NUTRITIONAL DEFICIENCIES AS A CAUSE OF ELEVATED BLOOD PRESSURE IN RATS (WITH ESPECIAL REFERENCE TO THE VITAMIN B_2 COMPLEX)*

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Many recent experimental observations are compatible with the idea that certain types of hypertension may be of metabolic origin and that the effective etiologic mechanism is diminished oxidative activity of the kidney. The close connection between some of the vitamins and metabolic processes suggests that vitamin deficiencies might cause a rise in blood pressure by limiting the oxidative capacity of the kidney. Such a possibility has been adopted as a working hypothesis in the present study, attention being given especially to deficiencies of vitamin B since this complex is known to furnish important components of several respiratory enzyme systems. The results indicate that a reduced intake of the entire complex is followed by a slight fall in the blood pressure of rats, whereas a deficiency of only the heat-stable portions of this complex leads to a reversible rise in blood pressure.

The discovery by Goldblatt (1) that constriction of the renal arteries is followed by persistent hypertension has been interpreted by some authorities as indicating that "arterial and arteriolar sclerosis of the kidney probably precedes and determines the existence of hypertension" (2). However, the occurrence of even a few cases of essential hypertension without arteriolar changes and the observation that hypertension itself may cause arteriolosclerosis (3) have kept alive the question of "whether arteriolar sclerosis in the kidney is cause or effect, or both, of hypertension" (2). The search for etiological factors which might cause hypertension by duplicating the physiologic consequences of renal ischemia and which might even lead to sclerosis of the arterioles cannot, therefore, be abandoned.

Within recent years, much experimental work has served to revive the old idea that essential hypertension may be a metabolic disease. Suggestive evidence, reviewed by Blalock (4), is to be had from the fact that the mediator of experimental hypertension is a blood-borne chemical, though whether the effective part of the mechanism is an excess of pressor substances or an insufficiency of anti-pressor substances has yet to be determined definitely. More direct evidence in favor of the possibility was supplied by Dicker (5), who showed that the formation of pressor

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substances by the kidney is in no way related to its excretory functions. The older objections to the theory of the metabolic origin of hypertension were thereby eliminated, for these objections had been based mainly on the fact that the majority of patients with hypertension do not show any impairment of renal excretory functions (6). Finally, the essential metabolic nature of experimental renal hypertension has been firmly established by the work of Rodbard (7) and of Rodbard and Katz (8). According to these authors, the chemical mediator of hypertension is a normal product of renal intermediate metabolism which is usually destroyed as rapidly as it is formed.

If the latter interpretation be correct, then the problem resolves itself into a search for the mechanism by which ischemia might cause metabolic abnormalities in the kidney. A clue of possible profit in this search is the finding that constriction of the renal arteries leads to diminished consumption of oxygen by the ischemic organ; this has been demonstrated both in the intact animal (Levy, Light, and Blalock (9)) and *in vitro* by the manometric studies of Gerbi, Rubenstein, and Goldblatt (10). The possibility therefore arises that the unknown metabolic defect may be dependent upon inadequate oxidation in the kidney. This suspicion is somewhat strengthened by the observation of Holtz and Heise (11) that in the absence of oxygen the incubation of *l*-dihydroxyphenylalanine (dopa) with renal cortex extract results in the formation of a pressor amine. Bing (12) and Bing and Zucker (13) have noted a similar reaction on perfusion of ischemic kidney, the amount of pressor substance being inversely proportional to the perfusion rate. These authors believe that this pressor amine results from the incomplete oxidation of dopa, and that the reaction is the prototype of perhaps many such reactions, all of which may contribute to the hypertensive mechanism. In the presence of oxygen, this pressor amine is oxidized further to a non-pressor substance, presumably by oxidative deaminization, a process which Kempner (14) has shown is impeded by low oxygen tensions.

The foregoing experimental observations admittedly would provide a plausible explanation of the hypertensive mechanism. Their acceptance as the actual explanation, however, necessitates the assumption that arteriolar or arterial sclerosis precedes the development of hypertension in every case; and, as stated above, there are valid objections against this assumption universally. In postulating that hypertension precedes the sclerosis, on the other hand, one is automatically abandoning organic interference with the blood supply as the primary cause of diminished oxidation in the kidney and is forced to look elsewhere for the explanation. Theoretically, one possible explanation hinges on the fact that biological oxidations are dependent not only on an adequate supply of oxygen but also on a multiplicity of enzyme systems the integrity of which is essential if oxidation is to proceed in a normal fashion. It is conceivable that the various oxidative functions of the kidney might be seriously compromised if there were a deficiency in these enzyme systems. This possibility is strengthened by the investigations of Schroeder and Adams (15), who demonstrated that tyrosinase has the capacity of inactivating renin and other pressor substances, as well as of combating experimental arterial hypertension.

In this connection, it is important to recall that certain components of these enzyme systems, such, for example, as the majority of the specific dehydrogenases, are intracellular and presumably an intimate part of the cell structure itself; others, such as the prosthetic groups of the di- and triphosphopyridine nucleotides and the flavins, are derived from exogenous sources. It is obvious that if these exogenous sources are cut off, the effectiveness of those systems involving coenzymes and flavoproteins must be reduced. The consequence would be a diminution of oxygen utilization by the body, including the kidney; and thus a condition would be created in the kidney duplicating that produced mechanically by the Goldblatt clamp.

This concept has provided a working hypothesis in the present study. Since the vitamin B complex is the source of several important components of the respiratory enzyme systems, it was chosen for the initial studies reported below. Analogous studies on a human subject, with similar results, have been reported by Elsom (16). The results of the present experiments are compatible with but by no means definitive proof of the working hypothesis just outlined.

Materials and Methods

Experimental Animals.—Most of the rats used in these experiments were obtained from a breeding colony of the Vanderbilt strain (17). Those used in Experiment 2 were from a piebald strain obtained from the School of Hygiene, Johns Hopkins University. At the beginning of each experiment they varied in age from 8 to 10 weeks.

Diets.—The rats were raised on a stock diet consisting of commercial dog chow checkers,[1] lettuce or other green vegetables, and white bread, and in addition received fresh milk for the 4 weeks following weaning.

The basic vitamin B-free diet was that described by György and Goldblatt (18), and consisted of 18 parts of vitamin-free casein,[2] 68 of commercial sucrose, 8 of melted butter fat, 4 of U. S. P. salt mixture No. 2,[3] and 2 of cod liver oil. As indicated in the protocols, various supplements were administered as follows: thiamin chloride in aqueous solution by dropper, in the amount of 40 μ gm. per rat per day; dried brewers' yeast,[4] either autoclaved or unautoclaved and in amounts as noted; crude aqueous extract of liver,[5] 1 ml. per rat per day; a concentrated extract of rice polishings,[6] 0.5 ml. per rat per day. The latter three ingredients were mixed with the foodstuff.

The term *vitamin B_2 complex* is used throughout this paper to denote the heat-stable fractions of the vitamin B complex.

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[1] Purina brand.

[2] Labco brand, The Borden Company, New York City.

[3] S. M. A. Corporation, Cleveland.

[4] Mead's brand.

[5] Valentine Meat Juice Company, Richmond.

[6] "Vitab" furnished gratis by National Oil Products Company, Harrison, New Jersey.

Laboratory Conditions.—The rats were kept in roomy cages equipped with wire mesh bottoms to prevent access to the feces. Scrupulous cleanliness was maintained in order to avoid epidemics. During the summer months the laboratory was air-conditioned, the temperature being kept constant at 70–75°F.

Description and Discussion of Methods of Estimating Blood Pressure.—The method described by Williams, Harrison, and Grollman (19) was used in estimating blood pressure. The readings so obtained are measures of mean pressures and are thus lower than the actual systolic levels. The method presented certain difficulties, arising from the fact that preliminary heating of the animals is required in order to produce a vigorous circulation through the tail. In general, the deficient rats require more heating for this purpose than do normal animals; but deficient animals withstand heating very poorly. Hence it was necessary carefully to standardize the duration of heating. In all except Experiment 1, the animals were handled in groups of four and were kept in the warmer box for 5 minutes and then transferred to the cooler box; from the latter, they were taken at random, and the readings on all four were completed within the ensuing 5 minutes. Only by strict adherence to these conditions can comparable pressure readings be obtained.

When large numbers of pressures were being determined, the temperature of the boxes of course varied somewhat, and since the boxes were opened frequently, thermostatic control was impractical. The varying temperatures were therefore compensated by alternating control and experimental groups of four animals with strict regularity.

It was found also that repeated heating of deficient rats has a rapidly cumulative effect. If pressure readings are attempted at too frequent intervals, many of the animals go into partial or complete collapse with resultant fall in arterial tension. Intervals of at least 3 days and preferably a week appear essential.

The accuracy of the above method of estimating blood pressures was checked by insertion of a cannula directly into the abdominal aorta, as described in Experiment 5. The findings indicate the reliability of the indirect method, provided the various precautions mentioned are observed meticulously.

EXPERIMENTAL

Influence of a Complete Deficiency of the Vitamin B Complex and of the Vitamin B_2 Complex on Blood Pressure

Experiment 1.—One group of 50 rats was placed on a diet completely devoid of all components of the vitamin B complex; after 2 weeks on this regime, thiamin chloride was administered. A second group of 50 animals was placed on an identical diet, and autoclaved yeast was mixed with the foodstuff in the proportion of 5 per cent; after 2 weeks of this regime, the amount of yeast was increased to 10 per cent; and after 1 week of this treatment, the animals were subdivided into two groups of 25 each, one group receiving a supplement of liver extract and the other group receiving a supplement of extract of rice polishings.

The results of this experiment are shown in Figs. 1 and 2. During the 1st week, the animals which received no vitamin B whatever registered a mod-

erate but significant rise in blood pressure (from an initial average of 119 mm. Hg to an average of 129 mm.). By the end of the 2nd week, however, the pressures had fallen to a point (average, 114 mm.) slightly below the initial level. When the diet was fortified by the addition of thiamin at this point, there was an immediate rise in pressure, which reached an average of 142 mm. after 2 weeks. By the end of the 5th week, although the pressures were still significantly high (138 mm.), the general condition of the animals was poor, they had begun to show gross evidences of riboflavin deficiency, they had lost weight, and 6 of the original 50 animals had died.

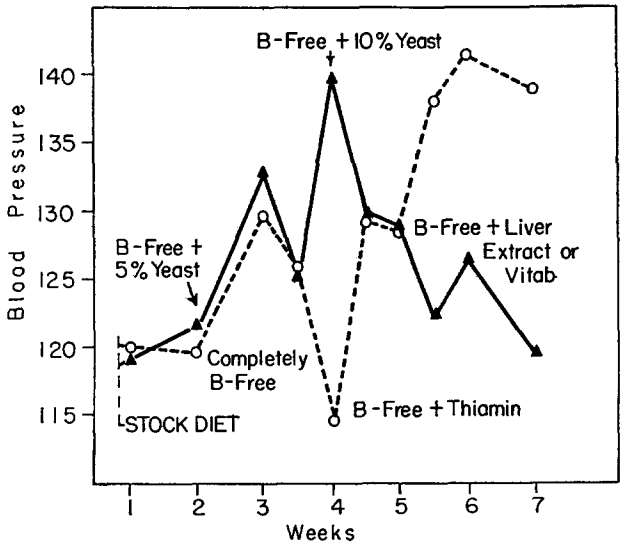

FIG. 1. The effects of deficiencies of various vitamin B fractions on the blood pressure of rats (Experiment 1). All readings represent mean pressures, which are lower than the actual systolic levels.

The second group of animals was intended to serve as controls. However, it was found that the addition of 5 per cent yeast was not sufficient to prevent a rise of blood pressure, for at the end of the 2nd week the average pressure had risen from an initial level of about 120 mm. to 139 mm. Hg. Increasing the yeast supplement to 10 per cent was followed by a significant drop in pressure (to an average of 129 mm.) though not to normal levels during the 1 week of treatment. The further addition of liver extract or extract of rice polishings was followed by prompt return of the pressures to the original levels (average, 119 mm.). Since the effect of these two supplements was identical, the results have been pooled on the accompanying charts.

Fig. 2 is a distribution curve of the pressure readings in these two groups of

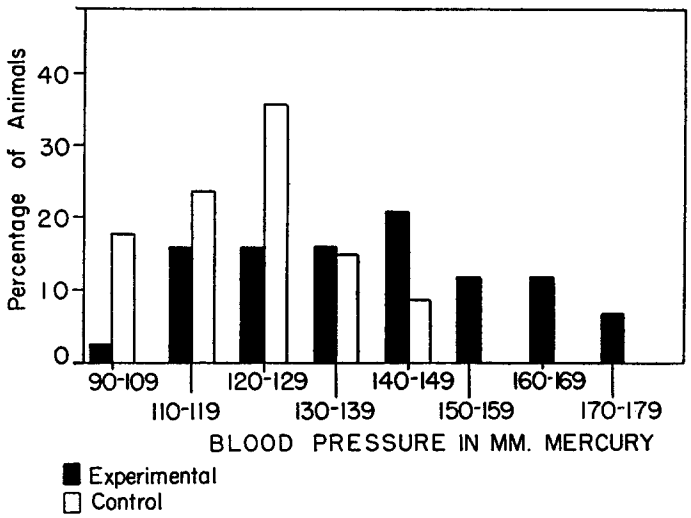

FIG. 2. Distribution curves of the blood pressure readings at the conclusion of Experiment 1 (Fig. 1). Hollow blocks represent well nourished group; solid blocks represent deficient group.

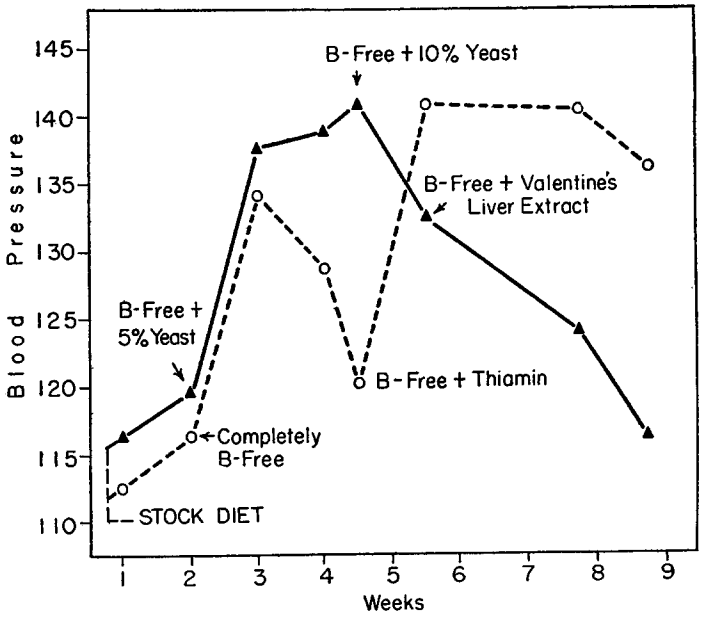

FIG. 3. The results of Experiment 2, the design of which was almost identical with that of Experiment 1 (Fig. 1).

animals at the end of the 5th week on the experimental diets. Fisher's t test shows that the difference in the blood pressures of the two groups was highly significant at the conclusion of the experiment ($P < 0.001$).

Experiment 2.—This experiment was identical in design with Experiment 1, except for the facts that it was performed in summer rather than winter and that the rats were of a piebald strain. Each of the two groups consisted of 50 animals.

The results of this experiment, which are shown in Fig. 3, closely parallel those obtained in Experiment 1. The difference in the blood pressures of the two groups was likewise highly significant at the end of the experiment ($P < 0.001$).

Influence of a Partial Deficiency of the Vitamin B_2 Complex on Blood Pressure

Vitamin deficiencies of the completeness represented by the first two experiments seldom occur under ordinary conditions of living. On the other hand, partial deficiencies, particularly of the vitamin B_2 complex, are common. Experiment 3 was therefore designed to determine the effect of such partial deficiencies on the blood pressure.

Experiment 3.—Five groups of 25 rats each were placed on the basic vitamin B-free diet, plus thiamin by dropper. One group received liver extract (controls); a second group received no supplement whatever; a third group received autoclaved yeast in the amount of 2.5 per cent of the diet by weight; a fourth group received a similar supplement of 5 per cent yeast; and a fifth group was permitted to eat yeast as desired.

The results of this experiment are shown in Fig. 4. The group receiving liver extract maintained consistently normal pressures throughout the experiment (average, 118.4 mm. Hg;[7] standard deviation of the mean, 0.676). The group receiving no vitamin B_2 complex whatever showed a consistently elevated pressure throughout (average, 135.4 mm.;[7] standard deviation of the mean, 0.844). The group receiving 2.5 per cent yeast likewise showed a consistently elevated pressure throughout (average, 142.2 mm.;[7] standard deviation of the mean, 0.898). The group receiving 5 per cent yeast also showed an elevated pressure, fluctuating between 135 and 140 mm.[8] The

[7] Snedecor's F test (24) shows that the variations of the weekly averages are no greater than would be expected from the variations of the individual pressures making up the averages. The average of the whole group is therefore used.

[8] The F test shows that the variations of the weekly averages are slightly greater than would be expected from the variations of the individual pressures making up the averages. Pooling the individual readings and striking an average of the whole group is therefore unjustified.

group which was allowed to eat autoclaved yeast as desired, while theoretically provided with an adequate source of the vitamin B_2 complex, refused to eat the fortified diet during the early weeks of the experiment, and during this time their pressure rose to an average of 139 mm.; gradually, however, as they ate the yeast better, their pressures dropped and at the end of the experiment the average was 124.5.[9]

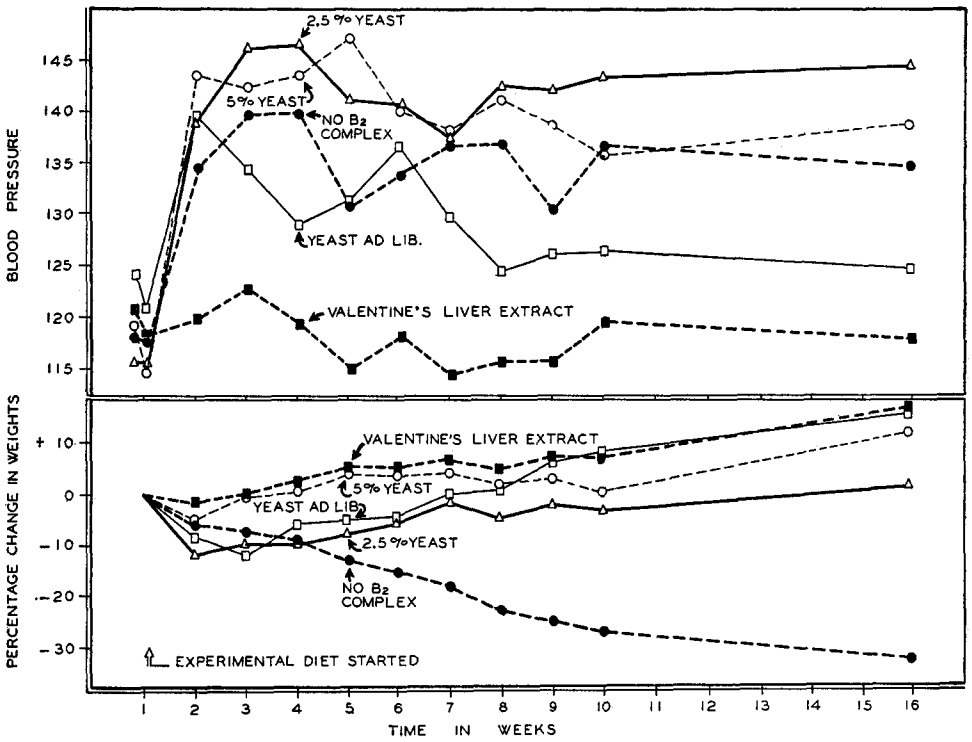

FIG. 4. The effects of partial deficiencies of the vitamin B_2 complex on the blood pressure and weights of rats (Experiment 3).

Fisher's t test shows that the differences in pressures of the groups receiving no vitamin B_2 complex, as well as those receiving 2.5 per cent yeast, are highly significant when compared with the controls ($P < 0.001$). Although the weekly averages cannot be pooled in the case of the 5 per cent yeast group, the t test shows a highly significant difference between each weekly average and the corresponding control ($P < 0.001$). The weekly averages of the

[9] The F test shows that the variations of the weekly averages are considerably greater than would be expected from the variations of the individual pressures making up the averages. Striking an average for the whole group is therefore unjustified.

group receiving yeast *ad libitum* also cannot be pooled. However, according to the *t* test, the differences between this latter and the control group are highly significant during the first 9 weeks of the experiment; but by the 10th week the pressures of this group have begun to fall closer to those of the controls (*P*, 0.02–0.01), and by the end of the experimental period there is no significant difference (*P*, 0.05–0.02).

Correlation of Changes in Blood Pressure and Weight.—A comparison of the blood pressure readings with gain or loss of weight, as indicated in Fig. 4, shows that the blood pressure changes were not related to the general nutritional status of the various animals. For example, the group receiving 5 per cent yeast gained a total of 10 per cent in weight, the group receiving no B_2 complex lost 33 per cent of their weight, and yet the blood pressures of both groups rose to approximately the same levels during the course of the experiment.

Influence of General Undernourishment and Consequent Secondary Vitamin Deficiencies on Blood Pressure

It is generally agreed that the functions of many of the various accessory food factors are so closely interrelated that ascribing a given effect to deficiency of a specific dietary factor or even group of factors is a difficult matter. The part played by secondary vitamin deficiencies in inhibiting intestinal absorption has been especially emphasized by Susan G. Smith (20). She was able to show, for example, that when dogs are maintained on a black tongue-producing diet, they develop black tongue despite concurrent administration of nicotinic acid by mouth; if, however, the nicotinic acid be administered intravenously, the dogs are promptly cured. Obviously, therefore, the black tongue-producing diet causes its characteristic effects not simply because of its inadequate content of pellagra-preventive factor but also because it leads to faulty utilization of this material, due apparently to poor intestinal absorption.

A similar situation has been shown to obtain in the case of vitamin A, for Underhill and Mendel (21) produced typical black tongue by means of a diet which Smith, Persons, and Harvey (22) showed to be deficient in vitamin A. Persons and Brown (23) were able to cure the black tongue so produced by the administration of nicotinic acid; huge doses were required by mouth, but small doses sufficed if given parenterally. Presumably, therefore, a deficiency of vitamin A produces black tongue by interfering with intestinal absorption.

Because of observations of this kind in nutritional studies, it was thought advisable to determine whether similar overlapping of vitamin functions obtained in the case of the dietary components under investigation in this study.

Experiment 4.—Ten pairs of rats of identical weight were selected. One group was placed on the usual vitamin B-free diet supplemented by thiamin. The other group was similarly treated but in addition received liver extract in the amount of 1 ml. per rat per day. The diets of this latter group were carefully weighed, and the total food consumption of each member was restricted to the amount consumed by his paired mate in the first group.

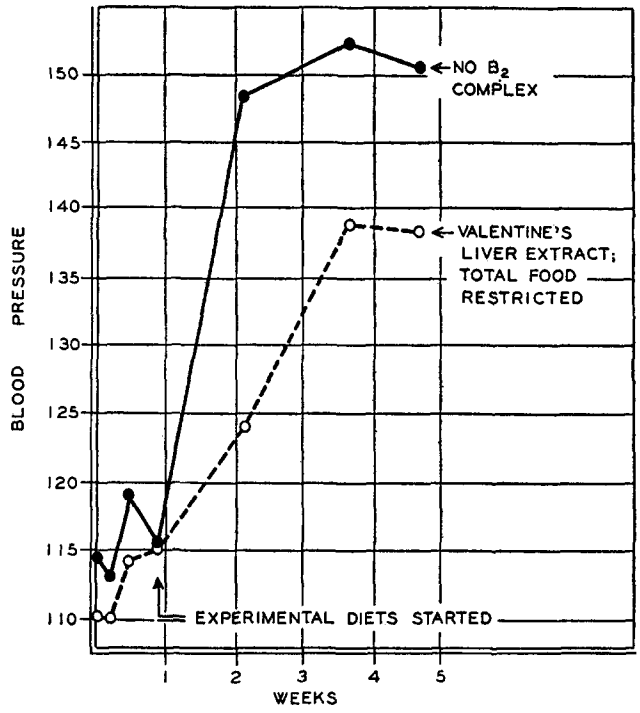

FIG. 5. The effects of restriction of total food intake and consequent secondary nutritional deficiencies on the blood pressure of rats (Experiment 4). Note that the rise under such conditions is slower and less marked than when the rats are deprived entirely of the vitamin B_2 complex.

The weights of the two groups remained practically constant throughout the course of the experiment. The effects on blood pressure are shown in Fig. 5. The rats receiving no vitamin B_2 complex showed the prompt and sustained rise in blood pressure seen in earlier experiments (from an initial average of 116 mm. to approximately 150 mm.). Those receiving a plentiful supply of liver extract but whose total food intake was restricted likewise experienced a rise in blood pressure, though the rise was much slower and less marked than in the former group (from an initial average of 110–115 mm. to 138 mm.).

As stressed by Susan G. Smith (20), relative speed and intensity of effect are important in deciding whether a vitamin is acting in a primary or secondary capacity. On the basis of these criteria, it would appear that the rapid and high rise of pressures seen in the group receiving no vitamin B_2 complex indicates that this complex is acting in a primary capacity; whereas the slower and less marked rise noted in the other group may be the result of secondary nutritional deficiencies.

A Check on the Reliability of the Indirect Method of Determining Blood Pressure

The indirect method of determining blood pressures in the foregoing experiments is subject to question because the preliminary heating introduces

TABLE I

Maximal Blood Pressure of Deficient and Well Nourished Rats As Determined by Insertion of a Cannula into the Abdominal Aorta

Blood pressures of deficient rats	Blood pressures of well nourished rats
mm. Hg	*mm. Hg*
155	135
135	128
135	125
140	125
155	110
135	110
165	120
130	115
	115
Average..... 143.75	120.33

df 15; *t* 4.4938; $P < 0.001$

an abnormal variable. Although this variable was minimized by heating all animals for identical periods of time and was thus a reduplicated error, it seemed advisable to check the accuracy of the indirect method by direct measurements of the pressure in the cannulated abdominal aorta.

Experiment 5.—Two groups of 10 rats each were placed on the usual vitamin B-free diet supplemented by thiamin. The first group received no other supplement, but the second group was given liver extract. After having been on these diets for 5 weeks, the animals were anesthetized by the intraperitoneal injection of nembutal. The abdomen was then opened and a cannula inserted into the abdominal aorta. A solution of heparin was injected to prevent clotting, and the cannula was connected with a mercury manometer.

The results of this experiment are detailed in Table I, in which are recorded the maximal pressures (mean) attained by the animals which survived the

procedure. The average maximal pressure of the deficient group was 143.8 mm. Hg (standard deviation, 12.75), while the average maximal pressure of the well nourished group was 120.3 mm. Hg (standard deviation, 8.57). These differences are of the same magnitude as those obtained by the indirect method used in the preceding experiments. The accuracy of the indirect method is therefore attested.

It was noted incidentally in the course of this experiment that the deficient animals were very intolerant of ether. A few whiffs of this agent, administered for the purpose of deepening the anesthetic effect of nembutal, were sufficient to cause immediate death. It was also observed that when the cannula was first inserted, the blood pressures of the deficient animals tended to be low and to rise gradually as the observations were continued for the ensuing 30 or 40 minutes. In contrast, the pressures of the well nourished group remained relatively constant throughout the period of observation. Presumably, therefore, the deficient animals were much more subject to shock than were the well fed rats.

DISCUSSION

The results of the above experiments indicate that a deficiency of the entire vitamin B complex is followed by a slight fall in the blood pressure of rats. A deficiency of only the heat-stable fractions, on the other hand, is followed by a significant and persistent rise in blood pressure, which can be combated successfully by restoring these factors to the diet. Partial deficiencies are even more effective than are complete deficiencies, probably because of the marked debilitating effects of the latter.

These studies also show that even an excessive intake of the vitamin B_2 complex does not protect the animals completely if the diet is otherwise deficient. It is known from studies on other deficiency states, such as canine black tongue, that various non-specific nutritional inadequacies may often duplicate the pathologic picture of a specific deficiency. There is good evidence that these non-specific nutritional factors exert their effects by interfering with the absorption and utilization of the specific vitamins. It is possible, therefore, that an analogous mechanism may be responsible for the rise of pressure seen in those animals whose total food intake was restricted.

At the present time, knowledge of the functions of the vitamin B complex is so incomplete that it is impossible accurately to define the mechanism whereby these deficiencies mediate a rise in blood pressure. One theoretical possibility is that some types of hypertension may be metabolic in origin, the crucial defect being incomplete oxidation in the kidney as a result of an inadequate supply of respiratory enzymes. Some components of these enzyme systems are an integral part of the structure of the cells themselves; others, such as the prosthetic groups of coenzymes I and II and the flavins,

have to come from exogenous sources. If the intake of these latter components be restricted by dietary manipulation, one would expect that the integrity of those systems to which they contribute would be impaired. As a consequence, the oxidative processes which depend on those systems would be retarded. The disturbance produced in this manner would, of course, be a generalized one, in which the kidney would share. The effect on this organ would be a slowing of the rate at which oxygen is consumed, with resultant impairment of its normal metabolic functions. This is the same physiologic abnormality as that produced by constriction of the renal arteries, though the two mechanisms are obviously different. This idea is advanced not as the proven explanation of the blood pressure rise seen in the present studies, but as a working hypothesis for further enquiries into the etiology and pathogenesis of the hypertensive states.

SUMMARY

The effects of dietary deficiencies on the blood pressure of rats were studied, with especial reference to vitamin B deficiencies. A deficiency of the entire vitamin B complex was followed by a slight fall in blood pressure. A deficiency of only the heat-stable fractions was followed by a significant and persistent rise in pressure, which could be reversed by restoring these factors to the diet. Partial deficiencies were followed by a higher rise of blood pressure than were complete deficiencies, perhaps because of the debilitating effects of the latter.

Even an excessive intake of the heat-stable fractions of the vitamin B complex did not prevent entirely a rise of pressure if the diet was otherwise deficient. Under the latter conditions, the rise in pressure was slower and less marked than in those animals with a deficiency of the vitamin B_2 complex only. It therefore appears that, while a deficiency of the vitamin B_2 complex plays a dominant rôle in causing a rise of blood pressure in rats, other dietary factors as yet undefined are also involved. From analogy with other deficiency states, it is possible that these undefined nutritional factors cause their effects by interfering with absorption and utilization of the vitamin B_2 complex.

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