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Extent and Control of Antibody Diversity

THE NINTH COLWORTH MEDAL LECTURE

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Antibodies are the key molecules in the recognition system that the body uses in order to assess its environment. Antibodies are immunoglobulins composed of two types of polypeptide chains [heavy (H) and light (L)] and having two or more combining sites that dictate the specificity of each antibody. Immune responses are controlled in their specificity by antibodies. Therefore antibody molecules must be made such that they can be specific for any possible chemical determinant. Antibody specific for a common bacterial antigen or the common-cold virus might be expected to develop during evolution. However, antibody specific for an artificial chemical, dreamt up in the laboratory, such as Nip,† cannot be seen to have any direct selective value.

Clearly we are dealing with a system for generating antibody diversity, and it is the ability to make as wide a variety of antibodies as possible that confers the selective advantage. Immunologists, like many other groups, invented their own GOD (Generator of Diversity) to explain their mystery. With a random system generating extensive diversity of antibodies there must be a finely tuned control over the expression of immune responses. The system must discriminate self from non-self and tolerate self. It must not over-react or have too low a threshold for responsiveness. We know that autoimmune diseases can occur, but that normally they are prevented by the immune system itself. From the experimental viewpoint antibody production is elicited only if the antigen is presented to the animal in an appropriate immunogenic form. Thus the immune system has the appropriate controls.

Antibody structure

There are various classes of antibodies, each of which performs a particular biological function, but any specificity of antibody can apparently be associated with any class of antibody. This is accomplished by the particular design of the antibody molecule. Each H-chain of a given class or L-chain of a given type has a constant region (C-region) of sequence common to all chains in that category. Specificity

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† Abbreviations: Nip, 4-hydroxy-5-iodo-3-nitrophenyl-acetyl; Dnp, 2,4-dinitrophenyl; IgG, immunoglobulin G.

resides in the N-terminal variable region of each chain (approximately positions 1–107 for L-chains and positions 1–121 for H-chains).

The variable-region (V-region) sequences are related to one another and they can be arranged in subgroups (Fig. 1); each V-region has certain residues in common with others of the same subgroup. It has been suggested that there is only one gene coding for each subgroup and that the variability stems from somatic mutation (Lederberg, 1959; Cohn, 1968) or recombination (Smithies, 1967; Edelman & Gally, 1967). V-regions have frequently been assigned to a given subgroup on the basis of limited N-terminal stretches of sequence, and as further analysis is made V-regions of one 'subgroup' often vary between each other more than would be expected from somatic-diversity theories (Hood & Talmage, 1970). This requires that the number of subgroups and hence the number of structural genes increase in order to preserve a somatic theory of diversity.

Separate genes for V- and C-regions

The best available explanation for the production of H- and L-chains is that each one is the product of two distinct genes (Dreyer & Bennett, 1965). There is considerable evidence that each C-region is encoded by a single structural gene per haploid genome. Mutations at C-genes segregate in a Mendelian fashion. Deletions of individual C-genes and new C-genes formed by unequal crossing over between adjacent C_H-genes (reviewed by Grubb, 1970) also point to single genes. On the other hand V-regions appear to be the products of a set of genes. Even if we accept one of the somatic-diversity theories the required number of V-genes increases as more subgroups of V-region sequences are discovered. By accepting separate V- and C-genes we can allow any number of V-genes to be shared by the singly represented C-genes. There are certain rules to this sharing: (1) there are three separate pools of V-genes grouped as shown in Fig. 1; one pool of V_H-genes is shared by all C_H-genes (although some preferential associations cannot be excluded by present data) and there are separate V_κ- and V_λ-gene pools; (2) integration of V- and C-gene information occurs at the DNA level (reviewed by Bevan et al., 1972); (3) integration of V- and C-genes is cis; only V- and C-genes present
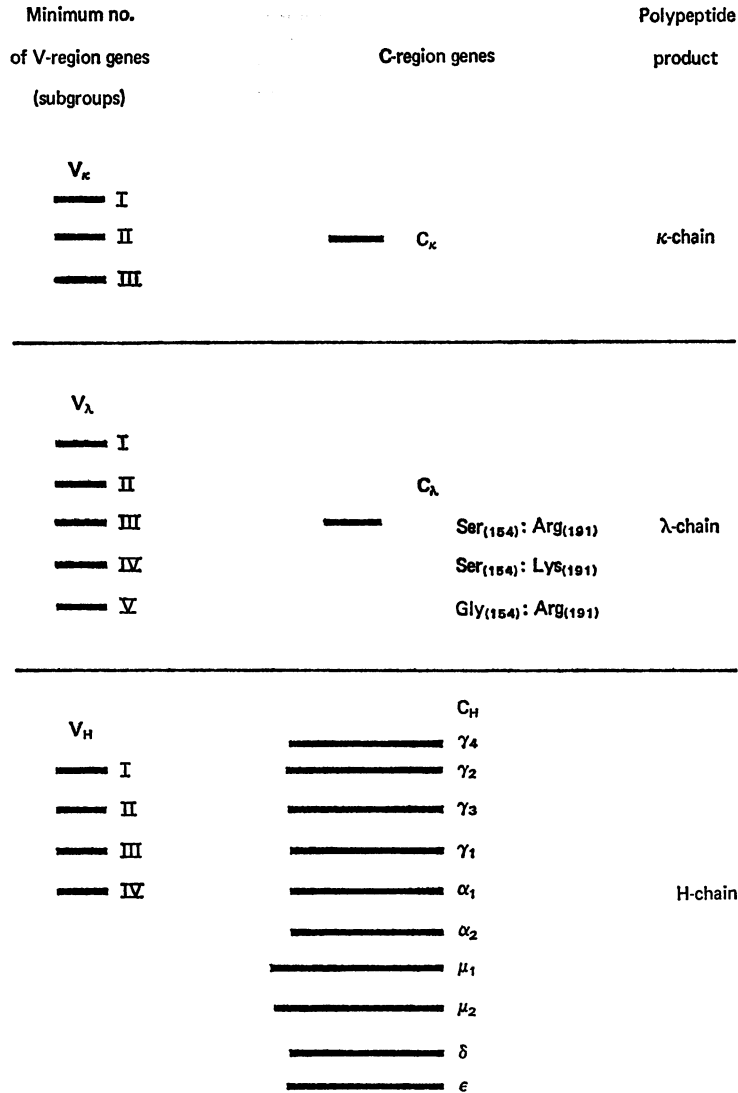
Fig. 1. *Minimum number of germ-line genes predicted from sequence data (adapted from Pink et al., 1971)*

Each C-region gene is assumed to be present as a single copy in the genome. The number of V-region subgroups is assigned from amino acid sequence data; the sequence differences between V-regions of the same subclass have been postulated to be due to somatic mutations of the minimal number of V-genes. It now appears that each 'subgroup' is encoded by a set of germ-line V-genes randomly duplicated and differing by accumulated mutations (see the text).

on the same chromosome can be co-expressed as a single polypeptide chain (Kindt *et al.*, 1970).

The alternative to somatic-diversity theories is a germ-line theory (Szilard, 1960; Dreyer & Bennett, 1965), which says that multiple germ-line genes encode antibody specificity (V-region genes). Distinguishing between the various hypotheses requires the estimation of the extent to which V-gene diversity is present in the germ-line. Genetics of antibody diversity is analogous to population genetics.

We know that the whole population of immuno-competent cells possesses the genetic information for the required range of antibodies, but we wish to know how much of that information is present in each cell.

To define the number of different antibodies requires that each one be identified. Amino acid sequence analysis has been the most extensively used and most rigorous criterion for identifying a given polypeptide chain. Even with modern techniques the determination of the complete sequences even of V-regions requires much material and time. As discussed below, we are using antibody isoelectric spectra as a criterion of identity of a monoclonal product. It provides a rapid screening method for many different serum samples each containing only small amounts of specific antibody. Most of our results discussed in this Lecture depend on this assay method.

Cellular basis of antibody diversity

One cell produces one type of antibody. Extensive production of a given type of antibody is accomplished by the growth of a clone of cells, each of which produces the same antibody. This is the essence of clonal selection theory (Burnet, 1959). The progenitor cells have surface receptors (presumably antibodies) for antigen, and interaction between antigen and receptor is a necessary step (though usually not of itself sufficient) for clonal expansion leading to antibody-secreting cells. The cells involved are lymphocytes and their progeny lymphocytes arise from bone-marrow stem-cells; some are processed in the thymus whereas others are processed in the bursa (in birds) or bursa-equivalent organ (in mammals). Bursa-derived B-lymphocytes are the progenitors of antibody-secreting cells (mediating humoral immunity). Thymus-derived T-lymphocytes mediate cellular immunity (e.g. graft rejection and delayed type hypersensitivity).

Bursa and thymus are both organs in which extensive and rapid cell division occurs, so these are excellent sites for the generation of a diverse population of lymphocytes. It is known that in the bursa B-lymphocytes acquire surface antibodies of different classes, M, G and A (Cooper et al., 1971).

Antibodies of any given specificity are usually heterogeneous, and this has plagued the study of their structure and their biosynthesis. Since this heterogeneity, or diversity, has a cellular basis, we are merely saying that most immune responses are multiclonal. Immunologists have for many years been separating single clones of cells but using a lymphoid-cell transfer system. Lymphocytes taken from, say, spleen or lymph node of a donor animal are used to repopulate X-irradiated syngeneic recipients. If a limiting dilution of donor cells is used

and the recipients are examined for their ability to make antibody to any single antigen, then the proportion of recipients able to make antibody of that specificity relative to the proportion not responding is as predicted by a Poisson distribution of single events. Statistically, therefore, one can predict monoclonality. Clonal identity could be established by characterizing the antibody produced by each clone. The isoelectric spectrum of the monoclonal antibody has proved to be a convenient marker for defining and following single clones of antibody-forming cells.

Antibody isoelectric spectra

The initial answer to the problem of antibody heterogeneity was to use the myeloma tumours. These and related lymphoid tumours are single neoplastic clones of antibody-secreting cells. Thus they provide large amounts of single species of immunoglobulin for amino acid sequence studies. Presumably each myeloma globulin has antibody activity, but its specificities are usually undetected. Mouse myeloma tumours provide an excellent source of cells, constitutively producing immunoglobulin, for biosynthetic studies. Since the myeloma protein is a monoclonal product it should be homogeneous. However, most myeloma proteins show microheterogeneity (Plate 1a). This property they share with most other 'pure' proteins, as discussed elsewhere (Williamson et al., 1972). Analytical isoelectric focusing in thin-layer polyacrylamide gels was developed by Zuhayr Awdeh for studying the origin of microheterogeneity of myeloma protein (Awdeh et al., 1968, 1970). It was established that the individual myeloma tumour synthesizes a single molecular species of immunoglobulin. This biosynthetically homogeneous product undergoes rapid postsynthetic charge alteration. After secretion further alterations of charge, due in most cases to deamidation of specific glutamine or asparagine residues, take place slowly in serum. The pattern of isoelectric bands, the isoelectric spectrum (Plate 1a), is characteristic of the immunoglobulin product of a single clone of cells.

The usefulness of analytical isoelectric focusing has been extended by the use of radioactive hapten or antigen as an overlay to give specific and sensitive detection of the isoelectric spectrum of antibodies focused from serum (Williamson, 1971). The presence of a given clonal product can be detected by analysis of a small sample of serum (Plate 1b). However, a secondary anti-Dnp response is multiclonal and an isoelectric spectrum of 20–30 lines is observed (Plate 2). The information required from such an analysis is the number of clones contributing to the response, but this is not directly discernible. A typical secondary-response spectrum can be constructed by summing the individual spectra of a small number of
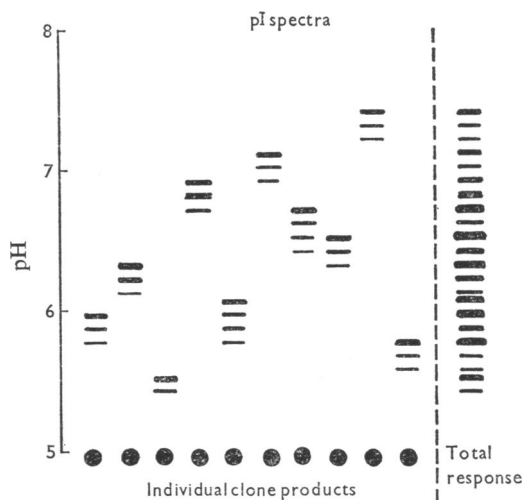
Fig. 2. *Schematic representation of the multiclonal origin of antibody heterogeneity analysed by isoelectric focusing*

The total response represents a heterogeneous antibody spectrum similar to those shown in Plate 2. This spectrum can be obtained by summation of the ten monoclonal antibody spectra drawn on the left.

monoclonal products (Fig. 2), but one cannot reverse this process.

Single clones of antibody-forming cells

Selection of single clones of antibody-forming cells is accomplished by combining the lymphoid-cell transfer system with the isoelectric-focusing assay method for identifying individual clonal products (Askonas *et al.*, 1970). Spleen cells from a donor mouse primed with hapten–protein conjugate are transferred in limiting numbers into irradiated syngeneic recipient mice; the homologous hapten–protein conjugate is injected with the cells, and about 10 days later the serum of each recipient is sampled and analysed. The isoelectric spectrum of anti-Dnp molecules present in each serum sample shows the number of clones of antibody-forming cells expanded in each recipient mouse. If the correct number of cells are transferred then each recipient will bear either one or two clones of anti-Dnp-forming cells or none at all (Plate 3). The distribution of clones follows a Poisson curve, and the single events that this curve predicts are single memory cells, resulting from the primary exposure to antigen in the donor mouse.

A host mouse containing a single anti-Dnp-forming clone can be rechallenged with Dnp-protein to boost the production of antibody by expanding the clone further. In this process memory cells are proliferated and differentiated into antibody-secreting cells (Fig. 3). At the same time memory cells must give rise to more memory cells by antigen-dependent regenerative proliferation, for if the spleen of this clone-bearing host is transferred together with antigen into a series of irradiated mice each of these new recipients produces the characteristic clonal antibody. Thus the clone is expanded and propagated by serial spleen-cell transfer. At each generation anti-Dnp production by the clone is strictly dependent on antigen (Dnp conjugated to the original protein carrier). In the absence of antigen clonal memory cells are long-lived (at least 1 month).

In addition to antigen the expansion of the clone depends on carrier-protein-specific T-lymphocytes. It is this requirement that accounts for the carrier specificity of clonal anti-Dnp production. Specific T-cells are originally present in excess, and since they too can proliferate and are thought to act catalytically they are not limiting for the first few transfer generations (Askonas & Williamson, 1972). The requirement for T-cells can be shown by killing the T-cells with a specific antiserum (anti-θ_{C3H}) and complement. The clonal memory cells that are B-lymphocytes remain viable but cannot give rise to antibody-forming cells on contact with antigen; for anti-Dnp production it is necessary to inject T-lymphocytes from a mouse primed against the carrier protein. Alternatively a partial turn-on of the clone can be achieved by using unprimed T-lymphocytes from another strain of mouse allogeneic (non-histocompatible) with respect to the original mouse strain (Kreth & Williamson, 1971). These findings led us to suggest a cell-surveillance model for T-cell involvement in antibody production. Using the idea that antigen bound to a lymphocyte receptor would display determinants making that lymphocyte appear foreign (Bainbridge, 1971), we envisage the transient interaction of T-cells with the foreign antigen determinants on B-cells. During this contact T-cells could stimulate B-cell proliferation by supplying a mitogenic factor, which has been detected in T-cell secretions. This model is compatible with the role of T-lymphocytes in cell surveillance [see *Transplant. Rev.* (1972) 7]. It remains to be seen whether this model approximates to reality. In any case the control steps for proliferation and differentiation of the B-cell clone remain to be defined at the molecular level.

A single clone of antibody-forming cells is the simplest humoral immune response that can be examined. For cellular studies many of the complexities of multiclonal responses are eliminated. However, for biochemical studies the small scale of the response is the biggest difficulty.
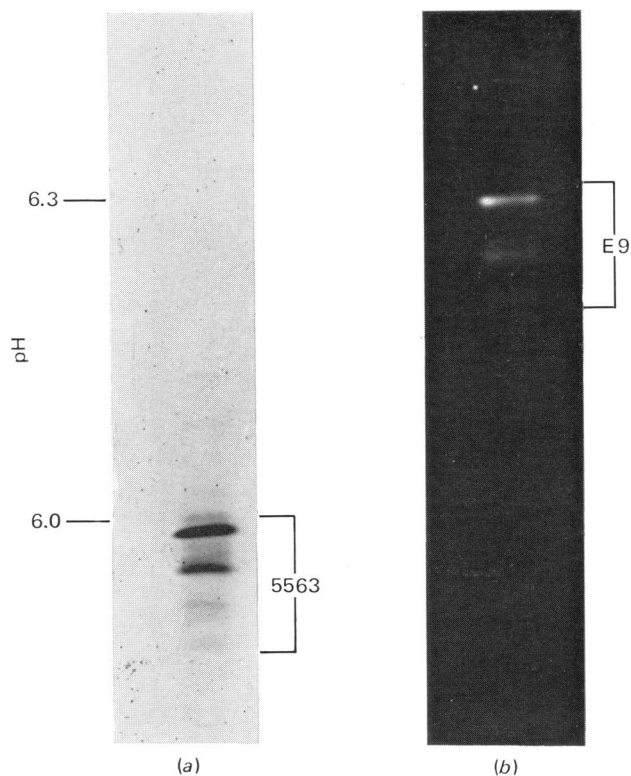
EXPLANATION OF PLATE I

Isoelectric spectra of monoclonal immunoglobulins

(*a*) Mouse G_{2a}-myeloma protein 5563; (*b*) mouse IgG_1 (anti-Dnp) produced by clone E9 (Askonas *et al.*, 1970). Isoelectric focusing was performed in a thin layer of polyacrylamide gel (Awdeh *et al.*, 1968). The anti-Dnp spectrum was detected by radioautography after overlay with ^{131}I-labelled hapten (Williamson, 1971).
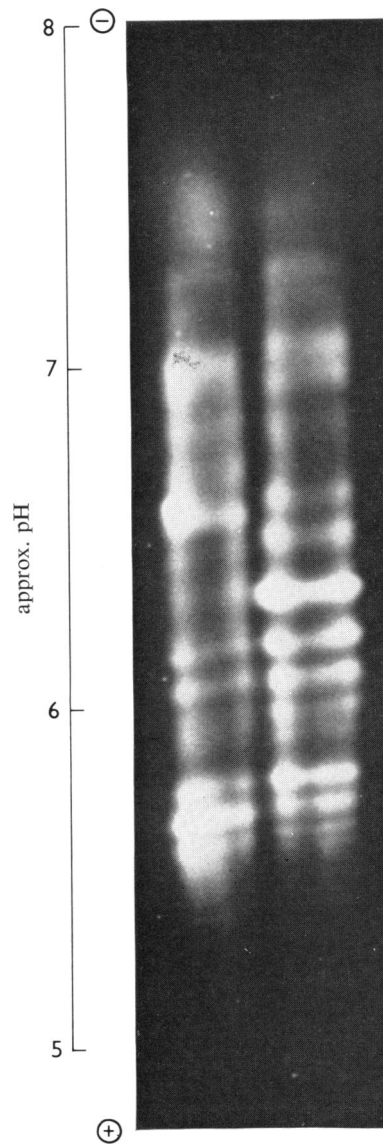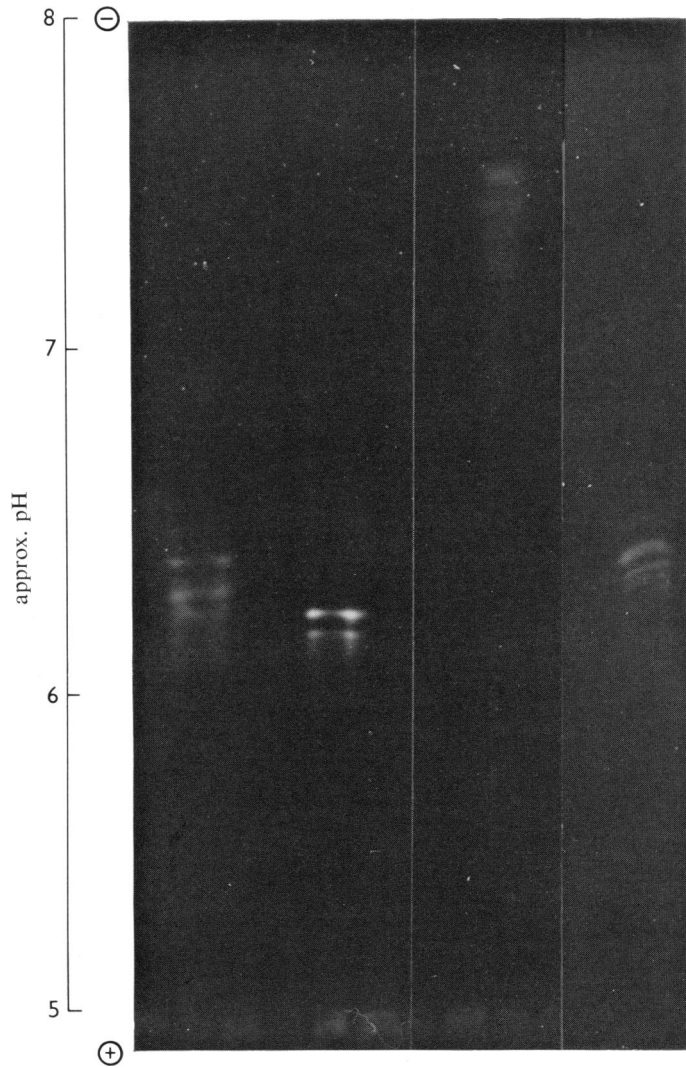
EXPLANATION OF PLATE 2

Isoelectric spectra of heterogeneous mouse anti-Dnp IgG

Sera were from two CBA/H mice 10 days after a secondary challenge with Dnp-bovine γ-globulin ($10\,\mu$g) in saline. Primary injection was Dnp-bovine γ-globulin ($100\,\mu$g) adsorbed on alum together with *Bacillus pertussis* (2×10^9 organisms). Sera were focused and anti-Dnp spectra detected as described in Plate 1 legend.

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EXPLANATION OF PLATE 3

Isoelectric spectra of individual monoclonal antibodies

The secondary response of a CBA/H mouse, primed as described in the legend to Plate 2, was distributed among a series of irradiated syngeneic recipients by transfer of limiting numbers of spleen cells together with antigen in saline. Spectra were analysed 10 days later. The experiment is similar to those described in Askonas *et al.* (1970).
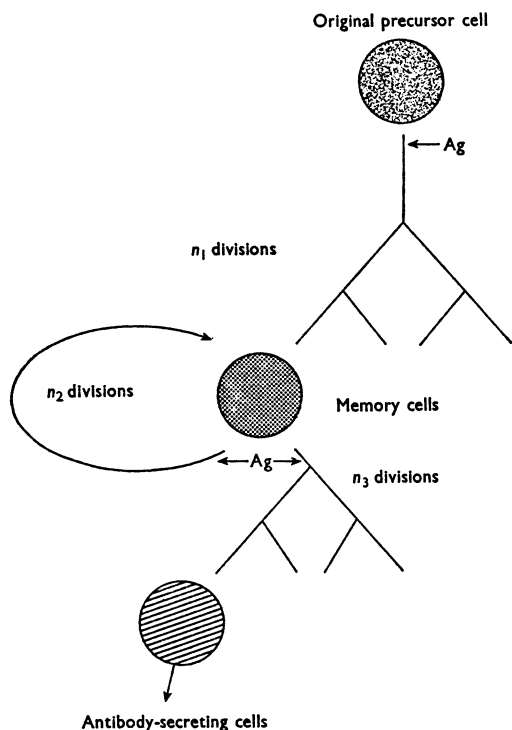
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Fig. 3. *Simple model of the development of an antibody-forming cell clone (taken from Williamson & Askonas, 1972)*

The clonal precursor cell is a small B-lymphocyte having surface receptors recognizing antigen (Ag). The three antigen-dependent proliferation steps, n_1, n_2 and n_3, all appear to require co-operating T-lymphocytes. Propagation of a single B-cell clone depends on the serial transfer of memory cells, and it is this population that appears to have a limited proliferation potential limiting the life-span of the clone.

Before leaving this brief description of the properties of single B-cell clones I should add that they each have a finite life-span. After several serial transfers memory cells fail to proliferate sufficiently to permit further propagation of the clone. The steady decline of clonal antibody production over the last few transfer generations (Williamson & Askonas, 1972) is strikingly analogous to the Hayflick phenomenon. Hayflick (1965) showed that cultured clones of human fibroblasts could undergo only a limited number of divisions even under supposedly optimal culture conditions. This system of cultured cells is used as a model for studies on cellular aging *in vitro*,

The limited proliferative potential of a B-cell clone propagated *in vivo* provides strong support for the idea of cellular aging or clonal senescence. This is the unexpected finding that can turn up in basic research; studies on aging were far from our thoughts when cloning of antibody-forming cells was undertaken.

Number of antibodies specific for a simple hapten

The potential for identifying individual monoclonal antibodies by their isoelectric spectra is illustrated by Plate 3. One can calculate that, by using our standard methodology to compare three-line antibody isoelectric spectra when samples are focused adjacent to one another, we should be able to distinguish in excess of 5×10^5 different antibodies of a single specificity (Williamson *et al.*, 1972). The problem is therefore to generate as many different antibodies of a given specificity as possible (i.e. express the genetic potential for antibody diversity); the number of different monoclonal antibodies can then be counted, the isoelectric spectra being used as the means of identification.

The first question that we wish to ask is: how many different antibodies against a simple hapten (in the first place Nip) is a single mouse potentially able to produce? This is unanswerable, because a single animal may not express its complete potential for production of anti-Nip at any chosen time or even over the whole of its life-span if it were possible to follow the response. The experimental solution is to use 'identical twins', as is done in human behavioural studies. With mice we have an unlimited number of inbred syngeneic animals that closely approximate to 'identical twins'. Our experimental answer will therefore be the number of different anti-Nip molecules for which the whole inbred strain of mice has the genetic potential. Clearly this number will also be the number of antibodies that a single inbred mouse is able to produce only if (1) all diversity is encoded in the germ-line and (2) all animals are truly genetically identical.

The basic experiment is the determination of the frequency with which the same antibody molecule recurs in independent events; with inbred animals we take this to mean the production of the same antibody by two or more individual animals. Dr. Kreth and I (Kreth & Williamson, 1972; H. W. Kreth & A. R. Williamson, unpublished work) chose to examine anti-Nip diversity in CBA/H mice. Mice were primed with Nip conjugated to bovine γ-globulin, chosen in order to give a heterogeneous presentation of Nip. The secondary anti-Nip response of each primed mouse was examined by using a spleen-cell transfer system as described above. Transfer of limiting cell numbers distributes the secondary response among

about 100 irradiated syngeneic recipients. Isoelectric focusing of the recipients' sera 10–12 days after transfer shows an average of one monoclonal anti-Nip per mouse (arranged by choosing the number of donor cells). Each clonal product is characterized by its isoelectric spectrum and comparison can be made between the individual spectra.

Each group of about 100 recipients, producing in sum about 100 monoclonal anti-Nip species, represents the secondary response of one donor CBA mouse. There is therefore a moderate repeat frequency (about 15%) for anti-Nip isoelectric spectra within one donor series; these repeats must be assumed to be non-independent events, most probably due to multiple memory cells from the same original clone. For independent events comparison must be made between donor series, and this limits experimentally the number of donors that can be compared. With 100 recipients per donor only four donors can be analysed. The repeat frequency of monoclonal spectra between donor series is low: in four donor series exhibiting a total of 337 unique anti-Nip spectra only five identical pairs of spectra were found between series. Even this low repeat frequency is still well within the resolving capabilities of our analytical techniques.

Application of a statistical analysis to these results allows one to calculate the minimum number of different anti-Nip molecules from which our sample was drawn (G. M. Wybrow & I. L. Berryman, unpublished work). The estimated minimum pool size for anti-Nip molecules in CBA/H mice is 8000. Since the number of repeat spectra is small and could easily vary by two or three pairs in either direction, this pool size cannot be defined within a few thousand molecules either way. However, the numbers are interesting for the purpose of further calculation.

We knew *a priori* that anti-Nip responses are usually very heterogeneous; now we have a rough quantitation for the number of anti-Nip molecules. Since all V_H-regions appear to be shared by all C_H-regions we can estimate the number of V_H–V_L combinations making anti-Nip combining sites by allowing for the different γ-chain subclasses represented in the pool of anti-Nip molecules; only κ L-chains were found. The minimum pool size for anti-Nip V_H–V_L combinations is decreased only to about 5000 (H. W. Kreth & A. R. Williamson, unpublished work). The question then is whether the genes coding for this set of V_H–V_L combinations are present in the germ-line of each CBA/H mouse. The minimum number of genes needed to generate 5000 V_H–V_L combinations is calculated by assuming an equal number of different V_H- and V_L-regions contributing to the diversity in all possible associations. In this case the total anti-Nip diversity in CBA/H mice can be encoded in 70 V_H- and 70 V_κ-genes.

Shared specificities

Although 140 V-genes is a small enough number to be carried in the germ-line it is still an incongruously large number of genes to devote to making anti-Nip combining sites. The simplest answer to this is in terms of multiple shared specificities. Each V_H–V_L combination is assumed to bind a variety of related and even apparently unrelated antigenic determinants. The precedent for this was pointed out by Glaser (1970) in his description of numerous proteins each of which binds several unrelated ligands. Shared specificities by individual antibodies do not contradict the known high specificity of immune antisera, since the latter contain a heterogeneous collection of antibodies having the chosen eliciting specificity in common. Talmage (1959) pointed out that 'In a mixture of a large number of different globulin molecules, the dominant reactivity will be that common to the largest number of molecules present'. Consequently it is predictable that homogeneous antibodies will be less likely to be monospecific than heterogeneous populations of antibody with a common specificity. The finding that myeloma proteins exhibit multiple specificities (Schubert *et al.*, 1968; Eisen, 1970) would therefore mean that they are not atypical immunoglobulins. The search for the single correct antigen for a myeloma globulin is meaningless; the range of shared specificities is the important observation.

The maximum diversity will be generated with the minimum number of V-genes if all V_H–V_L combinations are viable. Since the original combinations must be made at random, antigen playing no role until the V_H–V_L combination is expressed, the compatibility of all V_H-regions with V_L-regions should have selective value rather than the specificities of the combinations. Deleterious mutations in V-regions will be those limiting the range of combinations in which a given V-region can take part. The lower this number the less often will that V-region be expressed and the greater the chance that it will mutate or be deleted.

Number of antibodies specific for a complete antigenic determinant

A simple hapten, such as Nip, will constitute the immunodominant moiety of an antigenic determinant but will fill only a proportion (maybe less than 20%) of the antibody combining site (Kabat, 1966). A complete antigenic determinant consists of the hapten and the topographically adjacent carrier determinants. If hapten–carrier conjugates are devised so that there is only a single type of complete determinant, then we could expect that there would be fewer ways of designing complementary antibody combining sites. Natural antigens such as polysaccharides are based on simple repeating determinants,

and they often elicit antibodies of restricted hetero-geneity (Krause, 1970). Haber *et al.* (1967) discussed the idea of decreasing immunogenic complexity and presented binding-affinity evidence showing that haptens on simple carriers elicit antibodies of limited heterogeneity.

With simple immunogens it should be possible to analyse the isoelectric spectra of the resultant anti-bodies directly without resort to cell transfer systems. This would permit the use of larger animals and species where cell transfer is impractical.

One simple immunogen that has proved useful is di-Dnp-gramicidin-S. Gramicidin-S has much to recommend it as a carrier molecule: (1) it is a rigid cyclic decapeptide; (2) it has twofold symmetry, consisting of two identical sequences of five amino acids; (3) it has two free amino groups, one on either side of the rigid structure. Conjugation of the two amino groups with a hapten, in the first instance Dnp, gives two identical determinants symmetrically presented, each having Dnp as the immunodominant moiety and each sufficient to be a complete antigenic determinant. In rabbits di-Dnp-gramicidin-S can elicit a monoclonal response (Montgomery *et al.*, 1972). Not all rabbits respond, but so far responder animals have shown only monoclonal or diclonal antibody isoelectric spectra (P. C. Montgomery, personal communication). The homogeneous re-sponse persists for long periods, without any other clones being turned on in response to repeated injections of immunogen. In one rabbit the same monoclonal antibody was still being produced in response to di-Dnp-gramicidin-S 18 months after it was first elicited. Production of antibody remained dependent on repeated antigen injections, and the same antibody could be elicited even after a prolonged rest (5 months between antigen injections). These experiments lead one to speculate that perhaps each responding rabbit is only able to make one $V_H–V_L$ combination specific for the complete determinant represented by one half of di-Dnp-gramicidin-S. Non-responding rabbits might lack the genes for one or both of these V-regions. This remains to be proved.

Another system of simple immunogens is the set of α-Dnp-oligolysines that we are studying in col-laboration with S. Schlossman. These simple hapten–carrier conjugates are immunogenic in guinea pigs, giving a primary antibody response. Levin *et al.* (1971) showed that the antibody produced when tested in an antigen-binding assay against the set of α-Dnp-oligolysines of various chain lengths showed a slightly higher affinity for the homologous eliciting oligomer relative to all of the others. This exquisite specificity means that, although Dnp is immunodominant, the remainder of the antigen is contributing significantly to the combining energy; we have a set of complete determinants varying regularly in structure. To date,

our isoelectric-spectral analysis of the antibodies produced against α-Dnp-Lys$_{10}$ in strain-2 guinea pigs have shown that the primary responses are very restricted, usually mono-, di- or tri-clonal. Com-parison of isoelectric spectra is thus directly possible, and repeated monoclonal antibody spectra are found. Certain monoclonal antibodies are repeated with high frequency in each of a series of individual inbred guinea pigs. Not all animals make the same antibody against α-Dnp-Lys$_{10}$, but there would appear to be only a small number of $V_H–V_L$ com-binations of that specificity available in strain-2 guinea pigs. These preliminary findings already strongly support the theory that the information for these antibodies is encoded in germ-line genes.

Multiple germ-line V-genes

The discovery of V-regions and C-regions in immunoglobulin polypeptide chains led to somatic-diversity theories becoming fashionable, since this was seen as a way of maintaining the dogma one gene–one polypeptide chain. There is now, however, good evidence supporting the concept of separate V- and C-genes. As the sequences of more L- and H-chain V-regions are determined the concept of subgroups of V-region sequences, each requiring only a single germ-line gene, becomes less useful; a con-siderable number of subgroups becomes necessary to explain V-region sequences.

Recently evidence has been presented for the inheritance of V-regions based on following idiotypic antigens on anti-hapten antibodies in mice (Kuettner *et al.*, 1972) as well as on anti-carbohydrate anti-bodies both in rabbits (Eichmann & Kindt, 1971) and in mice (Eichmann, 1972). Idiotype is an anti-genic marker for one or a limited set of V-regions, so this evidence for heritability implies that the relevant V-regions are encoded in germ-line genes.

Taken together with our own evidence discussed above, the picture emerging is one in which the majority of antibody diversity resides in three pools or sets of germ-line V-genes, one set of V_H-genes, one set of V_κ-genes and one set of V_λ-genes. A reasonable guess might put of the order of 10^3 V-genes in each pool per haploid genome in order to account for the extent of antibody diversity. Both types of V_L-regions are complementary to V_H-regions, and the widely varying relative expression of κ- and λ-chains in different species might reflect differences in V_κ- and V_λ-gene pool sizes.

Sets of V-genes could be generated by repeated tandem duplication, with mutations introducing diversity. This is a basic mechanism of evolution and, as pointed out by Pink *et al.* (1971), it is a more likely way of generating V-genes than a saltatory duplication process. Tandem duplication is presumed to be an on-going process and so will constantly be

increasing the number of V-genes. There is, however, an opposite tendency to decrease the pool size; this would be supplied by haploid recombination, which would eliminate V-genes. A precedent for this is found in the behaviour of bacteriophage present in multiple copies in the bacterial genome (Gottesman & Yarmolinsky, 1968), and its possible relevance to V-gene sets was pointed out by C. Steinberg (personal communication). The pools of V-genes could be steadily changing in composition under the influence of these opposing processes. Expansion–contraction models of various types have been proposed (Milstein & Pink, 1970; Hood et al., 1970; Smith et al., 1971) to explain the presence of species-specific residues in V-regions and the V-region allotypic determinants found in rabbit H-chains. Smith et al. (1971) explained the divergence of V-gene sets between species by interchromosomal homologous but unequal cross-over events, but they point out that such multiple crossing over would not be compatible with the stable V-region allotypes. In the absence of novel mechanisms expansion–contraction via tandem duplication and haploid recombination is consistent with present knowledge. There is a good precedent for expansion and contraction of a set of multiple genes seen in *Drosophila*, where the number of rRNA genes can be dramatically increased in order to compensate for a chromosome deficiency (Tartof, 1971). Antibody diversity requires no mysterious generator of diversity in the model described above.

Expression of genetic potential

Immunology borrows precedents from other areas and also supplies analogies for other problems. Thus adaptive immunity and intelligence have often been compared. One of the more recent comparisons, by Cohn (1968), sought to explain both adaptive immunity and learning by a somatic theory. It now seems most likely, as I have tried to show here, that the large majority of antibody diversity is carried by germ-line genes. I do not wish to argue for or against the inheritance of intelligence. What I want to do is to point to the role of the environment irrespective of the genetic predisposition. The spectrum of antibodies produced by an individual depends on that person's history of antigenic stimulation. In early life passive antibody, acquired across the placenta and later via milk, helps the infant to survive its environment. If the newborn infant is put into a germ-free environment then no immune responses will be evoked and immunological memory will be missing. Return that infant at a later stage to the real world and it will appear immunologically inexperienced whatever the genetic potential for antibody production.

Intelligence is analogous. If the infant was maintained in an intellectual vacuum, a learning-free environment, then on exposure to the 'real world' of intelligence tests it would appear to be unintelligent. As with an immune response, a behavioural response such as intelligence reflects the early environment to which the child is exposed. Mittler (1971) expressed this as follows: 'genetic disposition towards a given pathology or behaviour can only be expressed in an appropriate environment whether pre- or post-natal'.

One could go on developing the analogies, but that is not necessary here. The effects of extreme environment are clear-cut. More subtle effects are harder to demonstrate. Twins are the usual experimental animals for behavioural-inheritance studies. Starting with the same genetic endowment, how do different environments influence the intelligence of twins kept apart? Different studies have led to different conclusions, but this may be attributed to the uncontrolled extent to which the various environments really differed. Studies of that sort led one to wonder about the detailed physical identity of twins. One of the most characteristic features of a person is their set of fingerprints. Do identical twins have identical fingerprints? Apparently not, to quote Penrose (1963): 'The general configurations of ridges agree, as hereditary traits should, on the hands of such pairs, and there are usually only slight differences in arrangement; but the details do not agree and are individually specific'.

I have diverged into this cursory discussion of inheritance of behavioural traits because I feel that the genetic control of antibody production teaches one a useful lesson. We should not be too ready to assign observed differences to differing genetic potential when we may be measuring mainly the effects of environmental factors on the expression of that potential. In immunology the 'numbers game' is fascinating and measuring the genetic potential for diversity is a challenge, but the most relevant medical questions concern the control of the expression of immune responses. I think the message is the same for studies on intelligence. Here it matters much less to what extent intelligence is heritable. We must understand the factors controlling the expression of behavioural traits such as the various forms of intelligence and create an educational environment in which each person has the opportunity to make use of his potential.

It is fun to make up stories about our experimental results and this is an important process in the progress of scientific thought. Bacon cautions us though that we should not 'give out a dream of our own imagination for a pattern of the world'.

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