VITAMINS AND HORMONES

ADVANCES IN RESEARCH AND APPLICATIONS

Edited by

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EDITORS' PREFACE

The reward for the tedious work of an editor of a scientific book comes from the expressions of appreciation by his colleagues. On this account the task of the Editors of *Vitamins and Hormones* continues to be particularly satisfying.

In this seventh volume are included seven articles on the subject of vitamins and five on hormones. The articles on anterior pituitary hormones are especially significant because they contain considerable data heretofore unpublished, representing a marked advance in our knowledge of protein hormones. Similarly, the extensive infrared spectrographic data on the steroids comprises partially unpublished reference material of great value.

With this volume we complete the group of articles on the nutritional requirements of the more important laboratory animals: primates other than man (Volume II); chick and mouse (Volume V); cotton rat and hamster (Volume VI); rat and guinea pig (Volume VII).

It is to be hoped that the present volume will be found as useful as its predecessors. Criticisms and constructive suggestions for future policy will be warmly welcomed.

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CONTENTS

Niacin in Amino Acid Metabolism

By W. A. Krehl, *Nutrition Laboratory, Department of Physiological Chemistry, Yale University, New Haven, Connecticut*

Diet and Aging

By Clive M. McCay, *Laboratory of Animal Nutrition, Cornell University, Ithaca, New York*

The Vitamin Requirements of the Growing Rat

By R. A. Brown and M. Sturtevant, *The Research Laboratories, Parke, Davis & Co., Detroit, Michigan*

Vitamin Requirements of the Guinea Pig

By G. J. Mannering, *The Research Laboratories, Parke, Davis & Co., Detroit, Michigan*

The Chemistry of Gonadotropic Hormones

By Choh Hao Li, *Institute of Experimental Biology, University of California, Berkeley, California*

The Chemistry and Physiology of Adenohypophyseal Luteotropin (Prolactin)

By Abraham White, *Department of Physiological Chemistry, School of Medicine, University of California at Los Angeles, Los Angeles, California*

Infrared Spectrometry Applied to Steroid Structure and Metabolism

By Norman Jones and Konrad Dobriner, *National Research Council of Canada, Ottawa, Canada and Sloan-Kettering Institute for Cancer Research, New York, New York*

Alloxan Diabetes

By C. Cabell Bailey, *Harvard Medical School, New England Deaconess Hospital, and Peter Bent Brigham Hospital, Boston, Massachusetts*

The Chemistry of the Hormones of the Posterior Lobe of the Pituitary Gland

By R. L. Stehle, *Department of Pharmacology, McGill University, Montreal, Canada*

The Actions of the Hormones of the Posterior Lobe of the Pituitary Gland upon the Circulation and the Secretion of Urine

By R. L. Stehle, *Department of Pharmacology, McGill University, Montreal, Canada*

Vitamin P

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CONTENTS

I. Capillary Resistance and Nutritional Status

1. Introductory

Since the studies of Hess and Fish in 1914, it has been held that capillary strength is associated specifically with the intake of ascorbic acid. It is inherent in this view that scurvy, and deficiency states of ascorbic acid short of clear-cut scurvy, are marked by unduly fragile capillaries. This is still the general view today, in spite of a considerable

body of evidence to the contrary which, taken together, seems overwhelming.* The subject has been reviewed in more detail recently by Munro *et al.* (1947), and it is, therefore, unnecessary to reopen the matter again. We agree with their conclusion that "there is very little evidence to associate capillary strength specifically with vitamin C intake in man" and would only add that this statement applies also the guinea pig.

In 1936 Armentano *et al.* reported that Hungarian red pepper and lemon juice contained a factor that could increase capillary strength and reduce capillary permeability in man, but was not ascorbic acid. As a result of animal experiments it was believed that the material responsible for these effects was a vitamin, and, because of its apparent effect on permeability and its presence in paprika, it was provisionally named vitamin P (Permeabilitäts-Vitamin). This claim has since been questioned on two counts, the one that no such substance exists, and the other that its action is pharmacological rather than that of a vitamin.

It is our purpose in this review to consider the evidence bearing upon these points and to summarize what is known about the chemical constitution and physiological properties of many of the materials which have now been reported to produce similar biological effects. The number and variety of these materials compel the view that the term vitamin P embraces a group of compounds not all of which are closely related chemically.

2. Capillary Permeability and Capillary Resistance

The term *capillary permeability* has frequently been used loosely in medical literature. Strictly speaking it should be restricted to that property of the capillary endothelium which is measured in terms of the volume of material passing per unit area per unit time per unit thickness per unit of effective pressure. For clinical purposes, however, such a definition is too rigorous, and capillary permeability may be thought of in terms of the volume of fluid filtered per unit mass of tissue per unit time. Of two methods available for assessing capillary permeability in man, that of Landis *et al.* (1932) was used by Armentano and his colleagues in spite of the fact that its limitations for this purpose had been clearly demonstrated by Landis *et al.* Although it is possible to demonstrate filtration of fluid in the human forearm when the outflow of venous

* For example: Abt *et al.* (1936); Bell *et al.* (1940); Brante (1939); Crandon *et al.* (1940); Difs (1940); Fox *et al.* (1940); Greene (1934); Goettsch (1935); Göthlin (1937); Heinemann (1941); Holland *et al.* (1947); Levcowich and Batchelder (1942); Liebmann *et al.* (1938); McNee and Reid (1942); Molitch (1935); Munro *et al.* (1942); O'Hara and Hauck (1936); Rapaport *et al.* (1940); Scarborough (1940, 1943); Scarborough and Gilchrist (1944); Turpeinen (1943); Weld (1936).

blood has been restricted, the results of successive observations in the same individual are such as to make real differences most difficult to evaluate (see for example Bing, 1938). So far as we know, the second method (Landis and Gibbon, 1933) has not been applied to studying the effect of vitamin P preparations on capillary permeability.

It is furthermore important to distinguish between capillary permeability and *capillary fragility*, which involves the passage of erythrocytes outside the vessels, either through the intercellular cement substance or as a result of the actual rupture of capillary walls. In the literature these terms have frequently been used synonymously whereas in fact the dissociation between them is often striking. Thus, in the various forms of purpura there is ample evidence of the presence of erythrocytes outside the vascular system (purpura), but no sign of the accumulation of fluid (edema). On the other hand tissue damage produced by heat or cold may result in considerable swelling of the injured area and the production of excessive tissue fluid, containing a high concentration of protein without any purpura. Until, therefore, more direct observations of both capillary permeability and capillary fragility have been made simultaneously in the same area, it is probably wise to consider permeability and fragility as different properties of the capillary wall and to judge vitamin P activity on the basis of effectiveness in increasing the capillary strength. For these reasons, and because of the doubt which surrounds many so-called determinations of capillary permeability in the clinic, we shall not refer to such studies again. That gross disturbances of capillary permeability and capillary fragility may occur simultaneously is, however, witnessed by cases of hemorrhagic whealing, where not only fluid rich in protein but also erythrocytes in large numbers are found in the hemorrhagic vesicles.

3. Tests of Capillary Resistance

The terms *capillary resistance*, capillary fragility, and capillary strength may be used interchangeably. The first, suggested originally by Hess, is the more generally used by workers in this field and is, on the whole, to be preferred because this property of the capillary walls is most conveniently examined by determining their ability to resist pressures applied to them. Apart from certain procedures of limited usefulness such as the snake venom reaction of Peck *et al.* (1936) and the "flicking test" of Jones and Tocantins (1933), all tests for assessing capillary resistance may be classified as either positive or negative pressure tests. Positive pressure tests are based upon the fact that when the venous return from a part (limb) is obstructed the pressure within the capillaries below the obstruction rises rapidly. In negative pressure tests the

capillaries are suddenly submitted to sub-atmospheric pressures applied to them through the skin. In both types of test the stress applied is sufficient to rupture capillaries, and the effect becomes evident as minute hemorrhages (petechiae) in the skin. The ease with which such petechiae can be produced serves as a measure of the strength of the capillaries (capillary resistance). It is clear that such tests are confined to the skin by the nature of the procedure.

It is not desirable here to consider in detail the merits and demerits of the many different methods that have been advocated for assessing capillary resistance, especially as the more commonly used have already been briefly dealt with by Munro *et al.* (1947). In the literature the two types of tests are referred to indiscriminately as though they measured the same thing. There is, however, evidence that this is not entirely true. Thus, Bell *et al.* (1942), using the Göthlin positive pressure test which involves venous obstruction for 15 minutes (Bell *et al.* 1940) and the negative pressure method described by Scarborough (1941b), found the correlation between results of the two tests to be barely significant for 142 healthy medical students. Scarborough (unpublished) has also examined the correlation between a rapid positive pressure test (venous obstruction for 5 minutes) and his negative pressure test in 120 healthy adolescents and has obtained correlation coefficients of under -0.3. This is in line with the experience of other workers (see Munro *et al.* 1947) and indicates that care must be exercised in comparing the results obtained by one method with those provided by another. It also increases one's confidence in a conclusion based upon results obtained by both types of test. It is necessary to refer to these discrepancies here because they may account, in part at least, for the different impressions of different workers as to the efficacy of preparations of vitamin P in elevating capillary resistance.

Even when these technical difficulties have been given due regard, there are still several factors for which allowance must be made in evaluating the results of both types of test. Although some of these were appreciated by early workers in this field (for example, Öhnell, 1928), they have frequently not received adequate attention. Among the normal physiological factors that may have to be considered, particularly with negative pressure methods, are diurnal and seasonal differences and variations due to altitude, humidity, age, exercise, skin temperature and menstruation, as well as alterations determined by differences in texture of the skin, method of application of pressure and part of body in which the test is made. Inflammation, fever, hypertension, certain metabolic disorders, such as hyperthyroidism and diabetes and conditions associated with cutaneous erythema may all influence the

results of capillary resistance tests. It is possible that differences in capillary resistance produced as the result of one or more such factors may be responsible for some of the so-called spontaneous variations by which certain workers have been surprised and discouraged. To add to the difficulties of those who approach the problem of vitamin P assay and the study of nutritional or hematological disorders associated with bleeding, there are two other factors which must be taken into account. The first is that any increase in capillary resistance produced as the result of the administration of an active substance is related to the initial level of capillary resistance before dosing (Scarborough and Wokes, 1946). The second is that the extravasation of blood into the tissues is itself followed by a sharp elevation in capillary resistance, which may be sustained for 3 or 4 days (Scarborough, 1941a).

4. Capillary Resistance and Scurvy

Before referring to the evidence for the existence of vitamin P, it is well to consider the background against which the results of more detailed studies should be viewed. The majority of workers have observed that in poorly nourished subjects the capillary resistance is significantly lower than in persons living on adequate diets, whether the tests be made by the positive or the negative pressure method. Although it is true, as emphasized by Munro *et al.* (1947), that capillary resistance is "normal" in at least two-thirds of undernourished subjects, this observation serves at least as well to suggest the desirability of re-evaluating what is to be correctly regarded as a normal capillary resistance as to discredit the facts referred to above, demonstrated so clearly by numerous observers at different times, using different techniques, in groups of healthy school children, children in institutions, students, healthy adults, hospital patients and old men. In frank scurvy positive pressure tests indicate unduly fragile capillaries in some 60 to 70% of cases, and Scarborough (unpublished) has found low capillary resistance in approximately 60% of 73 cases of scurvy by his negative pressure method. An observation that may, in part, account for the fact that a low capillary resistance is not invariably found in scurvy has been made by Scarborough (1941a), who reported that the extravasation of blood into the tissues can elevate capillary resistance.

That the low capillary resistance found in a significant proportion of subjects living on diets containing inadequate amounts of fruit and vegetables cannot be related to their impaired intake of ascorbic acid is strongly suggested by the following facts:

(a) There is no correlation between the plasma concentration of

ascorbic acid and capillary resistance as determined by either positive or negative tests.

(b) There is no significant relation between capillary resistance, measured either by positive or by negative pressure methods, and the results of saturation tests with ascorbic acid.

(c) We have never been able to detect any increase in capillary resistance following the administration of ascorbic acid alone to apparently healthy persons living on inadequate diets or to cases of frank scurvy. Most reports from other workers confirm our experience.

The effect of modifying the diet, as, e.g., by administration of fruit and fruit juices, in elevating the low capillary resistance found in persons subsisting on inadequate amounts of fruits and vegetables is not easy to gauge because the majority of such studies are not adequately controlled. It seems likely, however, that in some 60% of them the capillary resistance has been elevated by such means.

A reasonable interpretation of the results of all these studies is that a diet rich in fruit and vegetables contains some substance or substances that can maintain capillary resistance, and that in its absence capillary resistance tends to be low. On the basis of present concepts of the 'normal' some 60 to 70% of persons living on diets inadequate in this material exhibit signs of deficiency as revealed by unduly low capillary resistance. This substance is not ascorbic acid but, since it occurs in many foods which are themselves sources of ascorbic acid, evidence of ascorbic acid deficiency may often be expected in association with a low capillary resistance. The fact that frank scurvy may be present in certain individuals along with a capillary resistance that is not abnormally low may then be regarded as an expression of the balance between their needs and their intakes of the two materials. Whether this explanation or that advanced above be the more important in accounting for a high capillary resistance in certain cases of scurvy cannot be decided until the behavior of the capillary resistance during the onset of scurvy has been more closely studied. It may be mentioned, however, that Crandon, *et al.* (1940) found no change in capillary resistance by either a positive or a negative pressure method during the development of experimental scurvy in one subject and yet it is difficult to point to any source of vitamin P in their experimental diet.

5. "Citrin" and Hesperidin in Therapy

In 1936 Armentano (a and b) described in patients with bleeding diseases favorable therapeutic effects from the use of lemon juice and preparations of paprika ("vitapric") rich in ascorbic acid. Armentano *et al.* (1936) were struck by the observation, however, that similar results

could not be obtained with pure ascorbic acid. Considering, therefore, that some other active material must have been present in the fruit, they were able to obtain by repeated lead and barium precipitation an active fraction which was finally crystallized from methanol in a yield of about 2 g. from 200 kg. of lemons, or 70 liters of lemon juice. This material, which was believed to contain a mixture of flavones, was called "citrin" (citrus flavone). It was used in the treatment of seventeen patients, many of whom had a low capillary resistance as measured by a negative pressure test (Borbély, 1930). In four cases of thrombocytopenic purpura and in two of diabetes, "citrin" was without effect on capillary resistance, but in three cases of vascular purpura (with normal thrombocyte counts), one of myxedema and seven of various forms of infection, the capillary resistance was increased in all but one. In the cases of vascular purpura there was, in addition, a pronounced amelioration of the disease. In many of the more recent clinical studies on the effect of "citrin" and similar materials on capillary resistance and the bleeding diseases, due allowance has not been made for spontaneous variations in capillary resistance and spontaneous remissions of the disease. It is therefore well to emphasize that Armentano *et al.* were alive to this possibility, and by their selection of chronic cases and repeated determinations of capillary resistance anticipated this criticism of their work. There is no information in their report to show whether the patients were on inadequate diets before and during the treatment, but it is clear that in several cases the capillary resistance, although elevated by the administration of "citrin," was not abnormally low before treatment began. Bell *et al.* (1940) were unable to show that vitamin P (hesperidin) significantly increased the capillary resistance of apparently healthy persons with low capillary resistance (positive pressure test).

Struck by the apparent effect of hesperidin (a flavanone glycoside possessing vitamin P activity) in arresting the subcutaneous bleeding in three cases of arsenic or bismuth intoxication occurring in patients who had subsisted on inadequate diets for prolonged periods, Scarborough and Stewart (1938) studied the effects on capillary resistance (positive pressure method) of repeated periods of hesperidin administration in three cases with multiple vitamin deficiency disorders. The initial capillary resistance in all three was low before treatment began and was elevated in each by hesperidin. Discontinuance of hesperidin treatment was followed by a fall in capillary resistance. Similar results (Scarborough, 1940) were obtained in five cases, all with evidence of vitamin deficiency disorders and all with low capillary resistance (positive pressure test), again (Scarborough, 1945) in nine similar cases (negative pressure test) and in two cases of scurvy (1944, negative pressure test). The

only other workers who appear to have studied the effect of vitamin P preparations in cases of nutritional deficiency are Lazarus *et al.* (1948), using the negative pressure method of Scarborough: they got slight and inconsistent increases in capillary resistance following the administration either of ascorbic acid or of preparations of vitamin P.

The observation of Armentano and his colleagues that vitamin P preparations are capable of increasing capillary resistance in cases of vascular purpura has been repeated by Rusznyák (1937), Armentano (1940), Barcaglia (1940), Scarborough (1942a and unpublished cases) and Parrot *et al.* (1944). In allergic purpura, elevation of capillary resistance following treatment with vitamin P has been described by Jersild (1938), Hiramatsu (1939) and Kugelmass (1940). In other patients with unduly fragile capillaries, vitamin P preparations have also elevated capillary resistance (Jersild, 1939; Horne and Scarborough, 1940; Rapaport and Klein, 1941; Vacek, 1941; Griffith *et al.*, 1944; Shanno, 1946). Finally Hiramatsu (1941a) has reported greatly diminished bleeding after intraperitoneal hesperidin administration (50 mg. doses) in both thrombopenic and vascular purpura, especially the latter, produced by injection of antiplatelet and antiendothelial sera, respectively, into guinea pigs. These studies suggest that preparations of vitamin P can elevate the low capillary resistance found in persons subsisting on inadequate diets and in patients suffering from certain forms of purpura. That the action of such preparations is that of a vitamin has not been shown so clearly.

6. Effect of Vitamin P on Scorbutic Animals

The original suggestion that the action of "citrin" was that of a vitamin was based on observations of Armantano *et al.* (1936) that a group of scorbutic guinea pigs given the flavone preparation outlived a control group not given "citrin." Bentsáth *et al.* (1936, 1937) later reported that the survival time of guinea pigs fed the Sherman-La Mer-Campbell scorbutogenic diet was increased from an average of 28.5 to an average of 44 days when supplements of vitamin P ("citrin") were added to the diet. At autopsy the animals which had received "citrin" showed significantly fewer tissue hemorrhages than did their controls. It was concluded, therefore, that scurvy as ordinarily produced in the guinea pig represents a combined deficiency of ascorbic acid and vitamin P. Zacho (1939) and Todhunter *et al.* (1940) also reported that the amount of hemorrhage at autopsy was less in guinea pigs receiving sources of vitamin P than in animals maintained on the same scorbutogenic diet without supplements. It has, however, been shown by Zilva (1937) that the post-mortem findings described by Bentsáth *et al.* in the

animals that developed scurvy on a scorbutogenic diet plus supplements of vitamin P closely resemble the condition found in guinea pigs receiving minute doses (0.1 mg. daily) of ascorbic acid as supplements to a scorbutogenic diet. In dietary experiments with guinea pigs neither Zilva (1937) nor Moll (1937) could find any evidence for the existence of a vitamin P and the Hungarian group (Bentsáth and Das, 1937) and Szent-Györgyi (1937, 1938) have subsequently reported failure to repeat the guinea pig experiments upon which the discovery of a capillary resistance-increasing vitamin was principally based. Inability to delay or modify the onset of scurvy in guinea pigs by giving them supplements of vitamin P has been noted also by Detrick *et al.* (1940), Parrot *et al.* (1946a) and Hiramatsu (1940). The idea that vitamin P can modify the course of scurvy in the guinea pig, and that this disorder is due to a mixed deficiency of ascorbic acid and vitamin P, must therefore be discarded. It receives no support from clinical observations (for example, Scarborough, 1940).

In none of the above studies were any measurements made of capillary resistance. Zacho (1939) observed that in guinea pigs maintained on a scorbutogenic diet the capillary resistance fell progressively. The initial normal levels could not be restored until supplements of vitamin P ("citrin") were added to the diet. Bacharach *et al.* (1942) also found a fall in capillary resistance in guinea pigs maintained on scorbutogenic diets supplemented with ascorbic acid. This lowered capillary resistance was elevated by vitamin P, which was found in many fruits and vegetables (Bacharach and Coates, 1943) after these studies had led to an acceptable method for the biological assay of vitamin P potency (Bacharach and Coates, 1942). Using a slightly different technique, Bourne (1943b) has also shown that there was a marked fall (of at least 50%) in the capillary resistance of 54 guinea pigs on a scorbutogenic diet supplemented with 10 mg. per head per day of ascorbic acid. After five daily doses each of 10 mg. of "citrin" all fifteen animals tested showed increases in capillary resistance of between 60 and 100%. In those animals to which no "citrin" was given the capillary resistance remained at its low level. When citrin treatment was discontinued the animals which survived developed low capillary resistance again in 1 to 4 weeks. This work represents the most convincing evidence for the existence of a substance or group of substances which increases capillary resistance and strongly suggests that the action is that of a vitamin, at any rate for the guinea pig. Bourne's observations have been confirmed by Hughes and Parkes (1946) who found that in guinea pigs on a diet lacking greenstuff, but to which was added 5 mg. of ascorbic acid per animal per day, initially there might be no response to a negative pressure of 600 mm. Hg,