CLVI. THE PHYSIOLOGICAL RÔLE OF VITAMIN B. PART I.

THE RELATION OF VITAMIN B TO TISSUE OXIDATIONS.

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INTRODUCTION.

IN spite of a considerable amount of investigation and the accumulation of an extensive literature, the problem of the part played in the economy of the organism by the dietary principle known as vitamin B remains unsolved. Nevertheless, there has been no dearth of expressed opinions as to the rôle of this factor or regarding the cause of the nutritive failure which follows its withdrawal from the dietary. Indeed, the very multiplicity of theories and the wide field over which they range seems strong evidence that the actual facts have not been disclosed.

Reviewing the many hypotheses that have been advanced, and searching for some outstanding feature, one is impressed by the frequency with which investigators have expressed a belief that vitamin B is concerned in maintaining the normal level of activity of the oxidising mechanisms of the body, although there exists considerable diversity of opinion regarding the actual cause of the lowered oxidation.

In one direction there is a fairly general agreement that the animal deprived of vitamin B shows a markedly reduced oxygen utilisation [Raomino, 1916; Abderhalden, 1920, 1921, 1, 2; Anderson and Kulp, 1922; Caridroit, 1922; Mattill, 1923, 1, 2; Gulick, 1922, 1924; Japanese references cited by Shinoda, 1924, 1, 2]. Apart from a more or less general agreement on this point, there are not only divergent opinions regarding the significance of certain changes in the R.Q. that have been noted during the course of the condition induced by the deficiency, but also various suggestions as to the cause of the alleged lowered consumption of oxygen. The majority of investigators seem to have been inclined to attribute it, wholly or in part, to an actual reduction in the amount or the activity of the oxidising enzymes.

Amongst such suggestions is that advanced by Dutcher [1918], based on his estimations of catalase in normal and "beri-beri" tissues, that the vitamin stimulates the production of this enzyme. A somewhat similar view is taken by

Findlay [1921, 1] concerning a relationship between vitamin B and glyoxalase. The broader view is held by Hess [1921, 1922] who, insisting on the essential similarity between "beri-beri" induced by a diet of polished rice in pigeons and the condition following the administration of sub-lethal doses of cyanides, regards the nutritive failure in the former state as being due to a reduction in the amount or efficiency of the oxidising enzymes. From these views we may, bearing in mind Warburg's theories regarding the rôle of iron in biological oxidations, pass naturally to such suggestions as that one form of beri-beri in animals may be due to a deficiency of iron [McGowan and Crichton, 1924]. Numerous other theories might be quoted, many of which, such as the view that the animal suffering from a deprivation of vitamin B cannot utilise carbohydrates (see later references), could be loosely woven in with the theories of decreased tissue oxidation until an attractive hypothesis regarding the rôle of vitamin B had been built up.

However, the literature contained so many contradictions and discrepancies and a critical analysis revealed that so much of the experimental work recorded seemed unsatisfactory, that we were led to commence the investigation on which we are now reporting. Perhaps it should be remarked at this point that at the outset of the investigation we accepted as the foundation of our plans the one statement upon which there seemed to be a fair amount of agreement, namely, that deprivation of vitamin B leads to a reduction of the oxygen utilisation of the whole animal. In view of this fact, it seems the wiser plan to give an account of our experiments in the order in which they were carried out, rather than in what would seem the more logical sequence.

ANAEROBIC REDUCTION OF METHYLENE BLUE BY NORMAL AND "BERI-BERI" TISSUES.

The success which has followed the extensive use of methylene blue as a hydrogen acceptor to serve as an indicator in studies of anaerobic oxidation-reduction processes prompted us to compare the rates of reduction of this dyestuff by normal tissues and those from "beri-beri" animals under the conditions outlined by Thunberg [1920].

By employing the somewhat similar technique described by Lipschitz [1921], in which *m*-dinitrobenzene is used as the indicator, Hess [1921, 1922, 1923] observed that the rate of reduction was markedly reduced in tissues from "beri-beri" pigeons.

These results were confirmed by Hess and Messerle [1922] and by the work of Rohr [1923], who traced a parallel between the power of tissues to reduce this dyestuff and their antineuritic potency.

In our experiments a large number of observations were made on the time of reduction of methylene blue by the tissues before and after washing free from water-soluble extractable substances, and by washed tissue preparations in the presence of an artificial "metabolite" (succinate), the details of the Thunberg technique being closely followed in all respects.

The following results are typical of those obtained with tissues removed immediately after killing from a normal pigeon and one exhibiting typical symptoms of avian polyneuritis.

Table I. *Times of decoloration of methylene blue by pigeon breast muscle.*

Buffer p_H 7.5 (phosphate). Methylene blue 1 in 5000. Temp. 35°. Weight of tissue 0.2 g. K succinate $M/10$.				
	Buffer	K succinate	Methylene	Time
	cc.	$M/10$ cc.	blue cc.	
Unwashed tissue				
Normal	2.0	—	1.0	12.0)
	2.0	—	1.0	11.5) 12.2 mins.
	2.0	—	1.0	13.0)
"Beri-beri"	2.0	—	1.0	16.0)
	2.0	—	1.0	14.5) 14.3 mins.
	2.0	—	1.0	12.5)
Washed tissue				
Normal	1.4	—	0.2)	Over 2 hours
	1.4	—	0.2)	
	1.4	—	0.2)	
	1.0	0.4	0.2	
	1.0	0.4	0.2	12.0)
	1.0	0.4	0.2	11.5) 11.5 mins.
"Beri-beri"	1.4	—	0.2)	Over 2 hours
	1.4	—	0.2)	
	1.4	—	0.2)	
	1.0	0.4	0.2	
	1.0	0.4	0.2	12.0)
	1.0	0.4	0.2	11.5) 11.75 mins.

These results are confirmed by those in Table II, which are typical of those obtained in a number of studies on the leg muscles of normal rats and others in the final stages of the nutritive disorder induced by a diet deficient in vitamin B.

Table II. *Times of decoloration of methylene blue by rat leg muscle.*

Buffer p_H 7.5 (phosphate). Methylene blue 1 in 5000. Temp. 35°. Weight of tissue 0.2 g. K succinate $M/10$.				
	Buffer	K succinate	Methylene	Time
	cc.	$M/10$ cc.	blue cc.	
Unwashed tissue				
Normal	2.0	—	1.0	17.5)
	2.0	—	1.0	14.5) 16.3 mins.
	2.0	—	1.0	17.0)
"Beri-beri"	2.0	—	1.0	15.5)
	2.0	—	1.0	16.5) 16.5 mins.
	2.0	—	1.0	17.5)
Washed tissue				
Normal	1.4	—	0.2	86.0)
	1.4	—	0.2	72.0) 80.0 mins.
	1.4	—	0.2	81.0)
	1.0	0.4	0.2	19.0)
	1.0	0.4	0.2	20.0) 19.0 mins.
	1.0	0.4	0.2	18.5)
"Beri-beri"	1.4	—	0.2	95.5)
	1.4	—	0.2	91.0) 93.0 mins.
	1.0	0.4	0.2	15.0)
	1.0	0.4	0.2	14.5) 16.0 mins.
	1.0	0.4	0.2	18.5)

In our opinion these figures conclusively demonstrate that the system responsible for the decoloration of the dyestuff is operating with a normal efficiency in the muscle tissues of pigeons and rats suffering from vitamin B-deficiency.

In spite of the apparent normality of the "beri-beri" tissues as regards their power to reduce methylene blue, it seemed of interest to examine whether the addition of small quantities of yeast extract accelerated the change, particularly in view of the emphatic claim of Abderhalden and his colleagues, to which detailed reference will be made later, that such an addition promptly and markedly increases the respiration of isolated tissue preparations, especially those from "beri-beri" animals.

As will be seen from the figures in Table III, we could trace no stimulating effect on adding yeast extract to the Thunberg tests; the slight acceleration of reduction when the yeast extract was added to the washed tissue preparation alone was, of course, to have been expected, and merely indicated the activation of certain constituents of the extract capable of yielding hydrogen to the dyestuff.

Table III. *Times of decoloration of methylene blue by pigeon breast muscle.*

Effect of yeast extract on rate of decoloration of methylene blue by K succinate and washed muscle.				
Buffer p_H 7.5 (phosphate). Methylene blue 1 in 5000. Temp. 35°.				
Weight of tissue 0.2 g. K succinate $M/10$.				
Washed tissue	Buffer cc.	Methylene blue cc.	K succinate cc.	2.5 % yeast extract cc.
	1.4	0.2	—	—
	1.4	0.2	—	—
	1.4	0.2	—	—
	1.4	0.2	—	0.2
	1.4	0.2	—	0.2
	1.0	0.2	0.4	—
	1.0	0.2	0.4	—
	1.0	0.2	0.4	—
	1.0	0.2	0.4	0.2
	1.0	0.2	0.4	0.2
	1.0	0.2	0.4	0.2

About 3 hours

32 } 31 mins.
30 }

11 } 10.3 mins.

10 }
12 }

10 } 11 mins.
11 }

Convinced from these experiments that the oxidative activity of the muscle tissue, in so far as it can be revealed by a technique of this type, is normal in animals exhibiting the typical symptoms associated with the later stages of vitamin B-deficiency, it remained to be seen whether the same would be found to be true in the case of animals poisoned with cyanide. Hess [1921] has described how by the intra-muscular injection of sub-lethal doses of cyanides it is possible to produce a condition in pigeons which he alleges closely resembles that caused by prolonged feeding on polished rice. His views have been attacked by Abderhalden [1922] who refuses to admit that there is any real relation between the two conditions on the grounds that cyanides do not depress, but may actually raise, the gaseous metabolism during the period when the symptoms are apparent, and also because yeast extracts will not prevent or cure the condition brought about by cyanides.

A number of experiments have been made by us along these lines, and on some points we can confirm the observations recorded by Hess. The administration of doses of KCN of about 0.4 mg. per 100 g. of body-weight is followed after some 4 minutes by very rapid collapse and death. Smaller doses, 0.1–0.25 mg. per 100 g. weight, give rise to staggering some 4 or 5 minutes after injection, after which the bird sits still with eyes shut, ruffled feathers, and gives occasional gasps. Some 10 minutes later there is usually recovery. With intermediate doses, that is, 0.25–0.35 mg. per 100 g. weight, these symptoms are exaggerated and followed by convulsions resembling in some respects the spasms shown by “beri-beri” pigeons. From these the birds sometimes recover.

In Table IV are given the data obtained from a study of the breast muscle of a pigeon which had received three injections of a freshly prepared solution of potassium cyanide. The bird weighed 370 g. and received a dose of 0.35 cc. of freshly prepared 0.1 % solution of KCN at 10.52 a.m. At 11.25 a.m., having recovered, it received a dose of 0.6 cc. and at 11.37 a.m. a dose of 1.0 cc. This last dose produced severe convulsions during which the animal was killed and the breast muscle from the opposite side to that into which the injections had been made dissected out for immediate use.

Table IV. *Time of decoloration of methylene blue by “cyanide”
pigeon muscle.*

Three sub-lethal doses of KCN in the left breast (totalling 1.95 mg.). Weight of bird 370 g. Muscle cut from the <i>right</i> breast. Weight of tissue 0.2 g.			
Buffer	Methylene blue	K succinate	Time
cc.	cc.	cc.	
Unwashed tissue	2.0	1.0	12.0
	2.0	1.0	10.0
	2.0	1.0	9.5
	(Normal unwashed—12.2 mins.)		
Washed tissue	1.4	0.2	Over 2 hours
	1.4	0.2	
	1.4	0.2	
	1.0	0.2	14.5
	1.0	0.2	13.5
	1.0	0.2	14.5
(Normal washed—11.5) mins.			

It is doubtful whether the rate of reduction by washed tissue in the presence of succinate is actually slower than in the case of normal muscle as the figures are, in the light of our experience, scarcely outside the range of variation that may be expected in duplicate experiments.

It is not intended to discuss at this point the possible relations between the cyanide poisoning and “beri-beri,” but merely to remark that, having satisfied ourselves that muscle tissue removed from animals almost moribund in both conditions shows normal oxidative activity, in so far as this is revealed by the power to reduce methylene blue under anaerobic conditions in the presence of normal metabolites or added succinate, it seemed desirable to examine the actual oxygen consumption of the tissues by a direct method.

OXYGEN CONSUMPTION OF NORMAL AND "BERI-BERI" TISSUES.

In this connection the literature provided two opposing claims. Abderhalden and his colleagues in a series of papers record a large number of experiments to show that in the absence of extracts supposed to contain vitamin B the respiration of both plant and animal cells is greatly reduced. Abderhalden [1920, 1921, 1, 2; Abderhalden and Wertheimer, 1921, 1, 2, 1922] observed a reduced oxygen uptake of pigeons that had been fed on polished rice, and has many times since emphasised not only this point, but also his conviction that the oxygen utilisation rapidly rises after the administration of yeast preparations. Furthermore, Abderhalden and Schmidt [1920] presented data to show that whereas the respiration of tissues removed from pigeons fed on polished rice was much below normal, it could be raised by the addition *in vitro* of extracts believed to contain vitamin B. Somewhat similar but rather unconvincing results are reported by Shinoda [1924, 2].

In complete disagreement with this claim are the results of experiments by Roche [1925] who found that isolated tissues of both "beri-beri" and starving pigeons showed an oxygen uptake of the same order as that of tissues removed from the normally fed control animals. As will be seen, our observations entirely support Roche.

For studying the oxygen uptake of isolated tissue preparations we employed a modified Barcroft-Haldane apparatus of which the respiration vessels were specially constructed with flattened bottoms so as to expose a relatively large surface of a very shallow layer of tissue suspension to the air. The potash for absorbing carbon dioxide was contained in a bulb so blown on the side of the neck of the vessel as to give a relatively large absorbing surface without risk of splashing during shaking. From a number of experiments we have selected the typical curves shown in Figs. 1, 2, 3 and 4.

Fig. 1 illustrates the identical rate of absorption of oxygen by normal and "beri-beri" breast muscle of the pigeon under the conditions of the experiment; that is, suspended in phosphate buffer of p_H 7.4, and shaken vigorously in a bath at 37°. Much the same types of curves are shown in Fig. 4, where is also given the curve illustrating the rate of oxygen uptake of the breast muscle of a pigeon poisoned with potassium cyanide; actually in this case the bird described on p. 1233. Moreover, this chart illustrates how the addition of yeast extract failed in our experiments to make any alteration of the slope of the curve of the "beri-beri" muscle. Fig. 3 represents the rates of oxygen uptake of liver tissue from normal and "beri-beri" pigeons, which, if allowance is made for a slight lag of the "beri-beri" liver in the early stages of the experiment, are quite similar.

The same is also true of the muscle from acutely and chronically poisoned "cyanide" pigeons in Fig. 2, for in view of other results we have obtained, such as those in Fig. 4, we are not inclined to attach any importance to the slight divergencies of these curves. At any rate, considering that the tissues compared with the normal preparations were removed from animals showing

Fig. 1.

Breast muscle.

 p_H 7.5. T. 35°.

"Beri-beri" ————|———|

Normal ————•———•

Fig. 2.

Breast muscle.

—•—•— Normal.

- - - - - Chronic cyanide poisoning.

..... Acute cyanide poisoning.

Fig. 3.

Liver tissue

 p_H 7.5

Temp. 35°.

"Beri-Beri" ————|———|

Normal ————•———•

Fig. 4.

Breast muscle

—•—•— Normal.

- - - - - "Beri-beri."

○—○—○ Cyanide.

——* "Beri-beri" and marmite.

either severe symptoms of "beri-beri" or practically dying from cyanide poisoning, any reduction of oxidative activity which they show seems to us to be relatively insignificant, and out of all proportion to what one would expect from the striking figures presented by Abderhalden and his colleagues¹.

As will be appreciated from Figs. 1-4, our experiments led us definitely to reject the results of Abderhalden, and to conclude that the tissues themselves show no appreciable impairment of oxidative activity in vitamin B-deficiency.

How this conclusion stands with reference to much of the previous work on the subject will be left over until the discussion. Meanwhile, tracing the course our experiments followed, we shall describe how our failure to confirm Abderhalden led us to investigate the oxygen requirements of the intact animal in vitamin B-deficiency, for it seemed at this stage of our enquiry an entirely reasonable hypothesis that, whereas the tissues themselves might be in possession of a normal equipment of oxidative agents, there might well be in the whole organism, in the absence of vitamin B, some incoordination of the mechanism for controlling metabolism that would result in lowered gas exchange. To gain light on this point we made a prolonged study of the gaseous metabolism of rats in various states of nutrition.

INFLUENCE OF STATE OF NUTRITION ON OXYGEN UPTAKE OF RATS.

At an early stage we were obliged to recognise that, whereas it may be an easy matter to determine the respiratory quotients of a small animal, it is far from simple to obtain values for its oxygen uptake which are significant. The obvious source of trouble, and curiously it is one which many investigators in this field seem either to have neglected entirely or to have appreciated inadequately, is the difficulty of controlling the movements of the animal in the chamber. Preliminary studies with both normal and "beri-beri" animals gave such scattered values that, apart from a clear indication that the gas exchange was greatly depressed in the last stage of the vitamin B-deficiency, nothing of certain value could be deduced from them.

For estimating the oxygen utilisation of the rats we employed a closed circuit system in which was placed a recording spirometer. The general plan of the apparatus is shown in Fig. 5. The apparatus was, as far as possible, immersed in a bath at constant temperature. The animal having been placed in the chamber *A*, which also contains absorbents in the form of calcium chloride and soda lime beneath the platform, the air was caused to circulate by means of the pump *B*, consisting of a rubber bulb with inlet and outlet valves and compressed at the desired frequency by the eccentrically driven plunger *C*. By means of a three-way tap a known volume of oxygen could be passed by displacement with mercury into the circuit from the container *D*, and estimations of the rate of utilisation of the gas were made by noting the time taken for the writing lever of the spirometer, which rose on admission of the gas, to fall once again to the base line on the recording drum *E*.

¹ Estimations of moisture show that the results obtained by us cannot be attributed to changes in the water content of the tissues.

In spite of the fact that all rats were starved for at least 24 hours before making a record of their oxygen consumption, our earlier results showed so wide a variation that it seemed necessary to have some sort of base line to which our figures could be referred. This we obtained by a series of estimations of the oxygen consumption of rats starved for 24 hours and placed under amytal (ethyl-iso-amylbarbituric acid) anaesthesia. It is, of course, necessary to maintain the body temperature of the animal during amytal anaesthesia if normal values for the oxygen consumption are to be obtained, and in all experiments of this type, unless otherwise desired, the temperature of the bath was kept at 38°. The figures obtained on a number of rats of different weights are plotted in Fig. 6, and through them a reasonable curve may be traced. Our results confirm the opinion expressed by Deuel and Chambers [1925] that gaseous exchange experiments on animals under the influence of amytal provide trustworthy information concerning the normal body metabolism.

Fig. 5.

It is, of course, important to administer approximately the same dose of the anaesthetic to each animal. We usually employed 10 mg. per 100 g. of body-weight injected subcutaneously. This dose produced a light anaesthesia and enabled us to obtain good reproducible readings of oxygen consumption. The figures thus obtained are naturally on a slightly lower level than that of the values for what is usually regarded as the basal metabolism.

Accepting the results of the experiments with amytal, we were able to construct another curve, the upper one in Fig. 6, which was traced parallel to the "amytal" curve and traversing the area occupied by points representing the oxygen uptake of non-anaesthetised animals in all experiments where little or no movement of the rats in the chamber was observed. This seemed to us, in view of the very great difficulties with which we were faced, a justifiable means of obtaining some sort of estimate of the basal metabolism of rats of different weights; at any rate, it provided a method of which the error was probably smaller than the differences we expected to detect.

Later in this investigation the question of the effect of the environmental temperature on the intensity of the metabolism assumed some importance, and it was decided to compare the oxygen uptake of rats at 38° and 28°: the normal body temperature of the rat being about 38°. Generally speaking, we found in studying normal rats that the errors due to movement of the animals were greater than the difference between the metabolism at the two temperatures, so that the attempt to construct separate curves was abandoned.

Fig. 6.

Oxygen consumption of normal rats.

Temp. 38°

• Under amytal.

+ Unanaesthetised.

Oxygen consumption of rats deprived of vitamin B.

Groups of young rats were fed on a vitamin B-deficient diet employed in this laboratory, and composed as follows:

Extracted caseinogen	20 parts
„ rice starch	63 „
Salt mixture	5 „
Cod-liver oil	2 „
Hardened vegetable oil	10 „

After various periods on this diet they were starved for 24–36 hours and determinations of their oxygen consumption made. The results were widely varying values, from an analysis of which in the light of the curves in Fig. 6 it seemed clear that the gaseous exchange was not reduced appreciably in vitamin B-deficiency, except during a comparatively short period before death.

It seems necessary before presenting a selection of these results to draw attention to certain features of the clinical picture exhibited by rats deprived of vitamin B. The deprivation, as is well known, results in an almost immediate cessation of growth in the young animal, followed by a period during which its

weight either is maintained or slowly falls. The duration of this period is roughly proportional to the initial weight of the animal, and during this time the rat seldom shows obvious symptoms of ill health, other than loss of appetite, constipation, disinclination to exercise, ruffled fur, and a slight fall in pulse rate and body temperature. Quite suddenly the last phase of the disorder appears, body temperature shows a rapid fall, respiration becomes laboured, there may be incoordination of the hind limbs followed by convulsions and death; usually this phase lasts some 24 hours, seldom more than 48.

It is particularly the body temperature of these animals to which we wish to draw attention, because the fall that has been so often observed has been regarded by some authorities as characteristic of vitamin B-deficiency. To this question we will return again later, but its immediate interest is in connection with our observation that only during the last stage of the deprivation, when the body temperature of the animals was falling rapidly, were low values obtained for oxygen consumption markedly outside the range of variation of normal figures.

This is seen from the figures given in Table V, in which are recorded oxygen consumptions of rats fed on vitamin B-deficient diet for different periods and measured at an environmental temperature of 28° after 24 hours' starvation.

Table V. *Oxygen consumption of rats on vitamin B-deficient diet.*

Weight g.	Days on diet	Rectal temperature ° C.	Oxygen uptake	Approximate normal value (± 20)
			cc. per 100 g. per hour	
49	35	30.5	120	285
50	21	35.0	166	285
50	—	31.0	222	285
54	35	31.5	185	280
56	25	31.0	133	280
65	7	35.8	293	278
75	35	33.9	200	260
77	35	33.9	260	260
80	21	35.8	251	260
132	35	32.2	151	220
147	35	34.0	291	210
170	21	36.1	166	190
174	21	36.0	156	190
201	14	35.5	143	180
203	6	36.6	163	117

Generally speaking, it was observed that definitely low oxygen consumptions were recorded when the body temperature of the rats had fallen below 33°, and it remained for the significance of this fact to be considered.

The fall of body temperature in a warm-blooded animal might arise from one or more of several causes. On the one hand, it might be due to a breakdown of some part of the nervous or glandular apparatus for temperature regulation, whilst, on the other hand, it might follow a marked reduction in the intensity of the oxidations in the tissues. We have seen that the results of the experiments on the oxygen uptake of tissue suspensions are not in favour of the view

that the mechanisms for carrying out cellular oxidations are impaired in vitamin B-deficiency.

An attempt to throw additional light on this question was made by investigating the influence on metabolism of raising the environmental temperature of animals showing a reduced body temperature and oxygen uptake in the last stages of the condition induced by deficiency of vitamin B. In most cases after warming the animal up to its normal body temperature by placing it in a warm chamber for 2 or more hours, the oxygen consumption rose to a figure approaching the normal level; coincidentally there was very marked but only temporary, improvement in the general condition of the animal.

Table VI.

Weight g.	Rectal tem- perature at beginning of experiment ° C.	Temperature after warm- ing up ° C.	Oxygen uptake in cc. per 100 g. weight per hour		
			At 28°	At 38°	Normal ± 20
49	30.5	37.5	120	235	285
54	31.5	37.3	185	262	280
56	31.0	37.3	133	233	280

These results seem definitely to confirm those of the experiments on the oxygen utilisation of the isolated tissues in showing that mechanisms for cellular oxidations are unimpaired by vitamin B-deficiency. They are also obviously related to the interesting facts recorded by Peters [1924, 1925] that the well-known symptoms of vitamin B-deficiency in pigeons occur earlier in birds exposed to cold than in those kept in a warm room, and also that temporary cures of pigeons showing signs of imminent convulsions may be effected by bringing them into warmer surroundings. It occurred to us that by maintaining rats fed on vitamin B-deficient diets in an environment of a temperature approximating to their normal body temperature the final decline might be postponed. A few experiments, admittedly inadequate, seemed to indicate that such was not the case.

While considering how best to investigate the cause of the fall of body temperature in the vitamin B-deficient animals, we were more and more impressed with the necessity for controlling the influence on the organism of the greatly diminished food consumption which is so characteristic a consequence of deprivation of vitamin B, but before describing the results obtained in that part of the investigation, we may briefly refer in passing to a few tests which were made in order to determine whether the atrophy of the thyroid which numerous authorities have noted in vitamin B-starved animals [Funk and Douglas, 1914; McCarrison, 1919, 1, 2; Findlay, 1921, 2; and others] might in any manner be connected with the fall of body temperature. Dutcher [1920] thought that some relief of symptoms in pigeons followed administration of thyroid gland or thyroxine. The tests on thyroid administration were not carried very far, as the investigation passed to the more important path we have just mentioned, but confirmation was obtained of the observations pre-

viously reported by Drummond [1918], that oral administration of desiccated thyroid gland in daily doses of about 100 mg. tended, if anything, to hasten the appearance of the fatal decline. No improvement of symptoms or rise of body temperature was noted in the animals treated with the gland preparation.

RELATION OF VITAMIN B-DEFICIENCY TO FOOD CONSUMPTION.

Investigators in this field seem almost unanimous that deprivation of vitamin B leads quickly to a reduced food intake, which, as the condition progresses, may gradually fall until, in the later stages, the animals are to all intents and purposes starving. The curves in Fig. 7 show typical records of weight, body temperature and food intake of young rats fed on the vitamin B-deficient ration. When their temperature falls the animals are virtually starving, with Calorie intakes as low as 3-4 per 100 g. as compared with about

Fig. 7.

Rat 8623 ♂. Fed on vitamin B-deficient diet.

Rectal temp. ······
 Food intake ; ····· ;
 Body wt. —————

30 Calories per 100 g. required for maintenance at this age. Obviously, therefore, no decision whether the fall in body temperature and metabolism in the last phase of the disorder is due specifically to the absence of vitamin B or not could be reached until the behaviour of animals on restricted food intake or deprived of food had been studied.

In a previous communication [Drummond, 1918] a study of the food consumption of rats on normal diets and on others deficient in vitamin B was reported, in which it was shown that soon after the administration of a diet deficient in vitamin B the food consumption fell to a level approximately that required to supply the calorific requirements for maintenance.

On this basis a series of rats were fed daily on a complete artificial diet

(that described on p. 1238), plus 5 % yeast extract, in amounts of the order that it was estimated would have been voluntarily consumed had the animals been given an ample supply of the vitamin B-deficient ration. Control groups provided with an unlimited supply of the complete diet and the deficient ration were also observed. The general result of these tests was that the groups fed on the restricted ration of the complete dietary (approximately 1 g. daily per 10 g. body-weight) maintained both body-weight and temperature, whilst occasional estimations of oxygen consumption yielded values within the normal range. Typical charts illustrating these results are shown in Fig. 8.

Fig. 8.

Rectal temp.
 Body weight of rats on complete diet but receiving a restricted quantity.
 ↑ Rats starved. ↓ Rats fed.
 Oxygen consumptions in cc. per 100 g. per hr.; approximate normal value below.

In the smaller rats a decline in weight often occurred rather suddenly, and was accompanied by a rapid fall of temperature and followed by death.

The type of record obtained from the rats on the vitamin B-deficient diet is illustrated by the three pairs of curves in Fig. 9, each of which represents an animal of approximately the same initial weight as that of the corresponding animal in Fig. 8.

A general similarity of the records is immediately apparent on inspection, and it remains to study the response of animals to subsistence on water alone.

Typical charts of body-weights and temperatures of rats during inanition

are presented in Fig. 10, from which it can be seen that the latter seldom falls to any appreciable extent until shortly before death.

The fall of body temperature that occurs just before death as a result of deprivation of food is, as would be expected, accompanied by a fall in oxygen consumption, which, as in the case of the animals deprived of vitamin B, only becomes marked when the body temperature has dropped to about 33° or lower.

Fig. 9.

Rectal Temp. -----
 Body weight ----- of rats on B-deficient diet.
 ↑ Rats starved. ↓ Rats given food again.
 Oxygen consumptions in cc. per 100 g. per hr.; approximate normal value below.

Table VII. *Oxygen consumption of rats during inanition determined at 28°.*

Weight g.	Day of experiment	Rectal temperature ° C.	Approximate normal value (± 20)	
			Oxygen consumption cc. per 100 g. per hour	
62	3	31.0	142	280
64	4	30.0	90	280
64	6	30.5	84	280
75	4	35.0	363	260
73	4	35.2	274	260
70	5	36.4	238	265
92	6	38.3	271	250
113	7	30.0	76	240
116	8	37.5	323	240
119	8	34.4	187	235
125	7	28.5	40	225
130	8	34.4	185	225

By warming up the animals showing lowered metabolism, even when almost moribund, it was possible, as in the cases of vitamin B-deficiency described above, to increase their oxygen consumption to values approaching normal, and to effect a similar marked improvement of muscular tone and considerable increase in movement, but the improvement was only of short duration.

Fig. 10.

Rectal Temp.
 Body weight
 Rats during starvation.
 ↑ Rats starved. ↓ Rats fed.

Table VIII. *Effect of environmental temperature on oxygen consumption of rats in last stage of inanition.*

Weight g.	Days without food	Rectal temperature ° C.	O-con- sumption at 28°	Rectal tem- perature after warming in bath at 38° ° C.	O-con- sumption at 38°	Normal value
64	4	30.0	90	37.5	200	280
64	6	30.5	84	37.3	280	280
125	7	28.5	40	37.5	137	225
113	7	30.0	76	37.8	131	240

RELATION OF VITAMIN B-DEFICIENCY TO INANITION.

As several workers have pointed out [*e.g.* Walshe, 1918], total deprivation of food is actually a form of vitamin B-deficiency. Accordingly, a number of parallel experiments to those just described were carried out in which animals of similar weight were given daily from 0.5–1 g. of yeast extract as their sole

food together with ample supplies of water. The amount of utilisable food, from the standpoint of calories, supplied in this yeast extract was negligible.

In no essential did the behaviour of the animals in this group differ from those maintained on water alone (see Fig. 10). The provision of ample supplies of vitamin B neither prevented the final fall of body temperature, blood-sugar (see p. 1246) and metabolism nor prolonged life.

To our minds it seemed clear from these results that the lowered metabolism and body temperature observable during the last phase of the condition resulting from deficiency of vitamin B were merely indications that the animal was dying and that the immediate cause was general starvation. Before investigating the part played by the vitamin in inducing this state of affairs one important point remained to be examined. A number of investigators [cf. Funk and Schönborn, 1914; Shinoda, 1924, 1, 2; and others] have reported that vitamin B-deficiency brings about a condition of hyperglycaemia, and that, in pigeons at any rate, the blood sugar is high at the time that the characteristic convulsions appear. Unless the tissues had wholly or partly lost their power to oxidise blood-sugar, it was difficult to reconcile these claims with our own observations on the lowering of body temperature and metabolism. It was obviously necessary to make a study of the concentration of sugar in the blood of rats under the conditions of our experiments.

Blood-sugar level of rats on vitamin B-deficient diets.

Eggleton and Gross [1925] have described series of estimations of sugar in the blood of rats fed on the standard ration deficient in vitamin B used in this laboratory. They give the normal value of the blood-sugar level 24 hours after food as 0.09 % and the average after 40 days' subsistence on the deficient ration as 0.115 %. Later, in the final stage of the disorder, the concentration fell to the starvation level or lower. On these grounds they were, however, not convinced of the existence of a hyperglycaemia such as Funk and Schönborn had noted in pigeons.

In our experiments the estimations were made by the trustworthy method of Hagedorn and Jensen. A large series of determinations on normal rats-kept without food for at least 6 hours gave the average value 0.082, the extreme values being 0.073 and 0.091.

The analysis of the blood of a number of rats deprived of vitamin B gave results which make clear two points. In the first place, there appears to be a definite hyperglycaemia in the rat during the greater part of the period it is kept on a ration deficient in vitamin B. Secondly, there is a very marked fall in the blood-sugar level during the final stage of decline when the body temperature and metabolism are also reduced. The following are typical results.

For purposes of comparison, estimations were made of the blood-sugar level in starving animals. Admittedly these are too few to be of real value, but, as far as they go, they are most suggestive in that they indicate a definite hyperglycaemia in what may be termed the middle phase of starvation followed

Table IX.

Weight g.	Rectal temperature °C.	Days on B-deficient diet	% sugar in blood
58	34.0	29	0.105
58	34.2	31	0.083
45	34.4	31	0.115
57	34.1	32	0.116
47	34.5	56	0.109
65	34.3	41	0.154
70	33.9	41	0.140
44	33.5	22	0.078
73	35.5	45	0.156
62	35.0	29	0.146
Average for animals with body temperature above 33°			0.120
62	27.0	29	0.035
57	25.0	28	0.048
48	26.5	31	0.025
56	31.0	52	0.042
52	27.0	38	0.029
67	29.4	45	0.061
48	28.0	28	0.033
Average for animals with body temperature below 33°			0.039

by a rapid fall to very low values during the period when the body temperature drops. Morgulis and Edwards [1924] noted in starving dogs that the blood-sugar after a preliminary fall rose beyond normal values. The average values for starving rats of about 80 g. weight on the fourth day of the fast when their body temperatures were about 35° was found to be 0.125 %, whilst in the final period of decline with a body temperature of about 25° the blood-sugar level had fallen to an average of 0.023 %. Only a few estimations were made on the blood of rats whose body temperature had fallen below 30° as a result of deficiency of vitamin B, but had been restored to normal level by warming up in a chamber. The records of these experiments are given below.

Weight g.	Initial temperature °C.	Final temperature °C.	% blood- sugar
46	28.5	37.5	0.034
130	26.0	37.3	0.042
60	30.4	37.5	0.051
58	27.0	37.3	0.030
53	27.5	36.8	0.034
Average			0.038

These figures seem to indicate that the temporary improvement in muscle tone and general activity that accompanies the artificial restoration of body temperature is probably not associated with an appreciable rise in the blood sugar level.

GENERAL DISCUSSION.

Before attempting a review of the results that have been set out in this paper, and stating the conclusions to which we think they lead, it would be well to draw attention to certain difficulties which render the task less easy than it at first appears.

Some of these arise from the uncertainty whether one or more than one

substance present in the yeast extracts employed as a source of vitamin B is responsible for the effects noted, whilst others appear as soon as the pitfalls that await one in attempting to correlate results obtained in the study of two species differing so widely from each other as the pigeon and the rat are appreciated.

Turning in the first place to the popular opinion that vitamin B, or some substance of similar distribution in natural foods, is essential for the production or functioning of the oxidative mechanism of the tissues, it must be apparent that our experience leads us completely to reject such view. To account satisfactorily for the opposing results of other workers, in particular those of Abderhalden and Hess, is not altogether easy. The experiments of the former investigator seem, however, open to criticism, if only on the grounds of the extraordinarily wide variability of his own figures. His employment of Ringer's fluid as the medium in which to conduct his experiments on the respiration of tissue suspensions seems unwise, and it is possible that some of the increases noted on adding various extracts supposed to contain vitamin B were actually due to a "buffering" effect.

Admittedly this would not explain all his results, which we must leave with the simple statement that we are entirely unable to confirm them on most essential points.

Passing reference may also be made to the claim of Abderhalden and Wertheimer [1923] that the tissues of "beri-beri" animals give the characteristic nitroprusside reaction for the sulphydryl grouping with diminished intensity. A series of estimations of glutathione made on normal and "beri-beri" tissues by the method of Tunncliffe [1925] failed to reveal any differences. Our figures were as follows:

normal muscle	0.036 %	normal liver	0.144 %
"beri-beri" muscle	0.038 %	"beri-beri" liver	0.173 %

The researches of Hess and his collaborators fall into another category and must be criticised from an entirely different standpoint.

It is not easy to give an opinion on the significance of Hess's results on the administration of cyanides. As we have shown, the tissues from cyanide-poisoned animals showed no reduction of oxidative activity, owing, probably, to the great rapidity with which the salt in sub-lethal concentrations is detoxicated.

We are inclined to support Abderhalden's view that the condition of pigeons poisoned with cyanides is not related in any essential manner to that induced by a diet of polished rice, not for his reason that vitamin B will not effect the cure of a bird with cyanide poisoning, but rather on the grounds that it is unsafe to argue from a superficial similarity of symptoms, particularly the convulsions.

Our failure to obtain with methylene blue as an indicator results of the same general nature as Hess and his colleagues obtained with *m*-dinitrobenzene is, of course, no proof that there is any real contradiction, although

Lipschitz [1921] obtained with tissues and *m*-dinitrobenzene similar results to those Thunberg obtained with methylene blue.

We are indebted to our colleague, Mr R. K. Cannan, for the following expression of opinion as to the relation between the two groups of experiments.

"Whereas the 'methylene blue technique' has laid bare important catalytic properties of living cells *in vitro* and given quantitative expression to the specific activating powers of different cells [Quastel, 1926] its use in attempts to discriminate between the activity of normal and pathological tissue has not yet been accompanied by any great success. In the experiments reported in this paper there is no evidence—under the conditions of concentration of the tissue and reagents employed—of any difference between the rates at which normal and 'beri-beri' muscle activate succinate (as representative of Thunberg's 'metabolites') or the natural hydrogen donors of the tissue. A more detailed study of the kinetics of the reaction might have revealed minor distinctions, but it does not appear that we are at present in the position to interpret any but striking differences revealed by this technique.

"The observations of Hess remain to be explained, and there seems no present explanation for them. The use of *m*-dinitrobenzene as a hydrogen acceptor is not to be encouraged. The reaction *m*-dinitrobenzene—nitrophenylhydroxylamine is not reversible in the sense that the reduction of methylene blue is reversible and, moreover, does not instantaneously achieve equilibrium with the reductant of a truly reversible system. That is to say, following the argument of Cannan, Cohen and Clark [1926], we shall not expect *m*-dinitrobenzene to oxidise instantaneously the hydrogen donors activated by the tissue, so that the rates of reduction observed by Hess involved not only the velocity of hydrogen activation by the tissue but also the velocity of reaction of the added indicator. We are not in a position to disentangle these.

"Finally, since the *m*-dinitrobenzene system does not exhibit reversible potentials at an inert electrode, we have no measure of the region of reduction intensity which it may impose upon a system to which it is added. The discrepancy between the present results and those of Hess cannot therefore be accounted for by a difference in reduction potential attained, under anaerobic conditions, by normal and 'beri-beri' muscle betrayed by a *known* difference in potential of the H acceptors employed. Indeed, the reduction of *m*-dinitrobenzene probably involves more than one stage of hydrogen (or electron) transfer and is altogether unsuited as an indicator of biological oxidation-reduction. It were better, as Clark [1925] has emphasised, to discard all such indicators as are not electrochemically active and whose potentials have not been established, else the problem will be strewn with empirical data."

It is obvious, therefore, that in the face of our results we cannot subscribe to the view of Hess [1921]: "Wir erkennen somit als stoffliches Prinzip des sog. antineuritischen Vitaminfaktors die Bausteine oder die Muttersubstanz von Zellfermenten speziell der Atmungsfermente." At the same time, we must regard both the results of Dutcher [1918] on the catalase content of the tissues

in "beri-beri," and the observations of Rohr [1923] on the existence of a parallel between the oxidative activity and the vitamin B content of tissues with suspicion.

If, as a result of our observations on the rate of reduction of methylene blue and of utilisation of oxygen by muscle and liver, we accept the conclusion that the mechanisms for oxidations in these tissues are virtually unimpaired by withdrawal of vitamin B from the diet, it still remains for the recorded work on the respiratory metabolism of the whole animal to be considered. The originals of some of the earlier papers, in particular those of Raomino [1916] and Novarro [1920], have unfortunately not been available to us, and we are unable to express an opinion on their work and conclusions. Abderhalden [1921, 1, 2] reported greatly reduced gaseous exchange in pigeons nourished on the deficient diet of polished rice, and concluded from the influence of yeast extracts that the vitamin was necessary to prevent or cure a disorder of the cellular oxidations, although he did not subscribe to the more clearly defined views of Hess to which attention has already been drawn [Abderhalden, 1922; Abderhalden and Wertheimer, 1921, 1, 2, 1922]. Gulick [1922, 1924] observed that the basal metabolism of rats fed for 2 months on a vitamin B-deficient ration had fallen to 69 % of the normal value, but he also found a similar reduction in control animals which were given inadequate amounts of a complete diet. His conclusion that the fall was due to calorie starvation was supported by Anderson and Kulp's investigations [1922], as well as those of Farmer and Redenbaugh [1925].

Studies of the respiratory quotient in vitamin B-deficiency are numerous, and authorities are not in agreement whether the values obtained have any significance from the standpoint of the disorder.

Jansen and Mangkoewinoto [1920], Anderson and Kulp [1922], Mattill [1923, 1, 2], Fleming [1923], Okada, Sakurai and Kabishima [1923] seem to think that the values cannot in any way be regarded as characteristic of vitamin B-deficiency, whereas Raomino [1916] and Caridroit [1922] seem to attach more importance to the changes that may be observed. Actually, most investigators record values near 0.7 for animals in the advanced stage of vitamin B-deficiency; a fact that is not surprising when it is recalled that they are practically starving.

A more important point concerns the question whether there is or is not a reduction of general metabolism as a result of deprivation of vitamin B. It must be remembered that the investigations of Magnus Levy [1906], Lusk [1921] and Benedict [1919] have clearly demonstrated that there may be a fall in the level of basal metabolism amounting to as much as 20 or 30 % in conditions of under-nutrition associated with loss of body-weight. This can be ascribed to a reduction in the amount of what may be termed the "active metabolic tissue." Some reduction of the gaseous exchange might, therefore, be expected as a result of the loss of weight that results from vitamin B-deficiency.

The difficulties of obtaining satisfactory measurements of the basal metabolism of small animals such as rats are very great, unless one adopts the tedious procedure of waiting until the animals fall asleep in the chamber before measurements are made; forcibly to restrict the movements, as Farmer and Redenbaugh [1925] did by binding the wings and legs of pigeons with wide bandages, appears to us a most unsatisfactory attempt to overcome the difficulty. The errors that arise from variations in the muscular activity of the animals in the chamber, even when only small movements are visible, are, in our opinion, large and render difficult, if not dangerous, the interpretation of any but considerable differences in the oxygen consumption.

For that reason we cannot attach any significance to a large number of our results, or to an even larger number of those recorded in the literature, but, as we have remarked before, we obtained definitely low values from animals in the last stage of the vitamin B-deficiency.

Can these be regarded as supporting the view that the oxidative mechanisms are damaged?

A conclusive answer to this question is, in our opinion, provided by the experiments in which animals nearly moribund, with low body temperature and oxygen uptake, were restored to an almost normal metabolic activity by artificially warming their bodies. The recovery was associated with an extraordinary but transient improvement of muscular tone and increased activity and could scarcely have taken place if the oxidising mechanisms had been seriously inactivated.

There is, however, one more point adduced by the supporters of the "depressed oxidation" hypothesis to which attention must be given. That relates to the claim, many times advanced, that administration of extracts containing vitamin B will raise the body temperature of animals suffering from a deficiency of that substance.

If this were true it would certainly favour the view that the intracellular oxidations are stimulated by the administration of vitamin B, but the evidence is as yet far from convincing.

In our own experience with rats we have very occasionally observed a small and temporary rise of a degree or so in the body temperature of vitamin B-deficient animals after administration of yeast extract, but usually no response occurs. Such is not the case when sugar is given, for then the temperature rises slowly and steadily by several degrees and remains high for some hours. Positive results of this type recorded by other investigators who administered vitamin extracts alone to pigeons are, we think, possibly to be ascribed to another cause than a stimulation of intracellular oxidations. Almost all authorities are agreed that the pigeon, by the time it has reached the polyneuritic stage, although eating very little, may often carry in its crop considerable amounts of undigested food. It seems not improbable that the effect of the vitamin extracts, as far as heat production is concerned, is indirect in that their action is to increase the tone of the muscles of the alimentary

tract [cf. Gross, 1924], and to expel food from the crop. Further work is needed to clear this matter up satisfactorily.

Before dealing with the relation of the condition induced in rats by a deficiency of vitamin B to simple starvation, we might consider briefly the recurrent expression of opinion in the literature that the "beri-beri" animal cannot properly utilise carbohydrates. The evidence is conflicting and inconclusive. McCarrison [1919, 1, 2] supported the idea that digestion and assimilation of carbohydrates are imperfect in vitamin B-deficiency, and his views were supported by Magne and Simonnet [1922] who stated that the injection of glucose did not raise the R.Q. of a "beri-beri" animal, whereas it did in cases of inanition. They also state that the pigeon fed on vitamin B-free diet contains glycogen reserves which it cannot mobilise until the missing factor is supplied.

The idea that vitamin B-deficiency leads to imperfect digestion and absorption of carbohydrates, and perhaps to a lessened ability to mobilise and burn these substances, also lies behind the conclusions of Randoin and Simonnet [1923, 1924] who trace a quantitative relation between the quantity of vitamin B in the diet and the amount of carbohydrate absorbed.

Results entirely opposing those of Magne and Simonnet are reported by Mattill [1923, 1, 2] who found that the injection of cane sugar immediately raised the R.Q. of rats on a vitamin B-deficient diet from 0.75 to 0.9 or more; results which naturally led him to reject the idea that this malnourished organism was unable to oxidise carbohydrates. Support for his point of view is also provided by the experiments on carbohydrate absorption and utilisation of Eggleton and Gross [1925].

These discordant results really prolong the earlier controversy between Braddon and Cooper [1914] and Funk [1914], on the one hand, and Vedder [1918] on the other, regarding the existence of a relation between the amount of vitamin B and the carbohydrate metabolised.

A critical study of these and many other papers on the subject, together with our own work, has led us to doubt whether any such relationship exists [Reader and Drummond, 1926], but it is obvious that a definite decision must be postponed until more work has been carried out.

The idea that starvation is closely related to the conditions exhibited by animals deprived of vitamin B is by no means new, for it has been time and time again suggested by various authorities, although seldom with any clear definition.

Roche, to whose careful studies we have already referred [1925], remarks: "Si l'avitaminose ne saurait être uniquement réduite à l'inanition, il n'est cependant pas douteux qu'elle est, sinon partiellement conditionnée, tant au moins accompagnée par une alimentation insuffisante," and concluded from his observations that the hypothermy certainly is a result of inanition and not specifically due to vitamin B-deficiency.

That the temperature must fall when the available food reserves are exhausted is obvious, and it seems that this is the most satisfactory explanation

of the hypothermy that is observed just before death whether from inanition or vitamin B-deficiency. Bierry [1919] has shown that it is coincident with a fall in blood sugar.

The evidence on which is based the opposite view of Weil and Mouriquand [1926] that vitamin B-deficiency and inanition are fundamentally unrelated is unconvincing.

Some reference must now be made to two abnormal conditions that are found in the organism as a result of deprivation of vitamin B, namely, certain lesions of the nerves and hypertrophy of the adrenal glands.

The former lesion is regarded by many as a characteristic and specific consequence of a deficiency of vitamin B. It is not intended here to review the extensive literature on this subject as it will form the subject of a paper by our colleague Dr Woollard, but it must be borne in mind that reliable authorities have more than once observed typical "beri-beri" in starving birds, particularly when life is prolonged beyond the usual span by administration of more water than the animal would voluntarily consume [Chamberlain, Blomburgh and Kilbourne, 1911; Chamberlain and Vedder, 1911; Eijkman and v. Hoogenhuyze, 1916].

The failure of other investigators to make the same observation [Frazer and Stanton, 1911; Funk, 1914; McCarrison, 1919, 2; Tscherkes, 1922, 1923, and others] seems likely to be due to the fact that their animals may have succumbed before there had been time for the disease to develop. In our own experiments on starving rats we observed two cases which presented an appearance closely resembling that of the "beri-beri" rat with characteristic posture of the hind limbs. Dr Woollard has found on careful histological examination that practically all our starved rats showed nerve lesions indistinguishable from those found in the vitamin B-deficient groups. This important observation is being followed up.

The second abnormality to which reference must be made, since it has been related by some authorities to the hyperglycaemia found in "beri-beri" animals, is the hypertrophy of the adrenals first noted by McCarrison [1919, 2]. This condition, standing out by contrast with the almost general atrophy of the other tissues [Funk and Douglas, 1914] is not characteristic of vitamin B-deficiency, unless starvation be regarded as a form of this disorder, because Vincent and Hollenberg [1920], McCarrison [1919, 2] and Findlay [1921, 1, 2] have recorded it in cases of inanition; a fact that has been confirmed by Dr Woollard. What this hypertrophy signifies is yet to be discovered. Funk [1919] regarded it as the cause of the hyperglycaemia he and Schönborn first noticed, whilst we have found that both are present in simple starvation. Riddle [1923] records hypertrophy of the adrenals and hyperglycaemia coincident in pigeons during ovulation. Cramer [1920] thought that the lowered temperature in vitamin B-deficiency might be due to interference with the heat-regulating function of the adrenal.

It is probable, however, that work along the lines indicated by the

researches of Cannon, McIver and Bliss [1923] will throw light on the matter. It will be remembered that these investigators demonstrated that any condition tending to cause a reduction of the level of sugar in the blood will lead to increased output of adrenaline.

To one other aspect of this many-sided problem we wish to call attention and we have reserved it until the last because we believe it may turn out to be the most important of them all. We refer to the studies of the relation of vitamin B to appetite made by Osborne and Mendel [1917], Karr [1920] and particularly Cowgill and his colleagues [1921, 1923, 1, 2, 1925, 1926]. The discovery of what appears to be a quantitative relation between the amount of vitamin B and the total food intake is of great importance in that it may provide the master key to the whole complicated problem.

SUMMARY.

1. The oxidative activity of the muscle and liver tissue of rats and pigeons estimated either by direct measurements of oxygen consumption or by the rate of anaerobic reduction of methylene blue is not reduced as a result of the animals being deprived of vitamin B. This confirms the observations of Roche, but is in disagreement with those of Abderhalden, Hess and others.

2. The resting oxygen consumption of rats deprived of vitamin B remains at a normal level until the final phase of the condition of malnutrition induced by the deficiency. This phase, lasting 1 to 3 days, is characterised by a sharp fall of body-weight, rectal temperature and oxygen consumption.

3. Observations of food consumption indicate that a deficiency of vitamin B causes a progressive failure of appetite, leading eventually to almost complete abstention from food. The results of control experiments on animals deprived of food indicate that the fall of body temperature and oxygen consumption in the final phase of vitamin B-deficiency are probably the natural consequence of an exhaustion of reserve food materials.

4. That this view is probably correct, and that there is certainly no interference with the oxidative mechanisms of the tissues as a result of deprivation of vitamin B, is shown by the fact that animals exhibiting the premortal fall of body temperature and oxygen consumption will yield approximately normal values for the latter measurement after their temperature has been artificially raised to the normal range by warming in a bath. This recovery is, as would be expected, of very short duration.

5. Further evidence in support of this view is provided by studies of the concentration of reducing substance in the blood in rats in inanition and vitamin B-deficiency. In both conditions a state of hyperglycaemia develops—associated with a hypertrophy of the adrenal glands—but is followed by a sharp fall to values much below the normal level. The period during which this reduction occurs is that characterised by the fall of body temperature and oxygen consumption.

6. These results are discussed in the light of existing theories regarding the physiological action of vitamin B, and the conclusion is reached that, in the case of rats at any rate, the nutritive failure following a deficiency of the vitamin is virtually identical with that resulting from starvation.

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