1963

Antibody Production in Organ Cultures of Lymph-Node Fragments Following in Vitro Secondary Antigenic Stimulation

Charles T. Ambrose, *Harvard Medical School*

ANTIBODY PRODUCTION IN ORGAN CULTURES OF LYMPH-NODE FRAGMENTS FOLLOWING *IN VITRO* SECONDARY ANTIGENIC STIMULATION[1, 2]

CHARLES TESCH AMBROSE,[3] Department of Bacteriology and Immunology, Harvard Medical School, Boston, Massachusetts

SUMMARY

The technique for obtaining a secondary antibody response *in vitro* by stimulating cultured fragments of immune rabbit lymph nodes is described. A thin pad of glass wool overlying the fragments in a Leighton tube was used to hold the fragments in place and to replace plasma for this function. In addition, the glass wool fibers provided an increased surface area for cellular migration, which may be studied in living cultures or in fixed and stained preparations. Experiments leading to the development of chemically defined medium are described; the components of this medium that appear to substitute for serum include hydrocortisone and insulin. Studies on the inhibition of antibody production *in vitro* by bacteriostatic levels of chloramphenicol suggest that the drug acts at an early step which is probably peculiar to the initiation of the secondary response.—NCI Monogr. 11: 117–126, 1963.

SEVERAL YEARS ago Dr. Maria Michaelides and Dr. Albert H. Coons devised a method of stimulating the secondary response in organ cultures of immune rabbit lymph-node fragments (*1*). The work to be described here deals with two quite different applications of this culture technique. One has been to determine optimal nutritional requirements of this culture system first by finding a defined medium which enables some antibody production to occur *in vitro* and next by supplementing with known compounds this basic medium to enhance production further. The second application has been to study the effect of chloramphenicol on the inhibition of this *in vitro* response. These studies will soon be published in detail elsewhere.

MATERIALS AND METHODS

The basic technique originally devised is as follows: Normal adult male rabbits receive an injection in each hind foot pad of 10 to 12.5

[1] Presented at the 13th Annual Meeting of the Tissue Culture Association, Washington, D.C., May 29–31, 1962.

[2] Supported by research grant H-2255, National Heart Institute, National Institutes of Health, Public Health Service.

[3] The assistance of Miss Linda Chesnel and Mrs. Elaine Lacks is gratefully acknowledged.

653901—63——14

mg bovine serum albumin (BSA) and 100 to 167 Lf diphtheria toxoid—both antigens being dissolved in the same 0.1 ml of saline. Three to 12 months later, when the rabbit's circulating titers to these two antigens are low, the lymph nodes draining the injection sites are removed aseptically and cut into 1 mm^3 fragments. These fragments are incubated for 2 hours with a dilute antigen solution of 0.5 mg BSA and 5 Lf diphtheria toxoid per ml of medium and are then rinsed several times with Hanks' solution to remove excess antigens. This step constitutes the *in vitro* secondary stimulation. Generally a small group of fragments is simultaneously incubated in medium without antigens and serves as the nonstimulated controls. Twelve fragments are implanted in rabbit plasma (usually autologous plasma) in a roller tube. Each tube receives 1 ml of basal medium [4] containing 25 percent normal rabbit serum. The tubes are incubated at 37° C in a roller drum. The medium is replaced every 3 days and saved for later measurement of its antibody titer. Stimulated lymph-node cultures of this sort frequently continue to produce high titers of antibodies simultaneously to BSA and diphtheria toxoid for over 3 weeks. Finally, the antibody titers are measured by the hemagglutination method with tanned sheep erythrocytes [Stavitsky's modification of the Boyden procedure (*2*)].

The only significant deviation from this original technique has been replacement of the plasma clot by glass wool pads to hold the fragments in place. A thin pad of glass wool, precut to the shape of the inner flat surface of a Leighton tube, is inserted over the fragments once they are aligned in the tube. This inert matrix of spun glass contributes no nutritional factors to the culture, but fixes the fragments in place and provides increased surface area onto which cells may migrate out from the fragments. The live culture may be examined under low- and medium-power objectives any time during an experiment. Also the fragments and their outgrowth entangled in the glass wool may be fixed and stained like coverslip preparations. Plasma may be added to the glass wool, which also prevents loss of fragments from lysis and retraction of the clot.

DEVELOPMENT OF A CHEMICALLY DEFINED CULTURE MEDIUM

In the culture system, as originally devised, there are three uncontrolled variables affecting the titer of antibody produced. The first and most significant, of course, is the starting tissue itself, which must be obtained fresh from a rabbit for each experiment. The lymph-node fragments differ considerably from animal to animal in two respects: 1) in the degree of their primary *in vivo* antigenic stimulation and 2) in their potential for protein synthesis as a functon of intrinsic nutritional factors. The existence of this second difference seems apparent from the fact that

[4] Basal medium consists of 1 percent Eagle's amino acid mixture, 1 percent Eagle's vitamin mixture, 2.0 mM glutamine, Hanks' salt solution, glucose (75 mg/100 ml), phenol red (0.002 g/100 ml), 50 units per ml penicillin; and 50 μg per ml streptomycin sulfate. The final *p*H is adjusted to 7.4 with a sodium bicarbonate solution.

fragments from some rabbits produce moderate amounts of antibodies when cultured in mere basal medium, *i.e.*, medium free of serum, while fragments from other rabbits in the identical basal medium often show no response. Yet both sets of fragments produce good antibody responses in serum-containing medium, indicating they were both equally well primed by the initial *in vivo* antigen stimulation. A possible explanation for different responses in basal medium is the existence of an intrafragment pool (probably intracellular pool) of some nutritional factor(s) in the nodes of one rabbit and not in those of another.

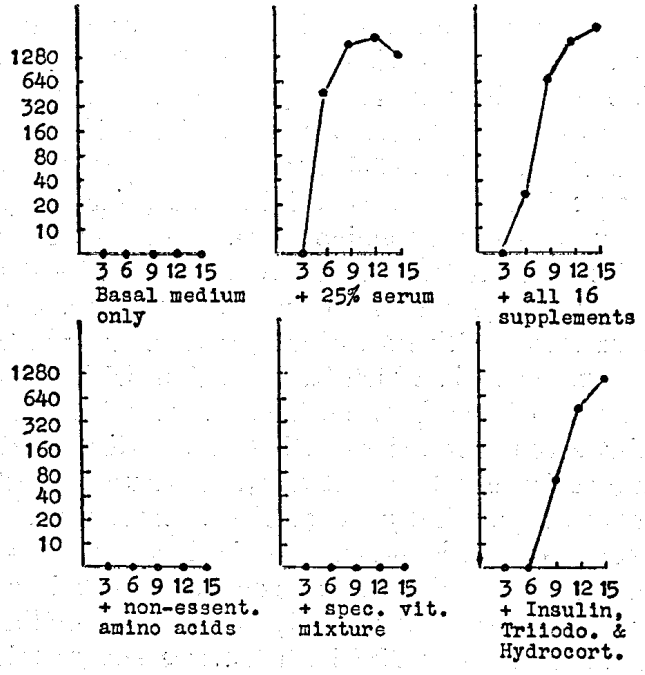
The remaining two variables affecting the *in vitro* response are the plasma clot and the serum used in the medium—both of which differ in the nutritional substances they contribute to the culture. We can eliminate the problem of plasma by replacing it with the glass wool pads described. Our interest in developing a completely defined mixture of substances to replace serum arose in the following indirect manner:

Studies preliminary to experiments on the incorporation of isotopically labeled amino acids in stimulated lymph-node cultures revealed that serum dialyzed ostensibly to remove free amino acids could not support antibody production. This deficiency could not be relieved by the presence of Eagle's mixture of 13 essential amino acids or by the addition of any of the "nonessential" amino acids. However, a serum dialysate could reconstitute the dialyzed serum to a considerable degree. In fact, the dialysate allowed good antibody production to occur in the absence of any dialyzed serum with its various proteins. More surprising, however, was an experiment in which basal medium with 12 percent normal rabbit serum allowed excellent antibody production, with 25 percent dialyzed rabbit serum allowed essentially no antibody production, and with the combination of 25 percent dialyzed serum plus 12 percent normal serum allowed practically no antibody production. One possible explanation consistent with these results is that serum contains a nutritionally essential component (or components) which is dialyzable and exists both in a free form and partially bound to a nondialyzable serum protein. On exhaustive dialysis [5] both the free and protein-bound fractions are lost from the serum. The dialyzable components in the dialysate can be returned to the dialyzed serum to render it again able to support antibody production, or the dialyzed component by itself can sustain antibody production independent of serum proteins. Furthermore, dialyzed serum may conceivably "deplete" normal serum if the dialyzed proteins of the former tightly bind the free components of the latter.

Certain hormones have these necessary properties, *i.e.*, they are dialyzable and exist in serum both free and bound to proteins. Three common ones are insulin (*3*), triiodothyronine (*4*), and hydrocortisone (*5*). We prepared a special mixture consisting of these three readily available hormones. At the time of these experiments we were also interested in the effects of vitamin B_{12}, several fat-soluble vitamins, and the nonessential

[5] In these experiments serum was dialyzed against running tap water for 72 hours at 5° C and then against several changes of normal saline-phosphate buffer at *p*H 7.0 over 24 hours at 5° C. We obtained the dialysates by dialyzing serum against an equal volume of distilled water with gentle shaking for 24 hours at 5° C.

amino acids. When this empirical mixture of 16 components [6] was added to the basal medium, this now serum-free medium resulted in production of antibody titers as high as did the basal medium supplemented with the serum dialysate or even with 25 percent normal rabbit serum. The next several experiments indicated that of the 16 substances included in the empirical mixture the hydrocortisone sodium succinate and the insulin (to a lesser degree) were the major active components (text-fig. 1). While individually neither the nonessential amino acids nor the special vitamin mixture sustained antibody production, yet some degree of synergism between them and the hydrocortisone and insulin was evident in still later experiments. The nature of the synergism, the range of optimal concentration of hydrocortisone and insulin, and the recognition of other dialyzable factors in serum are currently being investigated.



TEXT-FIGURE 1.—Effect of various supplements to basal medium on *in vitro* antibody production (anti-BSA). No plasma was used in these cultures. Each point in these graphs represents the average titer of 4 cultures.

INHIBITION OF ANTIBODY PRODUCTION BY CHLORAMPHENICOL

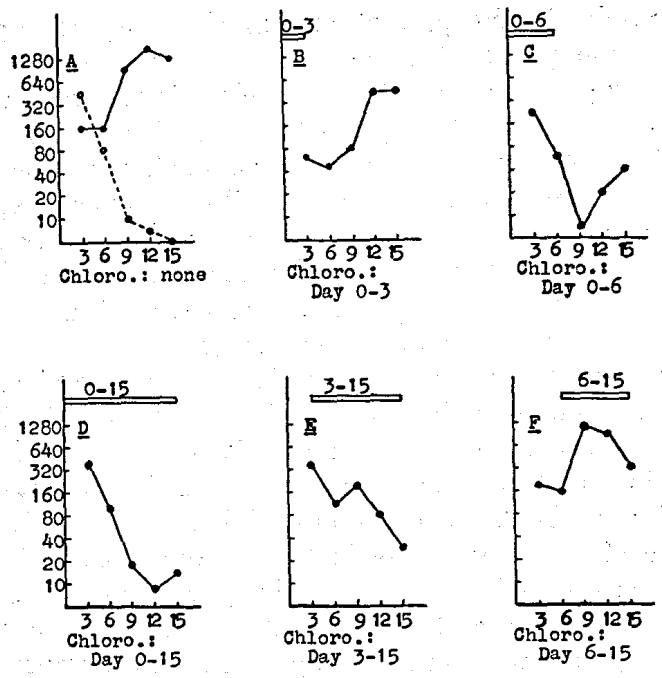
Our studies with chloramphenicol require a brief summary of some relevant data from the literature. In susceptible bacterial cultures and in cell-free bacterial systems the addition of 2 to 20 μg per ml of chlor-

[6] The 16 components of the empirical mixture included: 0.1 mM of each of the 6 nonessential amino acids (alanine, aspartic acid, asparagine, glycine, proline, and serine), 0.25 μg per ml vitamin A, 10 μg per ml vitamin E, 0.45 μg per ml vitamin K, 5 μg per ml linoleic acid, 5 μg per ml linolenic acid, 5×10^{-5} μg per ml thioctic acid, 2×10^{-4} μg per ml vitamin B_{12}, 0.01 μg per ml triiodothyronine, 0.1 μg per ml hydrocortisone sodium succinate, and 1.0 unit per ml crystalline zinc insulin.

amphenicol results in the prompt and nearly complete suppression of protein production (*6*, *7*). On the other hand, in mammalian cells this level of the drug has had no demonstrable effect. Indeed, only with very high levels (300 to 2500 μg/ml) has there been shown to be even partial inhibition of protein synthesis in mammalian cell cultures (*8*, *9*), in isolated thymus nuclei (*10–12*), or in cell-free systems (*9*, *13*). This apparent resistance of mammalian cells and systems to "bacteriostatic" levels of chloramphenicol has puzzled investigators interested in protein synthesis. However, we have found a clear effect of such low levels of chloramphenicol on the secondary response (*14*).

In one experiment various concentrations of chloramphenicol (5, 10, 20, 35, and 50 μg/ml) were included in the medium for the 2 weeks the cultures were followed. In cultures maintained in medium free of chloramphenicol, the lymph-node fragments produced titers averaging at their peak 1/5120 for anti-BSA and 1/320 for antidiphtheria toxoid. Increasing concentrations of chloramphenicol in the medium produced correspondingly more complete inhibition of antibody response. The anti-BSA response was inhibited over 95 percent by 20 μg per ml and over 99 percent inhibited by 50 μg per ml. The anti-diphtheria toxoid response, which in general was several twofold dilutions lower than the corresponding and simultaneous anti-BSA response, was completely suppressed by concentrations greater than 20 μg per ml.

Subsequent experiments showed that once the secondary response was well established, antibody production could not be appreciably stopped by a level of chloramphenicol which was completely inhibitory when continuously present in the medium from the time of the secondary stimulation. In text-figure 2 are plotted data of a typical experiment in which 50 μg per ml chloramphenicol was added to the medium on day 0 for various intervals or was added at different times after the secondary stimulation and maintained in the medium until the end of the experiment. The response of the cultures not receiving secondary stimulation *in vitro* and not exposed to the drug is shown as a *dotted* line in text-figure 2A. In these unstimulated cultures there was an initial high titer that rapidly fell in successive changes of medium; this initial peak represented the waning primary response to the antigen injected into the foot pad many months before. The response of the cultures stimulated *in vitro* and not exposed to the drug is depicted in this same figure as the *solid* line; a typical secondary response resulted in a peak antibody production around day 12. Stimulated cultures exposed to chloramphenicol throughout the 15 days (text-fig. 2D) showed a precipitous fall in antibody titers, which paralleled that in the unstimulated cultures. Exposure for the first 3 days only (text-fig. 2B) resulted in a delayed response which never quite reached that in the unexposed group of cultures. Exposure for the first 6 days (text-fig. 2C) caused a steep fall in titers, but a small response did occur 3 days after withdrawal of the drug from the medium. Exposure beginning on day 3 and continuing to the end of the experiment (text-fig. 2E) resulted in a slower fall in titer than in the cultures continuously



TEXT-FIGURE 2.—Effect of 50 μg per ml chloramphenicol on *in vitro* antibody production (anti-BSA). Chloramphenicol was present in the culture medium for various intervals starting at different times as indicated by the *bar* at the *top* of each graph. Each point represents the average titer of 3 to 4 cultures.

exposed for all 15 days. Finally, exposure beginning on day 6 (text-fig. 2F) allowed antibody production to continue for the next 6 days as well as it did in the unexposed control group of cultures.

The general pattern of response to chloramphenicol described in this experiment has been repeated in numerous others of the same design. These studies indicate that levels of chloramphenicol, which suppress protein synthesis in bacteria, also markedly inhibit antibody production in mammalian cells in culture when the exposure to the drug includes the period of 9 or 12 days immediately after the *in vitro* antigenic stimulation. These data suggest that chloramphenicol acts at a point in the metabolic sequence for antibody synthesis peculiar to the initiation of the secondary response.

REFERENCES

(*1*) MICHAELIDES, M. C.: Antibody production in tissue culture. Fed. Proc. 16: 426, 1957.

(*2*) STAVITSKY, A. B.: Micromethods for the study of proteins and antibodies. I. Procedure and general application of hemagglutination and hemagglutination-inhibition reactions with tannic acid and protein-treated red blood cells. J. Immunol. 72: 360–367, 1954.

(*3*) ANTONIADES, H. N.: Studies on the state of insulin in blood: The state and transport of insulin in blood. Endocrinology 68: 7–16, 1961.

(*4*) MYANT, N. B., and OSORIO, C.: A note on the binding of thyroid hormone by rabbit serum. J. Physiol. 160: 374–379, 1962.

(5) DAUGHADAY, W. H., and MARIZ, I. K.: Corticosteroid-binding globulin: its properties and quantitation. Metabolism 10: 936–950, 1961.
(6) BROCK, T. D.: Chloramphenicol. Bact. Rev. 25: 32–48, 1961.
(7) LAMBORG, M. R., and ZAMECNIK, P. C.: Amino acid incorporation into protein by extracts of E. coli. Biochim. et biophys. acta 42: 206–211, 1960.
(8) LEPAGE, G. A.: Effects of chloramphenicol on incorporation of glycine-2-C^{14} into mammalian tumor cell proteins and purines. Proc. Soc. Exper. Biol. & Med. 83: 724–726, 1953.
(9) SCHWEET, R., BISHOP, J., and MORRIS, A.: Protein synthesis with particular reference to hemoglobin synthesis—a review. Lab. Invest. 10: 992–1011, 1961.
(10) BREITMAN, T. R., and WEBSTER, G. C.: Effect of chloramphenicol on protein and nucleic acid synthesis in isolated thymus nuclei. Biochim. et biophys. acta 27: 408–409, 1958.
(11) HOPKINS, J. W.: Amino acid activation and transfer to ribonucleic acids in the cell nucleus. Proc. Nat. Acad. Sc. 45: 1461–1470, 1959.
(12) ALLFREY, V. G., and MIRSKY, A. E.: Amino acid transport into the cell nucleus and reactions governing nuclear protein synthesis. *In* Protein Biosynthesis (Harris, R. J. C., ed.). New York, Academic Press, Inc., 1961, pp. 49–81.
(13) VON EHRENSTEIN, G., and LIPMANN, F.: Experiments on hemoglobin biosynthesis Proc Nat. Acad. Sc. 47: 941–950, 1961.
(14) AMBROSE, C. T.: The effect of chloramphenicol on an entirely *in vitro* secondary antibody response. Fed. Proc. 21: 30, 1962.

DISCUSSION

ABRAM B. STAVITSKY,[1,2] Department of Microbiology, Western Reserve University School of Medicine, Cleveland, Ohio

Dr. Ambrose has reported some interesting and unique findings. Discussion of them is complicated by the following considerations: 1) There is some question about what he is measuring with his antibody assay and 2) he is studying phenomena whose mechanisms are poorly understood. Let me be more explicit.

The hemagglutination method measures antibody activity and is probably also a rough measure of antibody concentration. It is, therefore, possible that some of the antibody in the medium was not recently synthesized, but was made at some time in the past and released into the medium after the disruption of antibody-containing cells. The distinction between *de novo* synthesis of antibody and release of antibody can be made with certainty only by measurement of the extent of incorporation of radioactive amino acids into antibody. Table 1 illustrates the results of an experiment in which the secondary antibody response of the rabbit lymph node was stimulated *in vivo* and the node was set up in *in vitro* culture as in the experiment of Dr. Ambrose; the only exception was that the fragments of node were pinned to a stainless-steel wire grid with agar. It is clear that in this situation, which is admittedly quite different from Dr. Ambrose's, the hemagglutination titer is not a reliable measure of *de novo* antibody synthesis. I do not know why it is not a reliable gauge of synthesis, but suppose that in the presence of these purine and pyrimidine analogues some disruption of cells or cell permeability occurs, with release of intracellular antibody which was not made recently. However, over a period of years, we have tried with only intermittent success to get incorporation of C^{14} glycine into the antibody which appears in the medium in a system like the one Dr. Ambrose used. We have succeeded on occasion and I must, therefore, conclude that *de novo* antibody synthesis can occur under these conditions. That incorporation does not occur more consistently

[1] This investigation was supported by research grant E-1865 from the Allergy and Infectious Diseases Institute, National Institutes of Health, Public Health Service.
[2] The assistance of Mr. Weldon W. Harold is acknowledged.

must be a reflection of the relatively small amounts of antibody which are synthesized. Indeed it would be difficult to explain the data on any other basis; it is unlikely that the antibody which appears in the medium a few days to a week after the addition of antigen represents antibody that has persisted intracellularly from the last injection of antigen 3 to 12 months before. The rub, as I see it, comes in the interpretation of the titers of cultures 2 to 3 weeks old. The possibility remains that the antibody in the medium at this time represents a combination of newly produced antibody and antibody synthesized previously and released into the medium. A more sensitive isotopic assay and the development of conditions favoring the production of larger quantities of antibody are urgently needed.

TABLE 1.—Hemagglutination versus incorporation assay of *in vitro* antibody synthesis in presence of inhibitors

Lymph node* in medium plus	Hemagglutination titer†	cpm‡
—	640	1,488
500 μg 5-BRUD§	320	198
200 μg 6-AUR§	320	242
200 μg 8-AA§	320	284
200 μg 6-AT§	160	290

*Popliteal lymph nodes removed 3 days after third injection of bovine γ-globulin into hind foot pads of rabbit. Cultures set up of lymph-node fragments on stainless-steel grids in 1 ml of medium for 48 hours.

† Reciprocal of hemagglutination titer.

‡ Counts per minute of specific bovine γ-globulin-anti-γ-globulin precipitates.

§ 5-BRUD = 5-bromodeoxyruidine; 6-AUR = 6-azauracil riboside; 8-AA = 8-azaadenine; 6-AT = 6-azathymine.

Dr. Ambrose is studying the *in vitro* induction of the secondary antibody response, followed by continued intracellular synthesis of antibody and secretion of this antibody into the medium for a period of weeks. He has made some very useful observations on the factors in the medium that favor the secondary antibody response. However, it is difficult to know at what point(s) in this sequence of reactions the factors in serum, the special mixture and hormones, operate. In short-term, 24- to 48-hour cultures of lymph nodes cultured after *in vivo* induction of antibody synthesis, we found that optimal concentrations of certain amino acids had to be present in the medium for maximal synthesis (*1*). Addition to the medium of serum, serum ultrafiltrate, chick-embryo extract, purines and pyrimidines, certain lipides, carbohydrates, and coenzymes did not promote antibody synthesis in these cultures (*1*). In a similar study with spleen, Mountain (*2*) found that purines and pyrimidines, chick-embryo and liver extract, certain vitamins, and sucrose did not enhance antibody formation. Higher concentrations of sucrose, cysteine, cystine, and reduced glutathione inhibited antibody production (*2*). Working with isolated spleen cells, Vaughan *et al.* (*3*) added ribonucleosides, glucose, and ascorbic acid to the medium without affecting antibody synthesis, but higher concentrations of ascorbic acid, glutamine, and nucleosides inhibited antibody production. The nutritional requirements for successful long-term cultures are less well defined. From the work of Trowell (*4*, *5*) on organ cultures of rat lymph nodes *in vitro*, it appears that a simple synthetic medium consisting of certain amino acids, inorganic salts, glucose, thiamine, paraaminobenzoic acid, and insulin supports lymphoid cells in good condition as well as differentiation of reticulum cells to large lymphocytes. Dr. Ambrose also obtained antibody synthesis in the absence of serum if the special mixture was added to the basal medium.

Dr. Ambrose's findings on the effects of chloramphenicol on antibody formation in a mammalian cell system are unique. It is interesting that Trowell (*4*, *5*) had 0.06 percent chloramphenicol in his cultures—an amount greater than the highest concentration used by Dr. Ambrose—without any apparent effect on morphology or differentiation in his system. It would, therefore, be of interest to find out what biochemical, morphological, and physiological effects chloramphenicol has on the *in*

TABLE 2.—Synthesis of antibody in long-term cultures of rabbit lymph nodes

Days in culture*	cpm†
2	890
3	1, 070
5	760
7	820

*Rabbit popliteal lymph-node fragments derived from rabbits 3 days after the second injection of diphtheria toxoid. One μc of C^{14}-glycine was placed in the medium. Medium and isotope were replaced on days 2, 3, 5, and 7 of culture.

†Counts per minute of specific antigen-antibody precipitate derived from tissue-culture medium.

vitro antibody-forming system. I would agree that the data certainly suggest an effect on the inductive phase of the *in vitro* antibody response.

Therefore, further studies of normal and antibody-forming lymph-node cultures are a glaring requirement. I am perplexed about what goes on in the systems we have studied in which lymph nodes are removed from the rabbit 3 days after the second injection of antigen and then cultured in a manner similar to Dr. Ambrose's system, with the exception that the tissue fragments are stuck to a stainless-steel wire grid with agar. Under these conditions antibody synthesis continues for at least 1 to 3 weeks at a slowly declining rate, as shown in table 2. There is little evidence of mitosis *in vitro*, which is reminiscent of Trowell's observations on cultures of normal rat lymph nodes (*4*, *5*). Nevertheless, as shown in table 1, purine and pyrimidine analogues inhibit antibody synthesis almost completely and studies of *in vitro* incorporation of P^{32} into various cell fractions suggest a greatly increased synthesis of phospholipide, deoxyribonucleic acid (DNA), and ribonucleic acid (RNA) [table 3 (*6*, *7*)]. It is therefore surmised that these antibody-forming tissues are synthesizing and turning over substances other than antibody. Moreover, there seem to be important differences between antibody synthesis as it occurs *in vivo* and *in vitro*. The peak of antibody synthesis *in vivo* is 3 to 4 days after the second injection of antigen and by the 6th day only a low level of synthesis persists (*8*). Yet, lymph nodes removed during the peak of maximal synthesis continue to make antibody at only a slowly declining rate for 1 to 3 weeks *in vitro*. The difference may be that *in vivo* the immature plasma cell, which is capable of maximal antibody synthesis, differentiates into the mature plasma cell that is capable of only minimal synthesis, whereas *in vitro* this differentiation may not occur.

That Dr. Ambrose's studies raise more questions than they answer is a compliment rather than a criticism. I am certain that more detailed cytochemical, biochemical, and immunological studies of what is going on in his cultures will provide much useful information as well as many more incisive questions. Thus is progress made. The difficulty of the use of hemagglutination titration to measure *de novo* antibody synthesis in *in vitro* lymph-node cultures is stressed. It is, however, concluded that such synthesis does occur *in vitro* after the addition of antigen to lymph-node fragments from rabbits which were stimulated for the first time many months previously.

TABLE 3.—Incorporation of inorganic P^{32} into phospholipides, deoxyribonucleic acid, and ribonucleic acid of lymph nodes

Source of node*	Phospholipides (% dose/mg P)	RNA	DNA
Normal rabbit	3. 7	3. 0	0. 43
Normal rabbit	2. 3	1. 2	0. 26
Immune rabbit	6. 4	4. 6	1. 05
Immune rabbit	5. 6	4. 4	0. 71

*Immune rabbit—node removed from rabbit 3 days after second injection of diphtheria toxoid. Lymph-node slices incubated with inorganic 50 μc P^{32} for 5 hours *in vitro*.

Further biochemical, cytochemical, and immunochemical studies are required to understand what is occurring in organ cultures of antibody-forming tissues.

References

(1) WOLF, B., and STAVITSKY, A. B.: *In vitro* production of diphtheria antitoxin by tissues of immunized animals. II. Development of a synthetic medium which promotes antibody synthesis and the incorporation of radioactive amino acids into antibody. J. Immunol. 81: 404–413, 1958.

(2) MOUNTAIN, I. M.: Antibody production by spleen *in vitro*. I. Influence of cortisone and other chemicals. J. Immunol. 74: 270–277, 1955.

(3) VAUGHAN, J. H., DUTTON, A. H., DUTTON, R. W., GEORGE, M., and MARSTON, R. Q.: A study of antibody production *in vitro*. J. Immunol. 84: 258–267, 1960.

(4) TROWELL, O. A.: The culture of lymph nodes in synthetic media. Exper. Cell Res. 9: 258–276, 1955.

(5) ———: The culture of mature organs in a synthetic medium. Exper. Cell Res. 16: 118–147, 1949.

(6) HARRINGTON, H., and STAVITSKY, A. B.: Unpublished observations.

(7) DUTTON, R. W., DUTTON, A. H., GEORGE, M., MARSTON, R. Q., and VAUGHAN, J. H.: Phosphate metabolism of spleen cells in antibody formation. J. Immunol. 84: 268–272, 1960.

(8) STAVITSKY, A. B.: *In vitro* studies of the antibody response. *In* Advances in Immunology (Taliaferro, W. H., and Humphrey, J. H., eds.). New York, Academic Press, Inc., 1961, pp. 211–261.