CLXXIX. VITAMIN B DEFICIENCY IN THE RAT. BRADYCARDIA AS A DISTINCTIVE FEATURE[1].

By ALAN NIGEL DRURY[2], LESLIE JULIUS HARRIS[2] and CECIL MAUDSLEY (Melbourne).

From the Department of Pathology and the Nutritional Laboratory, University of Cambridge.

(*Received September 1st, 1930.*)

Drummond and others have emphasised the non-specific character of the known signs of vitamin B deficiency. "Practically all the abnormalities that have been described as being due to vitamin B deficiency... can with one exception (head retraction or convulsions) be attributed solely to the loss of appetite and resulting inanition that follows withdrawal of the vitamin" [Drummond, 1926–7]. "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" [Drummond and Marrian, 1926; see also Kon and Drummond, 1927, etc.]. In the present communication we are concerned with a distinctive abnormality which, although it occurs also in advanced starvation and inanition, cannot be adequately accounted for on the basis of the diminished food intake but appears to be due directly to the absence of the vitamin complex. The bradycardia which we have to describe is not seen in our control animals restricted to the same limited amount of food and it can be cured with dramatic rapidity by the simple administration of vitamin B when no increased food intake is permitted, or even during a period of complete abstention from food.

The rat has been so extensively employed in studying vitamin deficiency that reproducible results can now be readily obtained, but while results in the past have been judged mainly upon the appearance of the animal or upon body weight or such other gross changes as can be readily appreciated, attention now begins to be directed to changes in function. The heart presents the advantage that certain of its functions can be repeatedly and easily investigated. Electrocardiographic records can be obtained without undue disturbance of the animal, and from these the rate of the heart beat, the position at which it originates and the mode of spread of the contraction process through the heart can be accurately determined [Lewis, 1925]. The influence of abnormal diets upon two of its functions, namely rhythmicity and conductivity, can by this means be accurately studied. This consideration and the knowledge

[1] Communicated to the Biochemical Society, October 4, 1930 [Drury and Harris, 1930].

[2] Working on behalf of the Medical Research Council.

that cardiac abnormalities have been reported in clinical beriberi prompted us in 1928 to begin a comprehensive survey of the influence of various vitamin deficiencies or excesses upon these heart functions, and the present observations are an outcome of this work. Such studies may be expected to throw light not only upon the vitamin side of the question, but also upon the intimate functions of the heart itself.

Although the morphological cardiac changes which have been described in clinical cases of beriberi suggest the possibility of electrocardiographic irregularities also, none has, in fact, been recorded even during the height of the disease [Aalsmer and Wenckebach, 1929]. How far any such irregularities as may exist would be of significance here is difficult to decide, for other errors as well as simple vitamin B_1 deficiency are invariably present in clinical beriberi. The demonstration of heart block and bradycardia in pigeons fed upon polished rice of which a preliminary account has appeared [Carter and Drury, 1929] gave the study further impetus. The latter abnormality, however, is vagal in origin, whereas as we shall show the bradycardia in the rat is in no way dependent upon the integrity of the vagus nerves. To the present report we have appended a summary of our cardiographic findings in avitaminosis A, and D, and in hypervitaminosis D [for preliminary account see Harris and Moore, 1929, 1].

Method.

The electrocardiographic records have been obtained from unanaesthetised rats. In order to obtain satisfactory records the animals must be kept free from excessive movement, and to ensure this a specially designed board has been made upon which the animal can be quickly fixed and held secure upon its back[1]. The head of the rat is passed through an opening in a clamp, which is adjusted around the neck so that it is unable to withdraw it. The clamp consists essentially of two vertical brass plates with a triangular piece removed at the top of one and the bottom of the other. The upper piece, by means of a ratchet, can be raised or lowered, while the lower piece is fixed. By this means diamond-shaped openings of different sizes are obtained. This clamp is fixed on to a suitable board with side cleats. With the head firmly held in the clamp, threads with slip nooses are fixed to the fore and hind legs and fastened to the cleats. The rat, lying upon its back, is thus held firmly. After the observation is completed the threads are slipped off the limbs and the top vertical plate completely raised. The rat is allowed to free itself and is then picked up.

To obtain the electrical records two small needles are thrust under the skin of the right foreleg and the lower end of the thorax, about midway between the sternum and the left axillary line respectively[2]. These needles are passed about 2 mm. under the skin, and connected to a galvanometer string, the

[1] We are indebted to H. Hall of the Biochemical Laboratory for the construction of this apparatus.

[2] It was not considered necessary to record all the usual three leads, unless a definite and constant change was observed in this lead.

movement of which is recorded upon a moving paper camera. The point of insertion is marked by a dye so that the same position can be used at all subsequent examinations. Such needle electrodes are not "non-polarisable," and it is impossible accurately to standardise the string, and consequently the voltages of the curves cannot be compared, but information is available of the rate of the heart, the place of origin of the beat, the conduction between auricle and ventricle (the *P-R* interval) and conduction through the ventricular muscle (duration of the *QRS* complex) and the *T* wave. It has been found impracticable to use non-polarisable electrodes, owing to the high resistance of the fur and skin, which can only be lowered by prolonged soaking with saline. Comparative observations have been made, however, in anaesthetised rats of the curves obtained by the needle electrode method, with those obtained by non-polarisable electrodes fixed to right fore paw and left hind leg, and, as Table I (*a*) shows, both methods give identical results. In addition the *QRS* complex has been deformed by drugs and the records obtained show that the two methods in these circumstances also give similar measurements (Table I (*b*)). It can be safely assumed therefore that as far as the above measurements are concerned, the needle electrode gives the same results as the non-polarisable electrode, a conclusion which has been reached from the results already reported with the needle electrode in observations upon other animals [Verney, 1924].

Table I. *Comparison of needle electrode with non-polarisable electrode.*

(*a*) Normal rats anaesthetised.

Electrode used	Heart rate per minute	*P-R* interval in seconds	Duration of *QRS* interval in seconds
Rat 1. Non-polarisable	530	0·037	0·014
Needle	530	0·038	0·016
Rat 2. Non-polarisable	445	0·040	0·017
Needle	445	0·042	0·015

(*b*) Abnormal rats (anaesthetised) after injection of quinine sulphate.

Rat 3. Non-polarisable	381	0·052	0·032
Needle	381	0·051	0·031
Rat 4. Non-polarisable	290	0·060	0·038
Needle	290	0·058	0·037

In the experiments to be reported, rats of 40 to 80 g. initial weight on complete synthetic diet have been used; and although each experiment recorded has been controlled separately, a general consideration is desirable of the measurements from the electrocardiograms of young growing rats of such weights. The rats were held down upon their backs during the observation. The animal is of necessity subjected to the excitement of manipulation and is held in an unnatural position, but we have satisfied ourselves by a series of control tests that manipulation and the unusual position have very little influence upon the heart rate, and that the method employed gives an accurate estimate at least in so far as concerns young rats[1].

[1] Care is needed to avoid compression on the neck, which gives rise to a slow, irregular heart-beat, persisting for a long period.

The normal rat. In Table II the measurements of records from growing rats taken every seven days over a period of 42 days, and the average values of such measurements, are tabulated. It will be seen that the heart rate of each individual rat remains relatively constant over this period of time, the greatest variation in any one rat being 73 beats per minute. The average heart rate is from 503 to 530 per minute. The *P-R* interval is subject to very little variation, the greatest difference in any one rat being 0·0007 of a second. The average value is 0·036 to 0·041 of a second. The *QRS* interval also remains relatively constant. As it is impossible to ensure that the lie of the heart relative to the electrodes remains identical in the same rat on every occasion, slight alterations in the shape of the *QRS* complex occur and the greatest variations in the value are found when the form of the complexes is altered. The greatest difference in an individual rat is 0·005 of a second, while the average value is 0·009 to 0·012 of a second. The *T* wave shows little or no change throughout.

Table II. *Body weight in g., heart rate per minute, P-R and QRS intervals in seconds of growing rats on complete synthetic diet.*

Rat	Days ...	0	7	14	21	28	35	42
Female	Body weight	65	83	97	115	130	135	146
	Heart rate	490	480	500	514	540	529	490
	P-R	0·041	0·038	0·039	0·041	0·040	0·037	0·043
	QRS	0·014	0·014	—	—	0·013	0·012	0·013
Male	Body weight	56	75	97	110	137	153	166
	Heart rate	500	550	505	550	540	535	525
	P-R	0·039	0·035	0·033	0·036	0·036	0·034	0·035
	QRS	0·014	0·013	—	—	—	0·011	0·013
Male	Body weight	70	87	96	116	136	145	162
	Heart rate	525	491	533	525	540	562	533
	P-R	0·040	0·041	0·040	0·037	0·040	0·034	0·038
	QRS	0·013	0·010	0·012	0·008	0·010	0·012	0·011
Female	Body weight	78	87	95	106	117	124	142
	Heart rate	480	450	512	491	500	480	450
	P-R	0·040	0·042	0·037	0·039	0·040	0·037	0·042
	QRS	0·010	0·009	0·011	0·010	0·010	0·012	0·010
Female	Body weight	59	76	89	100	125	130	136
	Heart rate	550	533	540	558	534	533	500
	P-R	0·044	0·038	0·039	0·040	0·039	0·039	0·038
	QRS	0·010	0·011	0·008	0·009	0·010	0·009	0·007
Female	Body weight	57	67	77	84	100	103	105
	Heart rate	518	553	540	545	532	480	520
	P-R	0·041	0·034	0·037	0·038	0·039	0·035	0·033
	QRS	0·009	0·010	0·009	0·008	0·009	0·007	0·007
Average	Body weight	64	79	92	105	117	130	143
	Heart rate	513	510	525	530	530	520	503
	P-R	0·041	0·038	0·037	0·038	0·039	0·036	0·038
	QRS	0·012	0·011	0·010	0·009	0·010	0·011	0·010

The table shows that the values obtained for the heart rate, *P-R* interval, and *QRS* complex, remain relatively constant as the rats increase in weight from 64 g. to 143 g. Any definite change in any one of these values in rats of similar size occurring during a special experiment can, therefore, safely be ascribed to the experimental conditions imposed.

Observations have also been made upon rats dying from various causes other than vitamin deficiencies. Such moribund rats always exhibit a very slow heart rate which is often irregular and which sets in over a period of a few hours and reaches very low values; in fact, the lower the heart rate the nearer to death is the animal. The slow heart rate is sometimes associated with a lengthened *P-R* interval and on occasions with an incomplete heart block. This bradycardia of abrupt onset is also seen in dying rats on abnormal diets of all kinds and is an expression of the moribund condition of the animal and not a specific action of the diet.

Vitamin B deficiency.

The effect upon the electrocardiogram of absence of vitamin B from the diet has been tested upon a large number of rats. The composition of the diet was as follows: 20 % purified caseinogen, 55 % rice starch, 5 % salt mixture, 20 % arachis oil, supplemented with cod-liver oil, 2 drops per animal *per diem*. All the rats receiving this diet show a constant reaction. Coincidently with the fall in body weight, which usually begins after 2 weeks, a fall in heart rate sets in. During the next few days the heart rate rapidly decreases, reaching values of about 250–300 per minute. During this stage also the body weight steadily falls so that the two events run hand in hand (Fig. 1).

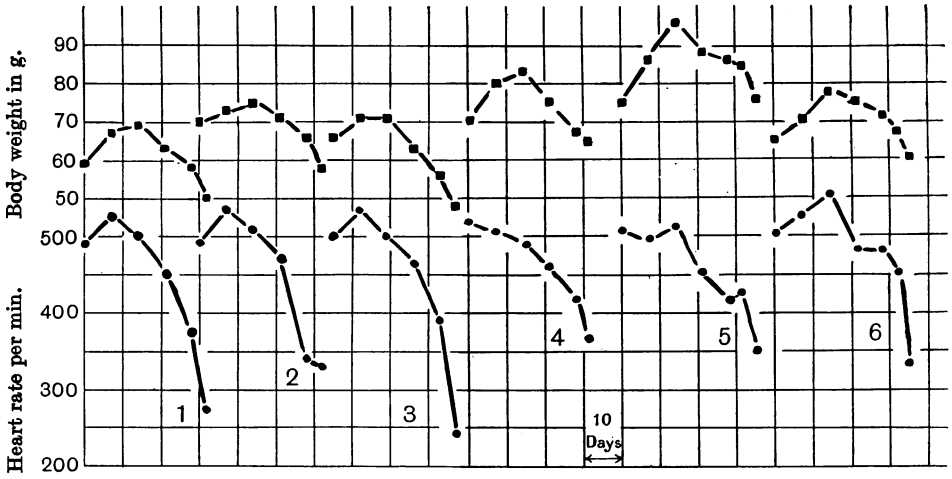

Fig. 1. Body weight and heart rate of rats fed upon synthetic diet free from vitamin B complex.

The measurements from six rats which are typical of all those receiving vitamin B-deficient diet are given in Table III.

It is seen from this table that although the heart rate falls considerably, even to half its original value, there is no change in the *P-R* or the *QRS* interval. This would indicate at first sight that the function of conductivity is unaffected, but it must be remembered that the slow heart rate allows a longer period of

Table III. *Body weight in g., heart rate per minute, P-R and QRS intervals in seconds of rats on a vitamin B deficient diet.*

Rat	Days ...	0	7	14	21	28	31	35
Female	Body weight	59	67	69	63	58	50	—
	Heart rate	490	525	500	450	375	273	—
	P-R	0·037	0·040	0·031	0·038	0·040	0·052	—
	QRS	0·013	0·013	—	0·010	0·015	0·019	—
Male	Body weight	70	73	75	71	66	58	—
	Heart rate	495	540	510	470	343	330	—
	P-R	0·042	0·034	0·034	0·042	0·034	0·039	—
	QRS	0·013	0·014	0·014	0·015	0·015	0·012	—
Female	Body weight	66	71	71	63	56	48	—
	Heart rate	470	540	500	465	390	240	—
	P-R	0·046	0·033	0·031	0·036	0·040	—	—
	QRS	0·012	0·015	0·013	0·014	0·012	—	—
Female	Body weight	70	80	83	75	67	65	—
	Heart rate	520	507	488	466	420	369	—
	P-R	0·039	0·040	0·042	0·043	0·038	0·037	—
	QRS	0·013	0·011	0·012	0·012	0·010	0·011	—
Male	Body weight	75	86	99	88	89	85	75
	Heart rate	507	496	514	450	415	428	343
	P-R	0·043	0·041	0·044	0·048	0·043	0·037	0·047
	QRS	0·009	0·011	0·011	0·009	0·009	0·011	0·010
Female	Body weight	65	70	78	75	71	67	60
	Heart rate	500	525	554	480	480	450	333
	P-R	0·037	0·037	0·036	0·038	0·040	0·036	0·046
	QRS	0·011	0·010	0·011	0·010	0·010	0·012	0·010

rest, so that an impairment of conduction might not be apparent under these circumstances. In some rats, a lengthened *P-R* interval is seen at the very low rates, but this is to be ascribed to the moribund condition of the animal rather than to any specific action of the deficiency, for it sets in suddenly, the lengthened intervals not being foreshadowed in the measurements obtained while the heart rate is steadily falling. The records show that there is no definite change in the size or direction of the *T* wave.

Controls with restricted food intakes. The close association of the fall in body weight with the onset of the bradycardia suggested the possibility that the cardiac change might be due not to any specific deficiency of the diet, but might occur in any condition in which the animal loses weight or might be due simply to insufficient food intake. To test this, a series of rats was taken, and grouped in pairs. One of each pair received the synthetic diet without vitamin B, the second of each pair (the control) received each day the same weight as the first ate the day previously, but of a complete synthetic diet containing 7·5 % marmite. Eight rats in all have been employed in these control tests, and all behaved consistently. The rats on the vitamin B-deficient diets soon showed some loss of appetite, ate less, and lost weight. The control rats on the restricted diets with marmite were found to lose weight likewise, in fact the weight curve of the paired rats usually coincided very closely. The electrical records showed that while the usual bradycardia developed in the rats on the vitamin B-deficient diet, the heart rate of the controls receiving limited amounts of the diets containing marmite remained within normal values (Table IV and

Fig. 2). It is evident from this experiment that the bradycardia is due to the absence from the diet of some factor present in marmite and is not due to inanition consequent upon the small intake of food. It is to be noted that the control rats upon the restricted diets may, if the intake of their pairs is very small, be brought ultimately to a moribund condition due to starvation. A bradycardia then sets in which confuses the result.

Table IV. *Body weights and heart rates of rats receiving (1) vitamin B-free diets and (2) identical amounts of complete synthetic diets.*

Rat	Days ...	0	7	14	21	28	Diet
Male	Body weight	79	85	85	79	62	Deficient in vitamin B
	Heart rate	514	540	525	430	300	
Male	Body weight	78	87	87	86	68	Restricted
	Heart rate	500	525	533	510	500	
Female	Body weight	71	73	73	67	55	Deficient
	Heart rate	488	500	471	408	300	
Female	Body weight	69	74	73	67	58	Restricted
	Heart rate	514	525	507	470	480	
Male	Body weight	73	58	65	58	44	Deficient
	Heart rate	525	500	480	450	320	
Male	Body weight	64	62	70	56	47	Restricted
	Heart rate	500	540	533	470	*500	
Male	Body weight	40	42	38	36	35	Deficient
	Heart rate	525	524	500	400	330	
Male	Body weight	40	41	37	37	38	Restricted
	Heart rate	540	545	510	510	510	
Male	Body weight	42	48	48	37	—	Deficient
	Heart rate	490	533	430	300	—	
Male	Body weight	46	48	48	45	41	Restricted
	Heart rate	525	500	553	550	536	
Female	Body weight	42	45	45	40	36	Deficient
	Heart rate	525	524	470	380	330	
Male	Body weight	43	49	51	47	41	Restricted
	Heart rate	540	545	560	540	504	

* 25th day.

Cure of the bradycardia with various sources of vitamin B[1]. When an extract of marmite[2] in doses of 0·75 cc. *per diem* was added to the diet of vitamin B-depleted rats with typical bradycardia, the heart rate rose rapidly and on the next day normal values were always recorded. In such experiments the animal quickly regained its appetite, its general condition improved, and it gained weight rapidly. In order to confirm the conclusion given in the last paragraph it was necessary to discover whether the rise in heart rate was due to any increase in the amount of food taken, or to the specific action of the vitamin, or possibly to inorganic substances present in the marmite. The influence of the amount of food taken can be eliminated by restricting the animals to the same limited amount of food as they had been consuming during the few preceding

[1] We are indebted to Miss Janet Clay for her assistance in this part of the work.

[2] [Harris and Moore, 1928, p. 1464.]

days when on the vitamin B-free diet, and that of the inorganic factors by using a very active yeast concentrate[1]. In an experiment conducted on these lines we find that the animal's condition and appetite improve, but the restriction in the food allowance results in the weight remaining stationary or rising only slightly (Fig. 2, Nos. 1 and 2). The heart rate, on the other hand,

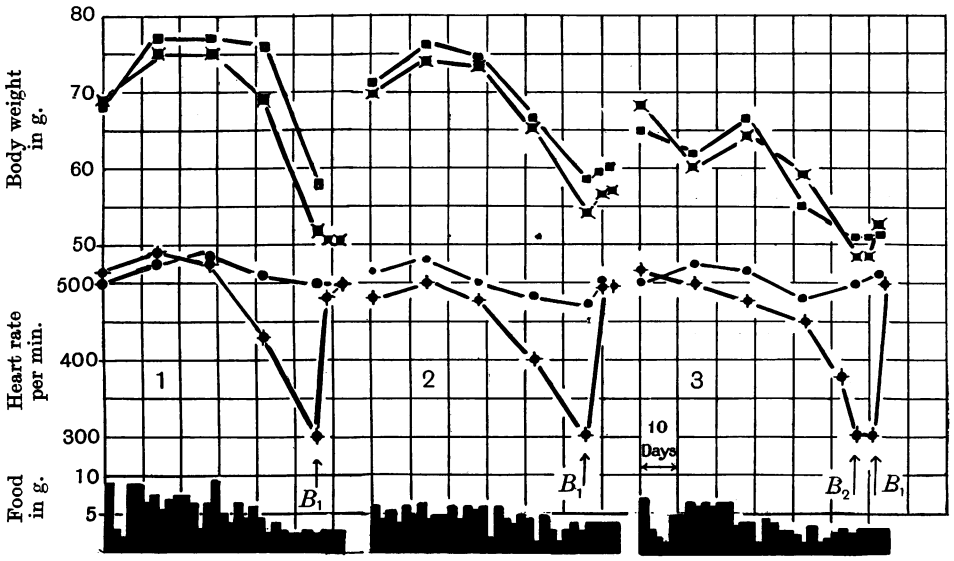

Fig. 2. Body weight and heart rates of paired rats, one upon a vitamin B-deficient diet, the other restricted to the same weight of a full synthetic diet. At B_1 in each instance, the vitamin B-depleted rat was given 0·15 cc. daily of yeast concentrate, no increase in food intake being permitted. At B_2 3 cc. of autoclaved marmite extract were given daily = 1 g. marmite. The food intake is shown at the bottom of the figure.

■ ●—Restricted diet. ✦ ✱—Vitamin B deficient diet.

returns to its normal value (Fig. 2). It would appear therefore that the amount of food taken is not the essential factor in the return of the heart rate to normal. Moreover, a concentrate of yeast is capable of restoring the normal heart rate without the occurrence of any coincident change in body weight.

Further evidence that an increased food intake is not essential for the alleviation of the bradycardia is provided by the demonstration of a return to normal rate within 3 to 4 hours following the administration of the concentrate, all food being withheld during the period. In studying the cardiac events immediately following the administration of substances rich in vitamin

[1] For this concentrate and the information concerning it we are indebted to Mr Guha of the Biochemical Laboratory, Cambridge. The concentrate was prepared as follows. Brewer's top yeast was extracted first with 97 % alcohol and then with 50 % alcohol. The two extracts were mixed and, after removal of alcohol, precipitated with neutral lead acetate. The filtrate, after removal of lead by hydrogen sulphide, produced good growth in rats, kept on a vitamin B_1-deficient diet, in a daily dose of 0·15 cc. which contained 35 mg. organic and 4·5 mg. inorganic matter. That the curative action is not due to the presence of salts is evident from the minuteness of the amount present in the active concentrate which is considerably exceeded by the amount consumed in the basal diet.

B_1, marmite and wheat germ as well as the yeast concentrate have been used. In these experiments the heart rate has been recorded before the administration of the substances and at hourly intervals afterwards. To eliminate any factor due to the intake of food after giving the concentrate, some of the rats have been allowed water only and no food during the first 6 hours following the administration of the concentrate. The concentrate has been used in two ways. Either 0·15 cc. has been pipetted into the mouth or the same amount has been diluted to 0·5 cc. with saline and injected into the peritoneal cavity. The wheat germ was readily and quickly eaten. The marmite was pipetted directly into the mouth. The results of the experiments in which only water has been allowed after administration of the vitamin are shown in Table V and Fig. 3, Nos. 1 and 2.

Table V. *Cure of bradycardia by administration of vitamin B during a period of starvation.*

	Heart rate per minute		
	Before	After*	Substance and dose
Male	300	300 (½) 400 (1½) 430 (2½) 500 (23)	0·15 cc. concentrate by mouth
Male	380	400 (½) 408 (1½) 410 (2½) 500 (23)	„ „
Female	350	415 (1) 440 (2) 450 (3) 505 (4) 510 (24)	Same dose injected intraperitoneally
Male	350	435 (1) 470 (2) 500 (3) 510 (4)	„ „

* Time in hours after administration of substance in brackets.

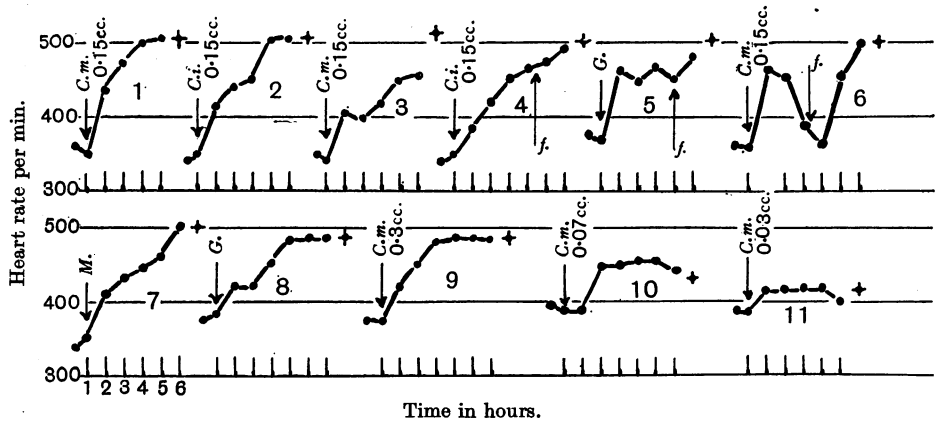

Fig. 3. Heart rates of vitamin B-depleted rats, before and for several successive hours after administration of one dose of a substance rich in vitamin B_1.
C.m. = yeast concentrate by mouth. *C.i.* = concentrate injected peritoneally. *G* = wheat germ. *M* = extract of marmite. Nos. 1 and 2: no food available till six hours after administration. Nos. 4, 5, 6: no food offered till arrow marked *f*. Nos. 3, 7, 8, 9, 10, 11: food available throughout. Water available in every instance.

From these measurements it is seen that the heart rate has risen considerably within the first hour; a few experiments indicate that at the end of the first half-hour there is an appreciable rise in rate, and that within 3–6 hours normal values are being recorded. The withholding of food after the administration of the concentrate may however sometimes give rise to certain

undesirable features. At the late stage of the avitaminosis at which it is selected, the rat is often feeding badly, but is feeding frequently; so that if food is withdrawn it quickly passes into a moribund condition due to starvation. This in itself gives rise to a bradycardia which confuses the result. This is clearly indicated by the experiments recorded below (Table VI, Fig. 3, Nos. 4, 5 and 6).

Table VI.

	Heart rate per minute		
	Before	After*	Substance and dose
Male	275	350 (1) 350 (2) 280 (3) Animal moribund	0·75 cc. marmite extract
Male	375	470 (1) 465 (2) 400 (3) Food given 370 (4) 460 (5) 500 (6) 500 (24)	0·15 cc. concentrate by mouth
Female	360	465 (1) 450 (2) 470 (3) 450 (4) Food given 480 (5) 500 (6) 525 (24)	0·75 cc. marmite extract

* Time after administration of substance in hours in brackets.

The recovery therefore is best studied in animals which are allowed restricted food, usually 4–6 g. daily, and which are able to eat at any time, if they desire. The measurements from such experiments (Table VII) show that full recovery is usually reached in 3–5 hours but that in some rats it may be delayed. (If a small dose of the concentrate is given, the heart rate fails to reach normal values, and reaches values which are approximately related to the size of the dose; see below.)

Table VII. *Curative experiments with restricted food allowance.*

	Heart rate per minute								
	Before	After*							Substance and dose
Male	360	405 (1)	400 (2)	420 (3)	450 (4)	455 (5)	525 (24)	—	0·15 cc. concentrate by mouth
Female	350	410 (1)	430 (2)	445 (3)	460 (4)	500 (5)	500 (24)	—	0·75 cc. extract marmite
Male	380	420 (1)	420 (2)	450 (3)	480 (4)	480 (5)	480 (6)	480 (24)	½ g. wheat germ
Male	375	420 (1)	450 (2)	480 (3)	490 (4)	480 (5)	480 (6)	480 (24)	0·30 cc. concentrate by mouth
Female	385	385 (1)	450 (2)	450 (3)	455 (4)	455 (5)	455 (6)	435 (24)	0·07 do.
Male	385	415 (1)	420 (2)	420 (3)	420 (4)	400 (5)	420 (24)	—	0·03 do.

* Time after administration of substance in hours in brackets.

The heart rate of rats cured in the manner described after remaining within normal limits for, say 1–3 days, begins to fall again, and 3 to 4 days later it is once more between 300 and 400 per minute. This enables the same dose to be repeated, or another substance to be tried. This has been performed upon several rats and the results are charted in Fig. 4.

Autoclaved marmite has been tried in a few cases as a substitute for vitamin B_1 concentrates (Fig. 2, No. 3 and Fig. 4, No. 6), but in no case has the bradycardia been cured. In one instance we used a preparation kindly provided by Mr Guha: the marmite had been autoclaved for 1½ hours at 115° at p_H 9·0; the amount given (3 cc.) was known to be adequate as a source of

vitamin B_2, from separate control experiments[1]. In another experiment the autoclaved marmite had been given us by Prof. R. A. Peters; this sample had been autoclaved for 1 hour at 115° at p_H 9·0.

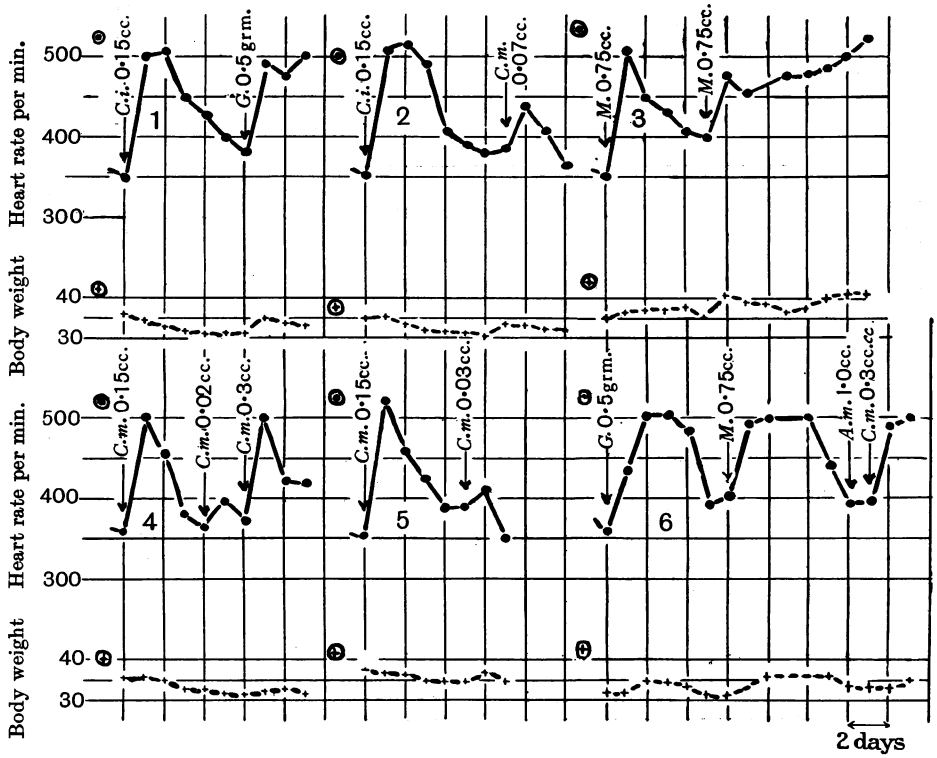

Fig. 4. Daily heart rates before and after the administration of single doses of substances rich in vitamin B_1.

C.m. = concentrate given by mouth. *C.i.* = concentrate injected intraperitoneally. *G* = wheat germ. *M* = extract of marmite. *A.m.* = autoclaved marmite. The heart rates and body weight before feeding the vitamin B-deficient diet are encircled.

With regard to the curative experiments with vitamin concentrates it may be undesirable to draw too definite conclusions of a qualitative nature without the use of a still larger number of animals, but there are certain points regarding the relation between the dosage and the degree of response which seem of interest. (1) After intraperitoneal injections of 0·15 cc. of the concentrate (Fig. 4, Nos. 1 and 2) the heart rate is within normal values for 2 days; after giving the same doses by mouth (Fig. 4, Nos. 4 and 5) normal values are only found during the day after. (2) To rats Nos. 4, 2 and 5 (Fig. 4) the concentrate was given on the same day in doses of 0·3, 0·07, and 0·03 cc. respectively; on the next day the heart rates were respectively 500, 430 and 410. The recovery of these rats during the hours immediately following the administration is shown in Fig. 3, Nos. 9, 10 and 11. It is evident that the degree of recovery is

[1] This sample contained very little vitamin B_1.

related to the amount of vitamin which is available, a result which was anticipated, since the fall in heart rate is gradual in onset.

The nature of the bradycardia in vitamin B deficiency.

The electrocardiographic records show that when the heart is beating at rates around 350, the bradycardia is sinus in origin. When lower rates are reached the *P* wave is lost, and it becomes difficult to decide whether A.V. rhythm has set in or not, no records having been obtained showing the change from the sinus bradycardia to the A.V. nodal bradycardia. In consequence of this, observations have been confined to animals in which the bradycardia is definitely sinus in origin.

It has already been reported [Carter and Drury, 1929] that pigeons fed on polished rice develop a heart block which is associated with a sinus bradycardia, and that this can be abolished by vagal section or atropinisation. It was anticipated that the sinus bradycardia in the rat would likewise be of vagal origin, but vagal section or complete atropinisation fails to return the heart rate to normal values.

The vagi have been sectioned in three animals and atropine injected into several others. Under urethane and ether anaesthesia the vagi were dissected in the neck. Records of the heart rate were then taken and the vagi sectioned, one after the other, records being taken at varying intervals afterwards (Table VIII).

Table VIII. *Bradycardia of avitaminosis B after vagal section.*

	Heart rate per minute				
	Before	Both vagi sectioned*			
1	300	330 (1)	350 (3)	330 (5)	328 (7)
2	350	320 (1)	310 (2)	300 (3)	290 (5)
3	320	310 (1)	310 (2)	300 (3)	290 (5)

* Time in minutes after section in brackets.

It is apparent that vagal section although producing in some rats a slight increase in rate by removal of vagal tone fails to restore the heart rate to the normal values. These observations are supported by the subcutaneous injection of atropine sulphate (Table IX).

Table IX. *Bradycardia of avitaminosis B after injection of atropine.*

	Heart rate per minute					
	Before	After*				Dose
1	343	330 (1)	323 (3)	333 (5)	—	0·2 cc. of 0·1 % solution†
2	250	240 (3)	240 (6)	230 (9)	225 (15)	0·2 cc. of 0·1 % solution‡
3	378	360 (1)	360 (3)	360 (5)	360 (8)	„ „

* Time in minutes in brackets. † Unanaesthetised. ‡ Anaesthetised.

Experiments were also performed which showed that after subcutaneous injection of 0·2 cc. of a 0·1 % solution of atropine neither electrical stimulation

of the vagus nerve, nor the intravenous injection of acetylcholine has any influence upon the heart rate; the vagus nerves being completely paralysed.

It is evident therefore that the sinus bradycardia, which occurs when rats are fed on a diet free from vitamin B, is in no way dependent upon the integrity of the vagus nerves, and in this respect differs from the heart block seen in pigeons fed upon polished rice.

Evidence has also been obtained by the fact that the bradycardia is not influenced by the injection of barium, that it is not related to the bradycardia produced by the injection of muscle adenylic acid [Drury and Szent-Györgyi, 1929].

In studying these bradycardias it is important that the temperature of the animal should be borne in mind and be kept carefully controlled during the observations. The rectal temperatures of rats depleted of vitamin B is often 34°–35° and, if the animal is moribund, may be even lower, and is readily influenced by external temperature. Experiments have been performed upon anaesthetised, normal, and vitamin B-deficient rats to determine the influence of rectal temperature upon the heart rate, and it is found that between the temperatures of 30°–40° a rise of one degree causes an increase in heart rate of approximately 15 beats. While the lowered temperature of the animal may conceivably help in producing the very low heart rates such as 250 per minute, which are occasionally seen, it is not responsible for the bradycardia with heart rates of 300 per minute and over, for these rates have been consistently found in rats whose rectal temperature was not allowed to fall below 37°–38°.

Vitamin A deficiency.

Baude and Deglaude [1924] have reported that there is no change in the heart rate in rats fed upon diets deficient in vitamin A, and our results serve to confirm and amplify their findings.

We have carried out tests upon two series of rats. The basal diet had the same composition as previously given, with the addition of 7·5 % of marmite extract as a source of vitamin B and 1 drop per animal per day of "radiostol" for vitamin D. All the experimental animals developed typical avitaminosis A.

Both series showed that the absence of vitamin A had no influence upon the rhythm of, or the conduction in, the heart, at least until the animal was almost *in extremis*. No change in the T wave was seen. The measurements from two rats which are typical of the series are given in Table X.

The controls of these series (receiving vitamin A in the form of a small supplement of red palm oil or carotene, or "radiostoleum" in place of "radiostol") increased in weight from an average of 52 g. to 148 g., and showed no change in the heart rate, the *P-R* or the *QRS* interval.

Table X. *Heart rates, etc., of rats receiving vitamin A-deficient diet.*

	Days ...	0	7	14	21	28	35	42	49	56	63	70	77	80
Female	Body weight	55	69	83	96	105	115	122	131	136	129	124	120	Dead
	Heart rate	520	600	600	565	525	571	553	543	533	525	500	480	
	P-R	0·037	0·033	0·033	0·037	0·037	0·033	0·034	0·034	0·032	0·035	0·036	0·037	
	QRS	0·013	0·009	0·010	0·009	0·009	0·008	0·007	0·008	0·008	0·015	0·013	0·008	
Male	Body weight	63	85	98	114	131	145	156	168	174	175	175	Dead	
	Heart rate	507	600	545	550	533	545	543	533	521	514	514		
	P-R	0·039	0·035	0·035	0·036	0·036	0·036	0·036	0·038	0·035	0·037	0·038		
	QRS	0·013	0·010	0·011	0·010	0·009	0·009	0·008	0·009	0·008	0·009	0·012		

Table XI. *Heart rates, etc., of rats on a rachitogenic vitamin D-deficient diet.*

	Days ...	0	7	14	21	28	35	42	49	56	63	70	77	84
Male	Body weight	73	80	88	99	105	110	114	124	132	129	132	130	130
	Heart rate	480	557	533	514	500	500	491	514	507	487	480	480	500
	P-R	0·038	0·033	0·036	0·035	0·039	0·036	0·036	0·037	0·037	0·038	0·038	0·036	0·035
	QRS	0·014	0·010	0·011	0·009	0·009	0·010	0·009	0·009	0·009	0·010	0·013	0·017	0·013
Female	Body weight	50	53	60	62	65	72	77	84	87	89	94	97	98
	Heart rate	545	540	525	540	525	566	533	545	550	520	562	514	525
	P-R	0·035	0·035	0·036	0·033	0·037	0·033	0·035	0·036	0·035	0·034	0·034	0·040	0·035
	QRS	0·013	0·009	0·011	0·011	0·008	0·009	0·009	0·008	0·008	0·013	0·011	0·015	0·013

Table XII. *Heart rates, etc., in hypervitaminosis D.*

	Days ...	7	14	21	28	35	42	49	56	63
Male	Body weight	72	74	74	69	69	73	73	63	64
	Heart rate	533	533	540	533	480	500	480	450	415
	P-R	0·036	0·037	0·034	0·034	0·037	0·040	0·034	0·036	0·044
	QRS	0·015	0·013	0·009	0·010	0·008	0·009	0·010	0·009	0·010
Female	Body weight	86	84	84	87	85	91	92	83	76
	Heart rate	562	533	500	525	480	500	480	430	471
	P-R	0·037	0·036	0·036	0·034	0·036	0·039	0·034	0·041	0·039
	QRS	0·013	0·011	0·009	0·009	0·009	0·008	00·07	0·008	0·010

Table XIII. *Electrocardiographic records of rats receiving diet deficient in vitamins A and D.*

	Days ...	0	7	14	21	28	35	42	49	56	63
Male	Body weight	60	63	81	99	113	123	133	137	129	—
	Heart rate	500	600	566	570	545	560	572	507	514*	—
	P-R	0·040	0·035	0·037	0·036	0·038	0·031	0·030	0·036	—	—
	QRS	0·013	0·010	0·008	0·008	0·009	0·007	0·007	0·008	—	—
Female	Body weight	55	60	73	85	95	95	100	99	92	90
	Heart rate	480	600	600	565	525	525	549	505	500	450†
	P-R	0·039	0·036	0·029	0·035	0·037	0·039	0·032	0·035	0·034	0·039
	QRS	0·009	0·009	0·008	0·008	0·008	0·010	0·007	0·008	0·008	0·015

* Died 58th day.

† Died 64th day.

Vitamin D.

Rats were placed on a Steenbock rachitogenic diet devoid of vitamin D. The measurements of two rats, typical of the series, are given in Table XI. Both these rats were killed on the 84th day and *post mortem* and histological examinations showed them to be suffering from typical severe rickets. The electrocardiographic measurements throughout show no abnormal variation, even at the stage when severe rickets had developed, and it appears evident that absence of vitamin D has no influence upon the rhythm of, or conduction in, the heart. No change in the T wave was found.

Hypervitaminosis D.

Agduhr and Stenstrom [1929] have reported changes in the ventricular portion of the electrocardiogram of mice fed upon excess of cod-liver oil which they consider typical of the condition and which they state can be related to the morphological changes in the heart muscle. We have ourselves been interested mainly in hypervitaminosis D induced by excessive administration of irradiated ergosterol, rather than in the effects of large doses of cod-liver oil which appear to have a different action [Harris and Moore, 1928; 1929, 1, 2]. The amount of irradiated ergosterol given was generally about 5 mg. (about 5×10^4 Coward units of vitamin D). The measurements of two rats typical for the series are given in Table XII.

It is seen that the heart rate tends to fall somewhat towards the end of the experiment. The fall in rate is in many cases quite slight, but all the rats on such diets at the end have heart rates which are below or on the lower limits of normality. The form of the electrocardiogram remains unchanged throughout. *Post mortem* examination of the rats showed the usual extensive deposits of calcium in the upper part of the aorta, and in the kidneys, etc. [Harris and Moore, 1929, 1].

Simultaneous deficiency of vitamins A and D.

A series of rats has been placed upon a diet which was deficient in both vitamins A and D. Measurements of two rats typical of the series are given (Table XIII). No change is found in the measurements or in the T wave.

Discussion.

Of the vitamin deficiencies tested, A and D, separately and combined, appear to exert no characteristic influence upon the rhythm of or the conduction in the heart or upon the T wave. Excess of vitamin D produces a very slight but definite bradycardia.

Deficiency of vitamin B has, however, a striking influence upon the rhythm, the heart rate being reduced from the normal values of 500–550 per minute to 350–300 per minute. The first point which emerges from our experiments is that the bradycardia although coincident in degree with the fall in body weight is

not part and parcel of such loss of weight, or of the diminished food intake, for control rats upon restricted diets losing weight at approximately the same rate fail to develop bradycardia until they are moribund. Again the bradycardia can be cleared up with rapidity by the administration of extracts rich in vitamin B, even during a short period of complete deprivation of all food, water alone being allowed. Indeed, a prolonged recovery of normal heart rates can be effected without gain in body weight by administering the vitamin while restricting the food intake to the amount consumed during the later stages of the avitaminosis. Reviewing our evidence we feel, therefore, that the bradycardia is not entirely a secondary effect resulting from diminished appetite and consequent inanition. A bradycardia, possibly indistinguishable experimentally, certainly develops when an animal is moribund as a result of extreme inanition or from other reasons. In the later stages of avitaminosis the animal is, it is true, not far removed from a moribund state due to starvation, but, as we have shown, the bradycardia begins to develop at a time long before the failing appetite can be regarded as a sufficient cause, and it can be terminated in the absence of food. In short we have a sign of advanced inanition, for which the degree of starvation is in itself an inadequate explanation. The growth failure, on the other hand, would seem to be adequately accounted for simply by the diminution in the weight of food eaten. The possibility may be borne in mind of the loss in appetite being secondary to the bradycardia.

It seems probable that the bradycardia (curative) test will prove of value in future work on the separation of the constituents of vitamin B. It is as sensitive as, and more specific than, a rat-growth test, and can be concluded in fewer hours than the latter requires days. We have seen that various sources of the vitamin B complex contain the antibradycardia substance, so that when only a rough assay for the vitamin complex as a whole is desired, without special reference to a particular constituent, the convenience of the new technique is again apparent.

As to the relation of the vitamin to the mechanism underlying the bradycardia, it is impossible, at this stage, to draw any definite conclusions. Normal cardiac rates are not reached for at least 3 hours after administration. The fact that several hours elapse suggests the possibility that certain changes must take place in the body before the rhythmicity is normal again. If the vitamin supplied a link in the metabolic process underlying rhythmicity one might expect the recovery to be even more abrupt, but, on the other hand, the recovery is too rapid for any organic degenerative process to underlie the impairment. A possible correlation may exist between the bradycardia and the accumulation of lactic acid in vitamin B depletion [Bickel, 1924, 1925; Collazo, 1923]: this accumulation is, according to Peters [1929], responsible for the opisthotonos, and its removal is accelerated by vitamin B. The well-known hypertrophy of the suprarenal gland seen in vitamin B deficiency [Marrian, 1928] might also be legitimately thought of here, with the possibility of some irregularity in the secretion of adrenaline.

We have seen that the component of the vitamin B complex responsible for the cure of the bradycardia is present in certain vitamin B_1 concentrates and that like vitamin B_1 it is comparatively heat-labile. Again certain sources of vitamin B_2 are inactive. But we consider that we have not yet examined a sufficient variety of preparations to enable us to identify it with a particular constituent of the complex, and in fact owing to the present position of the vitamin B problem we are of the opinion that such an attempt would be somewhat premature.

In the meantime we believe that the discovery of specific events such as has been described for the pigeon [Carter and Drury, 1929] and is here described for the rat must prove important to the problem, and a study of such phenomena may be expected to prove of use in the characterisation of the constituents of the vitamin B complex.

Summary.

Deprivation of vitamin B complex leads to a severe bradycardia in young rats. The slow heart rate is due, not to the lowered food consumption but directly to the absence of the vitamin; for, (1) control animals on restricted amounts of complete synthetic diets fail to develop the bradycardia until moribund, (2) the bradycardia can be cured rapidly in the entire absence of food by injection or ingestion of vitamin B concentrates, and (3) prolonged restoration to normal heart rate can be effected by adequate provision of vitamin B, even when the food intake is restricted to the amount consumed in the last stages of the avitaminosis and the animal fails to make up lost weight. The curative substance is present in various vitamin B_1 concentrates tested and is comparatively readily destroyed by heat. It gives a response roughly graded to the dose administered. The same animal can be used for testing different specimens rapidly in turn, and it is suggested that the bradycardia may prove useful as an index in the course of fractionation of the components of the vitamin B complex.

The bradycardia is of sinus origin and is not due to vagal influence. It is not related to the experimental non-vagal bradycardia produced experimentally by the injection of muscle adenylic acid. Deficiency of vitamin A, or of vitamin D, or of A and D combined, has no significant influence upon the heart. Excess of vitamin D produces constantly a very slight bradycardia.

REFERENCES.

Aalsmer and Wenckebach (1929). *Wien. Arch. inn. Med.* **16**, 193.
Agduhr and Stenstrom (1929). *Acta Paediatrica*, **8**, 493.
Baude and Deglaude (1924). *Compt. Rend. Soc. Biol.* **91**, 116.
Bickel (1924). *Biochem. Z.* **146**, 493.
—— (1925). *Biochem. Z.* **166**, 251.
Carter and Drury (1929). *J. Physiol.* **68**, *Proc.* i.
Collazo (1923). *Biochem. Z.* **136**, 278.
Drummond (1926–7). *Med. Res. Council Ann. Rep.* 74.
—— and Marrian (1926). *Biochem. J.* **20**, 1254.
Drury and Szent-Györgyi (1929). *J. Physiol.* **68**, 213.
Drury and Harris (1930). *Chem. Ind.* **49**, 851.
Harris and Moore (1928). *Biochem. J.* **22**, 1461.
—— —— (1929, 1). *Biochem. J.* **23**, 261.
—— —— (1929, 2). *Biochem. J.* **23**, 1141.
Kon and Drummond (1927). *Biochem. J.* **21**, 632.
Lewis (1925). The mechanism and graphic registration of the heart beat. (London.)
Marrian (1928). *Biochem. J.* **22**, 836.
Peters (1929). The Harben lectures for 1929. (London.) *J. State Med.* **37** and **38**.
Smith (1930). *U.S. Pub. Health Rep.* **45**, 116.
Verney (1924). *Arch. exp. Path. Pharm.* **104**, 170.