II. A STUDY OF THE WATER-SOLUBLE ACCESSORY GROWTH PROMOTING SUBSTANCE. II. ITS INFLUENCE UPON THE NUTRITION, AND NITROGEN METABOLISM OF THE RAT.

By JACK CECIL DRUMMOND.

From the Cancer Hospital Research Institute, London.

(Received Jan. 30th, 1918.)

IN a previous communication it was shown that certain properties of the water-soluble accessory substance are similar to those which are associated with the so-called "anti-neuritic vitamine" [Drummond, 1917]. This fact, taken into consideration with the observation of McCollum and Kennedy [1916] that many extracts known to contain the water-soluble growth factor are active in the prevention or cure of polyneuritis in birds, led to the suggestion being advanced that the two substances are identical. If this view is correct, it cannot but be apparent from recent research how very important a rôle is played by this dietary factor in the nutrition of the animal organism. The present paper presents a series of observations bearing more or less directly upon this subject, and although they do not represent the results of a systematic research, they have been collected together in the hope that they may assist in throwing light upon certain aspects of the question.

1. *Influence of a deficiency of the water-soluble accessory factor in the diet upon the nitrogen metabolism of the rat.*

Four healthy young male rats weighing approximately 90–100 grams each were selected for this study. They were placed in pairs in metabolism cages of the type designed by Dr Alexander Paine, Director of this Institute. Modifications of this apparatus have been described [Ackroyd and Hopkins, 1916], but the original form was found to satisfy the requirements of the present investigation. By preparing the dietaries in the form of stiff pastes, the animals are prevented from scattering the food about to any appreciable

extent. Fresh food and water were supplied every morning, the food-consumption being estimated by weighing the food containers before and after every 24 hour period. The 24 hours' urine was collected in a 100 cc. Erlenmeyer flask containing a few cc. of very dilute acetic acid. This, together with the cage washings, was filtered through glass-wool in order to remove fur and other extraneous matter, and made up to a known volume, usually 50 cc. The estimations were made by the usual methods; total nitrogen in faeces and urine by micro-Kjeldahl, urea by urease, ammonia by the aeration method of Folin, and creatinine and creatine by a modification of the micro-method used by Folin and Morris [1913] in their investigation of the normal protein metabolism of the rat. Two animals were fed throughout upon an artificial ration (Diet 1) which has been extensively utilised in this laboratory, and which is known to be adequate as regards all the known dietary components, since three generations of rats have been reared to maturity in the normal period upon it. The other two rats were fed for a short preliminary period upon the complete ration; then, when it had been ascertained that they were responding with a normal rate of growth, they were given Diet 2, which resembled Diet 1 in every way except for the absence of the water-soluble factor (yeast extract). The composition of the diets was as given below:

	Diet 1	Diet 2
Caseinogen ...	18 parts	18 parts
Butter-fat ...	20 „	20 „
Starch ...	46 „	52 „
Agar ...	5 „	5 „
Salt mixture ...	5 „	5 „
Yeast extract...	6 „	0 „

In order to render the diets of a pasty consistency a small quantity of distilled water was added in each case.

The tabulated results of the metabolism experiments are given in Tables I and II. These results will now be considered in detail, and other observations bearing upon each subject will be introduced where appropriate.

(a) *Nitrogen Metabolism.* The urinary analyses tabulated in Tables I and II do not show any striking abnormality, other than the appearance of creatinuria during the period upon the diet deficient in the water-soluble substance. This is not surprising in view of the slow decline in body-weight of the rats upon this ration caused by wasting of the skeletal muscles observed

TABLE I.

Metabolism of two normal rats fed upon a complete artificial dietary. (Diet 1.)

Day	Body weight g.	Rectal temperature °C.	Food consumed g.	N intake g.	Caloric intake	Total N in urine g.	% urea N	% ammonia N	% creatinine N	% creatinine + creatinine N	N in faeces g.	Total N excreted g.	N balance g.
1	94	118	31.0	0.861	117	0.491	—	—	—	—	0.050	0.541	+0.320
2	—	—	32.6	0.904	123	0.603	—	—	—	—	0.041	0.644	+0.260
3	—	—	26.2	0.665	99	0.251	80.0	11.5	0.65	0.70	0.046	0.297	+0.368
4	—	—	21.1	0.538	80	0.234	—	—	—	—	0.036	0.270	+0.268
5	109	131	22.5	0.571	85	0.210	76.7	11.1	1.03	1.04	0.042	0.252	+0.319
6	—	—	23.6	0.600	89	0.233	77.7	12.0	1.03	1.03	0.033	0.266	+0.334
7	120	141	33.3	0.846	126	0.255	—	—	—	—	0.027	0.282	+0.564
8	—	—	29.3	0.731	111	0.320	79.2	8.5	0.81	0.86	0.043	0.363	+0.368
9	—	—	32.9	0.822	124	0.307	80.1	10.0	0.94	0.99	0.047	0.354	+0.468
10	—	—	34.6	0.865	131	0.318	76.9	8.2	0.92	0.96	0.038	0.356	+0.509
11	128	151	37.7	0.943	143	0.384	—	—	—	—	0.055	0.439	+0.504
12	—	—	35.2	0.880	132	0.425	—	—	—	—	0.049	0.474	+0.406
13	—	—	28.4	0.710	107	0.396	—	—	—	—	0.048	0.444	+0.266
14	137	160	29.8	0.745	113	0.421	80.2	8.5	0.72	0.79	0.043	0.464	+0.281
15	—	—	35.8	0.902	136	0.406	80.1	8.6	0.95	0.96	0.053	0.459	+0.443
16	—	—	29.4	0.740	111	0.360	—	—	—	—	0.041	0.421	+0.319
17	149	169	34.8	0.878	132	0.356	—	—	—	—	0.047	0.403	+0.475
18	—	—	36.8	0.928	139	0.478	78.2	10.0	1.12	1.16	0.080	0.508	+0.420
19	157	178	38.7	0.972	146	0.529	78.9	8.9	1.03	1.03	0.047	0.576	+0.396

TABLE II.

Metabolism of two normal rats upon a diet deficient in water-soluble substance. (Diet 2.)
(Fore and after periods on Diet 1.)

Day	Diet	Body-weights g.	Rectal temperatures C.	Remarks	Food consumed g.	N intake g.	Caloric value g.	Total N in urine g.	% am- monia N	% creat- inine N	% creat- inine N	Total N ex- creted g.	N balance g.
1	1	84	86	38.0	37.9	—	—	—	—	—	—	—	—
2	1	93	101	—	—	—	—	—	—	—	—	—	—
3	1	93	101	—	—	—	—	—	—	—	—	—	—
4	1	106	116	38.1	38.0	—	—	—	—	—	—	—	—
5	1	106	116	38.1	38.0	—	—	—	—	—	—	—	—
6	1	—	—	—	—	—	—	—	—	—	—	—	—
7	2	109	119	38.3	38.3	—	—	—	—	—	—	—	—
8	2	107	110	38.2	38.5	—	—	—	—	—	—	—	—
9	2	107	110	38.2	38.5	—	—	—	—	—	—	—	—
10	2	—	—	—	—	—	—	—	—	—	—	—	—
11	2	—	—	—	—	—	—	—	—	—	—	—	—
12	2	106	107	38.0	37.7	—	—	—	—	—	—	—	—
13	2	—	—	—	—	—	—	—	—	—	—	—	—
14	2	—	—	—	—	—	—	—	—	—	—	—	—
15	2	—	—	—	—	—	—	—	—	—	—	—	—
16	2	—	—	—	—	—	—	—	—	—	—	—	—
17	2	106	107	37.6	37.3	—	—	—	—	—	—	—	—
18	2	—	—	—	—	—	—	—	—	—	—	—	—
19	2	—	—	—	—	—	—	—	—	—	—	—	—
20	2	104	106	37.4	37.4	—	—	—	—	—	—	—	—
21	2	—	—	—	—	—	—	—	—	—	—	—	—
22	2	—	—	—	—	—	—	—	—	—	—	—	—
23	2	103	102	37.2	37.0	—	—	—	—	—	—	—	—
24	2	100	99	—	—	—	—	—	—	—	—	—	—
25	2	—	—	—	—	—	—	—	—	—	—	—	—
26	2	—	—	—	—	—	—	—	—	—	—	—	—
27	2	100	98	37.0	36.8	—	—	—	—	—	—	—	—
28	2	—	—	—	—	—	—	—	—	—	—	—	—
29	2	97	96	—	—	—	—	—	—	—	—	—	—
30	2	—	—	—	—	—	—	—	—	—	—	—	—
31	2	97	92	—	—	—	—	—	—	—	—	—	—
32	2	—	—	—	—	—	—	—	—	—	—	—	—
33	2	—	—	—	—	—	—	—	—	—	—	—	—
34	2	94	92	36.2	35.9	—	—	—	—	—	—	—	—
35	1	96	—	36.0	—	—	—	—	—	—	—	—	—
36	1	106	—	—	—	—	—	—	—	—	—	—	—
37	1	111	—	—	—	—	—	—	—	—	—	—	—
38	1	124	—	—	—	—	—	—	—	—	—	—	—
39	1	130	—	—	—	—	—	—	—	—	—	—	—
40	1	130	—	—	—	—	—	—	—	—	—	—	—
41	1	136	—	—	—	—	—	—	—	—	—	—	—

P.M. examination rat 1: The skeletal musculature was markedly wasted. There was slight enlargement of the right heart, with congestion of the kidneys and liver. Thyroid gland appeared to be smaller than normal. Other organs normal.

P.M. examination rat 2: The animal presented a normal appearance. Thyroid gland was of normal size.

during that period. Small traces of creatine are often present in rats' urine upon normal diets [Folin and Morris, 1913].

(b) *Body-weight.* The two control animals fed upon the complete ration (Diet 1) showed a normal rate of growth throughout the experiment. Similarly the rats studied in Table II grew at a normal rate during the preliminary and final periods, when receiving that diet. During the period when they received the diet free from the water-soluble factor (Diet 2) there was complete inhibition of growth, and a very gradual decline in body-weight. A very large number of rats have been observed whilst being fed for considerable periods upon dietaries deficient in the water-soluble substance. In the case of young rats the withdrawal of this dietary component is almost invariably followed by an immediate cessation of growth. Such inhibition is usually followed by a period during which the animal either maintains a constant body-weight, or shows a very slow decline. Sooner or later there is a sudden and rapid drop in weight lasting a few days, terminated by death. If, during the periods of maintenance or decline, provided in the latter case that the condition of the rat is not extreme, the missing dietary factor is supplied in an adequate amount, the rat recovers and resumes a normal rate of growth. From a large number of observations it has been ascertained that the period of maintenance, which follows the restriction and precedes the final decline, is roughly proportional to the age of the animal when the restriction is imposed. Some average figures which illustrate this are given in Table III.

TABLE III.

Average body-weight of rat when water-soluble factor withheld from diet	Number of observations	Average duration in days of period of maintenance preceding final decline
50 g.	42	25
60 "	67	31
70 "	30	37
100 "	21	46
150 "	12	57
200 "	9	> 60

Naturally there are wide variations to be observed in animals of all ages, but the above averages are significant of the very great importance of the water-soluble accessory substance during the earlier stages of growth. Adult animals are able to live for considerable periods without receiving a supply of this dietary factor. During this period they usually show a slow decline in body-weight, which is associated at a later stage with an enfeebled resistance to disease. The ultimate decline is, however, inevitable. From the figures given in Table III it is reasonable to assume that the need of the

organism for the water-soluble factor during the very early stages of growth must be of an urgent nature. McCollum, Simmonds and Pitz [1916] found that the two dietary accessories, the "fat-soluble A" and the "water-soluble B" are only present in the milk of the nursing mother (rat) when they are supplied to her in her own dietary; it being, therefore, improbable that the animal organism can synthesise either substance. The experimental results of these authors regarding the latter substance have been confirmed by a series of experiments made in this laboratory. A number of healthy, full-grown female rats were selected from the breeding stock, all of which had reared at least one satisfactory litter previously. They were mated with reliable breeding males, and as soon as pregnancy was observed, they were removed to separate nesting cages provided with an ample supply of fine wood-shavings for bedding. At various stages, before and after parturition, individual females were placed upon the ration deficient in the water-soluble factor, and the influence of this deficiency upon the mother and young was closely noted. The results of this experiment are summarised in Table IV. From a consideration of the results contained in that table it will be seen that a restriction in the supply of the water-soluble factor in the diet of a pregnant rat may be followed by very serious consequences, both to herself and her offspring. This was particularly marked when the restriction was imposed before parturition. When imposed at, or after, delivery, the young usually lived, and in some cases even showed a subnormal rate of growth for a short period, but the fatal decline was not long in appearing. By returning the mother to a normal diet, the young could be saved, although the improvement in them was not immediately appreciable.

Further points concerning the relationship of the water-soluble factor to growth, will be considered in the later section which deals with its influence upon food-consumption.

(c) *Body-temperature.* The rectal temperature of the rats, whose metabolism was being studied, was taken regularly. It was found that a slow fall in body-temperature occurred in the two animals fed upon the inadequate ration. In order to confirm this observation, the body-temperatures of a large number of rats of various ages upon normal diets, and upon diets deficient in the water-soluble factor, were registered. The readings were taken at the same times during the day, as it was found that the normal rat shows considerable diurnal variation in body temperature. All the rats examined were under adult size (140 gms.), and the following average figures were obtained:

TABLE V.

Sex	Time	Average normal temperature	Average temperature after 10 days upon inadequate diet	Average temperature after 21 days upon inadequate diet
Male	10 a.m.	38.1°	37.8°	37.0°
	6 p.m.	38.5°	37.9°	37.2°
Female	10 a.m.	38.4°	38.0°	37.1°
	6 p.m.	38.7°	38.3°	37.5°

(d) *General Health and Conditions of the Organs.* Apart from the metabolism study reported in these pages, the researches carried out in this laboratory during the last year or two have provided an opportunity for observing the state of health of a large number of rats fed upon diets deficient in the factor being considered in this paper. Whilst many of these observations were merely of a cursory nature, the general appearance of the animal alone being recorded, others were more complete, including observations of body temperature, cellular content of the blood, careful post-mortem examinations, and histological examination of the individual organs.

For some time after the water-soluble factor has been withheld from the diet the general appearance of the animals is apparently normal. They are lively, vigorous, sleek-coated and bright-eyed. As the period of maintenance at constant body-weight draws to a close, the rats gradually lose this appearance. The fur loses its gloss and becomes ruffled, whilst the rats assume a hunched attitude, and become lethargic. During the final decline the rats appear pale and emaciated, feeling cold and thin to the touch, and they usually remain huddled in corners away from the light. Funk and Macallum [1915] have recorded that rats fed upon diets deficient in the water-soluble factor (yeast) showed a condition of spastic contraction resembling avian beri-beri, or infantile tetany, 24 or 48 hours before death. Only three cases showing symptoms at all suggestive of nerve disorders have been noted in this laboratory during the careful observation of a very large number of rats upon such diets. All three cases showed a uniformity of symptoms, usually beginning with an apparent difficulty in co-ordinating the movements of the hind limbs, which appeared about 24 hours before death. The inco-ordination became progressively worse, until there was partial paresis of the hindquarters. As the fatal termination approached, these animals periodically passed through violent convulsive spasms lasting a minute or two. During such attacks the loss of control over the hind quarters was complete, the head was rolled from side to side, and respiration became spasmodic, being assisted by violent diaphragmatic contractions. An attempt was made to

cure one rat showing these advanced symptoms, by an administration of a yeast extract, but it was unsuccessful, the animal dying an hour later.

A number of blood examinations were made upon rats during the period of maintenance. In practically every case normal values for cell counts and haemoglobin percentage were obtained. It is of interest to note that the cellular counts noted for the rats investigated in this work (black variety of *Mus norvegicus*) were uniformly lower than the average figures given for the albino rat by Taylor [1916]. His observation of the wide variations in the differential counts of normal rats was confirmed. The figures obtained are given in Table VI.

TABLE VI.

	Young adult rat normal diet; average of 12 observations	Young adult rats deficient diet; average of 14 determinations	Adult albino rat (Taylor)
Red corpuscles	$(8.9-7) \times 10^6$	$(7.8-9.5) \times 10^6$	$(9-10.5) \times 10^6$
White "	8,000-14,000	7,000-12,000	12,000-30,000
Polymorphonuclears	30	31	50
Large mononuclears	24	32	14
Small "	30	29	30
Transitional	15	7	5
Eosinophile	1	1	1

The cause underlying the fatal termination which invariably follows the absence of the water-soluble factor from the diet has not been ascertained. A considerable number of rats which have died from the consequences of this deficiency have been subjected to post-mortem examination. The majority presented an emaciated appearance, the wasting being limited chiefly to the skeletal musculature. Enlargement of the right heart was noted in a few cases, whilst a number of animals showed very small thyroid glands. A normal macroscopic appearance of the lungs, kidneys, pancreas, suprarenals, liver, spleen and alimentary tract was always observed. No examination of the nervous system was made. This may appear regrettable, when the possible relationship between this condition and the so-called avian polyneuritis is considered, but no opportunity for the study being made presented itself. Tissues removed from animals at post-mortem examination were subjected to histological examination. Testicles removed from adult males showed in several cases an inhibition of spermatogenesis. The results of the histological examination will furnish the subject of a separate communication.

Funk and Douglas [1914] examined the organs from a number of pigeons

which had developed symptoms of the so-called polyneuritis, as a result of continued feeding upon a diet of polished rice. They recorded complete atrophy of the thymus, whilst the other glands examined all showed a diminution in size, with microscopic evidence of degenerative change in the cells with higher function. The testes were atrophied and spermatogenesis was absent. The thyroid, although atrophied, did not show abnormalities regarding the colloid content.

That inadequacies in the diet of animals may adversely influence breeding is known to all who have had experience in feeding artificial rations.

That the inadequacy under consideration affects the male rat in this manner was shown by the following breeding trials. Six adult bucks, all known to be good breeders, were placed upon the diet deficient in the water-soluble substance (Diet 2). On the first day one animal was mated with two good breeding females who were receiving normal food, and was allowed to run with them for 48 hours. During this period the sexes were separated to receive their respective diets every morning and evening, and then allowed to run together again. Every seven days another buck was mated with two normally fed does for the same period, under exactly the same conditions. After having run with the females, each male rat was killed, the testicles being removed for histological examination. The results are given in summarised form below:

TABLE VII.

No. of male	Days upon inadequate dietary before given access to females	Result of mating
1	0	2 pregnancies, 15 young, all reared
2	7	1 pregnancy, 3 young, all reared
3	14	no pregnancy
4	21	"
5	28	"
6	35	"

(e) *Food-consumption*. From the figures given in Table II, it may be observed that a marked fall in the food-consumption of the rats occurred when the diet was deficient in the water-soluble factor. This has been repeatedly observed with large numbers of other rats, although it was more apparent in some cases than in others. The significance of the calorific intake in such studies as these has been made the subject of several investigations.

Hopkins [1912] submitted this question to a very complete examination and showed conclusively that growth was inhibited in young rats, by the absence of the accessory substances, at a time when the food intake of the

animals was more than sufficient quantitatively to maintain normal growth. He also found that whilst the absolute food-consumption of animals rapidly growing upon diets containing the accessory substances was greater than of those not growing upon the deficient diet, yet the consumption per unit live weight was, in comparable groups, nearly the same. He therefore concluded that any effect of the accessory substances upon the appetite must be secondary to a more direct effect upon the growth processes. The majority of investigators have observed that the absence of the water-soluble factor causes a lowered food-consumption [Funk and Macallum, 1915; Osborne and Mendel, 1917]. The latter authors have discussed the question in the light of some experiments they made, and concluded that yeast added to a diet deficient in the "water-soluble B" exerts some favourable influence upon the metabolism of the rat, causing it to eat more because its general condition is improved. This is confirmatory of the views expressed by Hopkins [1912].

A number of experiments bearing upon this subject were made during the course of the present investigation.

Two batches of twelve young rats were taken. One lot was fed upon the complete ration (Diet 1), the second batch receiving the inadequate diet (Diet 2). The food-consumption and body-weight of the animals were recorded daily, and were as follows:

TABLE VIII.

Day	Inadequate diet (3.95 cal. per g.)			Complete diet (3.34 cal. per g.)		
	Average wt. g.	Average food con- sumption g. per day	Average calorific in- take per 100 g. wt. per day	Average wt. g.	Average food con- sumption g. per day	Average calorific in- take per 100 g. wt. per day
1	89	6.6	29.4	90	13.6	50.5
5	91	6.3	27.5	110	14.3	45.0
9	90	6.7	29.4	123	15.6	42.4
13	87	6.4	29.2	135	16.7	40.2
17	86	6.0	27.6	149	17.1	38.4
21	85	5.7	26.6	156	18.0	38.6

As the calorific intake per 100 g. live weight was very low upon the inadequate diet, it was of importance to ascertain whether growth was possible upon such intake, provided that the accessory factor under consideration was present. The following ration was used:

Caseinogen ...	20 parts	Salt mixture	5 parts
Starch ...	25 "	Yeast extract	5 "
Butter-fat ...	20 "	Water ...	20 "
Agar ...	5 "		

The calorific value was approximately 3.7 calories per g. A series of healthy young rats were selected and weighed quantities of this food were supplied to them daily. The results are tabulated in Table IX.

TABLE IX.

	1st day	8th day	15th day	22nd day	29th day	36th day	43rd day
1. Food per day, g.*	8	8	8	8	8	20	20
Average calories per 100 g. live weight	35.2	27	24.3	22.5	21.8	52.8	45.8
Average yeast extract per 100 g. weight	0.48	0.37	0.33	0.30	0.29	1.77	1.54
Weight of rat, g.	84	109	122	132	136	140	161
2. Food per day, g.	8	8	8	8	8	24	24
Average calories per 100 g. live weight	35.2	27	24.8	22.9	22.5	63.6	55.9
Yeast extract per 100 g. live weight ...	0.48	0.37	0.33	0.30	0.30	0.72	0.63
Weight of rat, g.	84	110	120	130	132	139	159
3. Food per day, g.	8	8	8	8	8	20	24
Average calories per 100 g. live weight	36.6	28.8	26.5	26.1	24.4	62.0	62.5
Yeast extract per 100 g. live weight ...	0.48	0.38	0.36	0.36	0.33	0.78	0.71
Weight of rat, g.	81	103	112	114	122	129	142
4. Food per day, g.	8	8	4	5	10	10	10
Average calories per 100 g. live weight	37.5	31.5	16.0	19.0	36.8	33.0	28.4
Yeast extract per 100 g. live weight ...	0.48	0.42	0.21	0.19	0.60	0.53	0.46
Weight of rat, g.	79	95	93	97	100	112	130
5. Food per day, g.	8	8	4	5	10	10	10
Average calories per 100 g. live weight	33.0	26.5	13.5	17.4	33.0	29.8	26.8
Yeast extract per 100 g. live weight ...	0.46	0.36	0.18	0.23	0.54	0.48	0.43
Weight of rat, g.	88	112	110	106	112	124	138
6. Food per day, g.	8	8	4	5	10	10	10
Average calories per 100 g. live weight	32.6	24.8	13.3	17.2	33.0	30.8	28.4
Yeast extract per 100 g. live weight ...	0.44	0.33	0.18	0.23	0.54	0.50	0.46
Weight of rat, g.	91	120	112	108	112	120	130

* Weight of food recorded was given daily for week following.

The figures given in this table demonstrate that rats may show growth, at a corresponding period of development, upon a calorific intake lower than 29 calories per 100 g. live weight, provided that the accessory substance is supplied in adequate amount. Hopkins has stated [1912], "Only those perhaps who have had experience of feeding animals with excess of food, and have noted the amount eaten for considerable periods, will realise how well adjusted under normal circumstances is the instinctive appetite to the physiological needs."

Many opportunities for observing the truth of this statement have been met with during the course of the present research.

In the absence of the water-soluble accessory factor it is probable that the calorific intake is lowered approximately to that required for maintenance at the stage of development which has been reached. This may

be illustrated by the results obtained in a series of feeding experiments designed to ascertain whether food consumption is affected by the calorific value of the diet, when the water-soluble factor "B" is absent.

The following dietaries were prepared:

	High calorific diet	Low calorific diet
Caseinogen	20 parts	20 parts
Starch	35 "	45 "
Butter-fat	30 "	10 "
Salt mixture	5 "	5 "
Water	10 "	40 "
Calorific value per g.	5.06	3.04

TABLE X.

Low calorific diet (12 rats)					High calorific diet (12 rats)				
Days	Average body-weight g.	Average food consumed daily g.	Average daily calorific intake	Average calories per 100 g. live weight	Average body-weight g.	Average food consumed daily g.	Average daily calorific intake	Average calories per 100 g. live weight	
0	78	6.9	21.0	32.0	76	4.2	21.2	34.2	
4	79	9.0	27.3		72	5.7	28.9		
8	81	8.5	25.9		69	4.4	22.3		
12	76	8.4	25.6		69	5.2	26.4		
16	76	7.7	23.4		69	4.5	22.7		
20	74	7.6	23.1		69	5.0	25.4		
24	72	7.7	23.4		74	4.3	21.8		
28	71	8.2	24.9	69	5.1	25.9			
Animals given (diet 1) 5 % yeast extract diet (3.37 cala. per g.)					Animals given (diet 1) 5 % yeast extract diet (3.37 cala. per g.)				
29	72	13.5	45.6	63.4	70	12.0	40.5	57.9	
33	89	15.6	52.7	59.0	78	12.8	43.1	55.3	
37	101	16.0	54.0	53.4	91	13.3	44.9	49.4	
Animals given modified diet 1, 5 % meat extract instead of yeast extract (3.25 cala. per g.)									
38	106	11.3	36.7	42.6	109	14.6	49.2	45.0	
42	105	14.0	45.5		121	14.8	50.0	41.3	
46	104	15.0	48.7		130	16.5	55.7	42.8	
50	105	14.6	47.5		142	17.0	57.3	40.4	
54	105	14.2	46.1		150	18.1	61.1	40.7	

The food consumption of two closely similar batches of young rats fed upon these rations was noted, the results being given in Table X. It will be seen that the calorific intakes per 100 g. live weight of both groups approximate closely. The question of the influence of flavouring agents upon the palatability of the ration and food consumption has received attention from several investigators. Both Hopkins [1912], and Osborne and Mendel [1917], have shown, by administering the accessory substances separately from the main food supply, that the action of these substances is direct upon the

TABLE XI.

Day	Group 1 (8 rats)			Group 2 (6 rats)			Group 3 (6 rats)			Group 4 (4 rats)		
	Yeast g. per day	Average daily food intake, g.	Average weight g.	Yeast extract g. per day	Average daily food intake, g.	Average weight g.	Yeast extract g. per day	Average daily food intake, g.	Average weight g.	Yeast extract g. per day	Average daily food intake, g.	Average weight g.
0	0	7.2	86	0.25	8.9	90	0.5	10.0	80	1.0	13.2	86
2	0	8.1	89	0.25	8.9	90	0.5	12.4	86	1.0	14.6	89
4	0	7.4	84	0.25	10.0	92	0.5	14.1	89	1.0	15.0	94
6	0	7.6	87	0.25	9.4	93	0.5	14.2	90	1.0	15.7	102
8	0	7.9	87	0.25	9.7	92	0.5	14.1	92	1.0	17.0	105
10	0	8.0	89	0.25	9.8	94	0.5	15.0	96	1.0	16.0	110
12	0	8.2	87	0.25	9.2	96	0.5	13.2	99	1.0	16.7	116
14	0	8.2	85	0.25	9.7	96	0.5	13.0	100	1.0	17.2	122
16	0	7.7	85	0.25	9.9	95	0.5	12.7	104	1.0	15.0	129
18	0	7.4	84	0.25	10.0	94	0.5	13.2	110	1.0	17.9	134
20	0	7.1	86	0.25	9.7	96	0.5	14.5	112	1.0	19.2	137
22	0	6.7	84	0.25	11.1	97	0.25	12.0	110	1.0	18.0	142
24	0.5	10.2	86	0.75	15.0	105	0.25	12.0	110	1.0	20.0	149
26	0.5	11.1	88	0.75	16.7	112	0.25	13.0	109	1.0	21.2	150
28	0.5	14.4	90	0.75	17.0	119	0.25	12.0	109	1.0	19.1	157
30	0.5	13.0	93	0.75	18.7	122	0.25	12.2	108	0	17.2	156
32	0.5	12.9	96	0.75	18.5	130	0.25	12.2	109	0	15.4	150
34	0.5	14.2	98	1.0	19.8	138	0.25	11.7	107	0	15.0	150
36	0.5	15.0	110	1.0	19.4	147	0.25	10.7	105	0	15.6	149
38	0.5	15.1	114	1.0	19.0	150	0.25	9.8	105	0	14.3	147
40	0.5	14.6	116	1.0	20.0	150	0.25	9.9	104	0	14.0	146

growth processes. Commercial meat extract has been found to be deficient in the water-soluble accessory factor [Drummond, 1917]. The influence of this substance, acting as far as is known purely as a flavouring agent, upon food-consumption may be seen from Table X. An increase in calorific intake is apparent without any growth resulting.

Osborne and Mendel [1917] found that the amount of growth is proportional to the amount of yeast fed, the other components of the diet being adequate. Some experiments of this nature were made in this laboratory, mainly with the object of determining the minimum quantity of extract containing the accessory factor which would give growth. Four series of healthy young rats were selected, and placed upon the diet deficient in the water-soluble factor. A standard yeast extract was then prepared, measured quantities of which were administered *per os* to each rat daily by means of a small syringe before the animals received their morning meal. The results are tabulated in Table XI.

From Table XI the following average figures have been extracted (Table XII).

TABLE XII.

Approximate amount of yeast extract per 100 g. live weight given daily	Result	Animals yielding result
0	Maintenance or slow decline	Groups 1 and 4
0.25	Maintenance	Groups 2 and 3
0.50	Slow growth	Groups 1 and 3
0.60	Medium growth	Groups 1 and 3
0.70	Good growth	Group 2
1.0—1.5	Rapid growth	Group 4

These figures show reasonable agreement with some of the other results recorded in these pages. Thus, from Table IX, it will be seen that several rats receiving 8 g. of diet daily, containing 0.4 g. yeast extract, showed cessation of growth upon reaching an average weight of 135 g. that is when ingesting approximately 0.30 g. yeast extract per 100 g. body weight.

Although, as Hopkins has pointed out [1912], it is unwise to expend time upon determinations of this nature until a more or less pure substance has been isolated, it may yet be of interest to note certain facts regarding the composition of the yeast extract. The 0.3 g. contained 0.24 g. of solids, of which approximately 70 % was represented by products known to be inactive as regards growth-accelerating influence, such as inorganic salts. The remaining 0.064 g. contained the accessory factor under consideration, as well as considerable amounts of adenine and leucine.

2. *Concerning the presence of the Water-soluble Accessory Substance in actively growing Animal Tissues.*

The water-soluble accessory substance has not been found in association with extracts of animal tissues, such for example as commercial meat extract. Likewise fresh beef tissue has been found during the present research to be deficient in the factor. The question arose as to whether it might be present in tissues in the process of rapid growth. To test this, concentrated protein-free aqueous extracts were prepared from three such tissues, (a) rat-embryos, 30 days old; (b) rat sarcoma tissue; (c) Rous chicken sarcoma tissue. In no case did the administration of these extracts, or the feeding of the fresh tissues themselves correct the inadequacy of the diet deficient in the water-soluble B (Table XIII).

TABLE XIII.

Nature and percentage of substance added to inadequate diet	Average weight of 6 rats every seven days					Average period in days of maintenance before final decline
	0	7	14	21	28	
Inadequate ration alone	64	63	67	63	54	33
4 % beef tissue	56	59	62	59	49	31
5 % commercial meat extract	86	87	84	80	80	46
6 % aqueous extract rat embryo	58	60	63	56	52	30
10 % " " rat sarcoma	60	66	58	52	—	27
10 % " " fowl sarcoma	56	50	51	51	49	30
1 % desiccated pituitary gland	47	45	49	50	52	—
5 % desiccated pituitary gland	52	56	50	48	47	—
1 % desiccated anterior lobe pituitary	64	66	70	67	60	46
3 % desiccated anterior lobe pituitary	74	73	72	71	67	40
2 % desiccated thyroid gland	60	61	56	52	—	24
5 % desiccated thymus gland	77	78	71	70	70	40
2 % desiccated testicular tissue	46	45	52	47	41	30
2 % desiccated ovarian tissue	70	64	60	—	—	29

3. *Relationship of the Water-soluble Substance to the Glands of Internal Secretion.*

More than one author has made the suggestion that the accessory substances exert their influence upon growth by being indispensable for the functioning of one or more of the glands of internal secretion [Hopkins, 1912; Funk, 1914].

During the present investigation several such glands were added in the desiccated form to the diet deficient in the water-soluble accessory and administered to young rats, in order to determine whether any one of them would supply the missing factor. As may be seen from Table XIII, no such result was obtained in any case.

SUMMARY.

1. The nitrogen metabolism of rats fed upon an artificial diet deficient in the water-soluble accessory substance has been studied. The only apparent deviation from the normal noted was the appearance of creatinuria. It was accompanied by a slow wasting of the skeletal muscles.

2. The food consumption of rats fed upon the deficient diet is low, being in all probability reduced to that sufficient to supply the calorific requirements of maintenance.

3. An increased food consumption may be brought about by the addition of flavouring agents such as meat extract to the diet, but unless this agent contains the water-soluble substance no growth results.

4. The addition to the inadequate diet of extracts containing the water-soluble substance causes a greatly increased food intake, immediately followed by growth.

5. The amount of growth is, within certain limits, proportional to the amount of accessory substance added, provided that the diet is adequate in other respects.

6. There is evidence that the length of time that an animal (rat) is able to maintain itself upon a diet deficient in the water-soluble substance without suffering a serious loss of body-weight is directly proportional to the age at which the restriction is imposed.

7. Actively growing animal tissues (embryos, tumours) do not contain appreciable amounts of the "water-soluble B."

8. Desiccated pituitary gland (whole gland and anterior lobe), thyroid, thymus, testicle and ovarian tissues are also deficient in the "water-soluble B."

9. The cause of the fatal decline which inevitably follows a deficiency of the water-soluble substance has not been determined. Symptoms of nerve disorder were noted before death in three cases.

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