VITAMINS AND HORMONES

ADVANCES IN RESEARCH AND APPLICATIONS

Edited by

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Editors' Preface

The continued success of VITAMINS AND HORMONES indicates that these volumes are meeting a need for critical summaries of the progress of vitamin and hormone research.

Only those who have attempted to write a critical scientific review realize how much intense work and careful thought have entered into the preparation of the chapters contained in the present volume. The material has been gleaned from a variety of chemical, physical, biological and medical journals, then cautiously digested and carefully presented. The Editors wish to express their appreciation to each author for his contribution to the success of this publication.

Book reviews of previous volumes have offered constructive criticisms that have been helpful to the Editors in planning for future volumes of VITAMINS AND HORMONES. To some reviewers and readers it has seemed that each volume is a series of disconnected articles. Since there are many vitamins and hormones and since the research on each must be reviewed from the chemical, biological and clinical viewpoint before the subject is completely covered, the contents of any one volume must necessarily be somewhat unrelated. The subject matter of successive volumes will integrate more and more until VITAMINS AND HORMONES eventually becomes a complete reference to all active research in the vitamin and hormone field. Meanwhile, each chapter will serve as a thorough summary of progress in a small area of these fields and point the way toward which future research should be directed.

ROBERT S. HARRIS
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August, 1945
Cambridge, Massachusetts

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The Interrelation of Vitamins

By THOMAS MOORE

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I. INTRODUCTION

In discussions on the possibility of setting up standard values for the requirements of vitamins by man and animals most opinions lie between two extremes. According to one school of thought the problem presents little difficulty. On the principle that the speed of a navy is that of its slowest ship the deciding factor in determining the adequacy of any diet will, in the long run, be that vitamin which is present in the least amount in relation to the physiological requirement. The provision of extra supplies of other nutrients will not afford any compensation, and the requirement of each vitamin may therefore be considered separately as a simple question concerning that vitamin only. At the other extreme, the view is held that the requirement for one vitamin will be influenced so profoundly by the intakes of others that it is impossible to fix a minimum requirement which will remain constant when the other constituents of the diet are changed. Thus the standard value for the requirement of one particular vitamin, as estimated under experimental conditions with a basal diet which is intended to contain adequate amounts of all other vitamins, is considered to be liable to grave inaccuracy when the basal ration is replaced by an ordinary mixed diet of uncontrolled vitamin content.

The purpose of this article is to collect evidence on the "interrelation" of vitamins. This term has been widely interpreted to include not only instances in which the intake of one vitamin influences numerically the requirement of another, but also cases in which vitamins participate in related physiological or biochemical systems, or are concerned in the development and maintenance of the same tissues. Some notes on terminology may not be out of place. "Interrelation" has been used in a general sense to cover all aspects of interplay between vitamins. "Association" implies that two vitamins are concerned in the same sphere of influence, whether defined physiologically, biochemically, or morphologically, but does not infer a direct chemical or physiological connection, or imply that the requirement of one is necessarily influenced by the intake of the other. "Interaction" is reserved for instances in which a direct connection, either chemical or physiological, between two vitamins has been proved. For interaction in which the requirement of one vitamin is reduced by a liberal intake of another the term "synergism" as used by Hickman (1) may be appropriate. The term "secondary deficiency" means a deficiency of vitamin which is not merely due to a low dietary intake, but which is "conditioned" or aggravated by some other factor, such as an inadequate supply of another vitamin. It is not used in the sense accepted by some workers to designate the less conspicuous of two superimposed deficiencies, for which "concurrent" or "minor" would seem to be more apt descriptions.

Since no serious attempt to deal with the interrelation of vitamins seems hitherto to have been made, the author in his search for material has been denied the usual assistance afforded by the bibliographies of previous reviews. Apologies are offered to the authors of any relevant work which has inadvertently been overlooked.

II. The Interrelation of Vitamins in Vitro

It will be convenient to discuss instances of interrelations between vitamins under separate headings according to the circumstances under which they have been observed. Clearly, however, such classification will often depend on the particular aspect of the complete story which has so far come to light. An interrelation which has already been found *in vivo* may later be found *in vitro*, or *vice versa*, as indeed has already occurred in one instance. Classification is therefore based on the evidence at present available, and the distinctions drawn between different types of interrelation may not always be genuine.

1. Carotene, Vitamin A, Linoleic Acid, Tocopherol, and Ascorbic Acid

The potency of the tocopherols as antioxidants enables them to protect many of the readily oxidizable constituents of fats, including carotene and vitamin A. Some of the work in this field was reviewed briefly by Mason in Volume 2 of this series (1944). Golumbic (2) studied the action of tocopherols in stabilizing animal fats and related substances, and found that their antioxidant power was shared by hydroxychromans and hydroxycoumarans. Quackenbush, Cox, and Steenbock (3) found that α-tocopherol protected carotene when dissolved in ethyl linolate. It may be recalled that the oxidation of carotene in purified esters, with a corresponding loss in biological activity was reported many years ago by Hume and Smedley-Maclean (4). According to Quackenbush and his colleagues, tocopherol was no better than hydroquinone as an antioxidant for carotene *in vitro*, but was much more effective in protecting the carotene, and so securing full biological activity, when the solution in ethyl linolate was administered to rats. This relatively greater power of tocopherol in protecting carotene in the alimentary tract was ascribed to its remaining with the fatty phase during digestion, whereas hydroquinone would presumably be largely separated into the aqueous phase. Recently Hove (5) extracted a lipoxidase, capable *in vitro* of oxidizing carotene in the presence of linoleic acid, from the stomach of rats, but not from their intestines. This enzyme is inhibited by α-tocopherol. Hilditch (6) has found that tocopherol protects preformed vitamin A in margarine when it is exposed to air at room temperature.

Another vitamin which may be concerned in the oxidation of fats is ascorbic acid, which according to Golumbic and Mattill (7) enhances the activity of tocopherols in preventing the oxidation of certain vegetable fats. Aqueous colloidal solutions of carotene, without tocopherol, are also protected by ascorbic acid (8). It is clear, therefore, that the vitamins mentioned, and also the "essential" linoleic acid, may interact in fatty mediums either as substances liable to oxidation, or as antioxidants. It seems probable that future research will reveal other instances of interaction in fatty media, and possibly will implicate other vitamins. Already Pavcek and Shull (9) have shown that biotin is inactivated by rancid fats, from which it is protected by tocopherol. While in some cases the chemical behavior of the vitamins in fats may be related to their physiological activity, in others their reactions when exposed to oxidative influences may merely conform with those of the other constituents of the fat.

2. Asçorbic Acid and Riboflavin

Kon and Watson (10) found that the exposure of cows' milk to light caused a rapid destruction of ascorbic acid. Since the effective rays were found to be in the visible region, in which ascorbic acid has no absorption, it was obvious that the reaction was activated by some other substance or substances capable of absorbing visible light. Later it was found by Hopkins (11) that ascorbic acid is rapidly destroyed when exposed to sunlight in the presence of riboflavin. The riboflavin itself was converted to lumichrome and other products, which were however still capable of acting as sensitizers for the oxidation of ascorbic acid. Hand, Guthrie, and Sharp (12) showed that the photochemical oxidation of ascorbic acid in milk was due to the presence of riboflavin and could be prevented by the removal of oxygen.

III. The Association of Vitamins in Function

1. Ascorbic Acid, Vitamin P, and Capillary Resistance

The best example of combined action of vitamins in performing a single function, if the evidence so far available may be accepted, is perhaps to be seen in the interaction of ascorbic acid and vitamin P in preventing capillary hemorrhage. The early attempts of Bentsath, Rusznyak, and Szent-Györgyi (13, 14) to demonstrate that guinea pigs needed an additional vitamin to supplement the action of ascorbic acid did not carry conviction. Zilva (15) and Moll (16) were both unable to confirm the claims of Szent-Györgyi and his colleagues, who was himself unable to repeat his experiments (17). Scarborough (18) and Zacho (19), however, confirmed the action of vitamin P, in the form of citrin or other flavone pigments, in maintaining capillary resistance. Citrin alone considerably increased capillary resistance, but the simultaneous presence of both citrin and ascorbic acid was said to be necessary for the maintenance of resistance at its normal level. Bacharach, Coates, and Middleton (20) have proposed the introduction of a biological test in which capillary resistance in guinea pigs given adequate doses of ascorbic acid was found to vary consistently with graded doses of vitamin P, which was given either as crystalline hesperidin, or as an aqueous extract of orange peel and pulp. An experiment in the converse direction, to find whether capillary resistance is correlated with the ascorbic acid intake when vitamin P is controlled at an adequate level, seems highly desirable.

2. Vitamin A, Riboflavin, and Ascorbic Acid in Dark Adaptation

The preservation of normal powers of dark adaptation is another function in which, according to some workers, more than one vitamin is concerned. Kimble and Gordon (21) found that most individuals in their experiments who had poor dark adaptation and a low level of vitamin A in the blood could be brought into the normal ranges for both these criteria by dosing with vitamin A. Other individuals, however, failed to show the usual response. This led to the belief that other factors must be involved in the utilization of vitamin A for the synthesis of visual purple, and both riboflavin and ascorbic acid were tried as supplements to the action of vitamin A. Several otherwise normal subjects with poor dark adaptation and low blood vitamin A were given 200,000 I.U. of vitamin A daily as halibut liver oil, but failed to respond. It is perhaps rather difficult in the