f. physiol. Chem. lxxxvii. 21. 1913.