*THE MOLECULAR BASIS OF ANTIBODY FORMATION: A PARADOX**

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Communicated by Norman Davidson, July 15, 1965

When a foreign toxin or pathogen enters the circulatory system of a higher organism, it triggers the division of cells which are capable of making antibodies to react with the foreign substance.[1] The main circulating antibody which is produced in response to such a challenge is referred to as γ-globulin. Although perhaps as many as 10,000 different kinds of γ-globulin molecules may be produced, they all have remarkably similar structural properties including a common molecular weight of 150,000. As detailed knowledge of the structure of these molecules has accumulated, an apparent genetic paradox has arisen which will be the subject of this communication.

These antibody molecules have been shown to consist of four subunit polypeptide chains as illustrated in Figure 1*a*. Two of these chains are identical molecules of 25,000 molecular weight each and are referred to as the light (*L*) chains. The other two chains are identical molecules with twice the molecular weight of the *L* chains and are referred to as heavy (*H*) chains.[2–4] The amino acid sequence contained within these chains is such that the molecules fold into specific configurations in which they are capable of interacting with each other.[5, 6] This is shown diagrammatically in Figure 1*b* where light and heavy chains have folded and combined into a complex configuration of 75,000 molecular weight.[7] Figure 1*c* is a diagrammatic representation of the way in which such half molecules combine to form the 150,000 molecular weight γ-globulin structure. It can be seen that part of the subunit structure serves the function of causing the interaction of these chains, whereas other portions fold into shapes capable of interacting with the specific antigen. These sites which contain the antibody specificity are known to occur at both ends of the molecules with the dimensions indicated in Figure 1*c*.[8, 9] The model represented here resembles in many respects those which have been suggested by others[10, 11] and is consistent with data on the structure and function obtained through a variety of enzymatic, physical, and chemical methods.[12, 13]

The molecular heterogeneity of γ-globulin greatly handicaps chemical studies aimed at elucidating the molecular basis of structural differences. It has, however, been possible to take advantage of a malignant condition (multiple myeloma) of plasma cells, the antibody-producing cells. It appears that this disease results from the clonal proliferation of a single cell which has escaped from normal control of division. Such a cell line reproduces itself indefinitely while secreting a specific homogeneous protein. Although the protein which is produced by a given cell line may be any one of various types related to the immune globulins, the specific class of tumors which we shall discuss in this paper secrete only the light (*L*) chains of γ-globulin (Bence-Jones proteins). When such tumors are produced in the inbred strain of mice (BABL/c), the individual tumor clones can be transplanted separately and the protein produced remains constant through many generations.[14] Comparison of the protein products from many distinct mouse tumor lines by means of peptide mapping revealed that about one half of the amino acid sequence of these

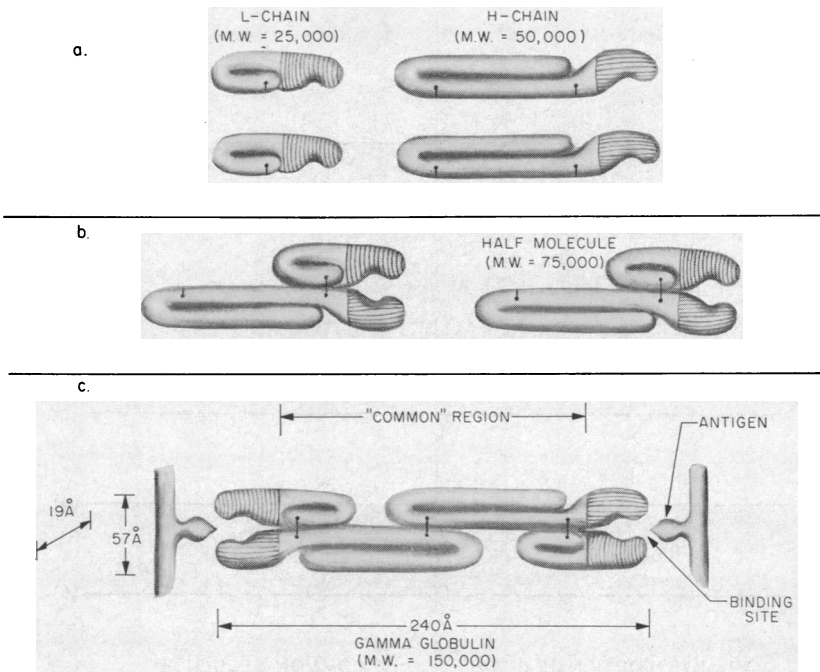

FIG. 1.—Diagrammatic representation of the multiple chain structure of rabbit gamma globulin (see text). Covalent, interchain disulfide linkages (●—●) serve to stabilize the complex structure after formation.

chains is always identical within this given class of molecules.[14, 15] In addition to the common amino acid sequence, another striking feature became evident, namely, that each of these proteins contained a different set of peptides which distinguished it from any of the other protein samples examined. In the limited number of samples obtained from human myeloma patients, the same pattern has emerged.[16–19] These results are diagrammed in Figure 2.

Initially the simplest explanation of these observations involved the assumption that the molecules under study were actually composed of two separate chains. Each chain could then be envisioned as having been synthesized under the control of a separate gene and then joined together through a covalent bond such as a disulfide linkage. We have carried out numerous experiments in an effort to detect disulfide linkages,[15] ester linkages,[20] or other nonpeptide bonds within these molecules. However, none of these procedures gave any indication that these proteins contain more than one chain. Studies of nonuniform radioactive labeling during protein synthesis should resolve this question with finality, including the possibility of a peptide bond joining two subchains.

An attempt to explain the genetic mechanism required to synthesize these proteins in a normal way (without subchain synthesis as discussed above) leads to a genetic paradox. The paradox results from the observation that one end of the light chain behaves as if it were made by the genetic code contained in any one of more than 1000 genes, while the other end of the *L* chains can be shown to be the product of a single gene. That this latter portion is under the control of a single locus is evidenced by the fact that it can undergo mutation (alloytypes),[21, 22]

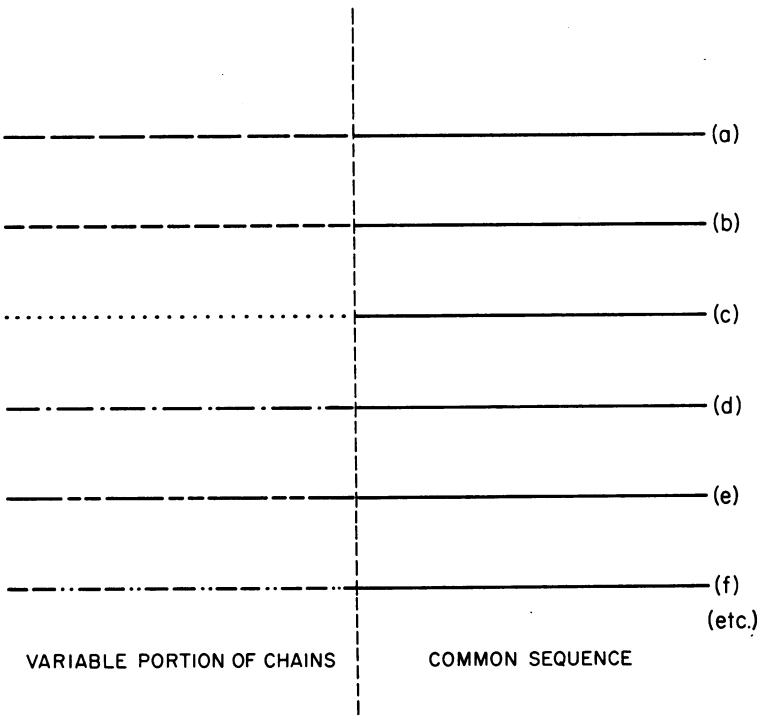

FIG. 2.—Graphical illustration of the general type of amino acid sequence variation found in *L*-chains of both mouse and man.

Mendelian segregation,[23] and that it is rigidly constant in amino acid sequence whenever produced.[14] These facts rule out the possibility that each of the complete polypeptide chains is synthesized under the genetic control of a separate and independent gene contained in the germ line.[24] It appears therefore that immunologically competent cells have evolved a pattern of somatic genetic behavior which is radically different from anything normally found in modern molecular genetics.

One way of resolving this apparent paradox would be to propose that the genetic material, adjacent to that which codes for the common part of the molecule, undergoes a kind of genetic scrambling process during the differentiation of specific cells. Many genetic and enzymatic mechanisms can be imagined which could result in such hypermutability. One particular explanation has been proposed by Smithies,[25] in which he suggests a scheme of multiple crossing over between complementary strands of the nucleic acid that codes for this part of the molecule. Other scrambling mechanisms can be envisioned which are capable of generating a much larger number of different polypeptide sequences, as would appear to be necessary. Such an alternative proposal can be constructed by taking advantage of genetic mechanisms known to occur in microorganisms. Several enzymes have been described that are capable of recognizing specific base sequences of unusual base structures within nucleic acids.[26, 27] One example is the enzyme which repairs ultraviolet damage to nucleic acids.[26] It is able to recognize a thymine dimer and hydrolyze one strand of the DNA at the correct position, whereupon the strand of nucleic acid is degraded for a significant stretch of nucleotides. Later the strand is resynthesized

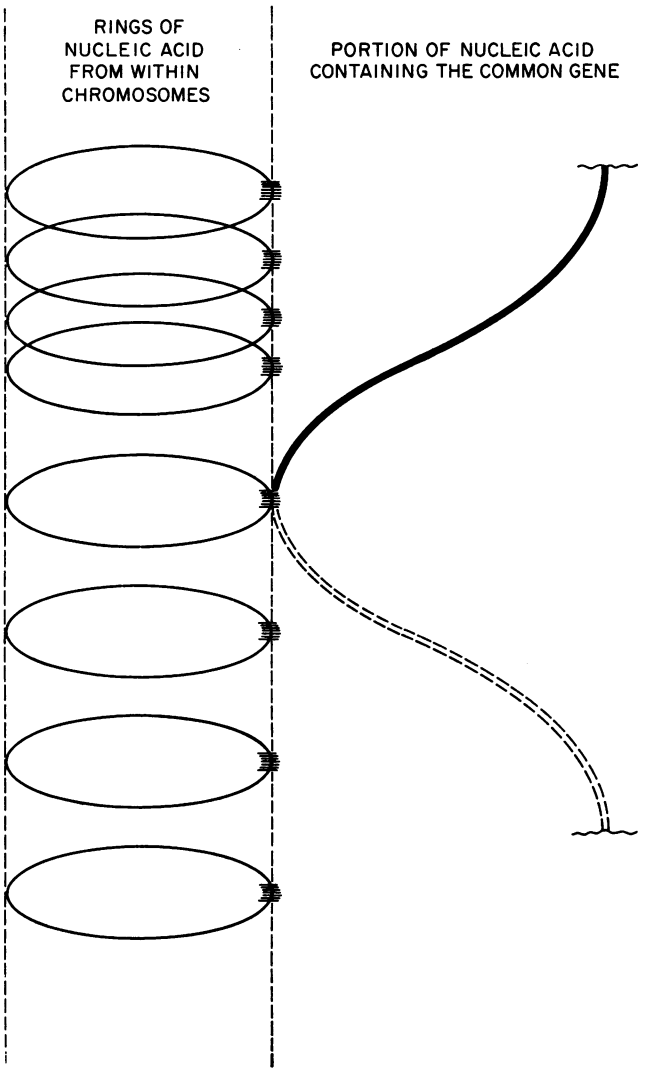

FIG. 3.—Diagram of the proposed genetic mechanism which accounts for the amino acid sequence variations found in *L*-chains. Genetic material which codes for the "variable" portion of *L*-chain molecules is inserted into that which codes for the "common" region of amino acid sequence by a mechanism similar to the insertion of the λ-virus into a bacterial chromosome (see text).

and the repair completed. A minor variation of this mechanism, wherein the resynthesizing enzyme is capable of making many errors in copying as it rebuilds the strand, provides an interesting possibility for a mechanism of mutation. While plausible, these scrambling processes seem unlikely in view of the apparent structural homology which occurs within the variable portion of the chains.[28] Observations of the heritable nature of the capacity to respond to particular antigens[29-31] also make it much less likely that a completely random process is involved.

We wish to propose an alternative theory which seems to resolve the paradox. We assume that the variable portion of these molecules results from genetic material which is present in the germ line and which is combined with the common gene during

the differentiation of the immunologically competent cells. Figure 3 illustrates this theory. Incorporation of a variable nucleic acid segment into the common gene locus might occur in exactly the same manner as that which is thought to occur in certain lysogenic bacteria.[32, 33] The temperate viruses, for example, phage lambda, pair with high efficiency at specific loci within the bacterial genome whereupon they are incorporated into the bacterial chromosome and are replicated in subsequent generations of the bacteria.[34, 35] In our theory we assumed that a very large number of rings of nucleic acid are stacked along the length of the chromosomes as illustrated on the left. Each ring is considered to code for a particular variable portion of a chain. Because of the homology within the variable sequence of the protein, we assume that many of these rings arose by gene duplication from a mutual gene ancestor. It is further suggested that the common gene contains a base sequence which is able to pair with any one of the large number of rings. This would result in a new stretch of combined genetic material coding for a particular chain of an immune globulin molecule in the differentiated cell. Evidence for nucleic acid rings similar to those which are well known in microbial organisms[36–39] has been given for mammalian chromosomes by Hotta and Bassel.[40] Additional examples in other organisms suggesting this type of specific combination of genetic material can be found in the literature.[41, 42] The kind of mechanism we suggest would require the presence of genetic material within mammalian cells which would appear under ordinary circumstances to be inactive. A mutation within any one ring would obviously not be detected by conventional genetic techniques, whereas one in the common segment would, as mentioned previously.[21] Such regions of genetic material in which mutations are rarely detected (e.g., heterochromatin) are known to occur. Although in the undifferentiated cell the variable rings are inactive, each has the potential for specific base pairing with the common nucleic acid segment. During maturation, when any one ring is incorporated into the common portion, it remains fixed.

The relative genetic stability observed in the cell lines which produce the chains of immune globulins is anticipated by this mechanism. It is envisioned to be exactly analogous to the genetic stability of *E. coli* K12 (λ) which only rarely dissociates to produce a λ-virus.

* This work was supported by grant GM-06965 from the U.S. Public Health Service.

[1] Urso, P., and T. Makinodan, *J. Immunol.*, **90**, 897 (1963).
[2] Edelman, G. M., and J. A. Gally, *J. Exptl. Med.*, **16**, 207 (1962).
[3] Pain, R. H., *Biochem. J.*, **88**, 234 (1963).
[4] Small, P. A., J. E. Kehn, and M. E. Lamm, *Science*, **142**, 393 (1963).
[5] Haber, E., these PROCEEDINGS, **52**, 1099 (1964).
[6] Whitney, P. L., and C. Tanford, these PROCEEDINGS, **53**, 524 (1965).
[7] Nisonoff, A., and D. J. Dixon, *Biochemistry*, **3**, 1338 (1964).
[8] Kratky, O., G. Porod, A. Sekora, and B. Paletta, *J. Polymer Sci.*, **16**, 163 (1955).
[9] Almeida, J., B. Cinader, and A. Hawatson, *J. Exptl. Med.*, **118**, 327 (1963).
[10] Fleischman, J. B., R. H. Pain, and R. R. Porter, *Arch. Biochem. Biophys.*, Suppl. 1, 174 (1962).
[11] Edelman, G. M., and J. A. Gally, these PROCEEDINGS, **51**, 846 (1964).
[12] Porter, R. R., *Biochem. J.*, **73**, 119 (1959).
[13] Nisonoff, A., F. C. Wissler, L. N. Lipman, and D. L. Woernley, *Arch. Biochem. Biophys.*, **89**, 230 (1960).
[14] Porter, M., W. J. Dreyer, E. L. Kuff, and K. R. McIntire, *J. Mol. Biol.*, **8**, 814 (1964).
[15] Bennett, J. C., L. Hood, W. J. Dreyer, and M. Potter, *J. Mol. Biol.*, **12**, 81 (1965).

[16] Putnam, F. W., C. W. Easley, and J. W. Helling, *Biochim. Biophys. Acta*, **28**, 231 (1963).
[17] Van Eijk, H. G., C. H. Monfoort, and H. G. K. Westenbrink, *Koninkl. Ned. Akad. Wetenschap. Proc., Ser. C*, **66**, 345 (1963).
[18] Hood, L., A. Blumenthal, L. Neidengard, and W. J. Dreyer, unpublished results.
[19] Putnam, F. W., and C. W. Easley, *J. Biol. Chem.*, **240**, 1626 (1965).
[20] Bennett, J. C., and W. J. Dreyer, unpublished data.
[21] Lawler, S. D., and S. Cohen, *Immunology*, **8**, 206 (1965).
[22] Small, P. A., R. A. Reisfeld, and S. Dray, *J. Mol. Biol.*, **11**, 713 (1965).
[23] Ropartz, C., J. Lenoir, and L. Rivat, *Nature (London)*, **189**, 586 (1961).
[24] Bennett, J. C., and W. J. Dreyer, *Ann. Rev. Biochem.*, **33**, 205 (1964).
[25] Smithies, O., *Nature (London)*, **199**, 1231 (1963).
[26] Boyce, R. P., and P. Howard-Flanders, these PROCEEDINGS, **51**, 293 (1964).
[27] Gold, M., R. Hausmann, U. Maitra, and J. Hurwitz, these PROCEEDINGS, **52**, 292 (1964).
[28] Titani, K., and F. W. Putnam, *Science*, **147**, 1304 (1965).
[29] Papermaster, B. W., R. M. Condie, J. Finstad, and R. A. Good, *J. Exptl. Med.*, **119**, 105 (1964).
[30] Finstad, J., B. W. Papermaster, and R. Good, *Lab. Invest.*, **13**, 490 (1964).
[31] Peterson, R. D. A., M. D. Cooper, and R. A. Good, *Am. J. Med.*, **38**, 579 (1965).
[32] Campbell, A. M., *Advan. Genet.*, **11**, 101 (1962).
[33] Ptashne, M., *J. Mol. Biol.*, **11**, 90 (1965).
[34] Jacob, F., and E. L. Wollman, in *The Chemical Basis of Heredity*, ed. W. D. McElroy and B. Glass (Baltimore: The Johns Hopkins Press, 1957).
[35] Jacob, F., and E. L. Wollman, *Ann. Inst. Pasteur*, **95**, 497 (1958).
[36] Fiers, W., and R. L. Sinsheimer, *J. Mol. Biol.*, **5**, 424 (1962).
[37] Ris, H., and B. L. Chandler, in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 28 (1963), p. 1.
[38] Vinograd, J., J. Lebowitz, R. Radloff, R. Watson, and P. Laipis, these PROCEEDINGS, **53**, 1104 (1965).
[39] Cairns, J., *J. Mol. Biol.*, **6**, 208 (1963).
[40] Hotta, Y., and A. Bassel, these PROCEEDINGS, **53**, 356 (1965).
[41] McClintock, B., in *Cold Spring Harbor Symposia on Quantitative Biology*, vol. 21 (1956), p. 197.
[42] Brink, R. A., in *The Role of Chromosomes in Development*, ed. M. Locke (New York: Academic Press, 1964), p. 183.

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*WATER AS THE SOURCE OF OXIDANT AND REDUCTANT IN BACTERIAL CHEMOSYNTHESIS**

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Communicated by R. H. Burris, July 29, 1965

The two vital aspects of chemoautotrophic metabolism are the generation of energy (ATP or the equivalent) and a simultaneous production of reducing power coupled to the enzymic oxidation of an inorganic substrate. Aleem and Nason[1] reported that in the obligately chemoautotrophic bacterial genus *Nitrobacter* the enzymic oxidation of nitrite is catalyzed by a cytochrome-containing electron transport particle via cytochrome *c* and cytochrome oxidase-like components. They subsequently demonstrated[2] the coupling of this oxidation with the generation of high-energy phosphate bonds which were identified as adenosine triphosphate (ATP). The over-all reaction is: