

VITAMINS AND HORMONES

ADVANCES IN RESEARCH AND APPLICATIONS

Edited by

ROBERT S. HARRIS *and* KENNETH V. THIMANN

Professor of Biochemistry of Nutrition,
Massachusetts Institute of Technol-
ogy, Cambridge, Mass.

Associate Professor of Plant Physiology,
Harvard University,
Cambridge, Mass.

VOLUME IV

1946

CONTRIBUTORS TO VOLUME IV

- MORTON S. BISKIND, *Endocrine Laboratory and Clinic, Beth Israel Hospital, New York, New York*
- F. W. CLEMENTS, *Division of Nutrition, Australian Institute of Anatomy, Canberra, Australia*
- ROY HERTZ, *National Institute of Health, Bethesda, Maryland*
- ALBERT G. HOGAN, *Department of Agricultural Chemistry, University of Missouri, Columbia, Missouri*
- BERNARDO A. HOUSSAY, *Instituto de Fisiologia, Universidad de Buenos Aires, Buenos Aires, Argentina*
- CHARLES D. KOCHAKIAN, *Department of Physiology and Vital Economics, School of Medicine and Dentistry, University of Rochester, Rochester, New York*
- J. J. PFIFFNER, *Research Laboratories, Parke Davis and Company, Detroit, Michigan*
- E. P. REINEKE, *Michigan State College of Agriculture and Applied Sciences, East Lansing, Michigan*
- HOWARD A. SCHNEIDER, *Rockefeller Institute, New York, New York*
- SIDNEY A. THAYER, *Laboratory of Biological Chemistry, St. Louis University School of Medicine, St. Louis, Missouri*

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.

KENNETH V. THIMANN
ROBERT S. HARRIS

August, 1946

CONTENTS

	Page
Contributors to Volume IV	v
Editors' Preface	vii

The Newer Hematopoietic Factors of the Vitamin B-Complex

BY J. J. PFIFFNER AND ALBERT G. HOGAN, *Research Laboratories, Parke Davis and Company, Detroit, Michigan, and Department of Agricultural Chemistry, University of Missouri, Columbia, Missouri*

I. Introduction	1
II. Norit Eluate Factor	2
III. Folic Acid	4
IV. <i>Lactobacillus casei</i> Factors	5
V. <i>Streptococcus lactis</i> R Factor	7
VI. Vitamin Bc	8
VII. Vitamin Bc Conjugate	11
VIII. Vitamin Bc Conjugase	13
IX. Other Nutritional Antianemia Factors	15
a. Vitamins B ₁₀ and B ₁₁	15
b. Factors R and S	16
c. Factor U	18
d. α - and β -Pyracins	18
e. Extrinsic Factor	19
X. Vitamin M and the Potential <i>Streptococcus lactis</i> R Stimulating Factor	19
XI. Relation of Sulfa Drugs to Nutritional Role of the Newer Hematopoietic Factors	22
XII. Xanthopterine	25
XIII. Summary	29
References	31

Nutrition and Resistance to Infection: The Strategic Situation

BY HOWARD A. SCHNEIDER, *Rockefeller Institute, New York, New York*

I. Introduction and Definitions	35
II. Nutrition and Susceptibility to Infection	41
III. Nutrition and Natural Resistance to Infection	47
1. Inanition	48
2. Malnutrition	49
a. Vitamin A	50
b. Vitamin B Complex	53
c. Vitamins C and D	57
d. Other Dietary Items	57
IV. Nutrition and Actively Acquired Resistance to Infection	62
V. Nutrition and Passively Acquired Resistance to Infection	62
VI. Nutrition and Processes Regarded as Contributing to Resistance to Infection	63
1. Nutrition and Antibody Formation	63
2. Nutrition and Phagocytic Activity	63
3. Nutrition in Relation to Serum-Complement	64

VII. Strategy and Prospects	64
References	68

Manifestations of Nutritional Deficiency in Infants

By F. W. CLEMENTS, *Division of Nutrition, Australian Institute of Anatomy, Canberra, Australia*

I. Introduction	73
II. Undernutrition	74
Clinical Manifestations of Deficiency	74
III. Protein	75
1. Physiology	75
2. Sources of Protein in Infancy	76
3. Biochemical Pathology of Deficiency States	76
4. Clinical Manifestations of Deficiency	76
IV. Water	78
1. Physiology	78
2. Sources and Requirements	78
3. Pathology	78
4. Clinical Manifestations of Deficiency	79
V. Vitamin A	79
1. Physiology	79
2. Sources of Vitamin A in Infancy	81
3. Pathology of Deficiency States	82
4. Biochemical Pathology of Deficiency States	83
5. Clinical Manifestations of Deficiency	83
a. The General Signs	84
b. Changes in the Eyes	84
c. Changes in the Skin	85
d. Other Systems	85
6. Relationship of Vitamin A Deficiency to Local Infections	86
VI. Thiamine	86
1. Physiology	86
2. Sources of Thiamine in Infancy	87
Requirements of Infants	87
3. Pathology of Deficiency States	88
4. Biochemical Pathology of Deficiency States	88
5. Clinical Manifestations of Deficiency	89
a. Partial Deficiency of Thiamine	89
b. Infantile Beri-beri	89
6. Radiographic Appearance of the Heart in Beri-beri	91
7. Electrocardiograph Tracings	91
VII. Riboflavin	91
1. Physiology	91
2. Sources of Riboflavin in Infancy	91
Requirements of Riboflavin in Infancy	91
3. Pathology of Deficiency States	91
4. Biochemical Pathology of Deficiency States	91
5. Clinical Manifestations of Ariboflavinosis	91

	<i>Page</i>
VIII. Niacin.	95
1. Physiology	95
2. Sources of Niacin in Infancy	96
Requirements of Niacin in Infancy.	96
3. Pathology of Infantile Pellagra	97
4. Biochemical Pathology in Infantile Pellagra	98
5. Clinical Manifestations of Infantile Pellagra	98
a. Age Incidence	98
b. Prodromal Signs	98
c. Skin Manifestations	99
d. Nervous Signs	100
IX. Ascorbic Acid	100
1. Physiology	100
The Relationship of Ascorbic Acid to Amino Acid Metabolism.	101
2. Sources of Ascorbic Acid in Infancy	101
Ascorbic Acid Requirements of Infants	102
3. Pathology of Deficiency States	103
4. Biochemical Pathology of Deficiency States	103
a. Plasma Ascorbic Acid	103
b. Serum Phosphatase in Scurvy	103
c. Serum Protein in Scurvy	103
5. Clinical Manifestations of Deficiency.	104
a. Age Incidence	104
b. Subclinical Scurvy	104
c. Clinical Scurvy	104
d. Limbs	105
e. The Ribs	105
f. Hemorrhages	105
g. Cardiorespiratory Sign	106
h. Anemia in Scurvy	106
6. Relationship of Ascorbic Acid Deficiency to other Diseases	106
a. Wound Repair	106
b. Union of Fractures	106
c. Infections	106
7. Radiographic Appearance of Bones in Scurvy	107
X. Vitamin D	107
1. Relevant Features of Calcium Metabolism	107
2. The Sources of Vitamin D in Infancy	108
Requirements of Vitamin D in Infancy	109
3. Pathology of Deficiency States	109
a. Bone.	109
b. Teeth	109
4. Biochemical Pathology of Deficiency States	109
a. Serum Calcium	109
b. Serum Phosphate	110
c. Serum Magnesium	110
d. Serum Phosphatase	110
5. Clinical Manifestations of Deficiency.	111
a. General Signs	111
b. Bony Changes	111
c. Nervous Disturbances	113

	<i>Page</i>
6. Radiographic Appearance of Bones in Rickets	113
XI. Vitamin E	114
XII. Vitamin K	114
1. Physiology	114
2. Sources of Vitamin K in Infancy	115
Requirements of Vitamin K in Infancy	116
3. Pathology of Deficiency States	116
4. Clinical Manifestations of Deficiency.	117
XIII. Iron	119
1. Physiology	119
2. Sources of Iron in Infancy	119
3. The Development of Nutritional Anemia in Infants	120
4. Biochemical Pathology of Infantile Anemia	120
5. Prevalence of Infantile Anemia	120
6. Clinical Manifestations of Deficiency.	121
XIV. Iodine	121
XV. Concluding Remarks	121
References	122

Effect of B Vitamins on the Endocrinological Aspects of Reproduction

By ROY HERTZ, *National Institute of Health, Bethesda, Maryland*

I. Introduction	135
II. Effects of Food Restriction on Gonadal Function	136
III. Relationship of Specific B-Complex Factors to Gonadal Function and Estrogen Metabolism	137
IV. B-Complex Factors and Lactation	140
V. Effects of B-Complex Content of the Maternal Diet on The Young	142
VI. General Considerations Concerning Vitamin-Hormone Interrelationships	143
References	145

Nutritional Therapy of Endocrine Disturbances

By MORTON S. BISKIND, *Endocrine Laboratory and Clinic, Beth Israel Hospital,
New York, New York*

I. Introduction	147
II. Syndromes Related to Excess Estrogen	148
1. Relation of Nutritional Deficiency to Inactivation of Estrogen in the Liver	148
2. "Functional" Uterine Bleeding, Cystic Mastitis, Premenstrual Ten- sion	152
3. Postpartum Subinvolution of the Uterus	161
4. Diminished Libido and Impotence in the Male	162
5. Implications for Industrial Toxicology	163
6. Prevention and Treatment of Neoplasms in Tissues Responsive to Estrogen	165
III. Infertility	167
IV. Thyroid Disturbances and Thyroid Therapy	168
V. Diabetes	170
VI. On the Technic of Nutritional Therapy	175
References	180

The Thyroid and Diabetes

By BERNARDO A. HOUSSAY, *Instituto de Fisiologia, Universidad de Buenos Aires, Buenos Aires, Argentina*

	Page
I. Relationship Between the Thyroid and the Intestinal Absorption of Sugars	188
II. Carbohydrate Metabolism in Hyperthyroidism	188
1. Blood Sugar	188
2. Tolerance Tests	189
3. Glycosuria	189
4. Glycogen	190
5. Mechanism of the Alterations Observed	190
6. Glucose Consumption	191
III. Carbohydrate Metabolism in Hypothyroidism	191
1. Blood Sugar	191
2. Glucose Consumption	191
3. Glycogen	191
IV. Sensitivity to Adrenalin	192
V. Sensitivity to Insulin	192
VI. Diabetogenic Action of the Thyroid Gland	192
1. Animals with Whole Pancreas	192
2. Animals with Partial Pancreatectomy	193
3. Action in Animals Previously Diabetic	194
4. Thyroid and Anterior Pituitary Association	194
5. Thyroid Action on Langerhans' Islets	195
6. Insulin Concentration in the Pancreas	195
7. Insulin Secretion	195
8. Characteristic Features of Thyroid and Metathyroid Diabetes	195
9. Mechanism of Thyroid and Metathyroid Diabetes	196
10. Sensitivity to Alloxan	197
VII. Diabetes and Hyperthyroidism in Man	197
1. Incidence of Hyperthyroidism in Diabetics	197
2. Incidence of Diabetes in Hyperthyroid Cases	197
3. Diagnosis	198
4. Pancreatic Lesions	198
5. Thyroid Administration	199
6. Treatment	199
VIII. Thyroid Deficiency and Pancreatic Diabetes	199
1. Dogs	199
2. Cats	200
3. Rats	200
4. Action of Thiouracil	202
IX. Phlorhizin Diabetes in Thyroidectomized Animals	202
1. Dogs	202
2. Rats	202
X. Alloxan Diabetes in Thyroidectomized Rats	202
1. Thyroidectomy in dogs with alloxan diabetes	202
XI. Thyroid Deficiency in Human Diabetes	203
1. Total Thyroidectomy	203
2. Myxedema and Diabetes	203
References	204

Thyroactive Iodinated Proteins

By E. P. REINEKE, *Michigan State College of Agriculture and Applied Sciences,
East Lansing, Michigan*

	Page
I. Introduction	207
II. The Iodination of Proteins	208
1. Iodination Methods	208
2. Iodine-Binding Groups in the Protein Molecule	209
III. Thyroidal Activity of Iodinated Proteins	211
1. Early Evidence of Thyroidal Activity	212
2. Hydrolysis and Concentration of the Active Substance	212
3. Formation of Iodinated Proteins	213
4. Methods of Forming Highly Active Iodinated Protein	214
a. Effect of Extent of Iodination	214
b. Relation of pH and Extent of Iodination to the Formation of Active Substance	216
c. Relation between Iodination and Incubation Temperature	217
d. Catalysis of Thyroxine Formation by Manganese Compounds	218
5. Proteins Suitable for Iodination	221
IV. The Isolation of Thyroxine from Iodinated Protein	222
1. Isolation of <i>dl</i> -Thyroxine	222
2. Isolation of <i>l</i> -Thyroxine	224
V. The Quantitative Assays of Thyroxine in Thyroactive Iodinated Proteins	227
1. Biological Assays	227
a. Stimulation of Metamorphosis in Frog Tadpoles	227
b. Assays Based on Elevation of the Metabolic Rate, and Decrease in Body Weight	228
c. The Relative Thyroidal Potency of <i>l</i> - and <i>dl</i> -Thyroxine	230
2. Chemical Determination of the Thyroxine Content of Thyroactive Iodinated Proteins	232
VI. The Formation of Thyroxine from Diiodotyrosine	234
VII. Mechanism of Thyroxine Formation	235
VIII. The Effect of Iodination on Physico-Chemical Properties of Proteins	239
1. Spectrographic Absorption	239
2. X-Ray Diffraction Pattern for Iodinated Amino Acids	240
3. The Effect of Iodination on the Dissociation Constant of Tyrosine	240
IX. Effect of Thyroactive Iodinated Proteins on Physiological Processes of Domestic Animals	241
1. Effect on Milk Secretion	241
2. Effect on Body Growth	244
3. Effect on Feather Growth	246
4. Effect on Egg Production	246
X. Discussion and Summary	248
References	249

The Protein Anabolic Effects of Steroid Hormones

By CHARLES D. KOCHAKIAN, *Department of Physiology and Vital Economics, School of
Medicine and Dentistry, University of Rochester, Rochester, New York*

I. Introduction	256
II. Nomenclature and Formulae of Steroid Hormones	257
III. Early Experiments with Crude Extracts of Testes	257

IV. The Demonstration that "Male Hormone" Extracts of Urine Cause Nitrogen Retention	259
V. The Effect of Steroid Hormones on Nitrogen Excretion in Urine	259
1. Experiments in Dogs	259
a. Δ^4 -Androstenedione-3,17	259
b. Testosterone, Testosterone Acetate and Propionate	261
c. Δ^5 -Androstenediol-3(β),17(α)	262
d. Estrogens and Progesterone	262
2. Experiments in Rats	262
a. Testosterone Propionate	262
3. Experiments in Man	263
a. Testosterone Propionate	264
b. Testosterone	270
c. 17-Methyltestosterone	271
d. 17-Ethyltestosterone	273
e. 17-Ethynyltestosterone (Anhydrohydroxyprogesterone, Pregnenolone)	273
f. Δ^4 -Androstenedione-3,17	273
g. Androsterone	273
h. Dehydroisoandrosterone and Acetate	274
i. Δ^5 -Androstenediol-3(β),17(α) and Diacetate	274
j. 17-Methyl- Δ^5 -Androstenediol-3(β),17(α)	274
k. Androstenediol-3(α),17(α) and Diacetate	275
l. 17-Methylandrostanediol-3(α),17(α)	275
m. Estrone	276
n. α -Estradiol and α -Estradiol Benzoate	276
o. Diethylstilbestrol and Dipalmitate	277
p. Progesterone	277
q. Δ^5 -Pregnenol-3(β),one-20	277
VI. The Effect of Steroid Hormones on the Nitrogen Constituents of Urine and Blood	277
1. Urea and Non-Protein Nitrogen	277
a. Dog	277
b. Man	278
2. Protein	279
3. Creatine-Creatinine	280
a. Dog	280
b. Rabbit	281
c. Rat	281
d. Monkey	282
e. Man	283
VII. The Lack of Effect of Steroid Hormones on Fecal Nitrogen Excretion.	287
VIII. The Effect of Steroid Hormones on Electrolyte and Water Metabolism	288
1. Dog	288
2. Rat	290
3. Rabbit	290
4. Mouse	290
5. Man	290
IX. The Effect of Steroid Hormones on Energy Metabolism.	292

	<i>Page</i>
1. Dog	292
2. Rat	293
3. Man	294
X. The Effect of Steroid Hormones on Tissue Formation	297
1. Body Weight	297
2. Accessory Sex Organs	298
3. Kidney and Other Organs	298
4. Skeletal Muscle	300
XI. The Mechanism of Action of the Anabolic Steroid Hormones	301
XII. Discussion and Summary	303
References	305

Methods of Bioassay of Animal Hormones

BY SIDNEY A. THAYER, *Laboratory of Biological Chemistry, St. Louis University School of Medicine, St. Louis, Missouri*

I. Introduction	312
II. Principles Which Should Govern Biological Methods.	313
1. The Product.	313
2. The Determination of Animal Variation	314
3. Choice of Suitable Standard	314
4. Response	314
5. Units	314
III. Statistical Analysis of Data	315
1. Accuracy of Results	315
2. Standard Deviation	316
3. Significant Difference	316
4. The Equation to the Regression Line	316
IV. The Gonadotropic Hormones	318
1. Assay of Anterior Pituitary Gland Extracts	319
2. Assay of Gonadotropic Substance of Pregnancy Urine (PU)	321
3. Equine Gonadotropins (PMS)	326
V. Growth Hormone.	328
VI. Adrenotropic Hormone.	330
1. Adrenal Hypertrophy of Intact Immature Rat	330
2. Assay of Adrenotropic Hormone in Hypophysectomized Rat	330
a. Repair of Adrenals of Hypophysectomized Rat	330
b. Maintenance of Adrenals of Hypophysectomized Rat	330
VII. Thyrotropic Hormone	331
VIII. Lactogenic Hormone (Prolactin)	333
1. Crop Gland Methods	333
a. Weight Method	333
b. Minimum Stimulation Method	333
c. Local Stimulation Method	333
2. Mammary Gland Method	335
IX. Bioassay of Adrenal Cortical Hormones	335
1. Introduction.	335
a. Survival	336
b. Growth of Young Rats	336

c. Survival of Adrenalectomized Rats in Low Environmental Temperature	336
d. Maintenance of a Normal Condition in Adrenalectomized Dogs	336
e. Sodium Retention	337
f. Deposition of Glycogen in Fasting Adrenalectomized Rats	337
g. Long Stimulation of Muscle	337
2. Deposition of Glycogen in Fasting Adrenalectomized Rats	337
a. Experimental	338
α. Animals	338
β. Diets	338
γ. Final Assay Procedure	338
δ. Extracts	339
ε. Standard	339
b. Comparative Activity of Seven Extracts of Adrenal Cortex	340
3. The Test of Renal Function in Adrenalectomized Dogs	341
a. Methods	341
b. Results	345
c. Discussion	345
4. Sodium Retention in Normal Dogs	346
a. Methods	346
b. Results	347
c. Discussion	348
5. Growth and Survival in Immature Adrenalectomized Rats	348
a. Methods	348
b. Results	349
c. Discussion	352
6. Comparisons of the Adrenal Cortical Potency of Seven Extracts Determined by Four Methods	353
7. Assay of Six Crystalline Hormones of the Adrenal Cortex	354
Discussion	357
References	358
Author Index	363
Subject Index	382
Cumulative Index of Vols. I-IV	404

The Newer Hematopoietic Factors of the Vitamin B-Complex

BY J. J. PFIFFNER AND ALBERT G. HOGAN

CONTENTS

	Page
I. Introduction	1
II. Norit Eluate Factor.	2
III. Folic Acid	4
IV. <i>Lactobacillus casei</i> Factors	5
V. <i>Streptococcus lactis</i> R Factor.	7
VI. Vitamin Bc	8
VII. Vitamin Bc Conjugate.	11
VIII. Vitamin Bc Conjugase.	13
IX. Other Nutritional Antianemia Factors	15
a. Vitamins B ₁₀ and B ₁₁	15
b. Factors R and S.	16
c. Factor U	18
d. α - and β -Pyracins.	18
e. Extrinsic Factor	19
X. Vitamin M and the potential <i>Streptococcus lactis</i> R Stimulating Factor	19
XI. Relation of Sulfa Drugs to Nutritional Role of the Newer Hematopoietic Factors	22
XII. Xanthopteryne.	25
XIII. Summary	29
References	31

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 B₁₀ and B₁₁, 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* R¹.

¹ Krueger and Peterson (45) have recently called attention to the work of Niven and co-workers (86) who have demonstrated that *Streptococcus lactis* R is an enterococcus, specifically *Streptococcus faecalis*. 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.