BRITISH MEDICAL JOURNAL

LONDON SATURDAY OCTOBER 10 1959

AUTO-IMMUNE DISEASE

I. MODERN IMMUNOLOGICAL CONCEPTS*

BY

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Medicine has been the mother of all the biological sciences. There are many reasons for this, but one of them is specially relevant to my present topic. It is simply that while the body functions smoothly things are taken for granted. It is only when things go wrong that it becomes possible to perceive that there is something in normal function which requires understanding. The central theme of these lectures is that the phenomena seen clinically in diseases of the so-called auto-immune group can be interpreted to throw light on the nature of immunological processes, and equally that recent developments in theoretical immunology are becoming highly relevant to clinical medicine.

We were all taught to regard antibody production and other immune responses as manifestations of a process of defence against invading micro-organisms or any other type of foreign material entering the tissues. In some way the body was enabled to recognize what was foreign and to swing into action the immunological responses needed to speed its removal. The meaning of foreignness was something that seemed to worry nobody. Only with the recognition that there are disease conditions in which antibody is actually directed against body components—acquired haemolytic anaemia, for instance—did a real sense of the importance of the body's ability to differentiate between self and not self come into being.

In Melbourne I am closely associated with our Clinical Research Unit, but only as an immunological consultant without clinical responsibility. My approach to the medical problems must necessarily be at the theoretical level, but at the present time I believe that it is better to try to present a working approach to generalization about the auto-immune diseases than to add to the volume of clinical descriptions and therapeutic trials. The approach will be based very largely on a concept of immunity which is still a heterodox one—although it has some distinguished proponents—which has been called the clonal selection theory (Burnet, 1959). The stimulus to adopt such a theory came largely from a consideration of the pathology of immunity, and perhaps its chief virtue over earlier theories is its direct relevance to the problems of auto-immune disease.

The Facts of Immunity

Every human being has an immunological individuality that differentiates him from every other person—with only the minor qualification that identical

twins are identical immunologically as well as in all other inheritable qualities. The most familiar evidence of this is the failure of a skin graft from another person to take, although autologous transfer of skin from one site to another on the patient is a standard technique.

These differences between individuals are of genetic origin, and one can express the basis of individuality as depending on two general concepts: (1) the chemical pattern of all the macromolecules of the body is genetically determined; and (2) random gene mutation will introduce possibilities for discontinuous changes in macromolecular pattern, many of which will have no relevance for survival but will introduce immunological differences.

When foreign and hence potentially dangerous material enters the body—classically as an invading micro-organism—it requires to be recognized as foreign; this recognition can only be based upon the fact that the alien material carries some molecular patterns which are not represented in the host. The classical immune responses obviously represent evolutionary adaptations needed to allow survival in a world teeming with potentially pathogenic micro-organisms. Equally obviously, any body component must not provoke the appearance of antibody or of cells which can react specifically to contact with it. Any defence force must know how to distinguish friend from enemy. The characteristic feature of what might be called the new immunology is its interest in the nature of the process by which this recognition of the difference between what is self and what is not self is mediated.

The possibility must not be excluded that some types of foreign material are so different from any found in a vertebrate body that other principles of recognition may come into play—for example, inability of body enzymes to break down certain chemical configurations.

There are several types of experimental material which bear on such differences. Skin-transplantation experiments in animals, usually mice, of known genetic constitution have been basic, but a variety of other types of transplantation, notably of malignant tumours and of bone-marrow suspensions, have also been used. Three findings from observations on cattle will provide a good introduction to the experimental approach.

1. If we take two randomly chosen calves and transfer pieces of skin from one site to another, we find that, as in human beings, autologous grafts will take place, while homologous grafts are rejected. When a graft from A is placed on B there is an initial phase during which the graft

*The first of two Croonian Lectures delivered before the Royal College of Physicians of London on June 16 and 18, 1959.

becomes vascularized, but within two weeks the graft area becomes inflamed and the foreign skin is rejected. If a second A to B graft is now tested the rejection is greatly accelerated. B has been modified by the experience of the first graft and is, as it were, immune to A, a phenomenon referred to as homograft immunity.

2. Calf twins are common enough, but identical twins are rare. If a pair can be found they will allow cross-transplantation. Grafts of A skin will take happily on B, and vice versa. This same mutual tolerance is observable in human identical twins, and depends on the genetic identity of the two subjects. With pure-line mice bred for many generations by brother-sister matings, genetic identity or near identity will also allow mutual tolerance of skin grafts.

3. It is well known that cattle twins usually develop a common placental circulation even when they are derived from two separate fertilized ova. When the sexes are different, hormonal interchange during embryonic life results in the female becoming a sterile "free-martin." If non-identical bovine twins are used for reciprocal skin grafts, takes occur as regularly as in identical twins (Anderson *et al.*, 1951). This is quite at variance with the findings in human beings, but it does call to mind a phenomenon observed earlier by Owen (1945) in such bovine twins—namely, that the pair would commonly show a sharing of two red blood cell groups between them. The two phenomena allow the formulation of an important provisional generalization—that cells present through embryonic life may persist into adulthood and be recognized as "self" even if they carry patterns genetically distinct from those of the other cells of the body.

The next step, due to Medawar and his colleagues (Billingham *et al.*, 1956), was to analyse the phenomena at the experimental level, using specially bred homozygous strains of mice. In discussing it I shall simplify the situation greatly and will gloss over the difficulties that made the results not as easily reproducible as could be wished. We have two strains of mice, A and B, A having white skin, B black. As proof of homozygosity, any A mouse will accept a graft from any other A mouse, the same holding for strain B. Grafts from A to B, or B to A, fail, and repeated homografts give rise to accelerated rejection—homograft immunity.

To imitate what happens in bovine twins, we can inoculate foetuses of A during the last week *in utero* with cells from strain B—for example, spleen or bone-marrow cells from adult B mice. After birth these mice treated *in utero* are found in a high proportion to accept a homograft of opposite type. It is possible to simplify matters by inoculating baby mice intravenously with the opposite type cells on the first day of life and still obtain the same tolerance of the homograft. We should be definite that when mouse A acquires tolerance for skin B it still retains its power to reject skin from strains C, D, and E. The effect is immunologically specific, and has been named by Medawar, "specific immune tolerance."

As I have hinted, the experiment is not always easy to accomplish, and two modifications may be mentioned which indicate the nature of some of the difficulties.

Suppose we have a mouse A rendered tolerant to B and carrying a healthy patch of black skin on its white coat. We transfer to this mouse lymph-node cells from a normal non-tolerant A mouse. They are accepted, and in a week or two the previously stable black graft begins to break down and is eventually rejected. The process can be accelerated if, instead of using a normal A mouse, we take the lymph node from an A mouse which has developed homograft immunity against B.

It is clear that some capacity has been *lost* by the tolerant mice which can be replaced by cells from normal or immunized animals.

I have spoken of A and B strains of mice as if any pair of strains would serve. Actually they have to be chosen rather carefully. If we take, for instance, strains B and C and inoculate spleen cells of C into newborn B, we find that the baby B mice fail to flourish, most develop diarrhoea and die within three weeks, and an occasional stunted specimen will survive and can be shown to be tolerant to C skin. This has been called "runt disease" or secondary disease, and has been ascribed, with what seems to be excellent reason, to the immunological attack of the *graft* on the host. The strongest evidence for this interpretation is that by using *embryonic* C cells tolerance can be induced without runt disease. Here we have a process by which two sets of embryonic tissues can both become tolerant of the foreignness of the other.

So far we have been concerned with situations in which tolerance or immunity is provoked by living cells from the same species of animal but of a different strain. Presumably these reactions are produced because of chemical differences between the strains in some of their cellular proteins or other macromolecules. The next step then is to find whether pure proteins foreign to the body can behave in similar fashion. We know that they can produce antibody when inoculated into grown animals, and in recent years several workers have produced a non-responsive state by administration of such antigens to very young animals.

Behaviour of Foreign Proteins

Smith and Bridges's (1958) studies of bovine serum albumin (B.S.A.) in rabbits inoculated on the first day of life have provided a relatively simple picture. If 100 mg. is administered the rabbit will fail to respond by antibody production to any further injection of that antigen for something over three months. If, however, the delay before testing extends for 200 days or more the second injection will provoke an antibody rise. By a study of the relation between size and dose and the time over which non-responsiveness to antibody extended, Smith and Bridges concluded that the effect of the first injection is to induce a state of non-responsiveness which persisted only so long as some of the foreign antigen was present in the body. By appropriate timing it was possible to maintain a rabbit quite inert to B.S.A., although reacting normally to any unrelated antigen. Smith and Bridges believe that their results indicate that tolerance in Medawar's sense is dependent on the continued persistence of the relevant antigens.

From all these observations it is clear that the adult animal can differentiate in some way between substances which have been present in the body since some stage of embryonic or neonatal life and substances foreign to the body. Everything indicates that this capacity for recognition is a function of the cells characteristically present in spleen and lymph nodes. It is a function of some or all of the mesenchymal cells of the body.

Our immediate problem is to find the best working hypothesis which will cover these facts and provide a basis for the phenomena from human disease that I shall consider in the second lecture.

Let us start from Smith and Bridges's data on B.S.A. In the non-responsive rabbit B.S.A. can be differentiated

(by this fact of non-reactivity) from a variety of other antigens, presumably from all other antigens not very closely related to B.S.A. B.S.A. is, in fact, treated as if it were part of the rabbit's own albumin—it is eliminated at the same rate, and by all tests is immunologically quite inert as long as tolerance lasts. Broadly speaking, there are only two possible mechanisms by which the differentiation of B.S.A. from other foreign antigens could be achieved. Antibody is produced by cells, and we can ascribe failure to produce an antibody despite presence of the normal stimulus to one or other of two alternatives: (1) the preparatory injection at birth may have inhibited or eliminated a set of cells (or clones of cells) which alone are capable of producing antibody against B.S.A.; or (2) if cells capable of producing one antibody can, under corresponding circumstances, produce any other antibody, then the preparatory injection must modify one region in all such cells while leaving many other regions available for reaction with other antigens.

Either interpretation, as well as any conceivable compromise or alternative, demands a very extensive store of "information" which in some way or another must be stored in the mesenchymal cells. "Information" is used here in a technical sense, which, however, is not very far from its normal one. So far we have been concerned only with experimental situations in which a foreign antigen or group of antigens has been used to trick the body into recognizing it as self; but, without attempting to elaborate the point, we can be equally certain that the same holds for all the multitude of potentially antigenic materials and chemical configurations to be found amongst the normal components of the body. None of them provoke antibody production, presumably because of a process equivalent to the production of tolerance to foreign cells. A particularly cogent example concerns the rare blood group "Bombay." Here there is a genetic block which prevents the production of any of the ABO antigens in the body. In their absence anti-A and anti-B and anti-H (O) agglutinins develop in the serum (Bhende *et al.*, 1952). In normal individuals, of course, one or more of the antigens are present and the corresponding antibodies are never produced.

We may state the first requirement of any comprehensive immunological theory, that it will account for the fact that *from the time of birth there is in the body an adequate supply of information to allow the recognition of vast numbers of antigenic configurations as either "self" or "not self."*

There are many other aspects of immunity, but from our special interest in auto-immune disease this is clearly the most important, just as in the earlier discussions of immunology it was probably the most neglected. Very briefly, the other important features of immunological phenomena to be considered are:

1. The specific complementary relationship of antigen and antibody on which all methods of demonstrating antibody depend.
2. The difference between primary and secondary types of immune response.
3. The existence of three qualitatively distinct types of immune response which can often be evoked by the same antigen—classical antibody production, "hay-fever type" antibody production, and delayed hypersensitivity.
4. The long persistence of immunological memory in the sense, for example, that an injection of tetanus toxoid will give a secondary type response in a man whose last injection

was 15 or 20 years previously—or that immunity to measles will last a lifetime without reinforcement by re-exposure to infection.

5. The development of delayed hypersensitivity and of effective immunity following virus disease in patients with congenital agammaglobulinaemia who cannot produce antibody.

Immunological Theories

This is not the place to discuss details of immunological theory beyond what is necessary to provide a framework of ideas against which to discuss immunopathology. The weakness of standard theories of antibody production is simply that they provide no point of approach to the problems of auto-immune disease. The clonal selection theory of immunity is still only something in the process of being shaped into usefulness. It has been fairly fully described at the experimental and theoretical level (Burnet, 1959), and only a summary account is given here.

Essentially, classical theory holds as self-evident that antigen entering the body as something completely foreign and unknown imprints its character on certain cells and enforces them to produce globulin molecules carrying a complementary pattern. This globulin, by uniting with the foreign material, may neutralize any toxic qualities it possesses and in general prepare the way for its accelerated elimination from the body. On classical theory the pattern of the antigen provides the information which is incorporated in the pattern of the antibody and makes it specifically reactive with the antigen. It is necessary to assume that, in addition to providing antibody pattern, antigen is also the stimulus which provokes the antibody-producing cells to proliferate and to synthesize and liberate antibody.

Clonal Selection

Clonal selection theory, on the other hand, allows the antigen no part in impressing pattern on the antibody-producing cell. The capacity to produce a given antibody is a genetically determined quality of certain clones of mesenchymal cells, the function of the antigen being to stimulate cells of these clones to proliferation and antibody production.

In these lectures I am concerned only with potential antigens which form part of the body, or which may be present in other individuals of the same or other vertebrate species. It does not necessarily follow that the concepts I am using are equally applicable to antigens that are completely remote from those of vertebrate tissues. It may be a fundamentally significant finding that with one slightly equivocal exception no one has demonstrated tolerance to a bacterial antigen.

Any biological theory will need *ad hoc* elaboration in various directions to cover the complications of reality, and this is no exception. The most important extension is to devise a way by which the information needed to differentiate between body components and foreign material can be derived—with at the back of our minds the need to have in reserve a possible interpretation for the breakdown in that "information service" in auto-immune disease.

The clonal selection approach is to generate a very large number of clones by allowing a high degree of somatic mutability in the early stages of embryonic development. If such mutation is at random many clones will be initiated which will correspond to potential antigenic determinants in the body. The central

feature of the hypothesis is that contact of immunologically competent cells with corresponding antigen during embryonic life results in the elimination of those cells. If the rate of mutation slows down sharply in the second half of embryonic life, this double process of mutation and elimination of clones corresponding to antigenic determinants in the body will automatically provide a range of residual clones capable of reacting with all foreign antigenic patterns. Figs. 1 and 2 may clarify this idea.

It is not wholly an *ad hoc* assumption that an immunologically competent cell, if embryonic, will react with the corresponding antigenic determinant and be destroyed, while if adult it will be stimulated to proliferate. Antigen-antibody contact is capable of a variety of damaging effects in the body, and it is well known that excess antigen can inhibit antibody production in the immunologically mature animal.

If somatic mutation can modify immunological pattern at random, some way to eliminate cells which have undergone an unfortunate mutation must exist.

FIG. 1.—The essential features of the clonal selection theory. Above: The changing reactivity of an immunologically competent cell to contact with the corresponding antigenic determinant: before birth inhibition or destruction with gradual change; after birth to proliferation and antibody liberation. Below: Postulated mutability of the relevant portion of the genome, at first so high as to be virtually a process of randomization.

FIG. 2.—An attempt to illustrate how immunological information could be generated. Each lower-case letter indicates an immunological pattern (potential antibody) corresponding to an antigen shown by upper-case letter. If from the stabilized random population all patterns corresponding to body components are eliminated, the residual population will be able to react only with foreign configurations—that is, it will be able to recognize antigenic determinations as “not self.”

Obviously if a mutant clone should arise which corresponds immunologically to a determinant in, say, the serum albumin molecule, it must be eliminated rather than fostered. A simple hypothesis which will provide such a homeostatic mechanism is to assume that once a cell has been stimulated to proliferate by an antigenic stimulus it will pass through a transient phase in which it is susceptible to destruction or inhibition on further contact with the same antigenic determinant. Whenever the antigenic determinant is freely present in the body and accessible to the corresponding immunologically competent cells, such destructive contacts would be inevitable. So far as one can judge, the recorded data on the reaction of animals to very large amounts of antigen contain nothing incompatible with such an hypothesis. On the other hand, there is, strictly speaking, no positive experimental evidence either of somatic mutation of the type postulated or for this type of homeostasis. With the rapid development of the experimental approach to cellular immunology both problems should, however, soon come within the range of experimental decision.

Cells Involved

The discussion so far has made no specific reference to the cells involved beyond the fact that they are of mesenchymal origin. The interrelationships of these cells and the correlations between morphological appearance and function are still controversial. There is, however, sufficient convergence of view to allow some general remarks to be made. In the first place, some of the studies on the repopulation of bone marrow and lymphoid tissue after x-irradiation and treatment with heterologous cells (Ford *et al.*, 1957) point very strongly to a unitarian origin of the whole group of mesenchymal cells, including granulocytic and erythrocytic series. The various determinants involved in the changes from one morphological form to another are almost completely unknown, but there is no doubt that such changes do occur. Within the range of cells, which include reticulum cell, large, medium, and small lymphocyte, plasma cell, macrophage, and fibroblast, almost every possible interconversion has been described. In any clonal approach it will be necessary to accept the possibility that within an immunologically competent clone representative cells may have the morphological appearance of any of the standard types.

On any interpretation the small lymphocyte must play an important part as the commonest of all mesenchymal cell types. Probably the most significant thing about the lymphocyte is its scant amount of cytoplasm in comparison with the nucleus, with the obvious suggestion that the nucleus is the significantly functional portion of the cell. A nucleus contains two things that may be important—genetic information and nucleic acids. Many have suggested that the lymphocyte might be a convenient means of bringing the building blocks required for nucleic acid synthesis to a given region of the body. Alternatively, one could regard the lymphocyte as a carrier of the information needed to allow it to transform into a functioning cell of more than one type, the cytoplasm being limited to functioning merely as support for various trigger mechanisms that will set such transformations in action. This is very much in line with the views of Yoffey, Farr, and others, but a majority of histologists would probably still consider the lymphocyte an end-cell. It is still only a hypothesis—but it is a good hypothesis pointing to many types

of experimental approach—that the main function of the lymphocyte is the carriage of immunological information.

The other cell type that requires discussion is the macrophage. Despite the prominence that was once given to cells of the reticulo-endothelial system in theories of antibody production, the present trend is to deny them any such function. Macrophages take up antigen but they do not produce antibody. There have been theories that a first stage of antibody synthesis takes place in the macrophage, the product then being transferred to a cell of the lymphoid series. There is, however, neither experimental evidence nor theoretical necessity for such an assumption. Modern work, such as, for example, that of Uhr, Salvin, and Pappenheimer (1957), points to the effectiveness of minute amounts of antigen as a stimulus, and it may well be that the main function of the macrophage is not to collect antigen in order to make antibody but to reduce the concentration of antigen to a level which will not embarrass the antibody-producing cells.

All workers are agreed that the production of antibody is a function of plasma cells and that the characteristic morphology of a plasma cell is no more than evidence that it is in fact actively synthesizing and liberating protein. Since the monograph of Fagraeus (1948) there has been no serious doubt on this point, but the final proof that plasma cells produce antibody was only provided last year by Nossal (1959) in Melbourne. He showed that plasma cells from an immune animal when isolated in microdrops as single cells produced antibody, while small lymphocytes from the same lymph-node suspension did not.

At the cellular level, the essence of the clonal selection theory is that contact of antigenic determinant with immunologically competent cells—presumably lymphocytes—can stimulate them in various ways: (a) to liberate pharmacologically active agents such as histamine and serotonin, and perhaps (b) to be rendered non-viable in the process; (c) to proliferate, producing more cells of the lymphoid series; and (d) to give rise to antibody-producing plasma cells. The type of response will depend on (1) the physiological state of the cell, (2) the concentration of the antigen, and (3) the site in which the stimulated cell lodges.

To those who have not been in touch with recent developments in immunology this may well seem a paradoxical and unconvincing way of looking at a phenomenon which has a much more straightforward interpretation. There are still many immunologists who would prefer the Haurowitz-Pauling hypothesis in one or another elaboration. On this view the antigen or its effective portion is retained in the antibody-producing cell, where it acts as the template against which each antibody molecule is synthesized. Unfortunately this is a point of view that makes no contact whatever with the phenomena of tolerance and of immuno-pathology. The clonal selection theory that I have presented will certainly have to be modified in the light of the experimental studies that it has already stimulated, and it may eventually be discarded, but any new theory or rehabilitation of an old one will have to have equal relevance to these key phenomena.

A Recapitulation

Let me in conclusion recapitulate this theory. Its major difference from earlier theories is its contention

that the antigen does not determine the pattern, the specificity of the antibody, in any more direct sense than the giraffe's habit of browsing on tree leaves has lengthened its neck. It is a Darwinian, not a Lamarckian, approach.

Perhaps we can put the difference between the two into modern terms. It is a "central dogma" of modern biochemical genetics that only genetic processes can modify the information in a genetic system. There is no presently conceivable way by which information from the environment can be incorporated into the nucleic acid of the chromosomes. The flow of information is always from the genetic to the biochemical, never in the opposite direction. The chain of command is pictured as deoxyribonucleic acid—ribonucleic acid protein (D.N.A.—R.N.A.—protein), and on this dogma there is no way in which an antigen can meaningfully influence the pattern of D.N.A.

To satisfy these requirements we have introduced the concept of a hypermutable segment of the genome in mesenchymal cells, especially during the early stages of differentiation. It is of interest that detailed study of bacteriophage mutability shows "hot spots" where mutations are much more frequent than at other loci (Stent, 1958). It is not unreasonable to believe, therefore, that the needs of survival might have led to the evolution of a "hot spot" of this type in the genome of mesenchymal cells. This allows the postulate that all conceivable patterns which can be taken by the rather small specific area of antibody globulin molecules could be represented in the genomes of cell clones arising in the course of embryonic life. Tolerance results from the elimination or inhibition of all cell clones which react with antigenic determinants present in the body.

Immediately after birth the body begins to make contact with antigens of all sorts, notably bacterial antigens derived from the alimentary canal. This initiates a process that continues through life. When antibody is needed it is automatically produced by specific stimulation of the cells in clones preadapted to react with the corresponding antigenic determinants. When the antigen disappears from the body the proliferative stimulus lapses and the clones slowly diminish in importance. Mutational change will also in most cases gradually erase the specific character of the clones unless some natural or artificial "booster" is encountered. So that by adult life the immunological experience of the body is incorporated not only in the antibodies that are circulating in the blood but in the clones of cells that make up the cell populations of spleen, lymph nodes, bone marrow, and the various local accumulations of lymphoid tissue.

From the point of view of human pathology, the great virtue of the clonal selection approach is its provision of a basis for the emergence of antibody or of immunologically active cells directed against body components. Almost all who have discussed the character of such a disease as acquired haemolytic anaemia are clear that the anomaly is not in the red cells which are damaged but in the cells which produce the abnormally reactive globulins found in the plasma. It is an essential feature of our hypothesis that mutation in immunological pattern is always in process. Random mutation is bound to produce a proportion of patterns reactive against body components, and in the normal individual there must be a mechanism by which cell clones of such character are prevented from

proliferating. It is convenient to refer to such clones and the immunological patterns they carry as "forbidden" clones or "forbidden" patterns, and to regard the mechanism by which they are eliminated as part of the general homeostatic control of body structure and function.

A wide variety of medical conditions in the body represent the partial failure of homeostatic mechanisms—auto-immune disease can be interpreted best as another example of partial and often temporary failure of one such mechanism. Instead of being eliminated certain forbidden clones of cells find opportunity to proliferate and, in reacting with the antigen involved, inflict damage on the body.

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[The second Croonian Lecture will appear in next week's issue.]

EXPERIMENTAL STUDY OF AN ERYTHROPOIETIC PRINCIPLE PRODUCED IN THE KIDNEY

BY

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The present paper is concerned with problems related to erythropoiesis. An attempt is made to extend our knowledge of the mechanism regulating the level of erythrocytes in the body, and to throw light on the pathogenesis of some types of anaemia. Special attention is given to anaemia developing in the uraemic condition.

Anaemia occurs in most types of human renal insufficiency. This form of anaemia, like that of chronic infection, appears to be due both to a reduction of the life-span of the red cell and to deficient marrow response (Wintrobe, 1956). That author states: "It has been repeatedly noted, however, that the rate of corpuscular elimination has not been such that it could not be met by increased production, were erythropoiesis accelerated to the full capacity of the bone marrow. It is noteworthy that erythropoiesis in the bone marrow may not be reduced below the normal, and, although there is usually no increase in reticulocytes, an occasional normoblast may be found in the blood smear. Nevertheless, red cell production does not accelerate sufficiently to meet the demand." The cause of the anaemia, however, in renal disease, as well as in chronic infection, hepatic disorder, hormonal disturbance, neoplastic disease, etc., is still obscure.

In the experiments young male mice of the WLO (White Label, Oslo) strain were used. A great number of the animals were rendered nephritic by local x-ray irradiation of the kidneys, done under ether anaesthesia. During the irradiation the whole animal was shielded with lead, with the exception of the exteriorized kidney, which was hanging down in a plastic chamber through which Ringer's solution at 37° C. was flowing. The physical data were: 50 kV, 2 mA, hvl 0.3 mm. Al. The distance from focus to mid-axis of the kidney was 31 mm. The dose rate was about 1,700 r/min. An even dose distribution was obtained by irradiation from both sides.

Reticulocytes were counted in Nile-blue Giemsa-stained blood smears; 2,000 cells were counted on each slide. As an immediate reticulocyte response of short duration may be regarded as an outflow of reticulocytes not related to cell production (unpublished personal material), only protracted reticulocytosis of several days' duration has been used as an indicator of red-cell production. The haemoglobin value was measured by means of Ljungberg's colorimeter. Blood-urea values were determined in all nephritic and some of the normal animals by means of a gas volumetric method (Horn, 1951). In some of the animals, erythrocytes, haematocrit, and CO₂-combining power ("alkali reserve") (Roughton and Scholander, 1943; Scholander and Roughton, 1943; Scholander *et al.*, 1947) were determined. With exception of the reticulocyte values, only a few of the results of these laboratory investigations are reported here: they are presented in the accompanying charts. To indicate the variation between the observations, ± 1 standard deviation has been calculated, and plotted in the graphs.

Evidence that an Erythropoietic Principle is Produced in the Kidney

The anaemia developing in uraemic mice with irradiated kidneys was found to vary from hypochromic to normochromic. Generally the reticulocyte count was slightly lower in the uraemic than in the normal mice. With increasing blood nitrogen accumulation, the bone marrow showed a hypoplastic erythropoiesis.

During a study, begun in 1956, on functional and anatomical changes in mice produced by x-ray irradiation of the exteriorized kidneys, the following observations were made: (1) By irradiation of the kidneys in mice a condition resembling the essential features of glomerulonephritis in man was produced. (2) In mice with total irradiated kidneys a definite but slight decrease in the haemoglobin values was noted, with only slight rise of the blood urea. (3) After unilateral nephrectomy alone, the blood urea rose from the normal 20 mg. per 100 ml. to about 30, and on removing one kidney and half of the other the blood urea rose to about 50 mg. per 100 ml. These animals, however, had only a mild, transient anaemia in spite of their raised blood urea. (4) In mice in which a small amount of kidney tissue was shielded during the irradiation, it was possible to produce uraemia with a blood-urea value about 150 mg. per 100 ml. without anaemia (Osnes, 1958).

These observations led to the following conclusions: The failure of the kidney as an excretory organ and the development of anaemia are parallel phenomena and independent of each other. These findings also indicate that the kidney may produce a principle of importance in erythropoiesis, and lack of this principle