On the Structure and Production of Antibodies

By E. SORKIN*

It is the purpose of this short article to discuss some aspects of the structure and production of antibodies based on some recent experimental facts and theories. Although there are numerous experimental results on the biological aspects of antibody formation, no decisive evidence for the understanding of the actual mechanism of antibody production has as yet been brought forward.

On the Role of Antibodies

It is necessary first to define the term antibody. Generally, antibodies are considered as globular proteins, especially γ-globulins which appear in the sera of animals and human beings upon introduction of a foreign substance, the antigen. A considerable change in the reactivity of the organism towards the antigen (e.g. virus, bacterium, soluble proteins, or polysaccharide) is established thereafter, as indicated by many of the classical phenomena of the immune response.

It is obvious that the ability of animals to form antibody must have been of survival value to the species. The constantly changing environmental conditions in the past and the present have almost certainly eliminated species or individuals not capable of antibody production. Nature has supplied us with a striking case of the significance of antibodies for immunity and survival, namely congenital agamma-globulinanemia. In this disease male children have, at a time when their lymphatic system should have reached immunological maturity, hardly any or no circulating γ-globulin or antibodies and they are extremely susceptible to bacterial infections. Upon immunization they seldom form circulating antibodies nor do they appear to produce any plasma cells. These facts are significant since these cells are usually assumed to be the cells responsible for antibody production. Without antibiotics and passive transfer of 'normal' γ-globulin these patients would succumb to bacterial infections. Naturally these facts should not blind us to overrating the significance of circulating antibodies as a defense mechanism against infectious agents as there are defense mechanisms known, which are not necessarily based on the presence of circulating antibodies or any antibody at all. Indeed the case of congenital agamma-globulinanemia shows that clinical immunity against several virus diseases can be established in these patients[1,2]. Although nothing definite is known about the origin of this type of immunity some unknown type of cell-fixed antibody might be involved.

One of the further functions of circulating antibodies, besides reacting with foreign agents and preparing them for phagocytosis, might be to confer their specifically directed reactivity against the antigen upon cells which had had no previous contact with it. This truly biological function ensues with the development of the capacity to react more efficiently upon second encounter with a virus, bacterium, or any antigen and presents itself as one of the basic phenomena of immunity .That it can be even harmful to the host as in anaphylaxis or other states of hypersensitivity does not detract from this fact.

Recent work has also established that antibodies are involved in several immunopathological processes where no external antigenic stimuli, at least directly, are responsible for the disease. The formation of 'auto-antibodies', that is antibodies directed against host's own cells or modified cellular components can cause a number of auto-immune diseases.

Some Facts about the Structure of Antibodies

Amino Acids. Antibodies are proteins, composed of the known twenty amino acids which are found in most other proteins. Since the molecular weight of human, rabbit, and several other antibodies is about 160000, they must contain some 1500 amino acid residues which have most likely the usual polypeptide backbone with their covalent bonds. No difference in amino acid composition has yet been found between so-called normal γ-globulins and antibodies[3].

Amino Acid Sequence. For an understanding of the correlation between structural features and biological activities and mode of production of antibodies we need to know the sequence of the amino acids. It is obvious from the number of amino acids in such a huge molecule that this is not feasible at the present. However, several N-terminal end-group determinations of intact rabbit antibodies have been performed. It was recognized[3-5] that both rabbit antibody formed against several different proteins and polysaccharides and inter rabbit γ-globulin had a single, common N-terminal amino acid-alanin-per molecule. Both PORTER[3] and McFADDEN and SMITH[5] proved that the N-terminal amino acid sequence is alanyl-leucyl-valyl-aspartyl-glutamic acid. It is difficult to use these results on five amino acids out of 1500 to conclude anything on the structure-activity relationship and sequence of amino acids in the various antibody molecules.

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1 R. A. GOOD, in *Immunopathology* (Ed. P. GRABAR and P. MIESCHER, Benno Schwabe & Co., Basel/Stuttgart 1959), p. 41.

2 D. GITLIN, C. A. JANEWAY, L. APT, and J. M. CRAIG, in *Cellular and Humoral Aspects of the Hypersensitive States* (Ed. H. S. LAWRENCE, Hoeber-Harper, New York 1959), p. 375.

3 R. R. PORTER, Biochem. J. *46*, 473 (1950).

4 E. S. ORLEANS, Nature *175*, 728 (1955).

5 M. L. McFADDEN, and E. L. SMITH, J. biol. Chem. *214*, 185 (1955).

Enzymatic Degradation of Antibodies. Work on numerous proteins has shown that the intact primary structure is not essential for the biological activity of proteins[6].

Extending earlier studies, PORTER[7] recently prepared rabbit γ-globulin from normal and immune rabbits and split them by digestion with crystalline papain. Three large fragments I, II, and III, which together formed 90% or more of the original molecules were recovered from the digest by chromatography on carboxymethylcellulose. The ratios of yield of the split products averaged 1:0.8:0.9 (I:II:III) and the relative sizes as determined in the ultracentrifuge were 1:1.05:1.6. Two of the fragments I and II of molecular weight 50000 to 55000 retained the power to combine with the antigen specifically and each contained probably one antibody combining site. The third piece (III) of molecular weight about 80000 crystallizes easily and showed no reaction with the antigen. However, it precipitated most of the antibody present in goat and rat anti-γ-globulin serum and therefore presumably contains much of the antigenic specificity of the original molecule.

These significant findings by PORTER have led to the conclusion that rabbit γ-globulin is formed of two pieces with very similar structure and joined to a third piece of quite different character. Table I summarizes these results.

In view of the distinctive characters of the three fractions the possibility was considered whether they might be synthesized separately and joined into the whole molecule in a separate step. Incorporation experiments with injected radioactive amino acids, however, showed no evidence for independent synthesis of these fractions.

No conclusion is possible from this work as to whether the primary structure in all rabbit antibodies is identical and whether the different antibody combining sites are only formed by refolding of the same polypeptide chains as suggested by PAULING[8]. But clearly this type of approach has important implications and further results can be awaited with interest.

The Immunospecificity of Antibodies

This aspect has been discussed recently by TALMAGE[9] and earlier by LANDSTEINER[10] and therefore only a few selected items will be mentioned here. One of the fundamental problems of antibody production is that stimulation of a host by an apparently unlimited number of antigenic substances should lead to the formation of a seemingly unlimited number of antibodies. Since there are probably millions of living species existing and each of them contains perhaps thousands of different antigenic proteins, enzymes, and polysaccharides, one host might be expected to distinguish between at least 10^9 molecular species. Furthermore, even one antigenic site or haptenic group can give rise to a recognized variation in the degree of complementarity of the antibody combining site. If we add to these the theoretical possibility of the huge number of hapten-protein combinations as antigens the recognition and specificity problem on the cellular and molecular synthetic level seems of a considerable order of magnitude, even if we consider the restrictions imposed by the well-known cross reactions. The most obvious explanation is offered by the hypothesis that an antigen conveys instructive specification on the γ-globulin molecule during some stage of its synthesis, be it by genetic modification (indirect template theory) or by direct interference, e. g. during the folding of the polypeptide chain (direct template theory). While the classical concept of immunospecificity and antibody production assumes that the specificity of antibody is due to instructive interference by the antigen and that the host does not provide the information, JERNE[11],

[6] R. L. HILL, J. R. KIMMEL, and E. L. SMITH, Ann. Rev. Biochem. *28*, 97 (1959).
[7] R. R. PORTER, Biochem. J. *73*, 119 (1959).
[8] L. PAULING, J. Amer. chem. Soc. *62*, 2643 (1940).
[9] D. W. TALMAGE, Science *129*, 1643 (1959).
[10] K. LANDSTEINER, *The Specificity of Serological Reactions* (Harvard University Press, Cambridge, Mass. 1945.)
[11] N. K. JERNE, Proc. nat. Acad. Sci., Wash. *41*, 849 (1955).

Table I: Some results of papain digestion of rabbit γ-globulin containing specific antibody (PORTER 1959)[7]

	Fragment I	Fragment II	Fragment III	Original
Molecular weight	50000–55000	50000–55000	80000	187600
Molar ratio	1	0.8	0.9	—
Antigenicity	none (in goat)	none (in goat)	strong (in goat)	strong
Antibody combining capacity (inhibition)	specific	specific	none	specific
Amino acid composition	as in II	as in I	very different from I and II	—
N-terminal amino acid	alanyl-	alanyl-	—	alanyl-
Carbohydrate	1.1%	0.25%	2.2%	1.1%
Heat stability	—	—	110°C; 1 h	—

TALMAGE[9], BURNET[12], and LEDERBERG[13] have recently developed alternative ideas. Their basic proposition is that antibody is natural globulin, produced by selected clones of cells. Furthermore TALMAGE[9] suggests that the specificity of the antibodies is due to a unique combination of antibodies, which were selected out from these natural globulins.

The Active Center of Antibodies

It is generally agreed that the number and size of the antibody combining sites are small and apart from some exceptions the number of the sites probably does not exceed two. LANDSTEINER has shown already that if the size of an artificial antigenic determinant is too large then antibodies are only formed against a proportion of the antigen. This might suggest a definite limit of the size in the antibody receptor site, which has been estimated by CAMPBELL and BULMAN[14] to be not larger than 700 Å². Only about 1% of the entire surface of the antibody molecule is likely to participate in its interaction with the determinant group of the antigen.

Nothing definite is known on the relative positions of the antibody combining sites, but for steric reasons they cannot be very close. We cannot discuss in the available space the interesting question of the shape of the active center, the number of amino acids necessary for binding a protein, a polysaccharide, or a hapten[15–17].

While it is possible that there are also electrostatic forces involved in the binding between protein antigens and their antibodies, there are probably only hydrogen bonds and van der Waal forces in the three dimensional network acting between carbohydrates and antibodies (see Fig. 1).

Future work will have to determine the fine structure and amino acid sequence of the (cavity-like?) active center in antibodies as it has been worked out already for several enzymes and hormones.

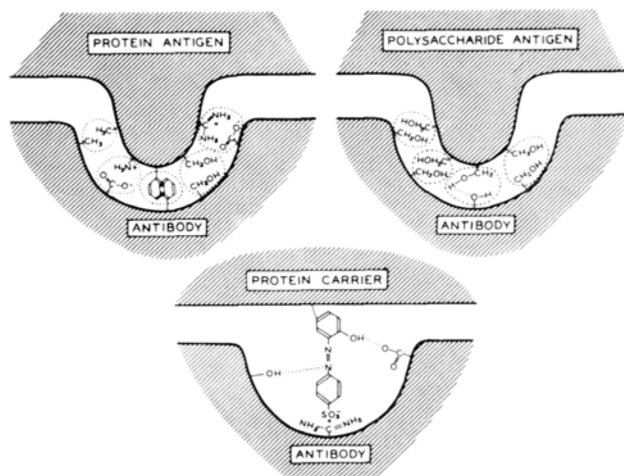

Fig. 1: Antigen-Antibody complexes stabilized by various types of bonds (hypothetical)

On the Biosynthesis of Antibodies

In discussing antibody synthesis it[18] is necessary to remember that antibodies are proteins which are not distinguished in their overall amino acid composition from many other proteins. There is not much reason to doubt that the 20 amino acids are built into the polypeptide chain of an antibody molecule, at least in part, by a similar mechanism as is now recognized for other proteins, but the experimental evidence for antibodies is still lacking. What has to be explained, however, is how and at what stage the molecule assumes its specificity. In other words, (1) does sequential information *eo ipso* determine antibody specificity? (2) Where does this information come from, is it genetically determined?[13] And (3) could or must antigen influence the process of information? But let us examine first some facts on the cellular origin and kinetics of antibody production.

Some Facts on the Cellular Origin of Antibodies

Earlier work, as summarized by COONS[19], has shown that the lymphoid tissues are the major site of formation of antibody. FAGRAEUS[20] and later many authors have demonstrated the relationship between the appearance of antibodies and plasma cells. She recognized that primitive so-called 'transitional cells' in the red pulp of spleen appeared on the third day of a secondary response and gave rise to immature and finally mature plasma cells on the fifth day or later. These and many other findings[19] are of significance because they demonstrate that antibody production is most likely parallel to a developmental process and takes place in a family of cells which is not recognizable morphologically before the antigenic stimulus is applied.

Intracellular antibody in lymphnode cells was demonstrated by COONS in granular parts of the cytoplasm and occasionally in the nucleus in a secondary response. Nothing definite is known yet, however, on the intracellular site of antibody synthesis, but ASKONAS[21] has obtained antibody from a microsomal fraction after elution at pH 9.5. It could not be established whether these particles were also the site of synthesis of the antibody.

[12] F. M. BURNET, *The Clonal Selection Theory of Acquired Immunity* (Cambridge University Press 1959).

[13] J. LEDERBERG, Science *129*, 1649 (1959).

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[19] A. H. COONS, Harvey Lectures (Academic Press, New York 1959), p. 113.

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The One Cell – One Antibody Hypothesis

It is well established[22] that the γ-globulin fraction in general and antibodies to a specific antigen are heterogenous. This poses the important question whether this heterogenity has its origin on the cellular or subcellular level. Is it possible that one cell can synthesize only one antibody, as demanded by the clonal cell selection theory, or can one cell produce two, three, or even any number of antibodies with different specificities? And if production of multiple specificities by one cell should be proven, is this due to instructive interference by antigen or due to a multiplicity of ribosomal templates?

The experimental evidence is conflicting at present.

Preliminary studies by COONS[19] with rabbits immunized with two antigens, namely diphtheria toxoid and hen's egg albumin showed, when tested with a mixture of both antigens in a secondary response, that there were approximately twice as many cells stained for both antibodies as in tests for a single antibody. It is assumed tentatively from these observations that cells might respond to a single antigenic stimulus and be capable of synthesizing one kind of antibody only.

NOSSAL and LEDERBERG[23,24] obtained similar results by a basically related method. Since lymph nodes are an important site of antibody production, they prepared single cell suspensions from popliteal lymph nodes of rats, simultaneously immunized against two Salmonella serotypes. Microdroplets of the cells were prepared and the cells in these tested for the presence of anti-flagellar antibody by their ability to immobilize motile Salmonella. They could find evidence of only monospecific cells producing one or the other anti-flagellar antibody.

ATTARDI *et al.*[25], however, have contradictory evidence. They have strong indications for bispecific antibody forming cells in rabbits serially immunized against two bacteriophages (T_2 and T_5). Cell suspensions obtained from popliteal lymph nodes of immunized rabbits were prepared, the cells dispensed in microdrops or pipettes to obtain single cells, and their antibody producing capacity tested by the neutralization of the phage T_2 and T_5 activity. They found that out of 925 lymph node cells examined, 92 cells (9.9%) produced detectable antibody. 5% of lymph node cells produced anti-T_2 antibody and 2.9% anti-T_5 antibody. Most significantly 18 out of 925 (1.9%) produced both anti-T_2 and anti-T_5 antibody.

It is perhaps worth mentioning here that recent experiments in our laboratory[26] stress the fact that this type of technique has to be evaluated carefully, since the capacity of cells to combine with antigen could be passively conferred from presumably antibody producing cells or rabbit or guinea pig antisera onto normal cells. We found that normal rabbit spleen cells which had been treated *in vitro* with certain rabbit or guinea pig antisera (e. g. against human serum albumin) and then washed are capable of specifically adsorbing isotope labelled antigen, e. g. I^{131}-HSA.

The antibody responsible for this type of effect appears to be distinct from the main precipitating antibody in the serum and the term 'cytophilic antibody' has been suggested. It is not impossible that this antibody could act as antigen receptor on cell surfaces and explain in part the difference between primary and secondary response.

Table II: Adsorption of antigen (I^{131}-Human Serum Albumin) by cells treated with rabbit antiserum or normal serum[26]

Type of cell	Treated with normal serum and washed 4 times	Treated with antiserum and washed 4 times
Rabbit spleen cells	2	556
Guinea pig spleen cells . .	3	648
Rabbit red cells	17	18

0.5 μg of labelled HSA was added to each tube (3,130 counts/min)

The Fate of Antigen in its Relation to Antibody Synthesis

Since the introduction of an antigen supplies the obvious stimulus for production of antibody, the important question remains, how this could happen. If, as is assumed by many workers, antigen supplies directly the information for the specificity of the antibodies it provokes, then clearly antigen or a determinant group thereof has to be present as long as actual antibody synthesis takes place. In some instances this would have to be for several years. Uptake therefore of antigen by the cells capable of antibody synthesis and its persistence is the basic condition for this assumption and also the cause of much difference in theories[27].

The overall experimental evidence, especially in recent years with isotope labelled antigen, is suggestive that antigenic groups can indeed persist intracellularly for some time[28,29]. Although these results indicate persistence of antigen, they do not provide direct evidence for the participation of antigen in transmitting information by acting as a template. The question also arises whether the low amounts of retained antigen,

[22] R. R. PORTER, Folia biol., Prague *5*, 310 (1958).
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[26] S. V. BOYDEN and E. SORKIN, Immunology, in press (1960).
[27] E. SORKIN, in *Symposium on the Mechanism of Antibody Formation* (Liblice, Czechoslovakia 1960), in press.
[28] F. HAUROWITZ, in *Immunity and Virus Infection*, (Ed. V. A. NAJJAR, J. WILEY, New York 1959), p. 18.
[29] D. H. CAMPBELL and J. S. GARVEY, Int. Arch. Allergy *12*, 70 (1958).

for example, a few hundred molecules per cell, are sufficiently high to account for continued stimulation of antibody production. POLLOCK's results[30] on enzyme induction are a case in point because they suggest that with the *Bacillus cereus* one inducing penicillin molecule is sufficient to result in the production of about 40 molecules of penicillinase/h. Also a few µg of an antigen, e. g. a pneumococcus polysaccharide, can give rise to the production of many grams of antibody.

Rates and Characteristics of Antibody Synthesis

It has been established for a long time that the induction period, the time until antibody is synthesized, is considerably shorter in a secondary response than in a primary one. Experiments *in vivo*[31–33] and *in vitro* (e. g.[34–36]) with labelled amino acids indicate that antibody is synthesized *de novo* from amino acids and this incorporation of the amino acids into antibody appears to occur only within a short period[37]. Incorporation *in vitro* into antibody was found between day 7 and 10 in spleens from rabbits after one injection of e. g. a soluble protein. The synthesis of antibody by spleen fragments *in vitro* took place essentially between day 3–5 after a booster injection and dropped very rapidly, a fact which agrees well with the findings on passive transfer of the antibody production by cells [38–41]. This production period during which immature plasma cells are mainly abundant gives way to the decline period with mature plasma cells in the tissues.

A detailed analysis of the kinetics of synthesis has been attempted by ASKONAS[42]. She investigated the mechanism of antibody secretion by studying the amino acid pool and incorporation of radioactive amino acids into the antibody by tissue fragments or perfused lungs of immunized animals. Intracellular antibody becomes labelled within a few minutes, but there was a delay of 30–45 min before any of the newly formed antibody was released by the cells and secreted into the extracellular compartments. It would seem that antibody is mixed with an intracellular pool from which secretion occurs continuously.

As is seen from Table III when cells were slightly damaged or broken no net synthesis of antibody could be found.

Further studies for the elucidation of the detailed mechanism of antibody synthesis will have to await results obtained with cell free systems.

Table III: Effect of cell damage on incorporation of C^{14}-glycin into Anti-ovalbumin antibody (ASKONAS 1958)[21]

Lymph gland	Wet weight (g)	Radioactivity (cts/min/cm²)
Slices	0.35	767
Cells with damaged cell walls	1	8
Homogenate	1	0

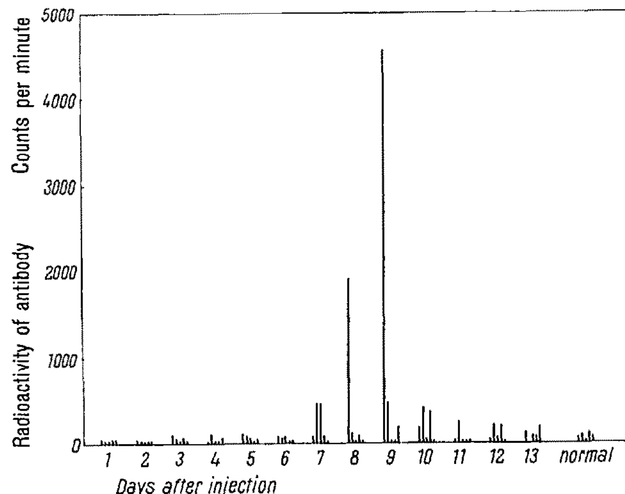

Fig. 2: Antibody synthesis *in vitro* by spleen cells: primary response

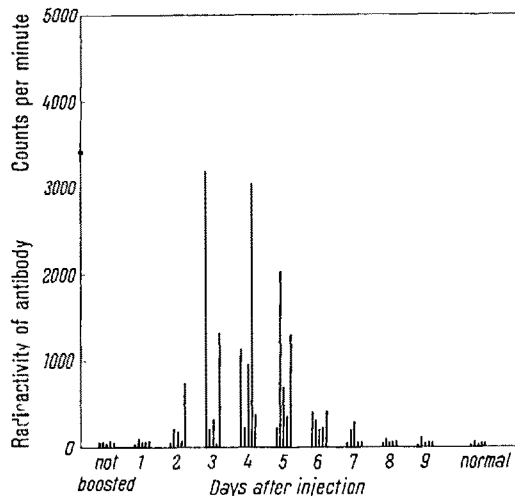

Fig. 3: Antibody synthesis *in vitro* by spleen cells: secondary response

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40 F. J. DIXON and W. O. WEIGLE, J. exp. Med. *105*, 75 (1957).
41 J. V. SPÄRCK, Acta path. microbiol. scand. *46*, 206 (1959).
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Recent Facts and Ideas on Protein Synthesis in Relation to Antibody Synthesis

The study of protein synthesis has made extraordinary progress during the last few years (e. g.[43,44]). In particular the finding that there are at least two ribonucleic acid types, (a) transfer RNA and (b) the microsomal RNA, have stimulated not only experiments but much speculation on the relationship between the genes (DNA), the RNA's and the proteins and the coding problem in general.

The recent findings by several laboratories suggest that possibly 20 different RNA-amino acid complexes of the type illustrated in Figure 4 exist for the 20 amino acids. The various transfer RNA-amino acid complexes are supposed to attach onto a ribosomal RNA-protein template. Each (theoretical) identifying triplet (or quartet?) in the transfer RNA should find its unique complement on this ribosomal ribonucleoprotein template. The amino acids are brought close enough together to react under peptide formation to form polypeptides and then proteins[43,44].

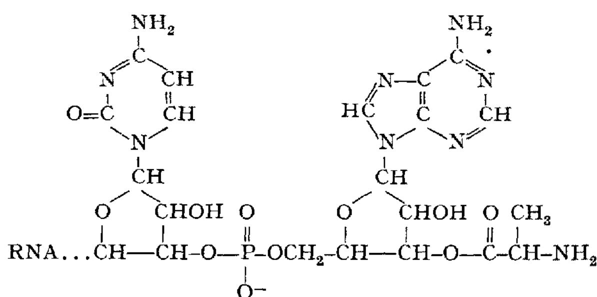

Fig. 4: Endgroup of transfer RNA (HOAGLAND 1959)[44]

What bearing have these and other facts and speculations on our problem of antibody synthesis? The crucial issue is obviously (1) are the specifications for the amino acid sequence of antibody laid down through a flow information from DNA to the ribosomal template (it is most likely not in transfer RNA's since these might be interchangeable in various tissues), and (2) is it likely from our present knowledge that sequence(?) and folding could be subjected to external (antigenic) influences of the most multiple kind as expressed by the innumerable antigenic agents? Or (3) is it more likely that the folding and three dimensional structure and specificity of antibody (or any protein) is based on the sequence of amino acids alone? While point 1 is likely to be correct but not proven, one has difficulties in imagining that the huge number of varied antigens discussed earlier could possibly act or interfere just at the time and place where the polypeptide chain folds.

If we assume that the reasoning under 3 is perhaps the correct one, and to which several workers will suscribe at the present[13] and agree on the dominating role of the DNA in the genes as the origin of information also for the RNA template, then the following hypothetical picture for antibody formation would emerge.

We have indicated by numbers (1,2) at what stage of antibody production antigen is supposed to interfere according to the instructive theories. It appears to this author at the present, that there is no contradictory evidence that structure and specificity of antibody molecules might be based on a flow of information from the genetic nuclear material (DNA) to a ribo-

[43] F. H. CRICK, Sympos. Soc. exp. Biol. *12*, 138 (1958).
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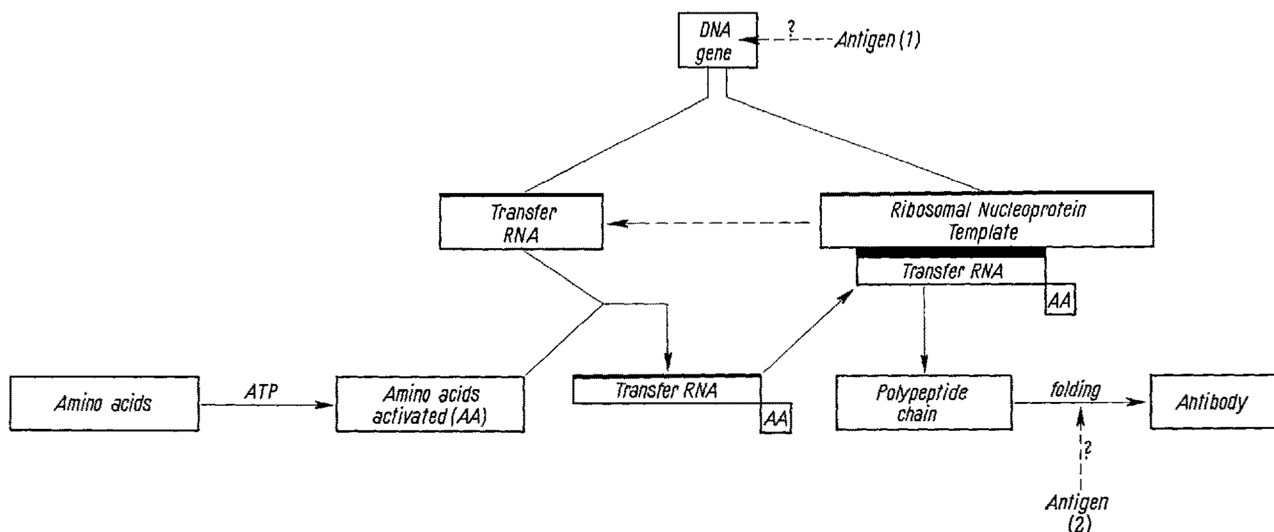

Fig. 5: Simplified hypothetical scheme of antibody synthesis, based on recent work and theories on protein synthesis
The two numbers indicate the suggested but unproven actions of antigen according to the indirect (1) and the direct template theories (2)

somal RNA template, which determines sequence of amino acids and coiling and folding of the polypeptide chain.

The function of the antigen has then to be sought elsewhere. Several possibilities come to mind, how antigen might interfere without imposing structural specification.

(1) Antigen could act by removing an inhibitor (natural antibody?) from the ribosomal template, thus permitting the assembly of RNA-amino acid complexes on the template to form new antibody. This it can only do in cells, potentially capable of synthesizing this antibody and carrying it naturally.

(2) Antigen could react with cell surface antibody (especially in secondary response) and intracellular antibody to produce cytotoxic complexes, perhaps with complement fixation, which could stimulate cell metabolism and eventually result in cellular proliferation and differentiation (see also [9,12,13]).

(3) Antigen might act either as such or in the form of e. g. peptide or carbohydrate fragments as the source of specific nutrition for the various kinds of cells producing antibody and stimulate their growth and thus give rise concomittantly to production of antibodies in these stimulated cells.

No information is available yet on any of thsee points, but they are liable to experimental testing.

Zusammenfassung

Einige Probleme der Struktur und Biosynthese der Antikörper werden auf der Basis neuerer Ergebnisse und Theorien diskutiert. Unter den vielen ungelösten Problemen werden hervorgehoben:

1. Die Frage nach der Aminosäuresequenz des «aktiven Zentrums» von Antikörpern.
2. Der biochemische Einfluss des Antigens auf die Zelldifferenzierung und die Faltung der Polypeptidketten.
3. Die Notwendigkeit der zellfreien Antikörpersynthese.
4. Die definitive *de novo* «Induktion» von Antikörper-Produktion *in vitro*.
5. Ein Entscheid über die Fähigkeit individueller Zellen, in ihrer Antikörpersynthese mono- oder multispezifisch zu reagieren.

How Specific is Immunity?

By HUBERT BLOCH*

The term, 'specificity', is derived from *species*. It characterizes properties as pertaining to a species, restricting them therefore to one kind. For instance, Diphtheria toxin is specific for the microorganism, *C. diphtheriae* since it is not known to occur in the cells of any other species. Likewise, the antibodies produced in an animal after the injection of diphtheria toxin must also be specific. They combine selectively with diphtheria toxin. As a consequence of this reaction, the toxin loses its toxicity. *In vivo*, the specific binding capacity of the antitoxic antibody results in protection against the effects of the toxin. It is by this mechanism that acquired immunity functions in diphtheria.

In connection with the study of infectious diseases, specificity has become an important element in our thinking. Pathogenic microorganisms are usually thought of as 'specific' agents causing 'specific' disease syndromes. In turn, under the impact of an infection, the diseased organism mobilizes 'specific' defense mechanisms resulting in 'specific' immunity to subsequent attacks. In a few diseases (of which diphtheria is an example), the chemical agent mediating the disease symptoms is known to be a toxin which can be isolated and purified. There is usually little or no chemical difference between the toxins obtained from various strains of toxin producers of the same species of microorganisms. The same antibody will therefore neutralize all of these products.

In most infectious diseases, however, the pathogenic effects cannot be attributed to a known toxin. Yet, infection may still result in specific immunity. Such is the case in measles or chicken pox. The pathogenic viruses being of a single stable type, immunity is acquired against this one type (and therefore specific). But examples of this sort are rare. In other virus diseases, e. g. influenza, identical clinical symptoms are caused by a great variety of viral agents which differ in their serologic types and leave little or no cross-immunity against infections with other types of the same virus. True, with respect to any one particular virus strain, the immune response as such is equally specific in these cases as it is with measles, but with regard to the manifestations of the disease caused by these viruses, there is very little specificity. Similar pathological symptoms are brought about by serologically different virus strains and the immunity resulting from infection with one type does not necessarily confer protection against subsequent attacks from viruses of other types.

There is among these viruses a high degree of variability. Mutants appear at a frequent rate and the serologic types prevailing in successive epidemics are sufficiently different from each other so as to provide no

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