LIX. A STUDY OF THE NITROGEN BALANCE IN VITAMIN B₂ DEFICIENCY IN THE RAT.

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It was reported in a previous communication [Kon, 1929] that rats kept on a diet deficient in vitamin B₂ show an increased carbon/nitrogen ratio in the urine as compared with individual controls receiving equal amounts of a diet considered to be complete. No such increase was noted in rats consuming a ration deficient in vitamin B₁.

The present paper deals with a quantitative study by the individual control method of the nitrogen metabolism of rats subsisting on diets low in the heat-stable factor (or factors) of yeast.

EXPERIMENTAL.

A litter of six rats of Wistar stock, bred in the laboratory on a slightly modified stock diet of Steenbock [1923] was used for the experiment. They were weaned at 21 days and, after a week on the stock diet, were run out for 17 days on our basal vitamin B-deficient diet 60 (identical with diet 540 of Evans [Kon, 1929]) and consisting of caseinogen (extracted with acidulated water) 25 parts, commercial cane-sugar 75 parts, and Steenbock's salt mixture No. 40, 4 parts. At the end of this time the litter was divided into three pairs, one member of each pair acting as an individual control to its mate. This method has been more amply described in earlier papers by Kon and Drummond [1927] and Kon [1929]. The animals were placed separately in modified Mitchell's cages [Kon, 1928] and offered the following diet: caseinogen (extracted with acidulated water) 10 parts, rice starch 70 parts, butter-fat 15 parts, and salts (Steenbock's mixture No. 40) 5 parts. The diet contained 1.5 % N and 10 % moisture. A quantity of it sufficient for the whole experiment was prepared and kept in cold storage at - 2°. This diet was selected for several reasons. Firstly in order to avoid spilling as completely as possible and to measure the food intake with a sufficient accuracy it was necessary to offer the food mixed with water to a paste. For this reason rice starch had to be used instead of sugar. Secondly it was thought best for the purpose of the experiment to select a diet low in nitrogen, and containing it at a concentration similar to those used in experiments on the biological value of proteins. Should any deviations from the normal path of nitrogen metabolism manifest themselves in the course of vitamin B₂ deficiency, they would be

most easily detected at such a level of nitrogen intake that most of it, under normal conditions, is used for repair of body tissues and for growth and a small percentage only, if any, burnt as such to cover the caloric requirements. The butter improved the texture of the diet and at the same time supplied vitamins A and D.

The experimental rats were given daily 0.4 cc. of an alcoholic extract from rice bran¹ equivalent to 3.2 g. of bran. This extract was shown in a special experiment to be lacking in vitamin B₂ (and possibly in some other factors supplied in autoclaved yeast). The control animals received an addition of 0.3 g. of dried brewer's yeast of tested potency. In order to equalise the intake of nitrogen an amount of purified caseinogen equivalent in nitrogen content to 0.3 g. of the yeast was placed daily in the porcelain dishes containing the rice bran extract for the experimental animals. The food was mixed with a little water to a pasty consistency to avoid scattering.

For the first four days the food was given to all rats *ad lib*. On the fifth day the residues left in the food cups by the experimental rats were dried at 105° to constant weight, the food intake of each experimental rat was measured and an equivalent amount of the diet was weighed out for the corresponding individual control. The dry residue was discarded from the cups of the experimental animals and fresh food was given slightly in excess of the intake. This procedure was from now on repeated daily, so that the controls were always receiving an amount of food equivalent to that eaten by their mates in the course of the preceding 24 hours. There was practically no scattering and the controls always consumed all the food offered. The yeast, rice bran extract and caseinogen were very readily taken.

The collection of urine and faeces was started after one week of quantitative feeding. This, as well as the analysis of the excreta, was carried out exactly as described in an earlier paper [Kon, 1928]. The rats were weighed once a week, the control animals 24 hours after the experimental ones. The actual metabolic experiment involved five separate collection periods and lasted 36 days, while the quantitative feeding was continued for a week longer (and started a week earlier). The first period lasted 7 days, the following ones 6 days each. At the conclusion of every period the rats were weighed and examined. To avoid any danger of nitrogen losses in the course of handling, an interval of 24 hours separated the periods.

Food consumption.

The food was weighed with an accuracy of 0.01 g. The residues left by the experimental rats were dried for 4–5 hours in an electric oven at 105°

¹ Prepared by extracting rice bran (supplied by Messrs Polski Przemysł Ryżowy in Gdynia) for three days with 25 % by weight alcohol, in the proportion of 4 litres to the kg., concentrating the extract *in vacuo* to a thick syrup, adding a sufficient amount of 95 % alcohol to make the concentration 80 % by weight, decanting the fluid, dissolving the residue in a small volume of hot water, bringing the concentration again to 80 % alcohol, combining the extracts and evaporating them *in vacuo* until 1 cc. is equivalent to 8 g. of the original bran.

until a constant weight was attained. The moisture content of the diet was checked at frequent intervals and was found to remain constant. As the food consumption of the control rats was quite quantitative and the scattering by all the animals negligible (while any food scattered could be easily recovered from the filter-paper on the bottom of a cage), it is felt that the daily difference between the food (diet) intake of a control rat and of its experimental mate did not exceed 0.03 g., the variations being obviously in both directions.

In addition to the diet every control rat consumed 0.3 g. of dried brewer's yeast containing 9.1 % water and 8.81 % N, while the experimental animals received 0.19 g. of caseinogen (with the exception of the first 4 days of the first period, when they were given 0.2 g.) of the same water content and 13.8 % N. The experimental animals were given in addition 0.4 cc. daily of the rice bran extract, containing 2 mg. of nitrogen and 0.164 g. of solid matter, mostly of a carbohydrate nature. This means that the experimental rats consumed daily slightly over 0.06 g. (0.064 g.) of solid matter more than did their controls.

General appearance of rats.

The experimental rats displayed progressively symptoms usually associated with a lack of vitamin B₂, namely rough coats, coagulated blood on paws and vibrissae and urine-stained abdominal fur. The eyes were not involved. One rat (No. 154) had a mild stomatitis. In contrast all the controls were quite normal in appearance and had normal sleek fur.

Weight curves of rats.

In every case the control rats made better gains than their experimental mates. Table I gives the weights at the beginning and end of the quantitative feeding period, while average growth curves for both sets are presented in Fig. 1.

During the whole of the experimental period every experimental rat con-

Fig. 1. Average growth curves of experimental and control rats.

Table I.

Rat no.	Sex	Initial weight g.	Final weight g.	Increase g.	Difference	Remarks
153	♀	56	74	18	+21	Experimental
152	♀	49	88	39		Control to 153
154	♂	65	75	10	+11	Experimental
155	♂	65	86	21		Control to 154
156	♂	56	72	16	+7	Experimental
151	♀	63	86	23		Control to 156

Table II. *Nitrogen balance.*

Period	Rat no.	Average weight	Food intake		Caseinogen intake		Yeast intake		Extract intake		Total intake		N in urine mg.	N in faeces mg.	Total N excreted mg.	N balance mg.
			g.	mg.	g.	mg.	g.	mg.	g.	mg.	g.	mg.				
I	153 E	59	31.45	524	1.24	189	—	—	1.15	14	33.84	727	631	32	663	+ 64
	152 C	59	31.45	524	—	—	1.91	185	—	—	33.36	709	414	65	479	+ 230
	154 E	63	26.37	439	1.24	189	—	—	1.15	14	28.76	642	589	37	626	+ 16
	155 C	62	26.37	439	—	—	1.91	185	—	—	28.28	624	550	65*	615	+ 9
	156 E	58	31.18	520	1.24	189	—	—	1.15	14	33.57	723	625	40	665	+ 58
II	151 C	62	31.18	520	—	—	1.91	185	—	—	33.09	705	521	65	586	+ 119
	153 E	59	26.72	445	1.03	157	—	—	0.98	12	28.73	614	565	50	615	- 1
III	152 C	63	26.72	445	—	—	1.64	159	—	—	28.36	604	467	58	525	+ 79
	154 E	63	23.61	393	1.03	157	—	—	0.98	12	25.62	562	481	31	512	+ 50
	155 C	65	23.61	393	—	—	1.64	159	—	—	25.25	552	473	33	506	+ 46
	156 E	59	24.42	407	1.03	157	—	—	0.98	12	26.43	576	547	48	595	- 19
	151 C	65	24.42	407	—	—	1.64	159	—	—	26.06	566	465	38	503	+ 63
IV	153 E	62	26.78	446	1.03	157	—	—	0.98	12	28.79	615	442	54	496	+ 119
	152 C	67	26.78	446	—	—	1.64	159	—	—	28.42	605	415	74	489	+ 116
	154 E	63	21.24	354	1.03	157	—	—	0.98	12	23.25	523	457	28	485	+ 38
	155 C	67	21.24	354	—	—	1.64	159	—	—	22.88	513	400	52	452	+ 61
	156 E	62	23.69	395	1.03	157	—	—	0.98	12	25.70	564	429	45	474	+ 90
V	151 C	68	23.69	395	—	—	1.64	159	—	—	25.33	554	421	75	496	+ 58
	153 E	67	24.45	407	1.03	157	—	—	0.98	12	26.46	576	468	38	506	+ 70
	152 C	73	24.45	407	—	—	1.64	159	—	—	26.09	566	392	49	441	+ 125
	154 E	65	22.78	380	1.03	157	—	—	0.98	12	24.79	549	418	35	453	+ 96
	155 C	70	22.78	380	—	—	1.64	159	—	—	24.42	539	415	60	475	+ 64
V	156 E	63	23.48	391	1.03	157	—	—	0.98	12	25.49	560	442	38	480	+ 80
	151 C	71	23.48	391	—	—	1.64	159	—	—	25.12	550	422	46	468	+ 82
	153 E	69	23.08	385	1.03	157	—	—	0.98	12	25.09	554	431	54	485	+ 69
	152 C	79	23.08	385	—	—	1.64	159	—	—	24.72	544	356	46	402	+ 142
	154 E	68	21.27	354	1.03	157	—	—	0.98	12	23.28	523	343	34	377	+ 146
V	155 C	76	21.27	354	—	—	1.64	159	—	—	22.91	513	343	54	397	+ 116
	156 E	65	20.30	338	1.03	157	—	—	0.98	12	22.31	507	425	28	453	+ 54
	151 C	76	20.30	338	—	—	1.64	159	—	—	21.94	497	377	62	439	+ 58

* Assumed; the determination was lost.

Table III. *Complete nitrogen balance (36 days).*

Rat no.	Initial weight g.	Final weight g.	Weight increase g.	Food intake		Caseinogen intake		Yeast intake		Extract intake		Total intake		Excreted N mg.	Balance N mg.
				g.	mg.	g.	mg.	g.	mg.	g.	mg.	g.	mg.		
153 E	58	71	13	152.59	2543	6.21	947	—	—	5.87	72	164.67	3189	+ 373	+ 799
152 C	56	81	25	152.59	2543	—	—	9.82	951	—	—	162.41	2696	+ 799	+ 409
154 E	64	69	5	135.22	2253	6.21	947	—	—	5.87	72	147.30	2863	+ 347	+ 307
155 C	61	78	17	135.22	2253	—	—	9.82	951	—	—	145.04	2857	+ 347	+ 439
156 E	58	67	9	142.61	2377	6.21	947	—	—	5.87	72	154.69	3089	+ 307	+ 389
151 C	60	78	18	142.61	2377	—	—	9.82	951	—	—	152.43	2889	+ 307	+ 439

sumed 3.36 g. solid matter (mostly assimilable carbohydrates) more than his control mate and, nevertheless, made a smaller gain.

Nitrogen balance.

Table II gives the intake and excretion of nitrogen during the five metabolic periods.

It will be recalled that between each metabolic period there was a gap of 24 hours when no collection of urine or faeces was made, the food intake however being recorded in the usual way. In order to calculate the nitrogen balance over the whole duration of the metabolic experiment, it was therefore necessary to include data for the nitrogen excreted in urine and faeces on the days when no actual measurements were made. These figures have been calculated on the probably correct assumption that the nitrogen excretion on the "gap" day would stand in the same ratio to the nitrogen intake as the actual figures obtained for the immediately preceding period. The results are presented in Table III.

An inspection of Tables II and III shows that for the entire period two control rats, Nos. 152 and 151, retained more nitrogen than their experimental mates, while the reverse was true for the control rat No. 155 (which nevertheless gained more in weight than his experimental mate). When the 30 separate measurements carried out in the course of the metabolic experiment are compared in 15 pairs it becomes evident that the control rats were favoured in 9 cases, while the experimental ones retained more nitrogen in 6. It will be noticed that the differences tend to become less marked as the experiment progresses, the values for the second and third pairs of rats running very closely together.

The excretion of faecal nitrogen is almost always higher in the controls (with 2 exceptions), probably due to the consumption of yeast and the production of more bulky faeces [Mitchell, 1924, 1]. It was thought that a clearer picture of the nitrogen metabolism of both groups of rats might be obtained if the biological value of the ingested nitrogen were calculated from the available data by means of the method of Mitchell [1924, 1; Mitchell and Carman, 1926, 2]. Such a calculation necessitates the use of figures for the "metabolic faecal nitrogen" and for the "endogenous urinary nitrogen," usually obtained by measuring the nitrogen excretion of rats during periods of nitrogen-free or of low egg-nitrogen diet. It was not thought safe to include such periods in the work described here, in order not to vitiate the results. It has therefore been necessary to introduce the following assumed factors, based on values obtained previously by Kon [1928] and by Mitchell [1924, 1; Mitchell and Carman, 1926, 2; Mitchell, Beadles and Kruger, 1927], for rats of approximately the same weight as those used in the present experiment. The assumed values are: 2 mg. of metabolic faecal nitrogen per g. of food consumed and 25 mg. of endogenous urinary nitrogen per day per 100 g. of weight. The results of such calculation are presented in Table IV.

Table IV. "*Biological value*" of the ingested nitrogen calculated according to Mitchell.

Period	Rat no.	Average weight	Food intake		Faecal N mg.	Body N in faeces mg.	Food N in faeces mg.	Ab-sorbed N mg.	N in urine mg.	Body N in urine mg.	Food N in urine mg.	Food N re-tained mg.	Bio-logical value
			g.	N mg.									
I	153 E	59	33.84	727	32	68	0	727	631	103	528	199	27
	152 C	59	33.36	709	65	67	0	709	414	103	311	398	56
	154 E	63	28.76	642	37	57	0	642	589	110	479	163	25
	155 C	62	28.28	624	65*	56	9	615	550	108	442	173	28
	156 E	58	33.57	723	40	67	0	723	625	101	524	199	27
	151 C	62	33.09	705	65	66	0	705	521	108	413	288	41
II	153 E	59	28.73	614	50	57	0	614	565	88	477	137	22
	152 C	63	28.36	604	58	57	1	603	467	94	373	230	38
	154 E	63	25.62	562	31	51	0	562	481	94	387	175	31
	155 C	65	25.25	552	33	50	0	552	473	97	376	176	32
	156 E	59	26.43	576	48	53	0	576	547	88	459	117	20
	151 C	65	26.06	566	38	52	0	566	465	97	368	198	35
III	153 E	62	28.79	615	54	58	0	615	442	93	349	266	43
	152 C	67	28.42	605	74	57	17	588	415	100	315	273	46
	154 E	63	23.25	523	28	46	0	523	457	94	363	160	31
	155 C	67	22.88	513	52	46	6	507	400	100	300	207	41
	156 E	62	25.70	564	45	51	0	564	429	93	336	228	40
	151 C	68	25.33	554	75	51	24	530	421	102	319	211	40
IV	153 E	67	26.46	576	38	53	0	576	468	100	438	208	36
	152 C	73	26.09	566	49	52	0	566	392	109	283	283	50
	154 E	65	24.79	549	35	50	0	549	418	97	321	228	41
	155 C	70	24.42	539	60	49	11	528	415	105	310	218	41
	156 E	63	25.49	560	38	51	0	560	442	94	348	212	38
	151 C	71	25.12	550	46	50	0	550	422	106	316	234	42
V	153 E	69	25.09	554	54	50	4	550	431	103	328	222	40
	152 C	79	24.72	544	46	49	0	544	356	118	238	306	56
	154 E	68	23.28	523	34	46	0	523	343	102	241	282	54
	155 C	76	22.91	513	54	46	8	505	343	114	229	276	54
	156 E	65	22.31	507	28	45	0	507	425	97	328	179	35
	151 C	76	21.94	497	62	44	18	479	377	114	263	216	45

* Assumed.

Table V gives the average biological values of the ingested nitrogen for all periods for the experimental and control rats. Out of the 15 pairs of

Table V.

Rat no.	Biological value	
153 E	34	
152 C	49	
154 E	36	General average for experimental rats: 34
155 C	39	
156 E	32	General average for control rats: 43
151 C	41	

biological values obtained 3 give equal values for both rats while the remaining 12 are in favour of the control rat, though in 5 cases the difference does not exceed 4, well within the experimental error.

It will be remembered that while the experimental rats derived their

nitrogen almost exclusively from caseinogen, the diet of the control rats contained a considerable proportion (over one-fourth of the total crude protein) of yeast proteins ($N \times 6.25$) in addition to caseinogen. It would be obviously quite simple to use a watery extract from yeast [Osborne and Wakeman, 1919] instead of yeast as a source of water-soluble vitamins for the control rats, but in the light of recent work [Kennedy and Palmer, 1928; Hunt, 1928; Sure, 1928] it seems doubtful whether such extracts contain all the factors originally present in yeast. Preliminary experiments carried out by the author (unpublished work) seemed to confirm this opinion and it was therefore thought safer to avoid the use of extracts.

It might be argued that a supplementary relation exists between caseinogen and yeast nitrogen, yielding a mixture of higher biological value than caseinogen itself and that the differences in weight, retention of nitrogen and biological value of the ingested nitrogen between the experimental rats and the controls might be wholly explained on this basis. The author is not aware of any published work on the biological value of such a combination or of yeast at a 12 % level (*i.e.* at approximately the level of protein used in the present work). He (unpublished experiments) has measured by the method of Osborne, Mendel and Ferry [1919] the biological value of the nitrogen of yeast and has found values of 1.48, 1.31 and 1.36 when fed at a level ($N \times 6.25$) of 8.4, 10.0 and 12.1 % respectively. Osborne, Mendel and Ferry found a maximal value for caseinogen of 2.25 when fed at 12 % level. It does not seem likely that this value would be raised by the addition of yeast. Admittedly this reasoning can only by indirect inference be applied to calculations based on Mitchell's method of evaluating the biological value of proteins. A more direct proof is found in the behaviour of rats Nos. 154 and 155. In this pair the control rat retained less nitrogen than the experimental one, while the calculated biological values of the ingested nitrogen are practically identical for both rats. Nevertheless the control animal gained 21 g. in weight and the experimental only 10 g., a difference equal to 17 % of the initial weight of the rats. It will be also recalled that in the previous communication [Kon, 1929] a distinct difference was reported between the weights of vitamin B₂-deficient and control rats while the former consumed a diet containing a high percentage of caseinogen (25 %), certainly more than necessary for optimal growth if taken in adequate amounts. At such level an increase of the efficiency of caseinogen by the addition of another protein is hardly to be expected.

The biological values calculated by the method of Mitchell for the experimental and control rats are both very markedly below the values found for caseinogen by Mitchell [1924, 2] at a 5 % and by Kon [1928] at an 8 % level, namely 71 and 68 respectively. Mitchell and Hamilton [1929, p. 537] believe that a variable food intake for rats of approximately the same size exerts no effect on the biological values obtained, provided the amount of food consumed is adequate for the maintenance of weight. Mitchell, Beadles and Kruger [1927] noted extremely low figures (zero in several cases) for rats

consuming inadequate amounts of a diet containing pork cracklings as a source of protein, while another group of rats consuming the food better gave a value of 25. The former rats lost weight. In the present experiment all the rats gained weight at a very slow rate, they should consequently, according to Mitchell and Hamilton [1929] give normal biological values (or at least the control rats). There is no doubt that the food intake was subnormal and that the control rats were prevented from growing at a more rapid rate only because their food was limited. It seems possible that at such low levels of food intake a part of the ingested protein may be used for energy purposes while the remainder is used for maintenance and even allows for growth at very slow rate. It is difficult to account otherwise for the very substantial difference between the biological values reported now and found previously for caseinogen.

A calculation of the biological value of the ingested nitrogen according to the method of Osborne, Mendel and Ferry [1919] can be made for the whole period of quantitative feeding covering 52 days. The following values are obtained (Table VI).

Table VI.

Rat no.	No. of days on diet	Consumption of		Gain in weight g.	Biological value
		Food	Protein (N $\times$ 6.25)		
153 E	52	236.68	32.09	18	0.56
152 C	52	233.32	31.41	39	1.24
154 E	52	212.25	29.54	10	0.34
155 C	52	208.88	28.86	21	0.73
156 E	52	224.02	30.77	16	0.52
151 C	52	220.66	30.10	23	0.76

The values obtained for the experimental rats are markedly lower than those found for the control animals. The difference is much more marked than when the biological value of the ingested protein is calculated according to the method of Mitchell. This result is readily understood in view of the very significant differences in weight gains between the two sets of rats. All the values are much below the figures cited previously for caseinogen fed under usual conditions at approximately the same level. This is due to the insufficient food intake.

Positive nitrogen balances have been obtained in the present paper for all the rats studied. If all of the retained nitrogen was used for building protoplasmic tissue then every gram of nitrogen should cause a gain in weight of 31.25 g. on the assumption that the deposited tissue would contain approximately 20 % protein (N $\times$ 6.25).

Table VII gives values calculated on this basis together with the actual gains made by the rats during the metabolic periods.

While for two experimental rats (Nos. 153 and 156) and for one control (No. 152) the calculated values coincide well with the actual gains, the nitrogen retained by rat No. 154 must have been used also for purposes other than the

Table VII.

Rat no.	N retained mg.	Calculated gain in protoplasm g.	Actual gain g.
153 E	373	11.7	13
152 C	799	24.9	25
154 E	409	12.8	5
155 C	347	10.8	17
156 E	307	9.6	9
151 C	439	13.7	18

formation of body protein, and the two control rats, Nos. 155 and 151, would have deposited fat in addition to the formation of the calculated amount of protoplasmic tissue. The only justifiable conclusion is that the gains made by the experimental rats and by the controls were of different composition.

DISCUSSION.

The results here reported were obtained with only a small group of animals (3-pairs) and, though they were rigidly controlled, great caution must be exercised in order not to draw unwarranted conclusions.

Reviewing the evidence on hand the following points seem to deserve special attention.

1. All three control animals made better weight gains than their experimental mates though the former consumed slightly less food. This observation is in agreement with earlier work [Kon, 1929] where, in a similarly controlled experiment, all six controls made better gains than their mates consuming a diet deficient (or low) in vitamin B₂.

2. The nitrogen balance experiments seem rather inconclusive—two experimental rats stored less nitrogen, while one deposited more than the respective controls—and a comparison of 15 pairs of nitrogen estimations shows that the experimental rats were favoured in 6, and the controls in 9, a result easily obtained by chance. The controls however stored on the whole decidedly more nitrogen than the experimental animals. When the biological value of the ingested nitrogen is calculated according to the method of Mitchell a slightly better efficiency is found in the case of the controls, 12 results being in favour of the latter, 3 even, and none in favour of the experimental rats—a result hardly due to chance.

3. There is a strong indication that the gains in weight made by the two sets of rats are of different composition.

4. To this may be added the evidence brought forward in the earlier paper [Kon, 1929] that the carbon/nitrogen ratio is slightly increased in the urine of experimental rats deprived of vitamin B₂, as compared with individual controls.

Is it possible to derive from these observations any conclusions concerning the rôle of vitamin B₂ in metabolic functions? In an effort to answer this question the points enumerated above will be subjected to a more detailed analysis.

(1) The phenomenon described under 1 is not characteristic for vitamin B₂ deficiency. It has been found by other investigators, in well controlled experiments, in the case of various deficiencies and is therefore not pathognomonic of the lack of vitamin B₂. Anderson and Smith [1924] in their study by the individual control method of the effect of acute scurvy on the subsequent nutrition and growth of guinea pigs came to the conclusion that "there are factors resulting in loss of body weight which operate in addition to fasting, and which depend on scurvy itself. In other words, inanition alone does not cause as severe a loss as when accompanied by lack of vitamin C." Mitchell and Carman [1926, 1] have shown by means of the same method that a ration composed mainly of maize, when supplemented with NaCl, induced 50 % faster gains in weight of both rats and chickens than the saltless ration. The authors remark that "the growth data of this experiment afford a striking demonstration of the fact that the utilisation of food energy by growing animals may be greatly impaired by an improper balance among indispensable dietary factors." Similar differences are reported by Mitchell and Beadles [1930] and Beadles, Braman and Mitchell [1930] in the case of diets deficient in cystine and by Jackson [1929] in a deficiency of tryptophan, while Burr and Burr [1930] made observations of the same nature in their study of the fat deficiency disease (individual controls were not employed in this study).

The estimation of the "physiological efficiency" of various proteins, as carried out by the earlier method of Osborne and Mendel [1916], is nothing else than the comparison of food mixtures based on a similar "individual control" method: the rats (all males) were of approximately the same body weight at the beginning of the experiment and were offered daily limited amounts of food "precisely alike in energy content, inorganic and accessory food ingredients, *etc.*" and differing only with regard to the proteins under investigation. The rats made widely divergent weight gains while consuming equal amounts of these rations. On commenting upon these excellent experiments Mitchell [1924, 3] very aptly remarks "these experiments suggest an interesting relation, hitherto uninvestigated as far as the author is aware, between the balance of nutrients in a ration and the utilisation of its energy."

We are here confronting a general phenomenon, namely the growth-limiting influence of the lack or insufficiency of any indispensable food constituent manifesting itself in an impaired utilisation of the food.

For a certain time some vitamins were referred to as "growth-promoting," but there is certainly no doubt that every essential food constituent is growth-promoting in the sense that its absence from the diet will sooner or later cause stoppage of growth. It seems that sufficient evidence was cited above to prove that, as a general rule, one of the ways in which this growth-inhibiting action will come into operation is through an impaired utilisation of the ingested energy, and that in this respect all indispensable nutrients, vitamins, minerals, or amino-acids, manifest a great similarity of action.

A deficiency of an essential ingredient of the diet may or may not have

a depressing influence on the appetite [Beadles, Braman and Mitchell, 1930], at least in the earlier periods of the deficiency. Ultimately probably the general derangement will be sufficiently pronounced to cause in every case a diminished food intake.

The most conspicuous association of an essential food constituent with appetite is certainly to be found in the case of the vitamin B complex and of vitamin B₁. The inanition sets in here so rapidly and is of such severity that no difference in the rate of the loss of weight can be found between "experimental" animals and controls, when the individual control method is used [Kon and Drummond, 1927; Kon, 1929].

(2) Passing now to the second point of interest relating to the study of vitamin B₂ deficiency as presented in this paper, namely to the nitrogen balance experiments, it seems that a less favourable balance would naturally be expected in the case of the experimental rats, having consumed the same amount of, but stored less, energy than the controls, and that therefore the results on the whole agree with the expectation. It was shown by Mitchell and Carman [1926, 1] that more favourable nitrogen balances were found for every one of their control rats and chicks receiving an addition of NaCl, as compared with the experimental ones. The evidence available does not seem to show that vitamin B₂ is any more connected with the metabolism of nitrogen than is sodium chloride.

(3) The question of the composition of the tissues added by the two sets of rats in the present experiment is certainly a very pertinent one. Little more can be said on this point than that the mere estimation of differences in weight does not yield sufficient information, and that in studies of this type, that is to say in the investigation of the relation of any indispensable nutrient to the metabolism of matter and energy, the actual chemical make-up and energy content of the added weight should be estimated as a matter of routine.

(4) Reviewing in turn the value of the finding reported in the previous paper [Kon, 1929], that in the absence of vitamin B₂ there is an increased excretion of carbon compounds as compared with nitrogenous bodies in the urine, it is necessary to ask how significant such an observation is. An alteration of the urinary C/N ratio in scorbutic guinea pigs has been reported by Jarussowa [1928], and Roche [1930] states that the ratio is increased in the terminal stage of vitamin B deficiency. Bickel [1924, 1930] is of the opinion that this symptom is present in disturbances of metabolism of a non-avitaminous character. Laclau and Marenzi [1930] report that the C/N ratio is increased in the urine of rats on a diet poor in cystine as compared with normally fed rats. Shipp and Zilva [1928] have criticised the experiments of Jarussowa and doubt whether the development of scurvy is associated with an increased C/N ratio in the urine. They admit however that their experiments are inconclusive in view of the discordant results obtained (there was a definite increase in the ratio in one of their guinea pigs while no such deviation was observed in the case of the other). While the question can be definitely solved

only by means of suitably controlled experiments (with equalised food intake for control and experimental animals), it seems at least doubtful at present whether the alteration of the urinary C/N ratio is characteristic for vitamin B₂ deficiency. It would be best to abstain from far-reaching conclusions, and in particular the author is of the opinion that, while the importance of vitamin B₂ for normal economy is self-evident, there are no sufficient grounds for making it responsible for any particular branch of metabolism.

In conclusion it may be said that while vitamin B₂ is undoubtedly in some way linked with the metabolic processes of the body, it is not at all evident whether this connection is to any extent of a different type from that of the general dependence of normal metabolic exchange upon the availability of any one of the indispensable food ingredients.

SUMMARY.

1. A study by the individual control method of the nitrogen balance in vitamin B₂ deficiency in the rat is reported.
2. The relation of vitamin B₂ to metabolism is discussed.

Note added, April 8th. Still and Koch (1928) have measured, by Mitchell's method, the biological values of diets containing one half of the nitrogen from yeast and one half from caseinogen (total N 2.9 %) and found no supplementary relation between the two proteins.

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