CXX. THE MINERAL CONTENT OF THE SKIN OF RATS SUFFERING FROM BERI-BERI, WITH INVESTIGATIONS ON THE FORMATION OF VITAMIN B₁ BY MOULDS.

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ON the subject of mineral metabolism, and still more on that of the mineral content of individual organs, our knowledge still consists of a series of isolated facts. The summaries of our knowledge on this subject, made by Klinker [1931], Heubner [1931], Reichel and Spiro [1927], Höber [1924], Zondek [1927], Spiro *et al.* [1929], all show the fundamental connection between mineral metabolism and the physico-chemical processes in and around the cell and the effect of this on cellular activity and development. Furthermore, the internal secretions exert an influence directly and indirectly through general metabolism and nourishment.

Owing to the complexity of this relationship, it is readily understandable that the figures cited as "normal values" are liable to show wide variations, dependent on the previous history of the animal and the conditions obtaining prior to the withdrawal of the tissue sample. Hence, for investigating the factors controlling the mineral content of a tissue, values taken for comparative purposes must of necessity be obtained under the most comparable conditions possible. For instance, in investigations by one of us [Jung, 1931, 1], on tuberculous subjects, quite different results were obtained from specimens collected *in vivo* and from subjects who had died from tuberculosis. In the case of the latter, the long cachexia plays a rôle not to be underestimated. At the commencement of the investigation in hand, namely the effect of avitaminoses B₁ and B₄ (termed collectively beri-beri) on the skin of the rat, no figures which might serve as "normal values" were to be found in the literature, and the more recent work of Loewy and Cronheim [1932] plainly emphasises the importance of comparing only such values as have been obtained under conditions as nearly identical as possible.

Very little is yet known regarding mineral metabolism in rat beri-beri. The clinical symptoms (rough hide with moist patches—coldness—a bluish colour, and red, swollen extremities in avitaminosis B₄), together with the general metabolic disturbances, would lead one to expect changes in the mineral constituents of the skin. Furthermore in this disease, which results from a deficiency of a very minute constituent of the diet, it is possible that changes of the internal secretions play a large part (*e.g.* suprarenals, thyroid *etc.*), so that one cannot say with any certainty where the primary disturbance sets in. Moreover, the sharp loss in weight and deficient growth due to avitaminoses B₁ and B₄ must also be taken into consideration. Unfortunately it was not possible to carry

out the necessary controls on as large a number of animals as one would have liked. Finally, this investigation was not designed to give the subject exhaustive treatment, but rather to determine as far as possible whether any specific conclusion could be drawn regarding the mineral content of the skin, which arose as a net result of these various complex influences. In carrying out these experiments, it was observed that a spontaneous formation of mould on the feeding cake appeared to inhibit the onset of beri-beri, and a further experiment was therefore conducted to elucidate this point.

VITAMIN B₁ FORMATION BY MOULDS.

In order to investigate the problem of whether moulds such as *Aspergillus niger*, which readily forms on the feeding cake, can produce this vitamin in sufficient amounts to satisfy rats and also to test whether the mould imparts the vitamin to the cake, and further, to ascertain in what part of the mould the vitamin is located, the following experiment was carried out. Ten animals were used, of which 5 at first only received vitamin B-deficient diet (BaW) until, after 27–44 days, they developed beri-beri. These animals were cured, with an immediate and rapid increase in weight, by the same basal diet on which *A. niger* had grown spontaneously. The animals still increased in weight well when the mould grew sparsely and for a time no blackening of the cake was obtainable. At this point, 3 animals were killed with ether for skin analysis. One of them, 2317, had received the mould-feeding for 26 days, the other two, for 56 and 50 days. The remaining animals showed a decrease in weight soon afterwards. This can be attributed on the one hand to scanty formation of the mould, and on the other hand to an overgrowth by a yellowish-green form of mould (*A. flavus*?). During this time, however, the cakes were always covered with mycelium. When for a few days no mould grew, the animals showed a sudden partial decrease in weight, but one of them, 2310, grew in spite of this. Renewed application of mould produced good growth once more. The animals developed unsatisfactory hides at this time, moulting and eczema formation occurring in places, which healed up after feeding with egg-white as the source of vitamin B₂, after the technique of Chick and Roscoe [1929]. The animals showed trembling of the head and heart-palpitations, were very nervously excited and had a severe thirst. This condition was cured by giving the animals the mould which had been cultured on rye-bread. This however did not produce the same rate of growth in the rats as that which grew on the basal diet; in fact one animal, 2308, even developed beri-beri. Although not studied quantitatively, in that the mould was only given in approximately equal quantities, this phase of the experiment seems to indicate that the mould when grown on rye-bread forms less vitamin B than when grown on the basal diet, for the animals' weights increased again as soon as they were given either the mould-covered basal diet or fresh basal diet + mould. That the mould yields little or no vitamin B to the cake itself is shown by results obtained during periods when only mould-cake was given from which the mould had been previously removed and which had been inactivated at 70° to prevent fresh conidia forming from the mycelium still present.

The sensitivity to lack of water was shown when, on one occasion, insufficient water was given. The animals lost 15–25 g. The success with which the rats thrive on the mould-covered food is shown by a young male that has now reached a weight of 219 g. after 236 days and is still growing. We are at present trying to use him as a breeding buck. The hide is still completely intact.

Five animals received the mould-covered basal diet throughout the experiment and grew well on it. In the case of 3 other animals it was only given after 49 days to one, and after 69 days to the other two. The growth of these animals ceased at once, they lost weight, and after 13–14 days beri-beri set in. One animal was successfully cured by mould feeding but this proved ineffective in another case. Three animals were taken for skin analysis.

It is intended to continue this investigation and grow the mould on artificial media, *e.g.* as described by Nielsen and Hartelius [1933], then once again on vitamin B-containing material such as wholemeal bread, but more especially, to carry out the work on a quantitative basis.

Up to the present it would appear that *A. niger* forms vitamin B chiefly in the conidia (spores), while the mycelium is relatively poor in it. It imparts very little vitamin B to the substrate. It seems to form less vitamin B when grown on rye-bread, and a yellowish-green mould (*A. flavus*?) appears to contain even less. There is evidence that not all the B-factors are formed in equal amounts (as judged by the symptoms; skin-exudate, derangements of balance, and trembling). The animals' severe thirst as well as their sensitivity to lack of water is quite remarkable.

In those groups of animals which had grown well, a very plentiful layer of fat occurred, especially in the lower part of the stomach and round the kidneys, similar to that described by Plimmer [1927] in relative vitamin B-deficiency. For the growth curves of these animals, see Fig. 1. The animals used in this experiment constitute groups 3 and 4.

The animals cited in Table III were used for skin analysis.

VITAMIN A CONTROL.

In order to be able to compare the results of the beri-beri experiments with the effect produced by vitamin A-deficiency, one animal, 2477, was taken, which, after 38 days of preparatory feeding, showed ophthalmic symptoms of vitamin A-deficiency.

The animal received the following basal diet: vitamin-free caseinogen (Wander), 18 %; coconut oil, 15 %; maize powder starch, 57 %; McCollum and Davis's salt mixture, 5 %; dry brewer's yeast, 5 %.

The diet was stirred into a paste with water, mixing 1 drop of "vigantol" with the fat per kg. diet, and the whole was dried at about 70° so as to form a cake. Shortly after the commencement of the experiment 0.5 g. of "Eimalzin" was administered. The ophthalmic conditions improved, with simultaneous increase in weight. Then the animal contracted a cold, producing a periodic bloody excretion, during the course of which rapid decrease of weight occurred, and the condition of the eyes got worse. Dyspnoea set in, the extremities became blue and the animal generally weak, while the excretion from the nose increased. It could only move shakily and on the last day could not even stand. The ophthalmic condition practically disappeared during the last few days.

Several symptomatic similarities to the B-avitaminosis appear in the clinical picture: *viz.* bluish extremities and derangement of balance. In this case the basal diet contained 5 % yeast, and 0.5 g. "Eimalzin" produced an increase of 50–60 g. in 35 days in the vitamin B experiment, on an average of 10 animals.

The vitamin A was at the lowest limit of its effectiveness, since out of 10 animals, only 5 produced an average growth of 29 g., the others did not increase in weight at all or else died.

EXPERIMENTAL.

Animal material. Rats of identical breed were used, which had all been fed on the same standard diet for several generations. The animals were initiated into the experiment at the age of 3–4 weeks. The following groups were investigated: Group 1, yeast controls; Group 2, vitamin B-deficient animals; Groups 3 and 4, the mould-fed animals cited above.

The animals received the following basal diet:

	BM	BaM
	%	%
Caseinogen, Merck's vitamin-free ...	20	20
Maize powder starch	60	55
Coconut oil	10	10
Cod-liver oil... ..	5	5
Salt mixture, McCollum and Davis ...	5	5
Yeast, autoclaved	—	5

BaW diet is the same as BaM except that caseinogen of Dr Wander was substituted for caseinogen Merck. Both have similar biological values.

The dried brewer's yeast was autoclaved at a weakly alkaline reaction for 5 hours at 120°. As shown by test with the international standard product for vitamin B₁, 5 % of this autoclaved yeast is not sufficient as a source of the extra B-factor. 8 % is the minimum sufficient. In these experiments, this perhaps must be taken into account with the other groups which received no yeast as supplementary diet.

Animal 1485 increased in weight very little. Since it was also sharply differentiated from the others as regards the mineral content of its skin (high figures for Cl and P but low for Na), it was considered separately in the calculation of the average values.

Table I. *Group 1. (Yeast controls.)*

Animal No.	1485	1488	1507	1511	1497	1500	1502
Mother's No.	456	456	509	509	740	740	740
Sex	m	m	m	f	f	m	m
Colour	gw	w	w	w	sw	sw	sw
Basal diet	BM	BM	BM	BM	BaM	BaM	BaM
Supplement (yeast)	0.05	0.2	0.2	0.05	0.1	0.1	0.025
Initial weight (g.)	48	47	55	66	39	51	42
Final weight (g.)	74	128	149	109	139	155	136
Increase in weight (g.)	26	81	94	43	100	104	94
Duration of exp. (days)	57	57	57	57	57	57	57
Age at end of exp. (days)	110	110	101	101	96	96	96

m = male, f = female, gw = grey white, w = white, sw = black white.

Table II. *Group 2. (Beri-beri animals.)*

Animal No.	1482	1638	1721	2149	2157	2655
Mother's No.	1195	777	767	1581	1533	1232
Sex	f	f	m	f	f	f
Colour	w	w	sw	sw	w	w
Exp. commenced (1932)	30. iii.	11. iv.	20. iv.	9. vi.	9. vi.	15. vii.
Basal diet	BaM	BaW	BaW	BaW	BaW	BaW
Onset of beri-beri (days)	None	51	42	48	48	50
Initial weight (g.)	36	40	51	37	45	41
Maximum weight (g.)	49	58	57	48	58	55
Weight at death (g.)	32	43	40	33	38	39
Age at death (days)	99	85	67	70	78	77

Table III. *Mould-fed animals.*

	Group 3. Cured			Group 4. Beri-beri			Group 5
Animal No.	2312	2315	2317	2309	2311	2314	A-control
Mother's No.	1236	1858	1858	750	1236	1858	2477
Sex	f	f	f	f	f	f	m
Colour	sw	sw	sw	sw	sw	sw	w
Basal diet	BaW	BaW	BaW	BaW	BaW	BaW	A-control
Exp. commenced (1932)	16-6	16-6	16-6	16-6	16-6	16-6	29-6
Onset of beri-beri (days)	33	40	44	13	34	37	(38-Ophth)
Age at death	110	110	90	84	110	110	112
Cause of death	E	E	E	sp	E	E	sp

E = Ether, sp = spontaneous, Ophth = ophthalmia, and remaining abbreviations the same as in Tables I and II. Weights are recorded in Fig. 1 below.

Fig. 1. Growth curves for mould-fed animals.

↑ food without mould.
 ○ beri-beri.
 + death.

E killed with ether.
 +m mould-diet with mould.
 -m mould-diet without mould.

The dried brewer's yeast was obtained from "Vitamin Hefe, A.G.," of Rheinfelden, Switzerland. 0.05 g. gives a minimum increase of 50 g. in 5 weeks, 0.025 g. of about 30 g. (according to the usual vitamin B₁ test, with an average of 10 animals).

Whereas the Group 1 animals were all taken for the experiment on April 13th, 1932, the animals of Group 2 were commenced at varying periods and the initial dates are therefore inserted.

Animal 1482 was weak from the commencement. Its weight increased from 36 to 49 g. in the period from day 35 to day 52, then it decreased suddenly. The animal died on the 66th day without sign of beri-beri. The initial increase in weight is attributed to spontaneous mould formation, which, in common with similar previous observations, was the incentive for carrying out the experiment on vitamin B-formation by moulds, cited above.

Animal 2655 showed the usual symptoms of beri-beri on September 2nd. On the 3rd its hide was rough and moist, symptoms became more severe and

death ensued on the 6th, or 4 days after onset of beri-beri cramp, whereas the remaining animals died at the most 1 day afterwards and showed beri-beri symptoms in even more pronounced forms.

The suprarenals of animal 1638 were weighed; they were, left, 6.94 mg.; right, 7.38 mg.; and from animal 1721 they weighed, left, 11.68 mg.; right, 10.48 mg.

Technique of skin analysis.

1. *Handling of the skin prior to analysis.* The skin was removed from the animal immediately after death, or when this was not possible, the animal was stored in the ice-chest until required. In any case, the removal of the skin took place within a few hours of the animal's decease.

The specimen of skin taken for analysis consisted of a complete band round the animal's body between the fore- and hind-legs, with the subcutaneous fatty layer adhering to it. The hair was removed with scissors and scalpels and the specimen immediately weighed out on to a watch-glass and dried in the oven at 100° to constant weight, this process providing also the estimation of moisture.

The dried specimen was then transferred quantitatively into an extraction cartridge and extracted with ether in a Soxhlet apparatus for 12 hours, previous preliminary estimations having shown that 12 hours was ample time for complete removal of the fatty constituents. The estimation of total ether-extractives is thus obtained and is set out in Table V (a), under the heading Fat. Only total ether-extractives were estimated; a detailed analysis of the fatty constituents was not undertaken. The first 3 specimens of ether-extract (Nos. 1638, 1721, 1482) were digested with nitric acid and the digest tested for chloride. No chloride was found.

The dried, fat-free skin was then weighed and transferred quantitatively into a quartz digestion flask, to which was added 10 cc. of *N*/50 silver nitrate and then 10 cc. fuming nitric acid. The digestion mixture was allowed to stand overnight and the digestion completed next day by boiling, a further quantity of nitric acid being added when necessary and finally 20 cc. of distilled water. The filtrate and washings were made up to 50 cc. According to Tschopp [1927], digestion in the presence of silver nitrate effects complete removal of the chloride by precipitation as silver chloride, which can then be determined by estimating the excess silver nitrate remaining in the solution. Therefore two portions of 10 cc. each were withdrawn from the 50 cc. ash solution and used for the chloride estimation in duplicate. The remaining 30 cc. was then treated with 1 cc. of *N*/10 HCl, to precipitate the excess silver remaining therein. 4 cc. of distilled water were then added, bringing the volume up to 35 cc. The silver chloride was removed by filtration.

The estimations of the remaining constituents were carried out in aliquot portions of this filtrate.

2. *Analytical methods.* The estimations were in most cases carried out in duplicate, the values, quoted in Table V, being the means of 2 estimations.

A negative control was also carried out, and the values obtained were deducted from the skin values where this proved necessary.

The methods of analysis selected were, in general, those of Rossenbeck [1930] and those employed in the more recently published work of Herrmannsdorfer and Jung [1932-33], as follows.

(a) Chloride [Tschopp, 1927; also Rossenbeck, 1930; Herrmannsdorfer and Jung, 1932-33].

(b) Sodium [Blanchetière, 1923; also Hirsch, 1931].

- (c) Potassium [Kramer and Tisdall, 1921, 1; also Rossenbeck, 1930; Herrmannsdorfer and Jung, 1932-33].
- (d) Calcium [Kramer and Tisdall, 1921, 2].
- (e) Total phosphorus [Bell and Doisy, 1920; also Rossenbeck, 1930; Herrmannsdorfer and Jung, 1932-33].
- (f) Magnesium [Denis, 1922; also St Gadiant, 1923; Herrmannsdorfer and Jung, 1932-33].

3. *Notes.* (a) It should be mentioned that in carrying out colorimetric phosphate estimations by the molybdenum-blue reaction, which is necessary for determinations of sodium, magnesium, and total phosphorus, by the above-mentioned methods, "Eikonogen" was used as the reducing agent in place of quinol. This is the Na salt of 1:2:6-aminonaphtholsulphonic acid ("Agfa" product) and yields very satisfactory results [see also Hirsch, 1931].

(b) The values for total phosphorus do not include lipin P.

(c) The amount of fresh skin obtainable (less hair) varied from 1 to 7 g., according to the size of the animal, and correspondingly, the amount of dry fat-free skin available for ashing varied from about 0.3 to 2 g.

RESULTS.

Moisture contents. In Group 1 the moisture content of the skin together with the subcutaneous tissue varied between 42.7 and 56.8 %, in Group 2, from 58.5 to 68.4 %, and is therefore significantly higher. The same can be seen in Groups 3 and 4. The moisture content in the beri-beri and A-control animals is only noticeably raised on account of the corresponding disappearance of the fat. This reciprocal effect is also seen by a comparison of these values and those of Loewy and Cronheim [1932], in that their moisture content corresponds with our Group 2, and the fat content is similarly low.

Fat content. By fat is implied the substances which are soluble in ether. The loss of flesh is significantly shown in the lowered fat content of the skin. Whereas in the well grown Groups 1 and 3, the fat content varied from 16.8 to 31.1 %, in the case of the poorly grown animal 1485, it was only 14.9 %, and in the case of animals with more marked loss of weight, it had dropped to minimum values of 1.1 and 0.6 % in Groups 1 and 2 respectively, and only one animal, 2149, was able to show 6.4 %.

Although the mineral constituents are in part dissolved in the tissue fluid, and the calculation on a fresh-weight and a fat-containing dry-weight basis has its justifications, the calculation on a fat-free dry weight basis is preferable, on account of the variations of fat-content, at least in tissues relatively rich in fat. The possibility of some of the mineral constituents being removed with the fat must also be borne in mind.

Sodium content. The Na contents of the skins of our Group 1 are somewhat high by comparison with the normal animals of Loewy and Cronheim [1932], although the 2 animals cited by these investigators form hardly a sufficient basis for "normal values." Brown [1926] obtained for dogs, values of 155-250 mg./100 g. dry-weight, and for rabbits 116-243 mg./100 g., values which are somewhat lower than those for dogs obtained by Meyer-Bisch and Bock [1927], who found 115-145 mg./100 g. before pancreas extirpation and 87-156 mg./100 g. afterwards, as fresh-weight values. Our values of 85-104 mg./100 g. fresh-weight, correspond approximately with these figures, if one is justified in comparing values from different animal species. The Na content for

human beings seems to be somewhat higher than for the animals investigated to date, as is shown by the analyses of Brown [1927] who found 118–188 mg./100 g. in the fresh tissue, and similar work by Jung [1931, 1] and Herrmannsdorfer and Jung [1932–33] in which 111–222 mg./100 g. fresh-weight was found.

If the Group 1 figures are taken as a basis, then the 2 poorly grown animals without beri-beri (Nos. 1485 and 1482) show a considerable decline, likewise the corresponding animals of Group 2, but without any significant changes seeming to be specific for beri-beri; this also applies to Group 4.

Potassium content. With this element important variations exist between the individual groups. Marked variations occur even within Group 1 itself, which also occurred in the work of Loewy and Cronheim [1932]. Our values lie approximately at the upper limit of the normal, both in comparison with their work and also with the work on mice by Börnstein [1926], who obtained values from 90 to 131 mg./100 g. for fresh cutis (similarities exist between the diet of these mice and that of our Group 1 animals). Stern [1932, 1], with guinea-pigs, obtained values of 351–590 mg./100 g. dry, fat-containing skin, and approximately 120–180 mg./100 g. fresh-weight (dry substance averaged 30 %), which was therefore of about the same order as our Group 1. It was not possible to ascertain the influence exerted by colour, sex or yeast administration (so far as that produced normal growth), except that the animals receiving autoclaved yeast in the basal diet showed somewhat higher results. This applies especially to No. 1502, in which, despite good growth the 0.025 g. yeast was perhaps not quite adequate.

The rise in K values in the other groups is very remarkable. No. 1482, which also had a low Na content and no beri-beri symptoms, forms an exception. The fact that for 2 animals of group 3 definitely raised values were found makes one consider that the time allowed to elapse during the investigation (*e.g.* only 26 days in the case of No. 2317) was perhaps not always sufficiently long to effect an appreciable change in the somewhat slow-acting mineral interchanges of the skin [*cf.* Stern, 1932, 1]. On the other hand, the mould did not always grow satisfactorily, as already mentioned, and the appearance of the animals, which justified this conclusion being drawn, certainly seemed to show that the supply of the B-factor was not at an optimum. Animals 2312 and 2315 correspond with this period of time. Unfortunately the work lacks a control group, which received the mould at its optimum growth from the start and similarly with Group 4. This latter group shows values agreeing very well with those of Group 2, and an increase compared with their corresponding animals (Group 3). The A-control animal shows a value of about the same order of magnitude. This subject will be further discussed later. Incidentally, Stern [1931] found almost equally remarkable relative rises in the K content of rabbit skins after irradiation, although the absolute values obtained were not so high. But here the irradiation had led to fairly heavy inflammation. He also found definite increases in the K values in human skin diseases (normal 157–367 mg./100 g.; inflamed, 312–635 mg./100 g.). Herrmannsdorfer and Jung [1932–33] found in normal skin, 136–244 mg./100 g. dry-weight, and 48–73 mg./100 g. fresh-weight, while one case of *Impetigo herpetiformis* (unpublished) showed a value of 150 mg./100 g. (fresh-weight). But even these values, resulting from inflammation, were not as high as ours.

Calcium content. This was normal in Groups 1, 3, 4 and 5, with a slight tendency to rise in Group 4, and in one Group 3 animal, 2317, which was investigated 26 days after beri-beri had supervened. But in Group 2, 4 out of the 5 animals which died of beri-beri showed a definite rise in the Ca values, while

the last one showed a decline. The rise in calcium is similar to that in potassium, so that the ratio K:Ca (in equivalents) of this group is similar to that of Group 1, whereas it is considerably raised in Groups 3, 4 and 5, and in the case of one animal (1502) of Group 1. The highest Ca value is shown by No. 1482, but similar rises of this value are also quoted in the figures of Loewy and Cronheim [1932].

Magnesium. Our values for Group 1 are on the average higher than those of Loewy and Cronheim [1932] for rats, and are approximately the same as their values for guinea-pigs, and also as those of Brown [1926; 1927] for dogs, rabbits and human beings, if compared on a fresh-weight basis. It is however, possible that the subcutaneous adipose tissue accounts to a certain extent for this rise, for in two cases investigated by one of us [Herrmannsdorfer and Jung, 1932-33], more magnesium was found in the subcutaneous tissue than in the skin separated from it (see Table IV). This explanation is supported by the fact that

Table IV.

Patient	Sodium		Potassium		Calcium		Magnesium		Phosphorus		Chlorine	
	cut.	subc.	cut.	subc.	cut.	subc.	cut.	subc.	cut.	subc.	cut.	subc.
Z.	596	633	167	204	57	49	7	20	79	72	205	268
S.	—	—	152	156	61	61	28	47	77	112	383	370

cut. = cutis, subc. = subcutaneous tissue. Calculated as mg./100 g. dry fat-free substance.

4 animals of Group 2, which had practically no fat, showed low Mg values compared with Group 1, when calculated on a dry, fat-free basis, but were of the same order of magnitude on a fresh-weight basis, and these animals yield values resembling those of Loewy and Cronheim [1932]. Groups 2, 3, 4 and 5 had no parallel rise of calcium corresponding with the rise of potassium but showed a rise of magnesium, so that the ratio Ca:Mg is lowered, while with the others it definitely rises.

Total base (in milli-equivalents). In the case of Group 1, this lies at about the upper limit of this expression for animals treated with reduced air-pressure by Loewy and Cronheim [1932], and is therefore somewhat high. It is also high compared with the values of Brown (according to Heubner's [1931] calculations), for rabbits, dogs and human beings, and somewhat higher than in the remaining groups. This is not merely the result of the loss of weight, as is proved by the fact that an increase also occurred with two animals of Group 3 and a small increase in two animals of Group 2. The value is lowered in the case of No. 1485. A comparison of the values for individual cations with the total base yields several interesting conclusions (see Table V). First of all, the relatively high potassium content is concerned with a relative lowering of the other percentages. This is particularly significant in the case of calcium, which is present in approximately normal or somewhat lowered amounts when this method of calculation is employed and rather lags behind the figures of Loewy and Cronheim [1932] for rats and those of Brown and Luithlen for rabbits, dogs and human beings (according to Heubner's [1931] calculations). Furthermore Jung [1931, 1] and Herrmannsdorfer and Jung [1932-33] found quite different ratio values for human beings, *viz.* sodium, 60 to 77 %; potassium, 11 to 22 %; calcium, 6 to 11 %, and magnesium, 4 to 7 %. Normal values for the percentage of calcium are only seen in four beri-beri animals and the two animals Nos. 1485 and 1482.

Table V (a).

Group	Rat No.	Water %	Fat %	Sodium		Potassium		Calcium		Magnesium		Chloride		Phosphorus		Total acids bases M.-E. %	Excess O. bases M.-E. %
				ff.dr. mg./100 g.	M.-E. % ff.dr.	ff.dr. mg./100 g.	M.-E. % ff.dr.	ff.dr. mg./100 g.	M.-E. % ff.dr.	ff.dr. mg./100 g.	M.-E. % ff.dr.	ff.dr. mg./100 g.	M.-E. % ff.dr.	ff.dr. mg./100 g.	M.-E. % ff.dr.		
1 Yeast contr.	1485	49.3	14.9	195	8.2	353	8.9	41.8	2.1	47.1	3.9	661	18.7	812	35.5	23.1	54.2
	1488	56	17.5	400	17.5	590	15	35.7	1.79	49.7	4.1	114	3.2	522	23.9	38.4	27.1
	1507	24.7	31.1	—	—	345	8.8	31.6	1.55	46.7	3.8	—	—	457	20.1	—	—
	1511	52.5	16.8	310	13.5	485	12.4	36.8	1.84	42.1	3.5	112	3.2	405	15.7	31.2	18.9
	1497	47.1	27.2	396	17.2	578	14.6	34.4	1.72	38.8	3.1	303	8.6	418	18.5	36.6	27.1
	1500	56.8	18.2	376	16.4	662	18.6	36.2	1.88	55.5	4.5	225	6.4	496	21.4	41.4	28.2
1502	52	21.7	330	14.4	872	22.2	23	1.15	51.2	4.2	372	10.6	442	19.4	41.9	30	+11.9
Averages	51.2	22.1	363	15.8	587	15.3	33	1.6	47	3.9	225	6.4	457	20	37.9	26.3	+11.6
2 Ber. beri	1482	63.2	1.4	133	5.7	718	18.2	220	10.4	387	32	369	10.8	684	29	66.1	+26.3
	1638	68.4	1.1	537	23	1261	32	101	5.4	23.4	1.9	336	9.4	763	37	62.3	+15.9
	1721	58.5	1.8	224	9.7	1986	50	190	9.4	22.8	1.9	278	7.8	515	23.5	31.2	+40.1
	2149	65.4	6.4	317	13.7	988	25	110	5.5	41.9	3.4	444	12.6	302	13.3	47.9	+22
	2157	64.1	1.5	282	12.3	1075	26	88	4.4	36.6	3	438	12.3	333	14.5	46.1	+19.3
	2655	62.8	2.4	224	10.6	2490	63	17	0.8	85.3	7	411	11.6	811	36	81.7	+34.1
Averages	63.9	2.7	319	14	1556	39	101	5	42	2.5	382	10.7	545	25	61.8	35.6	+25.9
3 Mould cured	2312	52.3	23.4	362	15.8	776	19.7	26	1.3	59.3	4.9	137	3.8	307	13.5	41.7	+24.4
	2315	58.4	18.3	478	20.8	1086	27.2	36	1.8	48	4	709	20	302	13.3	53.8	+20.5
	2317	57.5	21.1	513	22.2	1219	30.8	47	2.3	118	9.6	645	18.4	433	19.4	64.9	+27.1
	56	21	451	19.6	1027	26.2	36	1.8	75	6	496	14	347	15	53.5	26.2	+24
	Averages	(101)	(401)	(101)	(229)	(536)	(8)	(34)	(14.7)	(18)	(110)	(192)	(77)	(76)	(10)	(36.6)	(36.6)
	2309	65.2	1.2	372	16.2	1323	34	41	2	100	8.1	407	11.4	255	11.6	60.1	+37.1
4 Mould ber. beri	2311	64	0.7	515	22.8	1393	35.2	37	1.8	67.3	5.5	500	14.1	213	9.3	65.3	+41.9
	2314	67.5	0.6	417	17.8	1190	30.2	40	2	62.5	5.2	536	15.2	211	9.3	55.2	+30.7
	65.6	0.8	435	19	1302	33	39	2	73	6	547	13.6	226	10	60.2	23.6	+36.6
	Averages	(141)	(441)	(141)	(441)	(441)	(13)	(13)	(26)	(26)	(162)	(76)	(76)	(76)	(76)	(76)	(76)
	2477	65.9	1.4	323	14.1	1472	37.5	33	1.7	68.6	5.7	404	11.4	233	10.3	59	+37.3
	A-control	(106)	(106)	(106)	(485)	(485)	(11)	(11)	(22)	(22)	(133)	(133)	(133)	(133)	(133)	(133)	(133)

fr. = fresh, dr. = dry, ff.dr. = fat-free dry. M.-E. = Milli-equivalents. The average values in brackets are fresh-weight values in mg./100 g.

Table V (b).

Rat No.	Group	K : Na	K : Ca	Ca : Mg	Na : Mg			Na + Cl	K : P	P : Cl	Bases Acids
					Na	Mg	Na : Cl				
					Milli-equivalents						
2477	1	1.06	7.84	0.43	4.11	3.13	22	0.74	3.96	1.45	
	2	3.31	5.44	3.73	5.4	1.26	24.6	1.7	2.45	1.78	
	3	1.32	14.4	0.32	3.6	2.12	33.6	1.7	1.41	1.91	
	4	1.77	16.8	0.32	3.2	1.4	32.5	3.3	0.76	2.55	
	5	2.65	22.3	0.29	2.5	1.23	25.5	3.6	0.9	2.7	

These figures represent averages for each group.

The last two, however, show also a corresponding alkali deficit. By rearranging the table in such a way that the calcium equivalent is taken as unity (Tables VI and VII), and the other elements brought into line with it, a relative lowering of the three other cations is shown for the four beri-beri animals which had normal Ca values, while the 5th exhibits a marked rise of these ions, and the mould-fed animals and the A-control have particularly an increase of potassium. By this method of calculation, the ratio, Na:Ca:Mg shows a remarkable constancy in all the well-grown animals, even in those that subsequently underwent rapid loss of weight, while perhaps the deficient growth in a section of our animals forms the direct basis of the observed calcium increase in these cases. With these animals, the quotient K:Ca is remarkably similar to that of the controls. The drop in the magnesium value in these cases causes a considerable rise in the quotient Ca:Mg whereas this does not vary appreciably in the other cases. Only No. 2655, whose calcium was lowered in comparison to the enormously increased potassium content, had a lowered quotient. The ratio of alkali to alkali-earth is fairly constant. It is only lowered in the two animals Nos. 1485 and 1482. We are as yet far too scantily informed regarding the significance of these quotients. Table VI shows the large variations observed, which are to some extent the result of diet and of animal species, but are due in a large measure to factors not yet understood.

Table VI.

Equivalents of bases in the fat-free dry weights.

Rat No.	Sum M.-E. 100 g.	% of sum of M.-E.				Ratios for Ca = 1				Group
		Na	K	Ca	Mg	Na	K	Ca	Mg	
1485	23.1	35.6	38.5	9	16.9	4	4.3	1	1.9	1
1488	38.4	45.6	39.1	4.6	10.7	10	8.5	1	2.3	
1507	—	—	—	—	—	—	5.7	1	2.5	
1511	31.2	43.2	39.7	5.9	11.2	7.3	6.7	1	1.9	
1497	36.6	47.0	39.9	4.7	8.4	10	8.5	1	1.8	
1500	41.4	39.6	45	4.5	10.9	8.8	10	1	2.4	
1502	42	34.3	52.9	2.8	10	12	19.3	1	3.6	
Average	37.9	41.9	43.3	4.5	10.2	*9.3	*7.84	1	*2.2	
1482	61.1	8.6	27.5	15.7	48.2	0.5	1.75	1	3.1	2
1638	62.3	36.9	51.4	8.7	3	4.2	5.9	1	0.35	
1721	71.3	13.6	70.6	13.2	2.6	1	5.35	1	0.2	
2149	47.9	28.6	52.6	11.6	7.2	2.5	4.56	1	0.6	
2157	46.1	26.7	57.3	9.5	6.5	2.8	5.95	1	0.7	
2655	81.7	12.9	77.3	1	8.8	13	77	1	8.8	
Average	61.8	23.5	61.8	10.8	5.6	†2.1	†5.7	1	†0.52	
2312	41.7	37.9	47.2	3.2	11.7	11.8	15.4	1	3.6	3
2315	53.8	38.7	50.6	3.4	7.3	11.4	15	1	2.1	
2317	64.9	34.2	47.5	3.6	14.7	9.5	13.2	1	4.1	
Average	53.5	36.9	49.1	3.4	11.2	11.7	14.4	1	3.3	
2309	60.1	27	56.1	3.4	13.5	8	16.5	1	4	4
2311	65.3	34.9	53.9	2.8	8.4	12.5	19.1	1	3	
2314	55.2	32.3	54.8	3.6	9.3	9	15	1	2.6	
Average	60.2	31.4	54.9	3.3	8.8	9.5	16.6	1	3.2	
2477	59	23.9	63.6	2.8	9.7	8.5	22.3	1	3.5	5

* Without 1502.

† Without 2655.

Table VII.

Author	Animal species	Treatment	Ratios for Ca=1 (Milli-equivalents)			
			Na	K	Ca	Mg
Fawns-Jung	Rat	Yeast-control	4.0-12.0	4.3-19.3	1	1.8-3.6
		Beri-beri	0.5-13	1.75-77	1	0.2-0.8
		Mould-fed	9.5-11.8	13.2-15.4	1	2.1-4.1
		Mould-fed beri-beri	8-12.5	15-16.5	1	2.6-4
		A-avitaminosis	8.5	22.3	1	3.5
Loewy-Cronheim [1932]	Rat	Normal	4.2-5.8	1.8-4.8	1	0.7-1
		Diluted air	8-16.2	1.6-2.9	1	0.6-1.4
		Irradiated	6	1.9	1	1.1
		Dark	3.9-5.1	3.7-4.6	1	1.4-1.7
Börnstein [1926]	Mouse	—	—	1.6-1.92	1	—
Hayashi [1926]	Rabbit	Normal	7.5	4.7	1	0.8
		Cabbage-fed	7	4.4	1	0.9
		Oats-fed	7.5	6.5	1	0.9
		HCl-fed	5.6	6	1	0.9
		CaCl ₂ injection	5.6	3.5	1	0.6
Kaplansky [1928]	Rabbit	Normal	1-2.6	0.5-1.6	1	—
		Irradiated	0.7-4	0.8-2.7	1	—
Stern [1931]	Rabbit	Normal	—	2-5.4	1	—
		Irradiated	—	2.5-5.9	1	—
Brown [Heubner, 1931]	Rabbit	Normal averages	2.5	1.23	1	0.95
	Dog	Normal averages	4.2	2.84	1	1.06
	Man	Ill averages	6.8	2.65	1	1.06
Herrmannsdorfer- Jung [1932-33]	Man	Tuberculosis	6.8-12.3	1.2-2.7	1	0.2-0.78
Jung-Marx. Not published	Woman	<i>Impetigo herpetiformis</i>	—	3.5	1	0.4
Nathan and Stern [1928]	Man	Normal averages	—	5.4	1	—
		Dermatosis averages	—	6.1	1	—

Chlorine content. The chlorine content of Group 1 is very low. This might be attributed to the Cl content of the subcutaneous connective tissue [cf. Loewy and Cronheim, 1932]. This however cannot be generalised as the following facts seem to indicate that subcutaneous tissue need not necessarily have a much lower chlorine content than skin itself. Firstly, in Group 3, two animals which had a high fat content had higher chlorine values, while the other three had less; secondly, no noteworthy difference in chlorine content between cutis and subcutis was found in the two human skins cited in Table IV. Jung [1931, 1] was able to evolve a ratio for contrasting the plasma and connective tissue in musculature, by which it was established that in some cases the connective tissue contained an equal amount of chlorine to the plasma, in others less, but in a few cases significantly more. In the well-known work of Wahlgren [1909] and Padtberg [1910], the former found for dogs 376 mg./100 g. Cl in skin *plus* fatty tissue, and the latter 269 mg./100 g. in skin less fat. Atchley and Benedict [1927] give 132-240 mg./100 mg. (fresh-weight) as normal for dogs. Stern [1931] has recently shown that the skin can assimilate chlorine, for after administering "Afenil" (CaCl₂, urea) to rabbits, a marked rise of the skin Cl content was observed, whereas its calcium did not increase. The skin did not alter appreciably in its appearance, and there was no corresponding increase in water content. In one case in which the Cl content rose from 380 mg. to 1080 mg./100 g., the water, in fact, dropped from 73.3 % to 70.1 %. So also with our

animals, no parallel relation is shown between the water and chlorine contents, but it does occur between water and sodium chloride contents (the sum of Na and Cl equivalents), the fat contents being identical. In relation to the water content, fat appears to be more important than salt (NaCl), and further, chlorine can be retained "dry," but not sodium chloride.

It is noteworthy that the highest figures for the sodium chloride equivalent occur in the two groups of mould-fed animals, which as already mentioned showed an enormous thirst and great sensitivity to lack of water. But a large water requirement is generally observed with all animals participating in vitamin-B tests, especially in summer. Lack of water often produces bleeding, especially *via* the urogenital tract, which disappears immediately on ensuring an adequate supply of water. This sensitivity is seldom observed among animals being used for other investigations. The quotient Na:Cl varies somewhat irregularly.

Phosphate content. Only the non-ether-soluble phosphorus was estimated. But under this heading come many substances in which phosphorus is held in organic combination. Now since both the nucleic acids and the nucleotides as well as the hexosephosphoric acids have a dissociable hydrogen atom in the phosphoric acid group, they must be taken into account as regards acid-base regulation, whereas this is hardly the case with the phosphatides. One should therefore be justified in placing the various non-ether-soluble phosphoric fractions together and calculating them as acid. The calculation as equivalents is worked out at neutral reaction according to the system of Reichel and Spiro [1927]. The phosphorus generally shows a tendency to sink in the case of the beri-beri animals, especially in Group 4, but in Group 2 only two animals show a significant drop, while with two others, on the contrary, there is a rise. This is perhaps a result of poor growth since the phosphate value is also distinctly increased in the deficiently grown animals, 1482 and 1485.

The ratio P:Cl exhibits wide variations, if anything with a tendency to drop in Groups 2, 4 and 5, against which the ratio K:P shows a rise of about double the amount between Groups 1 and 2, and 3 and 4 respectively, while 3 has similar values to 2 and 5 to 4. The unusual animals, 1485 and 1482, have much diminished quotients.

Total acid content. The total acid shows no particular variations. A rise is seen in a few animals of Groups 2 and 3. It is particularly high in animal 1485.

Excess base. An increase in the amount of excess base is shown by Group 2 relative to Group 1, and by Group 4 relative to Group 3. The A-control has a value of the same order of magnitude as Group 4.

One single animal, No. 1485, shows a considerable excess of acid.

DISCUSSION.

In comparison with the control animals, the most striking change occurs in the potassium content, which is considerably increased in the beri-beri groups. This is not merely the result of emaciation, since the animals of Group 3 had equally high values, even in the presence of a good layer of fat, while one animal of Group 2 had a relatively low value. The values are higher than any yet obtained, even from skins affected by inflammation, and the relative increase corresponds to those obtained by Stern [1932, 2] from inflamed human skin and from the skin of irradiated rabbits as compared with normal skin. But with those of our animals taken for analysis, a visible change of the hide only occurred with No. 2655 which had a damp, rough skin shortly before the onset of beri-beri, and with the animals of Group 4, the hides of which were always rough. No

such signs of inflammation were observable at the time of the investigation. It should be mentioned that the high potassium content is significant as a preparatory step in the skin prior to an inflammatory reaction, for in the case of the mould-fed animals remaining in the experiment after the others had been removed for analysis, a deterioration of the skin was apparent, and about a month later quite a severe dermatitis occurred. It is noteworthy that the A-control animal, 2477, reacted in almost the same way as the Group 4 animals and to some extent like those of Group 2. Although only one single animal is concerned, the high potassium content is so striking that a discussion of the possible reasons for this is justified. Firstly, it should be mentioned that out of 14 animals receiving the same diet as No. 2477, in the vitamin A experiments, 9 developed a dermatitis 11–25 days after the commencement of the feeding and one of them indeed, only one day after. It is interesting that, sooner or later, a spontaneous healing took place, and the hair grew again. This was observed also in the case of three animals receiving 2 mg. cod-liver oil or 2.5γ carotene in olive oil per head. This tends to refute the theory that the dermatitis is due to a vitamin B deficiency in the basal diet and is cured when the supplementing substances are administered, for, as previously emphasised, the symptoms first appeared after the supplementary feeding was commenced.

The symptoms somewhat resemble those found by Burr and Burr [1929; 1930], which, together with the results of Burr and Brown [1931], were attributed to a deficiency of highly unsaturated fatty acids of high molecular weight, a suggestion which was disputed by Gregory and Drummond [1932]. In this deficiency disease, however, the dermatitis occurs more on the tail and hind-quarters, but with our animals almost exclusively on the foreparts, and only in one case in a vitamin B₁ investigation on the haunches, though this never occurred at all in the vitamin A experiments.

In its localisation it somewhat resembled the "pellagra" appearance. But it lacks the localisation around the eyes, previously observed [Jung, 1931, 2], and the clinical picture did not correspond with the description by Chick and Roscoe [1927; 1928], Findlay [1928], Sherman and Sandels [1929], and Scheunert and Lindner [1927]. Moreover, this dermatitis occurred in one animal that increased to 185 g. in 52 days, while receiving 0.1 g. yeast daily in a vitamin B₁ test. With one mould-fed animal, the dermatitis was cured by administration of egg-white, but in another case this proved ineffective.

It is therefore conjectured that as a result of a deficiency of any essential factor of diet, the skin becomes sensitive, and a dermatitis occurs as a result of subsequent metabolic changes (with the supplementary feeding). This is supported by the fact that spontaneous healing takes place concurrently with a satisfactory weight increase. It is not claimed that the changes of the skin observed with the various vitamin B groups and the vitamin A control are due to the same cause, for Nathan and Stern [1928] found identical disturbances in dermatoses of the most varying kinds, and Portmann [1927] found identical changes in the skin histologically after vitamin A-free, vitamin B-free and quantitatively insufficient diets.

One should at least comment on the extremely similar observation here, to that of Burr and Burr [1930], namely, the great thirst of our mould-fed animals and their sensitivity to lack of water. The skin of these animals showed to a certain extent an increase in the sodium, chlorine and water contents, which exhibited a relationship between the water content and the sum of Na + Cl, but independent of either of them taken singly. This relationship is in any case only valid under identical conditions of fat-content.

Have these changes anything to do with the deficiency of vitamins B₁ and B₄? It seems possible to answer this question in the positive and to state with justification that in rat beri-beri, the potassium content of the skin rises considerably, with small changes in the absolute values for sodium and magnesium, whereas to a certain extent the calcium alters correspondingly in such a way that the ratio K:Ca tends to remain constant. It is noteworthy that the specific animals which showed a markedly rough hide exhibited no rise in calcium. Phosphorus was definitely lower in Group 4 than in Group 3, but in the beri-beri Group 2, this was only the case with a few animals and then in relation to calcium. There is therefore a rise in the ratio K:P in most of these animals. It is unfortunate that it was not possible to estimate phosphorus in the ether extract as well, as one cannot tell if any change occurred in the lipin phosphorus, for, among others, Schmitz and Hiraoka [1928] have observed relationships between beri-beri and phosphatide metabolism.

The variations in the chemical constituents of the skin dealt with in this paper do not seem to be specific for beri-beri but are rather an expression of general changes in metabolism.

Whether or not this is due to changes in the internal secretions must at present be left an open question.

SUMMARY.

1. The mould, *Aspergillus niger*, can form vitamin B₁ from a vitamin B-free diet and cure and prevent beri-beri in rats. The vitamin is mostly contained in the conidia (spores) and only to a small extent in the mycelium and the basal diet on which the mould is grown.

2. Animals nourished on this mould-covered food appear nervously excitable and have a large water requirement and great sensitivity to lack of water.

3. The mineral content of the skin, after ether-extraction, was determined in groups of growing rats on various diets with the object of ascertaining the changes due to beri-beri.

4. In beri-beri a marked increase in potassium was found, and in some cases a corresponding rise of calcium. This is not the result of deficient growth, for it was much less marked in one rat that had no beri-beri.

5. Magnesium is raised wherever no corresponding calcium increase follows a rise in potassium, so that the ratio of univalent to bivalent bases is fairly constant.

6. A relationship has been shown between the sum of the equivalents of sodium and chloride and the water content, under similar conditions of fat content.

7. Phosphate generally showed a tendency to sink in beri-beri, chloride to rise.

8. A vitamin A control animal showed approximately the same ratio values as the beri-beri animals. In this connection it should be mentioned that a dermatitis was often observed in vitamin A tests, occurring first after supplemental feeding. Spontaneous healing occurred as weight increased, the condition thus resembling the beri-beri dermatitis.

9. Possible explanations for this are discussed.

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