THE EFFECTS OF LIFE-LONG SUBSISTENCE ON DIETS PROVIDING SUBOPTIMAL AMOUNTS OF THE "VITAMIN B COMPLEX"

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(With 4 Figures in the Text)

Introduction

It is clear from the rarity of pellagra or other forms of dermatitis of dietary origin and of conditions related closely to beri-beri that the ordinary individual in this country habitually consumes foods providing more of the "vitamin B complex" than does the diet of the malnourished workers in the "corn belt" of the U.S.A., or the simple fare of the poor rice-eating peasant in the Far East. But it does not necessarily follow that our modern diets supply a sufficiency of these vitamins. Indeed, some experts have maintained for years past that a large proportion of our population, particularly the poorer people, subsist, often for their lifetime, on diets which do not provide the physiologically requisite amount of the vitamin B complex (McCarrison, 1931; Marks, 1932; Plimmer & Plimmer, 1933; Drummond, 1934).

Of the five or six factors recognized to-day as components of the "B-complex", three are known to be required by man. These are B_1 (aneurin), lactoflavin and the antipellagra factor (P.-P), very recently identified with nicotinic acid or its amide; the last two were formerly associated in the accepted classification as B_2. Of B_1 it is possible to speak with considerable assurance. Its chemical constitution is known, at least one of its physiological functions in the tissues is understood, and there are reasonably accurate methods of estimating the amount present in foodstuffs. The constitutions of lactoflavin and nicotinic acid are known and there is evidence of their role in the body, but there are not yet available trustworthy estimates of the amount present in foods or of the requirements of the body. Another "antidermatitis" factor, provisionally referred to as B_6, is recognized as playing a part in the nutrition of rats. It is not known, however, whether it is concerned in human nutrition. Knowledge of the nature and distribution of B_6 is as yet fragmentary.

In surveying modern diets, therefore, it is natural for the moment to give

https://doi.org/10.1017/S0022172400011220 Published online by Cambridge University Press

most attention to the factor B_1, particularly as the comprehensive review by Cowgill (1934) indicates that a deficiency of this factor is often detectable. Our own analyses of modern English and other diets (Baker *et al.* 1935, 1936, 1937) provide strong support for the view that a considerable proportion of the population of Great Britain lives on meals which do not provide sufficient vitamin B_1. The evidence derived from this survey is summarized in Table I.

Table I. *Vitamin B_1 intake of adult man*

Class	International units of vitamin B_1 daily	Remarks
1	0–150	Beri-beri is almost certain to occur unless the higher intakes are associated with relatively high fat consumption
2	150–250	Beri-beri may occur if the diet is largely carbohydrate and the body weight is high. On 60 g. of fat or more, the individual will probably be protected, but will nevertheless be B_1 deficient
3	200–350	Intake of adult on typical "poverty" diet, composed of white bread, vitamin-poor meat such as beef, sugar and little dairy produce, liver or whole grain
4	250–500	Protective level for most adults on a good mixed diet. The upper level probably represents an adequate intake for most Europeans' diet
5	800–1200	Intake of adult if the bread of the "poverty" diet (3) were entirely whole-grain
6	1000–1500	Estimated requirements during pregnancy

One difficulty in making estimates of the vitamin B_1 requirements of man arises because it is not yet possible to make allowance for the influence of other constituents of the basal diet. It is known, for instance, that there is less demand for the vitamin when the diet is relatively rich in fat than when carbohydrate predominates (Evans & Lepkovsky, 1929). On the other hand, Sure and his co-workers find that there may be impaired assimilation of fats in B_1 deficiency (1935). Furthermore, it is established that the vitamin directly participates in the processes controlling one stage of carbohydrate breakdown, a fact which throws light on the direct relationships which have been traced between the requirements for the vitamin and the carbohydrate content of the diet or the total intake of calories (see Cowgill's review). Possibly in such relationships is found the explanation why a poor oriental peasant, living on a diet of rice with perhaps a little fish, rich in carbohydrate and providing daily some 10–12 g. fat and about 150 I.U. of vitamin B_1, falls a victim to beri-beri, whereas one of our own poor, consuming a diet little richer in this essential factor but supplying 70–80 g. of fat, is protected. Table II shows the estimated B_1 content of the diets consumed by the six representative income-level groups of Lloyd (1936) and considered by Orr in his recent survey (1936).

If these figures are accepted one can no longer question the view that much chronic if mild deficiency of vitamin B_1 can be encountered to-day. Moreover, if it is borne in mind that the best known sources of the other components of the B complex are relatively expensive foods (liver, eggs, milk), and that wheat germ, which also contains them, is present in a very small proportion of the

breads eaten to-day in England, it will be recognized that a similar deficiency of these other B factors is also common.

Although the literature contains a very large amount of information regarding the effects of a severe deprivation of B vitamins there is curiously little recorded about the consequences of living for a long time on a suboptimal

Table II. *Diets of Orr's six income levels, analysed for vitamin B_1 content*

Diet no.	I	II	III	IV	V	VI
Average amount spent per week per head on food	4*s.*	6*s.*	8*s.*	10*s.*	12*s.*	14*s.*
Units vitamin B_1 per day. Bread all white	290	357	402	430	465	528
Assuming 4–5% of the bread wholemeal*	—	—	414	442	—	—
Assuming 6–7% of the bread wholemeal*	—	—	—	—	508	543

* Approximations based on a limited survey we had made (see Baker & Drummond, 1937).

intake. There is, for example, little to tell us whether there are disorders common to-day but which were relatively rare one hundred years ago when the poorest people, for whom bread was virtually the staple food, had a daily intake of vitamin B_1 three to five times that of many families to-day. Clearly such information is needed, and as one step in that direction the experiment now to be described was begun.

Experimental

(1) *General plan*

The essential feature of the experiment was to maintain two comparable groups of rats for their whole lifetime on diets one of which was to be adequate in all respects whilst the other was to be deficient in the vitamin B complex but not to such an extent as to cause ill-health in a short time. It is important at this point to note that at the time when the investigation was begun (1931) the differentiation of the factors which together constitute the "vitamin B complex" was in an early stage. The factors B_1 and what was then termed B_2 (now regarded as composed of lactoflavin, nicotinic acid or its amide and possibly another factor) were accepted as entities, but the existence of yet other components of the complex was only vaguely suspected.

Actually, if the two diets employed are compared it will be seen that broadly speaking the experiment can be regarded as a test of the value of wheat germ in supplementing the vitamin deficiencies of white flour. In order to lessen the possible influence of environmental factors the experiment was duplicated in two laboratories. In all over 1000 rats were observed, approximately half being fed on each diet.

(2) *Composition of diets*

For the first three months the compositions of the diets were as given under A1 (adequate) and D1 (deficient). Later the composition was changed to those given under A2 and D2. The alterations were made to improve the balance of

certain nutrients, whilst the exclusion of the dried yeast from the basal deficient diet made it easier to regulate the amount of B_1 as it was desired to maintain this group in a state of chronic but not severe deprivation.

Table III

	A1	D1	A2	D2
Meat meal	10	10	3	4
Dried blood	2	2	4	7
Mineral mixture	2	2	0	0
White flour	66	80	71	82
Wheat germ (Bemax)	15	0	15	0
Dried yeast	0	1	0	0
Olive oil (or arachis oil)	3	3	3	3
Cod-liver oil	2	2	2	2
Common salt	1	1	1	1
Chalk	0·5	0·5	0·5	0·5
Bone flour	0·5	0·5	0·5	0·5

The cod-liver oil was mixed with the ground diet each week.

The analysis of the foods gave the figures:

	A1	D1	A2	D2
Protein	23·4	19·86	20·64	20·38
Fat	3·6	2·4	7·0	6·4
Carbohydrate	58·5	65·34	58·56	59·92
Minerals	5·0	4·3	2·3	2·7

The constituents of diets D1 and D2—excepting the yeast—supplied a certain amount of B vitamins. This was apparent from the growth which some of the animals made during periods when the yeast was not given. So far as we could then determine, the growth factors were associated with the meat meal. The general procedure, so far as the "deficient" animals were concerned, was to watch for a marked retardation of growth in young animals or for signs of deficiency or loss of weight in adults. When this occurred dried yeast was added to the deficient diet in amounts equivalent to from 0·5 to 2 % of the diet depending on the condition of the animal. The supplement was continued until a definite improvement had been brought about. In this manner the "deficient" animals were kept in a state of chronic undernourishment in respect to B vitamins during the whole of their lives.

The curves in Figs. 1 and 2 provide a comparison between the unrestricted growth on the adequate (A) diet and the controlled subnormal rate of development of the rats fed on the (D) diets.

(3) *Housing, records, etc.*

In most cases four adult rats were kept in each cage. Some larger cages contained six or eight. All were fitted with grids to prevent coprophagy. The diet was given *ad lib.* Weighings were made twice weekly during the period of growth and once weekly after maturity. Notes were made of matings, breeding records, appearance and state of health. The animals were kept until they died from natural causes, the only exceptions being a very few old and decrepit ones which were chloroformed because they became infested with vermin. Post-

mortem examinations were carried out on all carcases except those which had been partly devoured or which were to some extent decomposed. Several thousand preparations were preserved and subjected to microscopic examination.

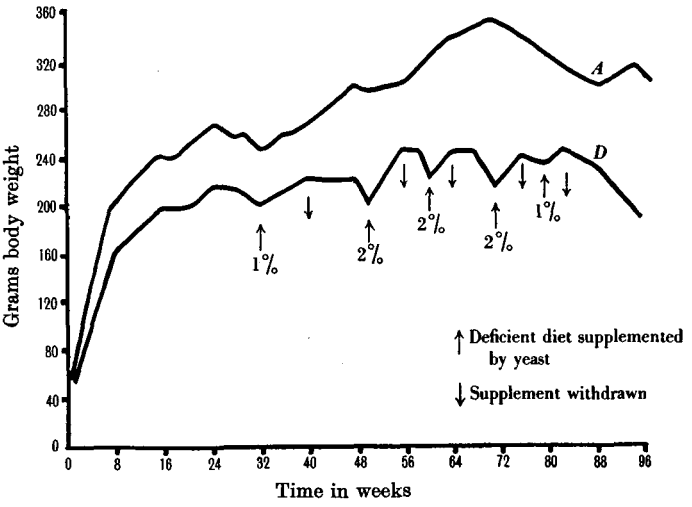

Fig. 1. Comparison of growth rates of male rats on (A) adequate diet and (D) deficient diet. It will be noticed how by adding, and then later withdrawing, supplements of yeast the weight of the deficient animals was maintained.

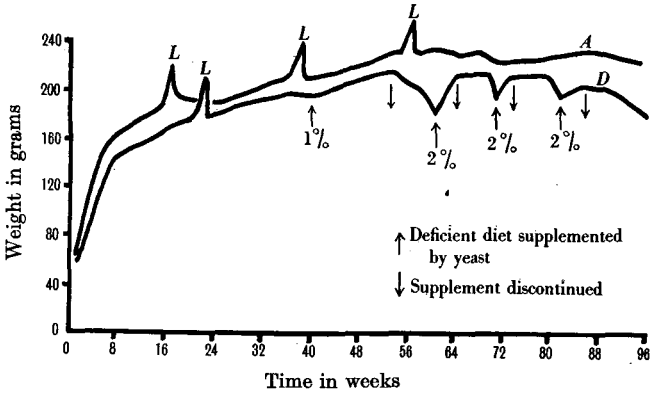

Fig. 2. Growth curves of female rats on (A) adequate and (D) deficient diets. (L) indicates birth of litters.

(4) *Records of the investigation*

(*a*) *Numbers.*

The total number of animals on which satisfactory post-mortem examinations were possible was 1039.

Diet	Laboratory U.C.	Laboratory V.L.
A	129	354
D	106	450

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(*b*) *Food consumption.*

All the animals were given the diets immediately after weaning. Accurate food consumption records were not kept, but general observation showed that the food consumption of the D groups was not quite as high as that of the controls. Anorexia is a well-known symptom of acute B deficiency in experimental animals. Its relation to gastric secretion and motility in B deficiency has been the subject of a number of investigations. The most striking observations are those of Webster & Armour (1932), Komarov (1934) and Cowgill & Gilman (1935), who found diminished secretion and a tendency to achlorhydria. Alvarez *et al.* (1936) were led by their studies to investigate B deficiency in man. Two volunteers lived for 6 weeks on a diet markedly deficient in vitamin B_1, but during this time no reduction in gastric acidity or loss of appetite was recorded.

(*c*) *Growth.*

The growth curves given in Figs. 1 and 2 are quite representative of the behaviour of the two groups. The rates of growth and maximum weights attained by the animals in group A were fully up to the normal records of the two laboratory colonies. The growth of the D rats, controlled as has been described by varying the amount of the dried yeast supplement, was sufficiently near that of the controls to justify the belief that these animals were being maintained in a state of mild vitamin B deprivation. This is also illustrated by the average figures for the 235 animals of one colony (U.C.).

	Initial weight	Body weight in g.	
Diet	g.	At 3 months	Maximum reached
A♂	54	223	309
D♂	58	164	249
A♀	59	165	270
D♀	58	141	193

(*d*) *Average duration of life.*

The experiment was undisturbed by any epidemic in either laboratory. This is of importance in considering the survival times of the two groups.

U.C. colony		V.L. colony	
Diet	Average duration of life in weeks	Diet	Average duration of life in weeks
A♂	84·2	A♂	116
D♂	64·0	D♂	88
A♀	85·1	A♀	104
D♀	73·4	D♀	92

We have no definite information about the normal average length of life of the piebald rat stock drawn upon for this experiment. Donaldson (1924) gives 3 years as an approximate estimate of the span of life of the albino Wistar rat,

whilst a few records by Slonaker (1912) for the same strain indicate about 136 weeks. The maximum duration of life in the V.L. colony was:

	♀		♂
A	38 months	A	41 months
D	39 months	D	40 months

The curves in Figs. 3 and 4 show the distribution of deaths amongst the male animals of the two groups.

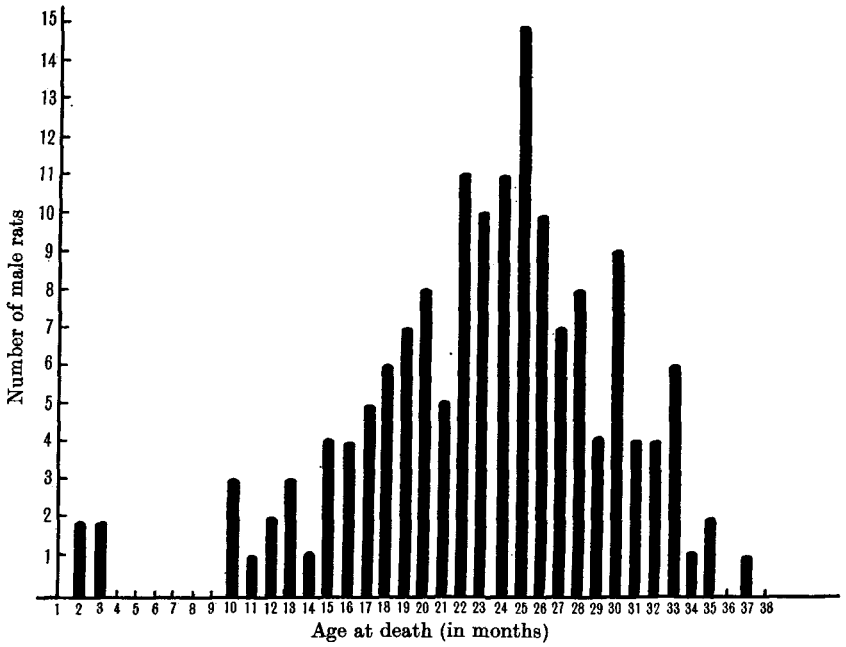

Fig. 3. Distribution of age at death of male rats on an adequate diet.

The average duration of life in both groups was severely affected by the incidence of chronic bronchiectasis. It is noticeable that although the group A animals lived on an average longer than the corresponding D animals yet the latter were able to survive quite a long time on their diminished vitamin B allowance. As the figures on p. 364 show, the rats of the U.C. colony were relatively more seriously affected by the chronic bronchiectasis than those of the V.L. colony. This doubtless explains the shorter survival times of the former.

(*e*) *Reproduction.*

For the greater part of the first year records were kept of the breeding in one colony (U.C.). The does were mated for the first time when the body weight was about 155 g. Records were also kept of the fertility of some of the bucks.

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		Matings	Positive	% positive	Number of young	Average number per litter	Number reared	% reared
A	Bucks	25	23	92	—	—	—	—
D	Bucks	24	22	90	—	—	—	—
A	Does	56	38	68	242	8·25	145	60
D	Does	59	34	58	207	6·05	19	9·1

These figures provide additional evidence that our D colony was in a state of mild B_1 deficiency. A greater deprivation would have affected the fertility, but the mating responses in both A and D groups are within the normal range.

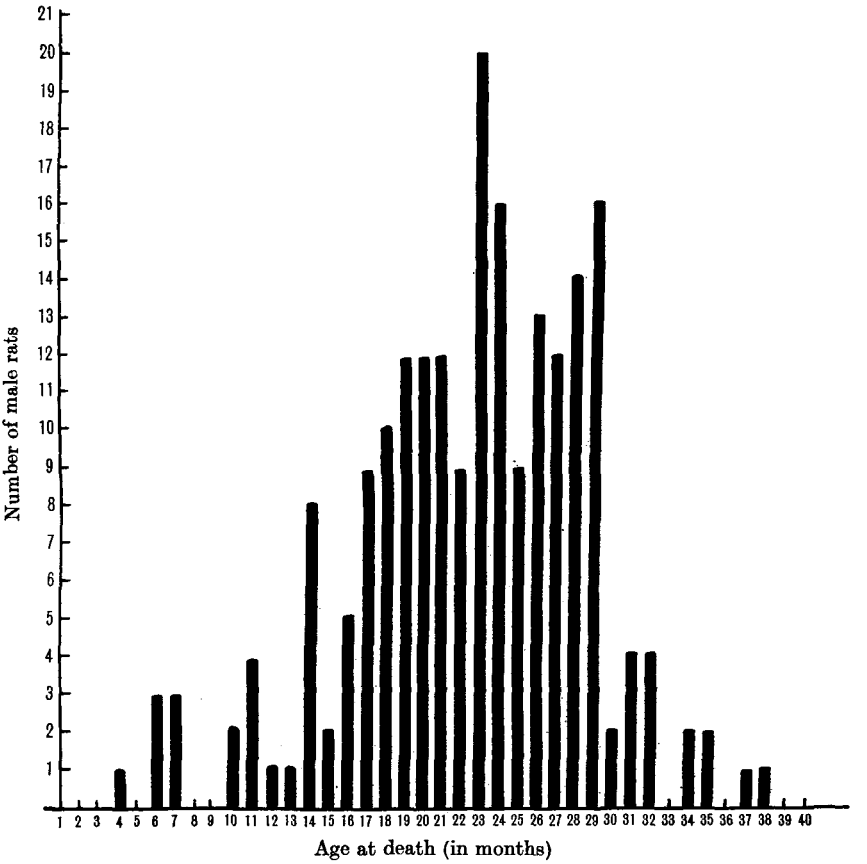

Fig. 4. Distribution of age at death of male rats on a deficient diet.

The effect of the deficiency is seen, however, in the smaller size of the litters born on the D diet and more strikingly still in the failure to rear young. The work of Sure (1927, 1928) has drawn attention to the greatly increased requirements of the mother for B_1 during lactation. In our experiment practically all the losses of young were due to a failure of the mother to rear them. Some that were allowed to suckle a normally nourished mother rapidly recovered and thrived. There were no signs that the breeding of the deficient animals was

impaired by vitamin E deficiency, although the diet did not contain wheat germ or any other recognized source of this factor. Sufficient may have been provided by the olive or arachis oil.'

(*f*) *Incidence of disease.*

There were no outbreaks of acute epidemics. For the first twelve months or so both the A and D animals in the two colonies U.C. and V.L. seemed in very good health. Those in the deficient group occasionally showed loss of hair or roughness of the coat, but this usually improved when for a time dried yeast was given. Later in the experiment the older animals of the D group did not respond so well to treatment and many developed patchy, staring coats and a harsh, scurfy skin. A number of rats in group A also developed a milder form of this condition. In the light of recent work, such, for example, as that of Birch *et al.* (1935), Lepkovsky *et al.* (1936) and Edgar & McCrae (1937), it seems likely that both A and D diets were, at their best, slightly deficient in one of the "antidermatitis" factors. We did not suspect this at the time.

Other diseases. No disease, other than bronchiectasis, was noted in any appreciable number of rats during their lifetime. Sporadic cases of pneumonia, of septic infections of abscesses, scabies, etc., were recorded, but no evidence that their incidence was related to the different characters of diets A and D was obtained.

(*g*) *Post-mortem records.*

In all cases a report of the post-mortem examination with naked-eye findings was made as soon as possible after death. Histological preparations were made only where uncertainty was felt about the nature of any abnormality found. On the whole, the deficient group (D) showed absence of body fat, noticeably round the kidneys. There was usually a fair amount of body fat in the A group. The post-mortem records are set out below (Tables IV–XIII), being divided into groups for convenience in treatment. Malignant conditions are considered separately.

Table IV. *Respiratory system*

	U.C. colony				V.L. colony			
	A		D		A		D	
	No.	%	No.	%	No.	%	No.	%
Lobar pneumonia	9	10·9	6	11·3	18	8·2	13	7·3
Bronchopneumonia	5		6		11		20	
Bronchiectasis	88	69	64	60	148	42	168	37
Emphysema	10	7·9	12	11·3	19	5·3	29	6·5
Oedema of lungs	7	5·4	4	3·9	13	3·7	6	1·4
Pleurisy with adhesions	—	—	—	—	—	—	3	0·8

The outstanding disorder is the bronchiectasis affecting so high a proportion of the animals of both groups. It is a condition common in many rat colonies. In this experiment it was responsible for much ill-health in the older rats. In

its early stages it could usually be recognized by the peculiar wheezing, a symptom which appeared in a large proportion of the rats between the ninth and twelfth month. As usual, the disease ran a slow course, but in time it caused a large number of deaths. The appearance of the lungs at the various stages and the extraordinary extent to which the lung tissue can be replaced by caseous or muco-purulent material before death ensues have been described by Passey *et al.* (1926). The incidence of the disease in our records was as follows:

	Colony U.C.		Colony V.L.	
	Number of cases	% group	Number of cases	% group
Diet A	88	69	148	42
Diet D	64	60	168	37

The difference between the incidence in the two laboratories is probably related to the difference between the average length of life previously noted. It is perhaps significant that environmental conditions were better in the case of the V.L. colony. It is quite clear that the character of the diets did not influence the incidence or the course of the disease. Passey *et al.* (1926) record that they observed bronchiectasis in rats on different types of diet to an extent varying from 40 to 68 %. The differences are probably not significant. They noticed, however, that the incidence of associated keratinization in cases with metaplasia was very much higher when the animals had been deprived or depleted of vitamin A. Our rats had ample supplies of vitamin A (2 % cod-liver oil of a good quality mixed fresh weekly) throughout their lives, and examination of livers after death showed that considerable reserves were usually present. From these observations and from the fact that the incidence of bronchiectasis was 68 % in one of Passey's experiments in which a liberal mixed diet containing liver, fish, green vegetables and cod-liver oil was used, it seems clear that vitamin A does not play an "anti-infective" role in protecting the rat from the organism primarily responsible for this chronic malady. It is interesting to note, however, that there was little evidence of keratinization associated with the metaplasia in the lungs we examined. This may be related to the vitamin A content of our diet. Our results clearly indicate that susceptibility of the laboratory rat to this common disease is not increased by mild vitamin B deficiency.

The bronchiectasis was in some cases associated with emphysema and in fewer cases with congestive heart failure.

The figures in Table V show no appreciable difference in the incidence of circulatory disturbances in the two groups. There is some indication of more frequent enlargement and dilatation of the heart on the deficient diet. It is doubtful whether the differences between the figures are significant, particularly because of the personal factor in judging whether enlargement is present. The heart is known to be affected in this manner when the deficiency is more severe.

Table V. *Circulatory system*

	U.C. colony				V.L. colony			
	A		D		A		D	
	No.	%	No.	%	No.	%	No.	%
Enlarged or dilated heart	9	7·0	19	18	50	14·1	64	14·1
Congestive failure	Not noted				10	2·8	26	5·7
Brown atrophy of heart muscle	4	3·1	3	2·9	17	4·8	17	3·7
Pericarditis	1	0·8	1	1·0	5	1·4	2	0·4
Cerebral haemorrhage	—	—	—	—	—	—	2	0·4
Infarcts	—	—	4	3·9	2	0·6	12	2·6
Mesenteric thrombosis	Not noted				5	1·4	6	1·3

Table VI. *Digestive system*

	U.C. colony				V.L. colony			
	A		D		A		D	
	No.	%	No.	%	No.	%	No.	%
Gastric distension	4	3·1	12	11·3	5	1·4	48	10·6
Gastric erosions	6	4·6	20	18·8	21	5·9	68	15·0
Chronic gastric ulcers	9	7·0	33	31·1	28	7·8	47	10·3
Hyperkeratosis	9	7·0	6	5·6	17	4·8	19	4·2
Hairballs	6	4·6	13	12·2	2	0·6	31	6·8
Intestinal distension	6	4·6	16	15·0	16	4·5	36	7·8
Intestinal ulcers	2	1·5	10	9·4	2	0·6	7	1·5
Caecal ulcers	2	1·5	28	26·4	6	1·7	14	3·1
Volvulus	Not noted				3	0·8	1	0·2
Congestion of intestinal wall	—	—	7	6·6	—	—	7	1·5
Intestinal obstruction	—	—	—	—	1	—	1	0·2
Perforated gastric ulcer	—	—	4	3·9	1	0·6	3	1·1
Perforated caecal ulcer	1	0·8	3	2·9	1		1	
Perforated duodenal ulcer	—	—	—	—	—		1	
Peritoneal adhesions	—	—	3	—	—	—	8	1·8
Liver: Fibrosis	1	0·8	4	3·9	—	—	5	1·1
Fatty change	3	2·3	3	2·9	13	3·6	9	2·0
Atrophy	1	0·8	—	—	2	0·6	1	0·2
Congestion	2	1·5	6	5·6	11	3·1	10	2·2
Enlargement (apart from cardiac failure)	2	1·8	—	—	10	2·8	—	—
Necrosis or abscesses	4	3·1	2	1·9	3	0·9	3	0·7
Non-malignant worm-cysts	18	13·8	27	25·4	49	13·7	57	12·5

The strikingly high incidence of gastric distension, erosions and related pathological conditions in the intestinal tract of animals suffering from chronic mild vitamin B deficiency is in line with the findings of a number of other workers. Chatterjee (1935) found that the movements of the excised duodenum of B-deficient rats and pigeons and its response to drugs were less in intensity and fewer in number than those of animals fed on complete natural diets or on full synthetic diets. Such atony is probably associated with the distension sometimes observed. Sparks & Collins (1935) found marked increase in the volume of the colon of adult rats maintained on a vitamin B-deficient diet for 2 months. Elsom (1934) found that loss of gastro-intestinal tone and mobility was one of the outstanding changes in a patient who received daily for 5 months a constant quantity of an experimental diet adequate except for moderate limitation of vitamin B_1. Cowgill *et al.*, as long ago as 1926,

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showed in dogs with permanent gastric fistulae that mild B deficiency produced either diminished gastric tone or absence of rhythmic contractions. Rapid improvement followed administration of vitamin B.

There have been some divergent findings in connexion with the incidence of gastric lesions in vitamin B_1 deficiency. Findlay (1928) found no gastric ulcers in young rats which had been completely deprived of vitamin B_1 for an average period of 32 days. This is more or less the duration of life to be expected in young rats fed on a diet completely lacking vitamin B_1, but it is probably too short a period for the appearance of gastric lesions.

Keith Simpson (1936), using rats 24 weeks old, claims to have kept them alive on a B_1-free diet for 180 days. The length of this survival seems to indicate that the diet was not as deficient as he believed. He found no dilatation, no haemorrhages, erosions or scars, but recorded microscopic congestion in the deeper layers of the gastric mucosa. He regards the changes in the stomach of the albino rat following complete deprivation of vitamin B_1 as "of little importance in the genesis of gastric ulcer", and criticizes other work on the grounds that where ulcers have been found, traces of vitamin B_1 have always been given. Our experiment was deliberately planned to allow rats to subsist for long periods so that the full effect of subnormal intake of the vitamin could be seen. In our experience rats *totally* deprived of vitamin B_1 die with or without convulsions in about 2 months and gastric lesions may or may not be found. It is the rat which survives for a long period on a restricted vitamin B intake which tends in middle life to develop erosions or ulcers. Dalldorf & Kellog (1932) observed ulcers in 73 % of their rats:

No. of rats	Diet	Findings
29	Little or no B_1	21 had one or more gastric ulcers
20	Complete diet or larger amounts of B_1	No ulcers
9	40 days' depletion followed by small doses of B_1 for growth test	7 gastric lesions

Average duration of deficiency when acute lesions were found ... 53 days.
Average duration of deficiency when chronic indurated ulcers were found ... 114 days.

Schiödt (1935), using 105 rats at a month old and an experimental period of 22 weeks during which he gave supplementary B_1 in the form of 0·5 g. of autolysed yeast weekly, or as Peter's concentrate, found haemorrhagic erosions of the gastric mucosa in 68 % and ulcers in 9·5 % of his animals.

The erosions are usually multiple, lying along the ridges of the glandular mucous membrane, but isolated ones or small groups may be found. The damage seldom extends deeper than the mucous layer. There is a close resemblance between the erosions found by us and those figured by Dalldorf & Kellogg and Schiödt. Definite ulcers were found in a considerable number of our animals on the D diet. They varied in size, the largest being as big as 0·25 in. in diameter and sometimes extended into the muscular layers. The base was often fibrous. Perforation occurred in several cases, but a carcinomatous change was only once detected.

The occurrence of hairballs in the stomach of the B-deficient animals was striking. Often several hard masses of hair, 0·25 in. in diameter, would be found in one stomach, usually associated with erosions of the mucosa. The accumulation of hair is due primarily, we think, to lowered tone of the stomach musculature. It must also be remembered that the undernourished rats lost hair from their coats more readily than the better fed controls.

Clemmenson (1933) arrested the growth of a group of young rats for more than 18 months by feeding them on a diet deficient in vitamin B, supplemented with regulated doses of dry yeast. At post-mortem examination a large proportion of these animals showed stomach disorders, erosions, ulcers, hairballs, etc., similar to those we observed.

The occurrence of hyperkeratosis in the pro-stomach leading to the appearance of small crater-like formations was noted in many of the animals: occasionally a true ulcer was found at the base. Its incidence was much the same in both groups, so vitamin B deficiency is probably of no significance in its causation. This interesting condition has been observed by several investigators. Some have found it associated with A deficiency (Fujimaki, 1926; Moll *et al.* 1933; Fridericia, 1934). It is doubtful whether it is caused by this dietary defect. The investigations of Aron & van der Rijst (1936) are against such a conclusion, whilst we think our own experiments clearly indicate that the condition may be found in animals obtaining plenty of A and possessing what are presumably adequate reserves in the liver.

The same question arises in respect to the caecal ulceration found in so many of our animals. It is, in our experience, rarely encountered in an adequately nourished animal, but in this experiment one colony (U.C.) showed rather a high incidence in the B-deficient group; in both colonies it was higher in this group than in the controls. Such ulceration is, however, found in animals fed on defective diets supplying what must be adequate B_1; Richards & Simpson (1934) reported a number of cases in animals deprived of A. It is uncertain, therefore, to what extent the condition can be regarded as primarily due to B deficiency. There is little of note in the records of liver abnormalities.

Table VII. *Urinary tract*

	U.C. colony				V.L. colony			
	A		D		A		D	
	No.	%	No.	%	No.	%	No.	%
Haemorrhage	6	4·6	4	3·9	1	0·3	3	0·7
Fibrosis	1	0·8	10	9·4	7	2·0	12	2·6
Cysts	—	—	—	—	—	—	2	0·4
Single abscess	1	0·8	2	1·9	5	1·4	6	1·3
Ascending infection	—	—	—	—	1	0·3	3	0·7
Fatty change	—	—	2	1·9	15	4·2	18	4·0
Cystitis	—	—	—	—	5	1·4	—	—
Early nephritis	17	13·1	16	15·0	—	—	—	—

There is nothing noteworthy in these figures from the standpoint of influence of vitamin-B factors on the tissues.

https://doi.org/10.1017/S0022172400011220 Published online by Cambridge University Press

Table VIII. *Reproductive system*

	U.C. colony				V.L. colony			
	A		D		A		D	
	No.	%	No.	%	No.	%	No.	%
Ovarian cysts	—	—	3	2·9	1	0·3	9	2·0
Ovarian abscess	2	1·9	3	2·9	4	1·2	7	1·5
Pyometra	3	2·3	8	7·5	6	1·7	12	2·6
Moles	—	—	—	—	2	0·6	3	0·7
Ante-partum haemorrhage	—	—	3	2·9	—	—	2	0·4
Procidentia	—	—	—	—	—	—	1	0·2

These are also of no significance.

Table IX. *Endocrine glands*

	U.C. colony				V.L. colony			
	A		D		A		D	
	No.	%	No.	%	No.	%	No.	%
Adrenal enlargement	5	3·9	20	18·8	21	5·9	64	14·1
Pituitary enlargement	Not noted				25	7·0	39	8·6

The figures in Table IX are interesting. Enlargement of the adrenal glands has been frequently noted in B deficiency and it needs no comment. The hypertrophy of the pituitary gland is of interest and is the subject of investigations now in progress.

It will be noticed that one colony (V.L.) suffered much less severely from caecal ulcers than did the other. This is not the only indication that the B-deficiency was in general more severe in the U.C. colony. It will be seen from the table on p. 361 that the duration of life was considerably greater in the V.L. colony and there was also considerably more baldness and skin complaints among the U.C. rats. The reason for the difference in the severity of the deficiency between the two colonies can be found in the fact that it was left to those looking after the rats to decide when it was desirable to administer a little yeast to the deficient group in order to prevent a decline in weight. It seems that this step was more frequently taken in the V.L. Laboratory than at U.C.

Table X. *Skin*

	U.C. colony				V.L. colony			
	A		D		A		D	
	No.	%	No.	%	No.	%	No.	%
Dermatitis	8	6·0	12	11·3	15	4·2	25	5·5
Baldness	12	9·3	21	20·0	4	1·2	9	2·0
Ulcerated	9	7·0	10	9·4	30	8·4	21	4·6

As we have remarked earlier, the dermatitis and loss of hair in some of the oldest rats may have been due to a slight deficiency of the "antidermatitis" factors recently differentiated from other components of the B complex.

Table XI. *Eye*

	U.C. colony				V.L. colony			
	A		D		A		D	
	No.	%	No.	%	No.	%	No.	%
Xerophthalmia	—	—	—	—	—	—	—	—
Conjunctivitis	—	—	6	5·6	—	—	2	—
Cataract	—	—	2	1·9	3	—	4	—

The absence of xerophthalmia and the rarity of conjunctivitis provide confirmatory evidence that there was no shortage of vitamin A during the course of the experiment. The few cases of cataract might be taken as indicating a slight insufficiency of lactoflavin in both A and D diets (Day *et al.*, 1931, Day & Langston, 1934), were it not for the fact that Bourne & Pyke (1935) have found that the strain of rat used in our two laboratories does not develop this condition when subjected to long continued deprivation of the B_2 complex.

Table XII. *Septic conditions*

	U.C. colony				V.L. colony			
	A		D		A		D	
	No.	%	No.	%	No.	%	No.	%
Pyaemia	7	5·4	7	6·6	25	7·0	24	5·3
Abscesses:								
Ear	5	3·9	6	5·6	12	3·4	32	7·0
Cerebral or subdural	—	—	—	—	13	3·6	26	5·7
Renal	1	0·8	4	3·9	5	1·4	2	0·4
Ovarian	2	1·5	2	1·9	4	1·2	7	1·5
Glandular	7	5·4	7	6·6	29	8·1	24	5·3
Bladder	—	—	—	—	—	—	3	0·7
Mediastinum	—	—	—	—	1	0·3	—	—
Cardiac muscle	—	—	—	—	3	0·9	5	1·1
Liver	—	—	1	1	—	—	1	0·2
Enlarged spleen:								
Associated with septic states	8	6·0	4	3·9	13	5·6	16	7·5
Other	4	3·0	4	3·9	7		18	

The incidence of septic conditions is, on the whole, low. It is quite negligible by comparison with the figures recorded by those who have observed animals suffering from A deficiency. Once again there is evidence that our rats were sufficiently supplied in that respect.

One of the most important questions we had in mind when the investigation was begun was that concerning the possible significance of vitamin-B deficiency in the genesis of cancer. We expected to find that the rats fed on diets defective in this respect would develop a variety of chronic lesions,

Table XIII. *Neoplasms*

	U.C. colony		V.L. colony	
	A	D	A	D
	(*a*) Primary malignant			
Lung	1	1	4	7
Adrenal	1	1	—	—
Liver	2	4	13	11
Uterus	1	1	2	—
Mediastinum	—	—	1	1
Muscle	—	—	2	1
Kidney	—	—	1	1
Stomach	—	—	—	4
Caecum	—	1	4	3
Thyroid	—	—	1	—
Total	5	8	28	28
	(*b*) Nonmalignant			
Fibroadenoma	4	3	10	8
Papilloma (uterus)	—	—	1	—
Hepatoma	—	—	—	2
Serous liver cyst	—	—	—	2
Papilloma (bladder)	—	1	—	1
Ovarian cyst	—	—	1	9
Implantation dermoid	—	—	1	—
Total	4	4	13	22

particularly of the gastro-intestinal tract. We wished to ascertain whether this would increase the incidence of malignancy, normally very low in the laboratory rat. Moreover, knowing our stock to be infected with *Cysticercus fasciolaris*, which is responsible for formation of liver cysts which sometimes give rise to sarcomata, we wanted to find out whether a deficiency of vitamin B in the diet would increase susceptibility to infection or the tendency to become malignant. It appeared to us that an experiment of this type might throw some light on the occurrence of cancerous changes in sites of chronic inflammation in man. Clearly, however, our results are negative. There is no difference which can be regarded as significant. Chronic inflammation associated with ulceration of the caecum and extensive fibrosis was more common in the animals on the deficient diet than in the control group, particularly in the U.C. colony. Nevertheless, the incidence of malignancy was equally low in both groups. Worm cysts were found in approximately the same proportion of animals in each group but cancerous changes arising therefrom were not more numerous in the "deficient" rats.

The incidence of liver tumours arising from cysts in our animals appears to be about the same as that found elsewhere. Bullock & Curtis (1930) found some 2400 cases in 10 years' observation of a rat colony which for at least 4 years had contained from 7000 to 10,000 animals.

There is a large and rather confusing literature dealing with the influence of vitamins on development and growth of tumours in laboratory animals. In view of the many conflicting statements it contains regarding the effect of vitamin B_1 on tumours little would be gained by reviewing it now, more

https://doi.org/10.1017/S0022172400011220 Published online by Cambridge University Press

particularly as a large proportion of the work can be criticized either because small numbers of animals or those artificially implanted with tumour grafts were employed. We regard our experiment as an entirely natural one and beyond criticism in respect to numbers. The results do not provide any support for the view that chronic mild vitamin B_1 deficiency raises the incidence or increases the malignancy of cancer in our strains of laboratory rats.

Summary

1. A comparative study has been made of the life history of two groups of rats, one of which (483 animals) was maintained on an adequate diet whilst the other (556 animals) was given a ration providing a suboptimal intake of "vitamin B". The chief defect of the second diet was probably in respect to vitamin B_1.

2. The duration of life was shortened and reproduction was adversely affected by the deficiency.

3. The examination of the post-mortem records provides clear proof of a significantly greater incidence of gastro-intestinal lesions, particularly ulcerations, in the animals on the deficient diet than in the control group.

4. The deficiency of B_1 did not influence the incidence or the severity of the chronic lung infection (bronchiectasis) common in laboratory rats.

5. The incidence of all other disorders was about the same in the two groups.

6. No evidence of increased susceptibility to tumours, malignant or innocent, was shown by the animals on the deficient diet. Cancerous developments arising at the site of chronic inflammatory processes (e.g. worm cysts in the liver, caecal ulcers of long standing, etc.) were not appreciably more numerous in one group than in the other.

Acknowledgements. We would like to pay tribute to the late Dr M. J. Rowlands with whom we were associated during a considerable part of this work. He helped to plan the investigation and followed and assisted its progress with enthusiasm until his untimely death in 1932.

The whole cost of this work was defrayed by a generous grant made by Messrs Vitamins, Ltd., to whom we are deeply grateful.

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(*MS. received for publication* 6. XI. 37.—Ed.)