dines, which is in contrast to the composition of the intact polymerized TMV-NA and hence it is likely that the chemical composition and not the size that influences the hyperchromic effect.

ACKNOWLEDGEMENT

I wish to thank Dr. W. M. STANLEY for his interest and encouragement during the course of this investigation.

SUMMARY

The effect of alkali, leaf ribonuclease and pancreatic ribonuclease on the changes in absorption of TMV-NA and its core was studied. It was found that alkali and leaf ribonuclease brought about increase in absorption ranging from 32 to 37%, while the pancreatic ribonuclease brought about a 15% increment. The increase in the absorption obtained following the treatment of the core with ribonuclease or *N* NaOH accounts for about 60% of the total increment brought about by the action of *N* NaOH or leaf ribonuclease on the intact TMV-NA. It was concluded that the polypurine nucleotide segments of TMV-NA are responsible for the major increase.

REFERENCES

1 G. OSTER AND H. GRIMSON, *Arch. Biochem.*, 24 (1949) 119.
2 M. OGUR AND G. ROSEN, *Arch. Biochem.*, 25 (1950) 262.
3 K. K. TSUBOI, *Biochim. Biophys. Acta*, 6 (1950) 202.
4 B. MAGASANIK AND E. CHARGAFF, *Biochim. Biophys. Acta*, 7 (1951) 396.
5 M. F. MALLETTE AND C. LAMANNA, *Arch. Biochem. Biophys.*, 51 (1954) 217.
6 M. HOLDEN AND N. W. PIRIE, *Biochim. Biophys. Acta*, 16 (1955) 317.
7 S. S. COHEN AND W. M. STANLEY, *J. Biol. Chem.*, 144 (1942) 589.
8 C. A. KNIGHT, *J. Biol. Chem.*, 197 (1952) 241.
9 W. FRISCH-NIGGEMEYER AND K. K. REDDI, *Biochim. Biophys. Acta*, in the press.
10 K. K. REDDI AND C. A. KNIGHT, *J. Biol. Chem.*, 221 (1956) 629.
11 E. J. KING, *Biochem. J.*, 26 (1932) 292.
12 K. K. REDDI, *Biochim. Biophys. Acta*, 24 (1957) 239.
13 E. CHARGAFF AND S. ZAMENHOFF, *J. Biol. Chem.*, 173 (1948) 327.
14 K. K. REDDI, *Biochim. Biophys. Acta*, in the press.
15 D. M. BROWN, C. A. DEKKER AND A. R. TODD, *J. Chem. Soc.*, (1952) 2715.
16 R. MARKHAM AND J. D. SMITH, *Biochem. J.*, 52 (1952) 558.
17 E. VOLKIN AND W. E. COHN, *J. Biol. Chem.*, 205 (1953) 767.

Received July 22nd, 1957

MECHANISMS OF ANTIBODY GLOBULIN SYNTHESIS BY LYMPHOID TISSUE *IN VITRO**

ABRAM B. STAVITSKY** AND BENJAMIN WOLF***

Department of Microbiology, Western Reserve University School of Medicine, Cleveland, Ohio (U.S.A.)

In a previous study[1] evidence was presented for *de novo* synthesis of diphtheria antibody when lymphoid tissue from immunized animals was incubated in a suitable medium. The increase in the antibody activity of the system during incubation apparently required intact cells and a source of energy since disruption of the tissue,

* This investigation was supported by a grant from the Life Insurance Medical Research Fund.
** This study was conducted during the tenure of an Established Investigatorship from the American Heart Association.
*** Predoctoral Fellow of the United States Public Health Service.

References p. 11.

anaerobic cultivation of tissue or metabolic inhibitors such as dinitrophenol or cyanide inhibited antibody production[1].

The purpose of the present investigation, which has been reported briefly[2,3], was to obtain evidence of net synthesis of antibody *in vitro* and to study some of the mechanisms of this synthesis. It was hoped especially to learn whether antibodies were derived from free intracellular amino acids or from more complex precursors such as proteins or polypeptides. Although the results of *in vivo* studies[4,5,6] suggested that antibody was derived from free amino acids, it was evident that this question could be analyzed more thoroughly with an *in vitro* system. In order to obtain maximal antibody formation we have employed lymph nodes removed from rabbits during the *secondary antibody response*; a greater response occurs following the second injection of protein than after the primary inoculation. Diphtheria toxoid has been used since it evokes a striking secondary response.

MATERIALS AND METHODS

Immunization and tissue culture

60 μg (20 Lf units) of alum-precipitated diptheria toxoid (Lederle Laboratories) were injected into the hind foot pads of 2.5–3 kilo rabbits. One month later the injections were repeated. At various times thereafter the regional popliteal lymph nodes were removed and cut into fragments about 3 mm^3. All procedures were done aseptically. About 20 mg of fragments (3 pieces) were placed in a 13 × 100 mm tube containing a medium. The tubes were stoppered, placed in a roller machine (12 rotation/hour) and incubated for 20–24 hours. Two media were employed, Solution V-614[7] supplemented with 20% (v/v) sterile rabbit serum, hereafter designated FS, and a chemically defined medium designated W*, which contained more amino acids and a higher concentration of each. After incubation the medium was decanted and clarified by centrifugation. Since 75–90% of the antibody was in the medium[1] after incubation the antibody content of the tissue at this time was not assayed. For analysis of the tissue before incubation it was homogenized in cold physiological saline, the homogenates clarified by centrifugation, and protein precipitated by the addition of an equal volume of 5% trichloracetic acid (TCA). The precipitate was dissolved in phosphate buffered saline, pH 7.4, and its antibody content determined by hemagglutination or quantitative precipitation. Its protein content was measured by the method of LOWRY *et al.*[9].

Antibody assays

Antibody was measured either by hemagglutination or the quantitative precipitin procedure. For hemagglutination "Purogenated" diphtheria toxoid (Lederle Laboratories) was adsorbed on sheep erythrocytes and the cell-toxoid complex agglutinated by antibody[10]. The potency of the antiserum was expressed as the reciprocal of the highest dilution which agglutinated the cells (serum with titer of 1/160 contains 160 units). The data are expressed on the basis of weight of tissue per tube and net antibody production (titer of medium after incubation minus titer of unincubated tissue homogenate) designated as hemagglutinating units per 10 mg of tissue. Each result was the average of triplicate cultures. The quantitative precipitin method[11] with purified diphtheria toxoid (K39/50 containing 2600 Lf units/mg protein N) was employed for more accurate determination of antibody production. The LOWRY method[9] was employed for the measurement of protein in the immune precipitates.

Isotope techniques

The methods of labeling the TCA-insoluble fraction of tissue will be discussed later. For determinations of radioactivity the globulins of the medium or tissue homogenate were precipitated with alcohol as precipitate A of the NICHOL-DEUTSCH method[12]. Radioactive substances, presumably

* The W medium consisted of Tyrode's solution[8] supplemented with the following compounds in mmoles: fructose diphosphate-0.00046, DL-α-aminobutyric 0.039, and these amino acids of L configuration, alanine-0.34, asparagine-0.023, aspartate-0.037, glutamate-0.034, glycine-0.10, hydroxyproline-0.015, ornithine-0.053, proline-0.04, serine-0.047, arginine-0.11, cystine-0.04, histidine-0.02, isoleucine-0.20, leucine-0.1, lysine-0.1, methionine-0.05, phenylalanine-0.05, tyrosine-0.1, tryptophan-0.01, valine-0.1, threonine-0.1. The medium also contained 100 units of penicillin G and 50 μg of streptomycin per ml. Glutamic (1.46 mmoles) was added just prior to use. The authors are very grateful to Dr. RICHARD HAFF for suggesting these concentrations of amino acids.

References p. 11.

complement, which might be adsorbed non-specifically on the antigen–antibody aggregate and give a false high value, were removed by a heterologous albumin-anti-egg albumin antibody system, essentially as described by KESTON AND KATCHEN[13]. This procedure did not remove any diphtheria antitoxin.

Antitoxin was precipitated by a concentration of K 39/50 toxoid which precipitated 96–99% of antibody as determined by testing the supernatant with the hemagglutination method. From 1–4% of the antibody was always demonstrable in the supernatant, presumably due to agglutination of the toxoid-cells by soluble antigen–antibody complexes. The precipitates were washed twice in cold saline containing 10 μg of methionine per 100 ml and dialyzed in the cold for 3 days against 2 changes of 1 ½ liters of saline. The precipitate was collected by centrifugation and redissolved in saline. 100–750 μg of protein were pipetted into a steel planchet, dried and radioactivity determined with a Nuclear flow counter. 3200–6400 counts were taken. The amount of antibody in the precipitate was calculated from the equivalence ratio of antibody to antigen (5.5)[11] so that the radioactivity of the antigen–antibody precipitate could then be recalculated in terms of the activity of antibody itself.

As a check on the ability of these methods to eliminate non-specific radioactivity, it was found that toxoid–antitoxin precipitates prepared after incubation of lymph nodes from non-immunized animals in a radioactive medium contained very little radioactivity (background or 1/3 greater).

EXPERIMENTAL

Optimal time for removal of nodes

The data in Fig. 1 indicate that maximal antibody production in FS medium is obtained with tissue removed from the rabbit 3 days after the reinjection of toxoid.

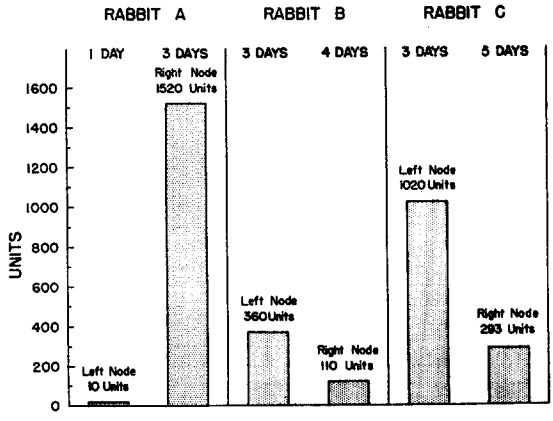

Fig. 1. *In vitro* antibody production by lymph nodes removed at various times after reinjection of diphtheria toxoid and cultivated in FS medium. All injections were made into the hind foot pads and nodes were removed surgically on days indicated for each rabbit.

No antibody was made by nodes removed 1 day after reinoculation. However, not shown is the fact that up to 150 units/10 mg tissue were produced by nodes removed 1 day after antigenic restimulation and cultivated in the W medium. Moreover, up to 500 units/10 mg tissue were formed on the W medium by nodes removed as early as 1 hour after reinjection of toxoid, if the node had been pretreated *in vivo* by the injection of 0.25 ml of sheep blood into the homolateral foot pad 3 days previously. Since much more antibody was made on both media by tissue ablated 3 days post-reinjection, this interval was used in the following experiments.

Net synthesis of antibody

In this experiment, summarized in Table I, the antibody content of tissue before incubation was compared with that of the medium after incubation. It is apparent

References p. 11.

that net synthesis of antibody occurred *in vitro*. The left node, which was pretreated with blood produced about 4 times as much antibody as the right. In 10 experiments employing nodes which were not pretreated an average of 400 μg of antibody was synthesized per gram of tissue.

TABLE I

NET SYNTHESIS OF ANTIBODY *in vitro*

Tissue	Tissue before incubation		Medium** after incubation	Amount antibody synthesized	Antibody synthesized per gram of tissue
	Wet wt.	Antibody content	Antibody content		
LLN*	210 mg	25 μg	225 μg	200 μg	950 μg
RLN	200 mg	20 μg	70 μg	50 μg	250 μg

LLN = left popliteal lymph node; RLN = right popliteal lymph node.
* 0.25 ml of sheep blood was injected into the left foot pad with second injection of toxoid.
** Medium was W solution.

Nature of precursors of antibody

In order to study the role of amino acids in antibody synthesis, attempts were made to deplete the lymph nodes of amino acid pools so that further antibody synthesis *in vitro* would be completely dependent upon an exogenous supply of amino acids. Thus far these efforts have resulted in loss by the tissues of most of their capacity for antibody formation. Therefore, *in vitro* antibody production as a function of the concentrations of amino acids in W medium was examined next. It was found that when the concentration of amino acids in the medium was doubled, there was at least a doubling in the amount of antibody produced. When the concentrations were tripled there was a decrease in antibody production compared to the basal medium, presumably due to damage to cells by the unphysiologically high concentrations of amino acids. However, a requirement for amino acids was shown by the fact that antibody production usually was far greater on any amino acid-containing medium than in balanced salt solutions.

A number of amino acid analogs were then tested as competitive inhibitors of antibody production. The data presented in Table II indicate that *dl*-ethionine inhibited antibody synthesis competitively since inhibition was reversed by homocysteine. Reversal also was accomplished by methionine, but methionine itself sometimes stimulated antibody production. Comparable concentrations of gamma ethyl amido glutamic acid* and *p*-fluorophenylalanine* were also inhibitory and their effects were reversed by glutamic acid and tyrosine plus phenylalanine respectively. Although it is known that amino acids can serve as precursors of antibody[4,5,6,13,2,3] and the foregoing data suggest a role for these compounds in *in vitro* antibody synthesis, it was possible that other types of precursors could be converted to antibody. An amino acid analog might block conversion of a precursor to antibody if this reaction involved the addition of the natural amino acid to the precursor. It was, therefore, desirable to label the potential TCA-insoluble tissue precursors such as peptides and proteins and ascertain whether any of the label appeared in the antibody synthesized by the tissue. It was essential that the TCA-soluble amino acid-containing

* Kindly supplied by Dr. HARLYN HALVORSON.

References p. 11.

TABLE II

COMPETITIVE INHIBITION OF ANTIBODY SYNTHESIS BY ETHIONINE

*Composition of medium**	*HA units per 10 mg tissue*	*% Inhibition*
W	128	—
W + DL Ethionine ($1 \cdot 10^{-3} M$)	23	82
W + DL Ethionine ($1 \cdot 10^{-3} M$) + L-Homocysteine ($7 \cdot 10^{-4} M$)	103	20
W + L-Homocysteine ($7.5 \cdot 10^{-4} M$)	116	9.5
HA-hemagglutinating		

* In the concentrations employed in these experiments neither the Ethionine or the Homocysteine affected the results of the hemagglutination titration.

fraction of tissue possess negligible radioactivity so that its contribution to activity of antibody not be detectable. It was also desirable to limit the total radioactivity of the TCA-insoluble fraction because from 8–15 % of cellular protein was catabolized during incubation and radioactive amino acids derived from protein would thereby be made available for incorporation into antibody. Incorporation of labeled amino acids into antibody also was minimized by dilution of these acids with unlabeled exogenous and endogenous amino acids. The experiments were carried out in the chemically defined W medium so there was no source of protein other than the labeled protein in the node. Since it was found that antibody and normal gamma globulin were synthesized while there was a net loss in total protein during incubation it is likely that little synthesis of proteins other than gamma globulins occurred. Therefore, conditions were suitable for detection of the conversion of potential labeled TCA-insoluble precursors to antibody. Since these precursors were labeled with ^{35}S only their conversion to S-containing proteins such as rabbit antibody[14] was detectable.

It was found that the desired level and specificity of labeling could not be achieved by exposing the tissues to radioactive amino acids *in vitro* since this resulted in appreciable radioactivity in the TCA-soluble fraction. However, the desired labeling was produced by injecting 500 μc of ^{35}S yeast protein hydrolysate (Abbott Laboratories) intraperitoneally into the immunized rabbit 16 hours before removal of the nodes for culture.

Table III presents the results of 2 experiments of this type. The data from the first are consistent with the interpretation that all of the radioactivity of the antibody in the medium can be accounted for by dilution of the activity of the antibody present before incubation. If the radioactivity had been derived from the pre-existing antibody and the bulk of the antibody was made from an unlabeled fraction of cellular protein, the remaining protein should have had a higher specific activity than initially. This explanation is excluded by the fact that the protein at the end of incubation had a somewhat lower (10 %) specific activity than initially. The discrepancy between calculated activity of 410 and the actual 360 may in part be due to breakdown during incubation of the antibody present initially. There was no apparent source of the radioactivity found at the end of incubation other than preformed labeled antibody since not enough radioactivity was found to be released during incubation to provide high specific activity precursors of antibody. Moreover, it was shown that the radioactive contribution to antibody of a TCA-soluble fraction with the activity of that in experiment 1 could not be detected.

References p. 11.

TABLE III

FAILURE OF CONVERSION OF TCA-INSOLUBLE COMPOUNDS TO ANTIBODY

Expt.***	Tissue before incubation*				Medium after incubation**		
	Antibody content	Sp. act. antibody***	Protein TCA-insol.	Sp. act. protein TCA-insol.	Antibody content	Sp. act. antibody***	Calc. sp. act. antibody§
	μg	c/m/100 μg	μg	c/m/100 μg	μg	c/m/100 μg	c/m/100 μg
1	25	3680	876	3360	225	360	410
2	0.94	§§	756	1002	70	0	§§

* c/m in Expt. 1: Total homogenate–39,232; total TCA-insoluble–29,424; total TCA-soluble–2800. c/m in Expt. 2: Total TCA insoluble–7720; total TCA-soluble–0.

** In Expt. 1 the tissues and media from 10 tubes which were incubated individually were pooled for these analyses. In Expt. 2 the contents of 8 tubes were similarly combined.

*** Antibody refers to amount calculated from optimal ratio (5.5) of antibody to antigen in specific precipitate.

§ On basis of 9-fold dilution of original specific activity of antibody by newly made antibody.

§§ Inaccurate since it depends upon hemagglutination rather than quantitative precipitation. This inaccuracy precluded any determination of specific activity of antibody before incubation or any calculation of specific activity of antibody in the medium after incubation.

The conclusion that antibody was not synthesized to any extent from pre-existing proteins and peptides was fortified by the results of Experiment 2. The specific activity of the antibody in the tissue could not be determined accurately. However, the antibody in the medium was co-precipitated by the addition of toxoid and antitoxin and the precipitate contained radioactivity comparable to that found in control experiments in which nodes from non-immunized animals were incubated in radioactive medium to which antibody was added.

In confirmation of previous observations[15,16], ^{35}S *l*-methionine was incorporated into antibody produced by nodes *in vitro*. In substantiation of earlier *in vivo*[6] and transplantation experiments[3,17], ^{35}S-labeled normal rabbit gamma globulin added to the medium in which nodes were incubated was not incorporated into the resulting antibody. However, it is conceivable that extracellular globulins cannot gain entrance into antibody-forming cells and are consequently unable to participate in this process.

DISCUSSION

Lymph nodes must remain in the animal for 3 days after the second injection of antigen in order to obtain maximal *in vitro* antibody synthesis. Since recent studies[18] implicate immature plasma cells in the formation of antibody, the residence of 3 days in the animal may be required for the development of adequate numbers of these cells in the node. The decrease in antibody production observed by cultivation of nodes removed more than 3 days after the reinjection of antigen (Fig. 1) may reflect the conversion of the active antibody-producing immature plasma cells into the less active plasma cell. Whatever the nature of the physiological events required for maximal antibody formation, thus far they have been observed to occur most effectively *in vivo*. However, these events apparently occur to some extent in vitro in the W but not the FS medium since antibody was synthesized *in vitro* by nodes ablated 1 day after antigenic restimulation and cultivated in the W medium.

References p. 11.

Antibody synthesis *in vitro* has been stimulated non-specifically in 6 out of 10 experiments by pre-treatment of the nodes *in vivo* with sheep blood. The injection of sheep blood also has promoted antibody synthesis by lymphoid cells upon transplantation from immunized to normal animals[19]. The manner whereby the blood affects these phenomena is not known, but the active factor appears to be in the plasma rather than cellular fraction of blood[19].

The present data indicate that a substantial net synthesis of antibody occurs *in vitro**. The amount of antibody produced is comparable to that made *in vivo* or following transplantation of antibody-producing cells to normal animals[2,3,20,21,22]. Thus far the antibody system and that in which serum albumin is synthesized by chicken liver slices[23] are the only mammalian systems in which a net synthesis of protein has been demonstrated unequivocally *in vitro*.

The requirement of amino acids for maximal antibody synthesis and the inhibition of this synthesis by several amino acid analogs point to an important role of these amino acids as precursors of antibody protein. It would be desirable, however, to extend the analog data to a wider variety of amino acids before this inhibition could be considered a general phenomenon. The most persuasive evidence for the role of amino acids in antibody formation is provided by the failure of conversion to antibody of pre-existing S-containing compounds other than amino acids (Table III). These data are consistent with previous findings in this laboratory[3,17] in which cells from immunized animals were transplanted to normal recipients and synthesized antibody in these animals. Analysis of this system revealed a tremendous discrepancy between the amount of total protein and gamma globulin in the transplanted cells and the amount of antibody produced by these non-proliferating cells. In a typical experiment the cells produced 1000–10,000 times as much antibody protein in the recipients as the protein they contained before transplantation. These data also concur with the finding[21,24,2,3] that the antibody formed by these transplanted cells apparently is not derived from pre-existing protein. However, due to limitations of experiments in whole animals it was not possible to exclude a small but nonetheless significant conversion to antibody of precursors more complex than amino acids. These conclusions also are in agreement with recent biochemical studies of protein synthesis in bacterial[25] and mammalian[26] tissues.

It is evident that the secondary antibody response does not depend upon the release of stored intracellular antibody or the conversion to antibody of pre-existing cellular proteins. However, the data provide no information as to the mechanisms whereby the amino acids are condensed to form antibody. It is possible, for instance, that this condensation involves the prior formation of small amounts of a rapidly-turning over precursor of the structural complexity of antibody.

In vitro incorporation of radioactive amino acids into antibody appears to parallel the actual net synthesis of antibody[24], but dissociation of these phenomena comparable to that apparently achieved in bacterial cells[27] has not been attempted.

It is clear from these and other[1,2,3,20] experiments that the *in vitro* antibody-synthesizing system possesses many advantages for the study of the mechanisms of synthesis of antibody and other proteins free from the complexities of the whole animal.

* After this study was completed and reported briefly[2,3], STEINER AND ANKER[20] reported the *in vitro* net synthesis of comparable amounts of antibody to bovine serum albumin by rabbit spleen cells.

References p. 11.

ACKNOWLEDGEMENTS

The author is grateful to Mrs. ANNE D. GARDNER and Mr. FRANK C. WILLIAMS for valuable technical assistance and to Dr. H. EARLE SWIM and Dr. CHARLES YANOFSKY for a thorough and constructive criticism of the manuscript.

SUMMARY

Lymph nodes from immunized rabbits effect a net synthesis of antibody on a synthetic medium *in vitro*. Several amino acid analogs inhibit this synthesis and their effects are reversed by the natural amino acids. An optimal concentration of amino acids in the medium is required for antibody formation. On the basis of isotope experiments it was concluded that cellular proteins were not converted to antibody *in vitro* and antibody was derived largely, if not exclusively, from free amino acids. Similarities between antibody synthesis and protein synthesis in microorganisms and mammalian tissues were indicated.

REFERENCES

[1] A. B. STAVITSKY, *J. Immunol.*, 75 (1955) 214.
[2] B. WOLF AND A. B. STAVITSKY, *Federation Proc.*, 15 (1956) 623.
[3] A. B. STAVITSKY, *Federation Proc.*, 16 (1957) 652.
[4] R. SCHOENHEIMER, S. RATNER, S. RITTENBERG AND M. HEIDELBERGER, *J. Biol. Chem.*, 144 (1942) 545.
[5] H. GREEN AND H. S. ANKER, *Biochim. Biophys. Acta*, 13 (1954) 365.
[6] P. GROS, J. COURSAGET AND M. MACHEBOEUF, *Bull. soc. chim. biol.*, 34 (1952) 1070.
[7] A. FISCHER, A. ASTRUP, G. EHRENSVARD AND V. OEHLEMSCHLAGER, *Proc. Soc. Exptl. Biol. Med.* 67 (1948) 40, 19.
[8] M. V. TYRODE, *Arch. intern. pharmacodynamie*, 20 (1910) 205.
[9] O. H. LOWRY, N. J. ROSEBROUGH, A. LEWIS, FARR, AND R. J. RANDALL, *J. Biol. Chem.*, 193 (1951) 265.
[10] A. B. STAVITSKY, *J. Immunol.*, 72 (1954) 360.
[11] E. A. KABAT, AND M. M. MAYER, *Experimental Immunochemistry*, Charles C. Thomas, Springfield, Illinois, 1948.
[12] J. C. NICHOL AND H. F. DEUTSCH, *J. Am. Chem. Soc.*, 70 (1948) 80.
[13] A. S. KESTON AND B. KATCHEN, *J. Biol. Chem.*, 76 (1956) 253.
[14] E. L. SMITH, M. L. MCFADDEN, A. STOCKELL AND V. BUETTNER-JANUSCH, *J. Biol. Chem.*, 214 (1955) 197.
[15] H. M. RANNEY AND I. M. LONDON, *Federation Proc.*, 10 (1951) 562.
[16] A. KESTON AND J. C. DREYFUS, *Federation Proc.*, 10 (1951) 206.
[17] A. B. STAVITSKY, *Federation Proc.*, 15 (1956) 615.
[18] E. H. LEDUC, A. H. COONS AND J. M. CONNOLLY, *J. Exptl. Med.*, 102 (1955) 61.
[19] A. B. STAVITSKY, *J. Immunol.*, (1957) in the press.
[20] D. F. STEINER AND H. S. ANKER, *Proc. Natl. Acad. Sci. U.S.*, 42 (1956) 580.
[21] W. H. TALIAFERRO AND D. W. TALMAGE, *J. Infectious Diseases*, 97 (1955) 88.
[22] J. C. ROBERTS, JR. AND F. J. DIXON, *J. Exptl. Med.*, 102 (1955) 379.
[23] T. PETERS, JR. AND C. B. ANFINSEN, *J. Biol. Chem.*, 186 (1950) 805.
[24] A. B. STAVITSKY, manuscript in progress.
[25] D. S. HOGNESS, M. COHN AND J. MONOD, *Biochim. Biophys. Acta*, 16 (1955) 99.
[26] R. B. LOFTFIELD AND A. HARRIS, *J. Biol. Chem.*, 219 (1956) 151.
[27] E. F. GALE AND J. C. FOLKES, *Biochem. J.*, 55 (1953) 721.

Received June 27th, 1957