VITAMINS AND HORMONES

ADVANCES IN RESEARCH AND APPLICATIONS

Edited by

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Editors' Preface

With the end of the war and the gradual untangling of the biological and medical problems of the postwar world, we shall doubtless see a large upsurge in the volume of research. The return of workers to peace-time research from their temporary diversion to war problems, and the reinstatement of universities and research institutions abroad will both contribute to this. The need for critical reviews in this and other fields will, therefore, doubtless prove greater than ever.

The reestablishment of normal international scientific relations will also make it possible for Vitamins and Hormones to reflect more fully world scientific opinion. Despite the influence of war conditions on the first four volumes, contributions have been published from England (several), Switzerland, Palestine, Argentina and Australia, (besides the United States and Canada), and it is our hope that a larger number of colleagues abroad will be able to participate in the future.

An interesting feature of the present trend is the increasing interrelationship between vitamin and hormone research. This is exemplified in three of the articles in the present volume, and it justifies the Editors' initial feeling that the bringing of reviews of these two fields under one cover would prove realistic and helpful.

The present volume has been compiled in the unsettling "aftermath" atmosphere, and delays and difficulties have been inevitable. The Editors wish to express their thanks to the contributors, whose patience and concentration under such conditions has led to the production of very valuable reviews.

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I. Introduction

Many years ago Castle (17, 95), in his work on pernicious anemia, demonstrated a parallel distribution in nature of the "extrinsic factor" and the vitamin B complex. Since that time an extensive literature, largely clinical, has sprung up on the relationship of various known and unknown members of the B complex to hematopoiesis. In recent years numerous studies in the fields of animal and bacterial nutrition, carried on in many different laboratories, have yielded results which direct attention to a group of new compounds which are intimately concerned with growth and the formation of both red and white blood cells. This article represents an attempt to correlate the findings of these recent studies in the light of present day knowledge and to review the field with particular emphasis on the problem of identification and chemical and nutritional interrelation of these newer hemopoietic factors. No effort has been made to survey the literature on the relationship of the better known members of the B complex to hemopoiesis nor on the general problems of the nutrition of the chick, rat, monkey or lactic acid bacteria.

In retrospect it would appear that the earliest observations on the hemato-

poietic activity of this group of then unknown substances were made by Lucy Wills (111) in 1931. She observed the striking effect of liver and yeast extracts on the macrocytic anemia of pregnancy which occurs commonly in India. The effect was not obtained with purified antipernicious anemia principle. She and Bilimoria (113) reproduced the nutritional deficiency in monkeys. These latter observations were extended by Day and his co-workers (24) and others (118, 104). Because of difficulties of assay in man and monkeys little progress was made in concentrating the active factors. Observations on the development of nutritional anemia in chicks (32, 54, 70) speeded up isolation work which was accelerated by the application of microbiological (70, 87, 62, 92, 37) and enzymatic-microbiological methods (5, 71).

In the literature these newer hematopoietic and related factors have been referred to as vitamin M, norit eluate factor, vitamin Bc, folic acid, *Streptococcus lactis* R factor, *Lactobacillus casei* factor, new *Lactobacillus casei* factor, vitamins B_{10} and B_{11}, vitamin Bc conjugate, *Streptococcus lactis* R stimulating factor, potential *Streptococcus lactis* R stimulating factor, potential folic acid, and folic acid complex. These terms were adopted for convenience by various groups of workers to indicate a substance or substances which could be defined by some measurable biological effect. Certain of these factors have been isolated as crystalline compounds, some have been obtained as concentrates, while still others are known only in crude natural vitamin carriers. Analysis of the literature is rendered more difficult, particularly for those not actively working in the field, by the fact that some workers have adopted the terms of others and altered the connotation. When different avenues of research, each with its own terminology, become confluent it is to be expected that there will be a certain temporary confusion in nomenclature. Although certain of the above factors are known as chemical entities and identity in some instances suspected, in no case has the identity of any two been unequivocally established by accepted chemical methods. In reviewing the facts, therefore, the authors will try as much as possible to employ the terminology adopted by those whose results are under discussion. In this way it is hoped to avoid further confusion which might arise as a result of premature assumption concerning chemical identity. The development of a system of nomenclature acceptable both to chemists and physiologists will no doubt follow in the wake of further chemical progress.

II. Norit Eluate Factor

In 1939 Snell and Peterson (86) reported in abstract form that liver or yeast extract was necessary for the growth of *L. casei* in a hydrolyzed casein medium. Earlier in their studies on the nutritional requirements of this and related organisms they had demonstrated the indispensability

of riboflavin, pantothenic acid and nicotinic acid for growth. They found that liver or yeast extract could be separated into two indispensable fractions by treatment with norit in acid solution and elution of the adsorbate with pyridine-alcohol mixtures. A number of properties of the factor in the eluate were given at that time. The following year (87) they pointed out in their detailed paper that the fraction not adsorbed by norit could be largely replaced in the medium by pyridoxine but that the filtrate also contained some other unknown growth factor. This second filtrate factor they later showed to be biotin (35). They were unable to find a known compound which would give a growth response comparable to that obtained with the norit eluate, and they referred to the unknown substance(s) as the norit eluate factor. The best sources were liver, yeast, malt sprouts and cereal grains. A study of the properties of the factor in yeast concentrates led them to suggest that the substance was a rather strongly basic compound, having some acidic properties and possibly being of a purine nature. Their purest preparation produced half maximum fermentation in a concentration of 0.055 γ per cc. of medium. In a subsequent paper in 1941 Hutchings, Bohonos and Peterson (35) described a simplified method of concentrating the norit eluate factor in liver extract about 100 to 200 times. They showed that the active principle could be inactivated with ethanolic HCl and that the activity could be regenerated in 50% yield with sodium carbonate. Along with this evidence, pointing to the presence of a carboxyl group in the norit eluate factor, they also presented evidence indicating the presence of an amino group since their concentrate lost activity on treatment with nitrous acid, acetic anhydride and benzoyl chloride. A number of other properties of the factor were given but no preparation was described which had greater activity than the products described a year previously. Hutchings *et al.* (34) demonstrated that concentrates of the norit eluate factor contained a chick growth factor and that the concentration of both factors ran parallel, that is, they were both adsorbed on norit and superfiltrol and could be eluted with ammonia in aqueous alcohol. Inactivation experiments demonstrated that the norit eluate factor and the chick growth factor were sensitive to the same reagents.

Peterson and his students (35) used *L. casei* as the test organism in their fractionation work. They recognized, however, the necessity of the norit eluate factor for the growth of *Lactobacillus delbrückii*, *Propionibacterium pentosaceum* and *Streptococcus lactis* R[1].

[1] Krueger and Peterson (45) have recently called attention to the work of Niven and co-workers (66) who have demonstrated that *Streptococcus lactis* R is an enterococcus, specifically *Streptococcus fecalis*. During the past few years the term *Streptococcus lactis* R and the initials SLR have been incorporated into the designation for several unidentified nutritional factors. For the sake of clarity in discussing these factors the term *S. lactis* R is used throughout this article.

III. Folic Acid

In 1941 Mitchell, Snell and Williams (62) reported the preparation of a concentrate from spinach which was very active in stimulating the growth of *S. lactis* R. The basis of their test medium was a hydrolyzed casein digest similar to that employed by Snell and Peterson (87). The medium was supplemented with a number of purines and pyrimidines including adenine, guanine, xanthine and uracil (85, 61).

In concentrating the growth factor Mitchell *et al.* (62) used methods involving successive adsorptions and elutions from norit, fractionation of lead and silver precipitates, followed by chromatographic fractionation on fullers' earth. Their most active preparations produced half maximum growth in a concentration of 0.00012 γ per cc. These workers felt that they had a growth factor in nearly pure form and suggested the name *folic acid* for the factor since their source material was green foliage. Folic acid was defined as "the active principle required for the growth of *S. lactis* R under specified conditions" (85). Their concentrates also stimulated the growth of *L. delbrückii* and *L. casei*. When fed to rats their spinach concentrates appeared to have a slight effect on the rate of growth but the limited number of test animals rendered the observations of questionable significance.

In a series of papers which appeared in 1944 Mitchell, Snell and Williams (63, 27, 64, 59) presented the results of their fractionation work in detail. Starting with large quantities of fresh spinach, they succeeded in concentrating the *S. lactis* growth activity to a point where the product was 137,000 times as active as their microbiological standard (Wilson's Liver Extract B).[2] Products of such high potency however were not characterized. The best concentration procedure involved repeated adsorption on charcoal and elution with aqueous ammonia or aniline. This was followed by precipitation of the activity with lead and regeneration of the precipitate with ammonium sulfate; precipitation of the silver salt and regeneration with ammonium chloride; adsorption on fullers' earth at pH 1 and elution with ammonia water; adsorption on alumina and fractional elution with dilute methanol and dilute methanol containing 2% of ammonia. Further purification was effected by chilling an acidified aqueous solution of the concentrate. The insoluble fraction was again chromatographed on alumina. The more potent eluates were combined and sub-

[2] According to Williams' method (109) of expressing potency of folic acid concentrates, crystalline vitamin Bc has a potency in the neighborhood of 200,000. To convert assay results in the literature expressed in terms of "folic acid of potency 40,000" into terms of crystalline vitamin Bc it is necessary to divide by 5. If the microbiological growth activity in the spinach concentrates is due to a single compound and if that compound (folic acid) is identical with crystalline vitamin Bc from liver then material of potency 137,000 would represent a product of about 65–70% purity.