genetics, which could have advanced rapidly with the rediscovery of Mendel's work, failed to achieve this advance until many years later, and there is some irony in the fact that during these wasted years it was the work of the anti-Mendelians that was outstanding.

The clarification of the status of familial disease made available a mass of clinical observations that in turn could elucidate Mendelian principles. Over the years came recognition of the specific problems of Mendelian ratios in the relatively small numbers presented by human families. There also came the concepts of expression and of penetrance, particularly in dominant disorders, and the concepts of incomplete recessiveness and the carrier state. The multiplicity of blood groups brought much elucidation on multiple allelomorphs. The interaction between heredity and environment seen in an extreme form in the fractures induced by minimal trauma in fragilitas ossium could also be seen in more subtle aspects in other disorders, including possibly tuberculosis and other infectious diseases which were operative only in a susceptible genetic constitution. Haemolytic jaundice of infants was seen not to be strictly genetic in character but a form of induced autolysis because of genetic incompatibility.

To-day both the significance and the scope of medical genetics are increasing rapidly. As the infectious diseases are being brought under control, the constitutional factors of disease are once again assuming a dominant place in pathology, and it is becoming increasingly obvious that these are largely determined genetically. Moreover, an extensive range of clinical appearances previously considered environmental in origin have come to be recognized as merely different stages of hereditary diseases which run an evolutionary course in post-natal life and thus present different aspects in different members of a family.

But present-day medical genetics is no longer a matter of collecting and interpreting pedigrees. Statistical methods have made possible the assessment of the frequency of pathogenic genes and of mutation rates in the population, so that genetically determined disease is no longer an altogether unknown quantity. Recognition of the inborn errors of metabolism of Garrod have led to intensive studies of enzyme systems at the cellular level, and there is hope that the uncovering of biochemical faults as the mechanism of genetic disorders may open therapeutic possibilities. Some such possibilities are already being effected, as, for instance, the relative control of galactosaemia by excluding galactose in the infant's food.

Faulty protein synthesis is yet another possible basis of genetic disorders. This is seen most strikingly in the current studies on sickle-cell anaemia and the other haemoglobinopathies, and it is likely that many other hereditary anomalies, including such classical affections as retinitis pigmentosa, may come to be explained in this manner.

Ever since the gene came to be located in the chromosome, the possibility of chromosomal anomalies as a cause of hereditary disease has been under consideration. Until recently such anomalies—observed microscopically—were held to be incompatible with life if they were present in any but a minimal form. It is now clear that chromosomal anomalies are in fact a substantial cause of pathological states. Down's syndrome (mongolism) is an outstanding example.

The gene itself has followed the atom in ceasing to be the irreducible particle of matter. There is evidence that the gene—which may have a thickness of a millionth of a millimetre—is itself a complex of thinner segments each with its specific effect. The intensive chemical studies on deoxyribose-nucleic acid (D.N.A.) have bearings on both chromosomes and genes.

A Fundamental Problem

The nature of the gene is a problem that goes to the root of all biological thought. Since each cell in the body carries a full complement of chromosomes—so that incidentally each cell in the body is either male or female, and demonstrably so histologically—there must occur in the course of repeated divisions of the fertilized ovum not only development of particular cells giving particular tissues and organs but suppression within such cells of all other potentialities. The puzzle of life therefore begins at a subcellular level, and an understanding of the pathways of gene action has become a fundamental problem in biology, which itself is reaching out towards the control of the energy locked in the gene. To a hypothetical philosophical biologist hereditary diseases represent Nature's experiments in gene action, and as such diseases in man are so intensively studied daily at thousands of centres they would give endless material for genetic investigations, for the study of anomalously acting genes is an excellent exercise in attempts to understand the normal. It is possible that ultimately Mendel's contribution to medicine will be seen to lie not so much in the opening of a new chapter in pathology as in the steering of medicine well into the main stream of biological thought.

Somatic Mutation and Chronic Disease*

Sir MACFARLANE BURNET,† O.M., M.D., F.R.C.P., F.R.S.

Brit. med. J., 1965, 1, 338–342

It is common to divide disease into that for which environmental factors are responsible and that which is genetic in origin, or, more correctly, to consider the relative environmental and genetic components that are involved in any given clinical situation. Mechanical trauma, poisoning, and most infections are almost wholly environmental; haemophilia and phenylketonuria are almost wholly genetic; and there is a range of conditions in which the joint effect of heredity and environment is clearly recognizable—for example, tuberculosis and poliomyelitis among infections, primaquine sensitivity among poisonings.

There is, however, another category of disease processes which seem to depend on something additional to the environmental

and genetic factors—namely, somatic mutation. For many years it has been recognized that the age-specific incidence of several types of cancer showed a strikingly simple pattern when plotted against age. If both age-specific incidence and age were plotted logarithmically the points fell on a straight line. This can be interpreted only on the assumption that the condition is determined by the random occurrence of events occurring with equal likelihood over the time-span being considered. The only random event which it is reasonable to consider in a

* Given as a Sommer Memorial Lecture at Portland, Oregon, on 23 April 1964.

† From the Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia.

multicellular biological system is somatic mutation. The results with malignant disease have been interpreted either as representing a series of five or six sequential mutations in a cell line not associated with proliferative advantage until the final cancer-producing mutation, or as two or more sequential mutations with proliferative advantage of some degree at each step (Armitage and Doll, 1957).

Such an approach to common diseases by a study of the changing incidence with age and sex has been a commonplace of epidemiology in relation to infectious diseases (Burnet, 1952). If the form of age-incidence of a disease is reproducibly similar in different environments, this must be regarded as one of its characteristics and therefore calls for interpretation. Since it can be expressed graphically as a definite curve, it is susceptible to mathematical treatment and may therefore be relevant as evidence to test this or that hypothesis of aetiology. It is to the credit of Burch, of Leeds, that he has developed this new and potentially powerful approach to an understanding of the aetiology of well-defined diseases of obscure origin.

The "clonal selection" approach to malignant disease is of quite respectable antiquity, although it has never been acceptable to a majority of oncologists, mainly perhaps because of the therapeutic pessimism engendered by the idea. There can be no doubt that in the genesis of any particular cancer environmental and physiological factors are concerned. It is equally certain, however, that these extrinsic factors express themselves as modifiers of the central process of somatic mutation or its equivalent. Somatic mutation was, however, not considered in relation to other aspects of human pathology until recently. It was a corollary to the clonal selection theory of immunity to believe that autoimmune disease was due to the activity of "forbidden clones" of lymphoid cells stimulated to proliferate by contact with the corresponding autoantigen and therefore behaving in many ways like a conditioned neoplasm (Burnet, 1959). The implications of this point of view have recently been examined in mathematical papers by Burch (1963) and Burch and Rowell (1963), who find that the epidemiology of rheumatoid arthritis and of several other autoimmune diseases is in accord with a model based on the emergence of forbidden clones by a process of somatic mutation. Subsequently Burch has found that similar reasoning applied to the age and sex incidence of both manic-depressive psychosis and schizophrenia points to an "autoimmune" aetiology. Our own interest in the New Guinea disease "kuru" has also been stimulated by the interesting resemblance of the adult age and sex incidence of kuru to that of systemic lupus. It is difficult, however, to believe that schizophrenia and kuru are both autoimmune diseases in any conventional sense.

In considering chronic disease it is impossible not to make some attempt to bring the general question of ageing into the picture. In many ways the major causes of "natural death," cancer, inadequacy of resistance to infection, and cerebral or cardiac accidents associated with vascular disease can probably all be regarded as more or less accidental intensifications of the general ageing process. In recent years there has been much speculation on whether somatic mutation plus secondary autoimmune processes plays a significant part in ageing (see Comfort, 1963). Perhaps the role of autoimmunity has been over-emphasized, but I believe that a strong case can be made for regarding somatic mutation as an important basic factor in ageing.

If ageing and chronic disease are to be regarded in part as the results of somatic mutation it is equally necessary to emphasize the converse of this hypothesis—namely, that there must be some highly efficient mechanism by which the germ cells do not age. This is too complex a subject to discuss here. In general terms, we can probably assume that the processes of meiosis and fertilization include an inbuilt scrutiny for chromosomal damage of any sort. In some way there is a high selective advantage in normality for the germ cells.

Types of Cell Renewal

Somatic cells can be divided into three major groups from the point of their renewal.

1. Cells which are expendable and constantly being renewed from stem cells. (a) Epidermal and various types of internal lining epithelia of which the epithelium of the small intestine is probably the most active. (b) The blood cells, erythrocytes, granulocytes, and lymphoid cells—we should include plasma cells, mast cells, and macrophages as derivatives of the lymphoid series.

2. Tissue cells which virtually cease to multiply once adult life is reached but which, on demand, can proliferate freely. The liver is the classic example, but there are probably many other tissues with similar capacity. In addition there are organs which on hormonal stimulus show mitotic activity and in which there is presumably a moderate cell turnover under ordinary conditions of life. The adrenal cortex and pancreatic islet tissue fall in this group.

3. Tissues of which the prototype is the central nervous system, in which there is no cellular proliferation. This, of course, does not preclude the continuous replacement of the organelles and molecules of the persisting cell.

Nature of Somatic Mutation

The essence of cell division is the accurate duplication of the whole genetic apparatus, in particular the base-by-base replication of the whole chromosomal deoxyribonucleic acid (D.N.A.) with its contained genetic information. No complex biological process is completely free of the possibility of error. In the case of D.N.A. replication it is almost universally accepted that the possibility of error is the main or only source of the raw material for evolution. In the absence of known mutagenic agents the chance of error at any nucleotide is of the order of 10^{-8} per replication (Orgel, 1963). Since this holds equally for somatic and germinal cells there must be a constant appearance of somatic mutations in all replicating cells of higher organisms.

If there are between 10^{13} and 10^{14} cells in the body there must be 10^{11} to 10^{12} mitoses per day. In very broad outline we may say that any mutation which we know can occur probably takes place to the extent of 10^{-6} to 10^{-7} per replication. If this is true there may be 10^5 mutations toward every potential type of unit genetic change taking place every day in a human adult. Genetic damage is not necessarily confined to replicating cells even in bacteria. Curtis (1963) has found that with ageing in mice there is a steady increase in the proportion of mitoses in liver cells showing microscopically visible abnormalities. The adult liver normally has very little cell replacement or mitosis, but when a standard degree of partial necrosis is produced by the administration of carbon tetrachloride large numbers of mitoses are seen in the regenerating liver three days later. The abnormalities noted with age chiefly take the form of "bridges" between the separating sets of chromosomes in mitosis. The number of these can be increased greatly by administration of a dose of x rays or γ -radiation sufficient to give a measurable shortening of life. The proportion seen at any given age of normal mice is also inversely related to the longevity of the strain, being notably low in long-lived C57 mice. It is of equal interest that chemical mutagens such as nitrogen mustard, which provoke many karyotypic abnormalities in bone-marrow, intestinal epithelium, and other expendable cells, do not reduce life-span or produce chromosomal damage in the liver.

Bridging during mitosis is only indirect evidence of mutation in the cells concerned, and one cannot altogether exclude the possibility that changes elsewhere in the body are responsible perhaps for a change in the viscosity or other physical quality of the intracellular environment. Nevertheless, the positive

correlation with life-shortening factors is impressive, and so far as the general process of ageing is concerned we must probably follow Curtis in ascribing a major role to random damage to the genome of non-replicating cells. The central nervous system is of special interest from this point of view, but at present possible changes are inaccessible to experimental demonstration. The gradual reduction in the number of neurones with age may represent the removal of cells which have become non-functional as a result of mutational damage to the genome.

However, from our present point of view we are concerned, not with a slow development of functional inadequacy whose only clinical effect is a shortening of the life-span, but with processes manifesting themselves in recognizable disease. We are specially interested in the possibilities of disease mediated by the highly mobile, expendable, and renewable cells of the lymphoid series. Curtis is probably correct that for such cells the average random somatic mutation will result in its rapid competitive elimination. The only significant mutations will be those which, in one way or another, produce a selective proliferative advantage.

Genetic Significance of Differentiation

At this point we may consider briefly the genetic significance of differentiation in relation to the effect of somatic mutation. Every cell capable of growth and replication must retain a large proportion of its genome in functional activity, but since its activities and potentialities are enormously less than those of the fertilized ovum there must also be a large proportion of repressed and non-functional areas. Mutation here will be without detectable effect. Mutation in a functional region will result in some inadequacy, particularly if it occurs in a gene whose allele is genetically aberrant. Whatever the functional effect of a somatic mutation, whether it is lethal to the cell or induces the cell to secrete a toxic product or to cease producing its normal product, there will be no observable effect if this occurs in a single cell among the many million cells in the tissue concerned. With a mutation occurring with a rate of the general order of 10^{-6} to 10^{-7} per replication it is never likely to make any direct difference to the functioning of an organ. It is almost inconceivable that any observable effect will arise from one cell in a million that is out of step with the rest.

The only way in which events of the nature of somatic mutation, "events random in respect of time," can have physiological or pathological effects is by their production of something which as a result of the mutation can increase largely in amount. The most direct way is that the cell in question should give rise to many more descendants, so that eventually these will form a significantly large fraction of the population; but at least one other mechanism can be imagined. Perhaps the simplest situation is when the somatic mutation occurs during an early stage in the primary segmentation of the ovum. An interesting example of this sort involving "fleece mosaics" in sheep has been described by Fraser and Short (1958). If we exclude such developmental mosaics the possibilities are:

1. That the mutant cell has a proliferative advantage over its congeners (a) because of capacity to override local morphological controls, (b) by greater metabolic effectiveness in a competitive situation even though still fully responsive to local controls, or (c) by the emergence of a capacity to be stimulated to proliferation by a substance or condition normally present in the internal environment.
2. That the mutant cell liberates an agent which is capable of affecting other cells previously normal so that they in their turn produce the agent and are functionally modified or destroyed in the process.

Such a process has been suggested as a possible interpretation of scrapie in sheep; but this is at present highly speculative, and I shall not mention this alternative further.

Proliferative Advantage to Mutant Cells

There is relatively little known about the process by which the shape and size of organs remain approximately constant and the size and distribution of mobile cells conform to standard. There must obviously be a wide range of homeostatic processes, each fitted to control some particular set or system of cell populations. In the small intestine we have a continuous normal process of rapid epithelial proliferation by which, as new cells arise by mitosis in the depths of the crypt, they move upwards, and, in general, for each mitosis one cell moves off the villus and is lost, while another becomes available, either for mitosis or for upward migration. On the average the total number of cells per villus remains constant, and in a sense we can regard each mitosis as asymmetrical, one daughter staying as a stem cell, the other migrating and eventually being lost.

Despite the activity of mitosis in the epithelium of the small intestine, this is an extremely infrequent site of malignancy, and we can probably accept Curtis's view that there is here strong competition for survival as stem cells; any atypical mutants will be discarded automatically. In general, mutants pointed towards malignancy do not have a more rapid turnover than their normal congeners. Their abnormality is that, having been produced, they are not susceptible to the controls which see that unwanted cells move out of the way. Under a condition of partial maturation arrest they will accumulate even though their actual replication may be slower than that of normal cells. We are not concerned here with malignancy, but similar considerations arise whenever, by one means or another, cells become unresponsive to normal controls.

The first of these to be discussed is based on the proposition now rather generally accepted that immunologically competent cells capable of producing antibody against antigen X are stimulated to proliferate by contact with X. If a lymphoid cell "happened to carry" capacity to produce antibody against one of the plasma proteins it could therefore be stimulated to proliferate in its normal environment and give rise to a clone of cells behaving more or less like a malignant clone. This appears to be the state of affairs in such a disease as systemic lupus, and our approach to autoimmune disease at the general level is to ask why this does not always occur and what is the nature of the abnormality which allows it.

The Forbidden-clone Concept in Autoimmune Disease

On a number of occasions I have discussed extensively the nature of autoimmune disease in terms of the concept of forbidden clones. This is based initially on the view that the normal tolerance of body components—their non-antigenicity—is of the same nature as experimental immune tolerance and that autoimmune disease is a partial breakdown of the natural tolerance to all accessible body components. Any theoretical formulation of autoimmune disease will necessarily depend, therefore, on the way the process of natural tolerance is interpreted.

In the clonal selection theory (Burnet, 1959) it is assumed that the pattern of gamma-globulin produced by a cell depends on the genetic information stored in the appropriate segments of the genome as modified in accord with the relatively frequent occurrence of mutation in the relevant segments. Cells are selected for proliferation from the available population by their possession of immune patterns which can react with substances, antigenic determinants, present in accessible form in the body. This reactivity is basically a cellular characteristic and may well normally be limited to one specific group of closely related antigenic determinants. No serious modification of the argument would be necessary if the number of antigenic determinants with which a cell could react was usually 1 or 2 and that the actual numbers might be distributed randomly over 0, 1, 2, 3, and 4. Tolerance is assumed to result from the process by which any cell carrying a capacity to react with an antigenic

determinant is inhibited or destroyed by such interaction when it occurs under appropriate circumstances in the early life of the cell.

The accumulation of random patterns must represent some type of mutational process, but details at the molecular level are unknown. Smithies (1963) has, however, produced an attractive hypothesis that the variability of gamma-globulin is based upon the existence of frequent inversions of short sections of the chromatids concerned. This hypothesis seems to fit the requirements of clonal selection admirably, but again there is as yet no serious experimental justification for the hypothesis and it may require eventual radical modification.

It is reasonable to locate a large proportion of the early development of random patterns and the elimination of self-reactive cell lines in the thymus. This is by far the most active producer of lymphocytes in the perinatal period. With the development of peripheral centres of lymphoid-cell proliferation it is likely that these processes will be progressively transferred from the thymus. There is evidence that the effective emergence of a new immunological pattern slows down after early life, but, to some extent at least, throughout life the general requirements for randomized pattern and selective elimination of self-reactivity seem inescapable.

The general result is that once cells have passed the test of "immunological homeostasis by destruction of self-reactive clones" they are free to react by proliferation and antibody production on contact with their corresponding antigenic determinants. The homeostatic barrier ensures that cells which would be stimulated to multiply by antigenic determinants carried on body components are absent from the lymphoid-cell population.

A forbidden clone is derived from a cell which, because of somatic mutation or its equivalent, can pass the homeostatic barrier although it is reactive with a self-component.

The implications of this hypothesis of autoimmune disease include the following, which have been verified either in human material or in the spontaneously autoimmune strain of mice NZB.

1. Cells should be capable of growth in the thymus as well as in spleen or lymph nodes (myasthenia gravis, S.L.E., and NZB mice).
2. Cells should be capable of growth in organs containing the self-antigen concerned (germinal centres and lymph follicles in thyroid).
3. Cytotoxic agents selectively active against proliferating cells should have therapeutic effects (mercaptopurine).
4. Some germinal centres should show evidence of production of a relevant antibody (rheumatoid arthritis).
5. Antibody can be produced against antigenic determinants common to all vertebrates and therefore antigenic (under normal circumstances) in none (Anti-D.N.A. in S.L.E.).

No other interpretation of autoimmune disease can provide a similarly straightforward interpretation of these findings.

As already mentioned, Burch has used the forbidden-clone concept to interpret the age and sex incidence of rheumatoid arthritis and other autoimmune diseases. I shall discuss this work only briefly and superficially.

By definition, mutations are rare events occurring in body cells in a fashion which is random in relation to time. If such events, either alone or in association with other random or definitive events, give rise to symptoms or disease a more or less characteristic age incidence of the disease in question will be generated.

Burch's contention is that, with adequate mathematical logic, it should be possible to deduce from age-incidence curves the nature of the relationships involved and hence to throw some light on aetiology. His approach seems to be based on the following assumptions:

- (a) That there are no effects of environment on the incidence of the condition, or, more strictly, that any such effects are randomly distributed over the whole life-span.

- (b) That diagnostic manifestations of a disease result at an approximately constant incubation period after the appearance of an initiating event.

- (c) That, in general, the initiating event depends on the presence of mutant genes in the individual genome and on the occurrence of somatic mutation in cells of the type concerned. In most instances, more than one somatic mutation is required.

Most human autoimmune diseases have a higher incidence in women than in men, so they must be related directly or indirectly to the sex chromosomes. Systemic lupus with its predominant impact in young women, according to Burch and Rowell (1963), requires three mutant genes on the X chromosome and three separate somatic mutations on that chromosome.

I am not competent to criticize Burch's work in detail. One has the impression that the accuracy of the available statistical material is inadequate to justify such precise interpretations, but there can be no possible objection to his main contention that this is a legitimate field for scientific study.

Dr. Holmes and I (1964) have recently completed the first attempt that has been made to apply Burch's methods to an experimental situation in a laboratory animal. We have estimated the specific age incidence of the conversion of the "autoimmune" mouse strain NZB to a positive Coombs test. This has been done in both sexes for homozygous NZB and the F1 hybrid with C3H. The results show a striking regularity, and I hope that eventually some of my mathematical colleagues will interpret them. In approximate figures the age of 50% conversion is for NZB males 5 months, females 6½ months; and for F1 (NZB × C3H) females 18 months, males 24 months.

Nature of Ageing

From the point of view of ageing, malignant disease and autoimmune disease are important, but it is more than probable that increasing knowledge will provide other less obvious mechanisms by which somatic mutation leads to shortening of life.

There are two basic aspects of somatic mutation to be considered in relation to age: (1) mutations which in one way or another diminish functional efficiency but are not lethal to cells of stable organs will accumulate arithmetically through life. (2) Any form of primary mutant with a proliferative advantage will become increasingly available for sequential mutation.

A third aspect, which has not yet been properly established, is the possible importance of immunological responses to the appearance of mutant cells in the body, and it is with possibilities in this area that the remainder of this talk is concerned.

Autoimmune Disease

This has already been discussed as a manifestation of somatic mutation. Here we are concerned only to mention the suggestion by Burch (personal communication, 1963) that the large group of cardiovascular-renal degenerative diseases could have an autoimmune basis. This has a very slender basis on the shape of specific age-incidence curves, but current opinion, that endovascular lesions are the most important cause of the immunological rejection of renal homografts in man, tends to strengthen the idea.

Immune Surveillance

Thomas (1959) was the first to suggest that the whole homograft immunity system may be derived from what I have subsequently called a surveillance mechanism whose function is to recognize and eliminate cells which, by somatic mutation or

otherwise, have become foreign to the body. Since a significant proportion of such mutants would be unduly apt to give rise to malignant disease there is a strong evolutionary reason for the development of such a mechanism. If the existence of the mechanism is admitted there are two ways in which it might influence ageing.

(a) The possibility has often been raised that if antibody or specific cellular activity develops against a modified body component there may develop a capacity to act also against the unmodified component. In other words, autoimmune activity is so initiated and could have correspondingly damaging effects. Elsewhere I have discussed this hypothesis and given reasons for regarding the postulated process as being more characteristic of artificial experimental situations than of natural ones (Burnet, 1963).

(b) On the forbidden-clone hypothesis, it is not excluded that the mutation giving rise to the pathogenic quality may also change the cells concerned so that they become "recognizably foreign" and therefore capable of provoking an immunological reaction against the clone. This has not been demonstrated in any specific situation, but suggestions of its existence come from various quarters—for example, see Burch (1963). If the general efficiency of the surveillance mechanism weakens with age, possibly in part with the atrophy of the thymus, we could expect a gradual increase in the number of forbidden clones, perhaps particularly those directed against vascular endothelium.

Possible Role of the Thymus in Ageing

It is clear that in mammals, at least, the thymus plays a key role in the development of immunity, but we have no real clue to the meaning of its progressive atrophy with age.

Experimental studies in mice recently discussed by Metcalf (1964) indicate that the thymus is an organ which, once established, is remarkably autonomous in retaining size and structure. When infant isologous thymuses are grafted they develop into normally structured organs, although appropriate experiments with chromosomal markers strongly suggest that the whole lymphoid element is within a few weeks completely replaced by host cells. This is, presumably, merely a manifestation of a rapid normal turnover of lymphocytes in the thymus. Under stress the thymus is subject to storms of lymphocytic destruction and repopulation.

Following Metcalf (1964), we can assume that the specific functions of the thymus are controlled by its own reticulo-epithelial tissue, including the characteristic P.A.S.-positive phagocytic cells of the cortex. These are the cells which, by hypothesis, produce both the lymphocytosis-stimulating factor of Metcalf and the Mowbray factor which form the "nests" within which lymphocytes proliferate and which control the entry and exit of lymphocytes and stem cells.

I should like to close with the suggestion that when we know more about the thymus we may know more about the mechanism of ageing. We know that the thymus regresses and finally atrophies with age, that it is responsible for the activity of lymphocytes as immunologically competent cells in early life, and that it has important, if incompletely understood, associations with autoimmune disease. I have mentioned already what is now a widely held view, that in early life the thymus is responsible for most of the new immunological patterns that are carried by lymphoid cells, and, what is perhaps an individual belief of my own, that most of the elimination of unwanted forbidden clones also takes place in the thymus during early life.

There are several immunological characteristics of ageing which, though at present based on rather fragmentary evidence, are probably general. The best-established is the increasing inability to respond to a new antigen as the animal ages. This seems to hold in human beings and contrasts with the often improved response of old people against antigens experienced in the past. The second is the increased frequency of auto-

immune manifestations in old age and chronic illness. If the existence of a surveillance mechanism is accepted this may in fact be a manifestation of the first disability.

Theoretically there are two ways in which the appearance and proliferation of forbidden clones can be prevented or controlled. The first is by the standard process of elimination of reactive cells at an appropriate stage of physiological immaturity by contact with antigen. The second will be expected if the mutation inducing the forbidden clone simultaneously results in the appearance of a new antigen. This will provoke the appearance of antibody-carrying or antibody-producing cells which will eliminate cells of that clone. In the older animal it may be impossible to produce a range of patterns wide enough to include any capable of developing in this fashion. It is equally possible that in the older organism with absent thymus and relatively inactive lymphoid tissue the first type of control may be ineffective as well.

Only indirectly connected with the phenomena of ageing, but important in the present connexion, is the growing evidence that the thymus may be a target organ in autoimmune disease, notably S.L.E. This is a hypothesis we are trying to explore at the experimental level in NZB mice and their hybrids. If, as we believe, the thymus is an important controlling organ one can conceive the development under some circumstances of a vicious circle which could in acute form present as systemic lupus. The possibility that a slower but self-perpetuating immunological attack on the thymus is associated with and perhaps is partly responsible for normal ageing may be a thought worth following in the future.

Summary

The gist of this review is that somatic mutation appears to be a very important factor in the genesis of a wide variety of chronic disease and in the process of ageing itself.

The nature of somatic mutation (a) in expendable actively replicating cells and (b) in non-replicating tissue cells is discussed and the consequences considered.

Malignant disease is included only *en passant* but autoimmune disease is discussed extensively from the standpoint of the forbidden-clone hypothesis, which makes it virtually equivalent to conditioned malignancy.

Various possibilities are discussed by which somatic mutation in one or very small numbers of cells may be "magnified" sufficiently to produce demonstrable effects.

There is much to suggest that somatic mutation, particularly as expressed through autoimmune disease, may be of special importance in the process of ageing.

The requirements for an autoimmune theory of ageing are: (1) that forbidden clones capable of minor attack on a variety of tissues may arise with greater frequency with advancing age; and (2) that forbidden clones are themselves subject to partial or complete control and that this control may diminish in effectiveness with advancing age.

REFERENCES

- Armitage, P., and Doll, R. (1957). *Brit. J. Cancer*, **11**, 161.
- Burch, P. R. J. (1963). *Lancet*, **1**, 1253.
- and Rowell, N. R. (1963). *Ibid.*, **2**, 507.
- Burnet, F. M. (1952). *Aust. Ann. Med.*, **1**, 93.
- (1959). *The Clonal Selection Theory of Acquired Immunity*. Cambridge Univ. Press, London.
- (1963). *Brit. med. Bull.*, **19**, 245.
- Comfort, A. (1963). *Lancet*, **2**, 138.
- Curtis, H. J. (1963). *Science*, **141**, 686.
- Fraser, A. S., and Short, B. F. (1958). *Aust. J. biol. Sci.*, **2**, 200.
- Holmes, Margaret C., and Burnet, F. M. (1964). *Heredity*. In press.
- Metcalf, D. (1964). In Minneapolis Symposium, *Immunobiology of Thymus*.
- Orgel, L. E. (1963). *Proc. Nat. Acad. Sci. (Wash.)*, **49**, 517.
- Smithies, O. (1963). *Nature (Lond.)*, **199**, 1231.
- Thomas, L. (1959). In *Cellular and Humoral Aspects of the Hyper-sensitive States*, edited by H. S. Lawrence, p. 530. Hoeber, New York.