rotatory dispersion in the absence of the field was in good agreement with the measurements of Doty and Yang.[8,9] In the presence of the electric field an increase in optical rotation was observed at all wave lengths. The change was found to be proportional to the square of the electric field strength as predicted; an easily measurable change of about 0.1° occurred for a field of about 2,000 volts/cm.

As the electrical orientation term is not known, quantitative values of $[\alpha_{33}]$ and $[\alpha_{11}]$ cannot be obtained. However, an estimate of this term from electrical birefringence studies[10] allows us to make the following conclusions. Both $[\alpha_{33}]$ and $[\alpha_{11}]$ are very large in absolute magnitude, but $[\alpha_{33}]$ is positive and $[\alpha_{11}]$ is negative. Both values increase in absolute magnitude as the wave length decreases; however $[\alpha_{11}]$ increases faster, thus leading to the change in sign of the average optical activity $[\alpha]_0$ at $\lambda = 425$ mμ. These conclusions are in good agreement with Moffitt's prediction.[11]

(8) P. Doty and J. T. Yang, THIS JOURNAL, **78**, 498 (1956).
(9) J. T. Yang and P. Doty, *ibid.*, **79**, 761 (1957).
(10) I. Tinoco, Jr., *ibid.*, in press.
(11) W. E. Moffitt, *Proc. Natl. Acad. Sci., U. S.*, **42**, 736 (1956).

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VITAMIN B_{12} AND PROTEIN BIOSYNTHESIS. II. EFFECT OF VITAMIN B_{12} ON AMINO ACID INCORPORATION IN MICROSOMAL PREPARATIONS

Sir:

It has been reported from this laboratory that, while vitamin B_{12} has no effect on nucleic acid biosynthesis,[1] there is a decreased incorporation of C^{14}-labeled amino acids into liver protein in vitamin B_{12}-deficient animals,[2,3] indicating that vitamin B_{12} is involved in the incorporation of amino acids into protein.

Following these findings with the whole animal, the incorporation of C^{14}-labeled amino acids into protein has been studied *in vitro* using the microsomal fraction of liver and of spleen from both B_{12}-deficient and normal rats.

The rats were killed by decapitation and the livers removed and microsomes prepared by the procedure of Zamecnik and Keller.[4] This microsome fraction was freed only of mitochondria and nuclei since, as Keller and Zamecnik[5] have reported, the supernatant fraction is essential along with microsomes for the incorporation of amino acids into protein. These preparations containing microsomes and supernatant were then incubated under oxygen (95% O_2, 5% CO_2) with fructose diphosphate, C^{14}-amino acid, ATP[6] and GTP[6] for one hour in the Dubnoff shaker. They were then inactivated by the addition of TCA,[6] and the proteins were isolated and counted at infinite thickness in a GM gas-flow counter. The results are given in Table I.

(1) S. R. Wagle and B. C. Johnson, *Federation Proc.*, **16**, 401 (1957).
(2) S. R. Wagle and B. C. Johnson, *Arch. Biochem. Biophys.*, in press.
(3) Presented in part before annual meeting, National Vitamin Foundation, March 6, 1957, New York; B. C. Johnson, *Am. J. Clin. Nutrition*, in press.
(4) P. C. Zamecnik and E. B. Keller, *J. Biol. Chem.*, **209**, 337 (1954).
(5) E. B. Keller and P. C. Zamecnik, *ibid.*, **221**, 45 (1956).

TABLE I

INCORPORATION OF C^{14}-AMINO ACIDS INTO PROTEIN BY MICROSOME PREPARATIONS[a] FROM THE LIVER AND THE SPLEEN OF VITAMIN B_{12}-DEFICIENT AND NORMAL RATS[b]

	Liver microsome preparation, c.p.m./mg. protein		Spleen microsome preparation, c.p.m./mg. protein	
B_{12} status	$-B_{12}$	$+B_{12}$	$-B_{12}$	$+B_{12}$
$C^{14}H_3$-Methionine	16	76	26	94
2-C^{14}-Alanine	12	57	21	69

[a] Complete system contained 0.1 μM. FDP (6), 0.5 μM. ATP, 0.25 μM. GTP, 0.5 ml. of microsome preparation and labeled amino acid, made to 1 ml. with 0.15 molar KCl. [b] Each figure is the mean for three rats and run in duplicate. Excellent agreement was obtained between replicate animals.

In another series of experiments, the enzyme preparations were supplemented with vitamin B_{12}. The results are given in Table II.

TABLE II

THE EFFECT OF ADDITION OF VITAMIN B_{12} TO LIVER AND SPLEEN MICROSOME PREPARATIONS[a] ON THE INCORPORATION OF C^{14}-AMINO ACIDS INTO PROTEIN[b]

B_{12} status of animals	B_{12} added to microsome prep.	Liver microsome preparation, c.p.m./mg. protein		Spleen microsome preparation, c.p.m./mg. protein	
		$-B_{12}$	$+B_{12}$	$-B_{12}$	$+B_{12}$
C^{14}-Methionine	None	19	64	14	81
C^{14}-Methionine	50 mμg	53	73	51	88
2-C^{14}-Alanine	None	12	44	21	67
2-C^{14}-Alanine	50 mμg	40	66	48	90

[a] See Table I. [b] Each figure is the mean for two rats, run in duplicate. Agreement was excellent between animals.

Table I clearly shows that there is much less incorporation of labeled amino acids into protein in the case of microsome preparations prepared from the livers and spleens of vitamin B_{12}-deficient rats than in those from normal animals. These results agree with our previous data on intact animals.[2] In an additional experiment, in which the effect of level of substrate (labeled amino acid) concentration on incorporation was studied, it was found that an increase in the incorporation of amino acids was obtained with microsome preparations (both liver and spleen) from B_{12}-normal animals, upon incubation with increasing levels of amino acid, but that these substrate increments had no effect in preparations from deficient animals. This indicates an enzymatic block in protein synthesis in deficient animals. Again, when the microsome preparations, particularly from the deficient animals, were supplemented with crystalline vitamin B_{12} increased incorporation of the amino acids occurred, bringing the incorporation almost to a normal level (the stepwise increase obtained with graded levels of vitamin B_{12} and also amino acid increment data will be reported in full

(6) ATP = adenosine triphosphate; GTP = guanosine triphosphate; TCA = trichloroacetic acid; FDP = fructose diphosphate.

later). All these data indicate that vitamin B_{12} is a co-factor for the incorporation of amino acids into protein–protein biosynthesis. A study of the distribution of radioactivity among the liver subcellular fractions following injection of 1 microcurie of Co^{60}-labeled vitamin B_{12} showed a high percentage of radioactivity in the microsomes (46%) and the supernatant fraction (25%) as related to the radioactivity of all the fractions. This high percentage in these fractions most implicated in protein synthesis is in agreement with the hypothesis that vitamin B_{12} is a co-factor for protein synthesis indicated by the amino acid incorporation studies. Work on the isolation of the enzyme containing B_{12} is in progress.

This work was supported in part by a grant-in-aid from the National Vitamin Foundation, New York. We are grateful to Dr. C. Rosenblum of Merck and Co., Inc., for a generous gift of Co^{60}-labeled vitamin B_{12}.

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BOOK REVIEWS

Lectures in Immunochemistry. By MICHAEL HEIDELBERGER, Emeritus Professor of Immunochemistry, College of Physicians and Surgeons, Columbia University, New York; Visiting Professor, Institute of Microbiology, Rutgers University, New Brunswick, New Jersey. Academic Press, Inc., Publishers, 111 Fifth Avenue, New York 3, N. Y. 1956. ix + 150 pp. 14.5 × 22 cm. Price, $4.00.

The author of this short volume is a chemist who began his distinguished scientific career at the Rockefeller Institute for Medical Research in 1912. It was he who, together with the great bacteriologist Oswald T. Avery, isolated the capsular polysaccharides of several pneumococcal types and showed that they were endowed with immunological specificity. This was an achievement of no small magnitude, as subsequent events revealed.

Immunology was a lively field of investigation during the two decades prior to this discovery. Although the science was relatively new, chemists were already beginning to make important contributions to an understanding of the processes involved in immune phenomena. The monumental work of Karl Landsteiner concerning the specificity of proteins, and his discovery of the specific blood groups, an achievement for which he later received the Nobel Prize, were but two of the great contributions of this period. The classical studies of Arrhenius and of Madson on the quantitative aspects of the toxin-antitoxin reaction had brought a new interpretation of a phenomenon which, but a few years before, had scarcely been conceived of as a chemical reaction.

The discovery in the early twenties of the specific bacterial polysaccharides and of the role which they played in antipneumococcal immunity added a new, imposing milestone to the progress which both the chemist and the bacteriologist have made to our understanding of immune reactions.

Six of the lectures found in this volume, delivered in 1954 at the University of Tokyo, are a summation of the contributions which the author has made to the modern field of chemical immunology. These lectures deal for the most part with his quantitative studies on the precipitin and agglutination reactions, with the chemical nature of complement, and its role in the hemolytic system, and with the relationship between the chemical constitution and specificity of proteins and of carbohydrates. Three other lectures are included in the volume. Two of these, delivered in Europe, have to do with an evaluation of antipneumococcal immunity in humans following administration of the pneumococcal polysaccharides, while the third, delivered in New Brunswick, New Jersey, presents a study of the serological properties of native and denatured proteins.

For those who wish to obtain a broad background in the field of chemical immunology and a knowledge of the developments which have occurred in this field during the past several decades, or for those who wish to gain a broad scope in this specialized and many faceted field of biochemistry, this is not the book. However, for those who wish to learn something of the individual contributions of a contemporary and distinguished biochemist in a rapidly expanding field the volume is to be recommended warmly, although it is regrettable that many of the statements in the lectures, and in particular those regarding the work of others, are not better documented.

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Enzyme Antigen and Virus. A Study of Macromolecular Pattern in Action. By F. MACFARLANE BURNET, Kt., F.R.S., F.R.C.P. Cambridge University Press, American Branch, 32 East 57th Street, New York 22, N. Y. 1956. viii + 193 pp. 13 × 18.5 cm. Price $3.50.

This monograph is an extension and revision of the author's earlier monograph ("The Production of Antibodies"[1]). It is concerned with the process of biological replication of specific patterns. Burnet is primarily interested in the replication of active proteins and presents evidence for the thesis that protein is synthesized by or on a RNA template. The content of the monograph is best summarized by the titles of the chapters and their contents.

I. Introduction: Enzyme Action and Protein Synthesis. (1) Enzyme specificity; (2) Adaptive enzymes in microorganisms; (3) Chemical aspects of the biosynthesis of protein; (4) The nature of adaptive enzyme synthesis.

II. Antibody Production. (1) The self-marker concept; (2) Antibody production after the elimination of antigen; (3) The site of antibody production; (4) Theoretical approach to antibody production; (5) Weaknesses of the present hypothesis.

III. The Self-marker Hypothesis in Relation to Cellular Proliferation and Control. (1) Immunological aspects of tumour transplantation; (2) The implications of cutaneous sensitization to simple compounds; (3) Application of Weiss's concepts of cell control to the self-marker hypothesis; (4) Summary.

IV. Virus Multiplication. Influenza Virus Multiplication: (1) Nucleic acid in relation to influenza virus; (2) An attempted visualization of the structure of influenza virus particles; (3) Process of infection; (4) Interference; (5) Incompleteness; (6) The dynamics of influenza virus multiplication in the allantoic cavity; (7) Recombination phenomena; (8) Mutation; (9) Summary.

V. The Scope of Biological Generalization. (1) Information theory in biology; (2) The application of pattern concepts to biological problems.

The monograph outlines very effectively much of the current thought on protein synthesis, antibody production and virus multiplication and, as a monograph should, it presents

(1) F. M. Burnet and F. Fenner, "The Production of Antibodies," Macmillan, Melbourne, 1949.