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NUCLEIC ACIDS AND THEIR ROLE IN FUTURE CHEMOTHERAPY OF TUMOURS AND VIRUS DISEASES*

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It is my intention in this lecture to review some of our present knowledge about the connexion between nucleic acids, viruses, and cancer, to consider this in the light of our understanding of nucleic acid chemistry, and to see whether any conclusions can yet be reached about the course of future chemotherapeutic work in the fields of cancer and virus diseases. I wish to make it abundantly clear that I speak as a chemist whose interests have lain in the structural chemistry of the nucleic acids; I am in no sense a biologist, and my only claim to speak on this subject rests on my belief that we have now reached a stage in which the chemist must bring his experience and outlook to bear on these vital problems, as well as the biologist, the medical scientist, and the physicist. Only by the joint effort of all can we pass through the present phase of searching in a haystack of complexities and emerge with the needle of true understanding of what may well be some of life's innermost secrets. But in the present circumstances I am well aware that the task I have set myself is not an easy one, for there is much conflicting evidence and much that we do not know. Moreover, my discussion may well be coloured somewhat by my personal and professional prejudices and my very incomplete knowledge of the biological aspects of the subject. I can only hope that my attempted synthesis may be of some interest and value, at least to the non-specialist.

Chemical Nature of Genes

One of the most striking and important recent advances in genetics has been the recognition of the chemical nature of the genes or units of heredity. During the second decade of this century it was recognized by the geneticists that the genes are carried in the chromosomes. These latter are composed of deoxyribonucleic acid (D.N.A.) and protein combined in some way not yet wholly understood, and it was for many years believed that genetic specificity was associated entirely with the proteins of the living cell. Attention first became focused on the nucleic acids as genetic material as a result of a series of studies starting with the observation of Griffith (1928) that he could transform one specific S-type pneumococcus into another specific S-type by injecting non-virulent R-forms into mice together with large amounts of heat-killed S-pneumococci of a type other than that of the organism from which the R-cells were derived. Living virulent S-organisms of the same type as the heat-killed S-forms were then recovered from the animals.

Later on, other workers found that the addition of an extract of one type of capsulated pneumococcus to a culture of a non-capsulated rough form would convert the latter into the same type of capsulated organism which provided the extract. Finally, Avery, McLeod, and McCarty (1944) showed that the substance in these extracts responsible for this effect was D.N.A. All subsequent work has confirmed that the primary genetic material in this organism is D.N.A. The evidence in higher forms of life than bacteria is perhaps not so clear-cut despite the interesting experiments of Benoit, Leroy, Vendrely, and Vendrely (1957a, 1957b, 1958), but it is at least now widely accepted as a working hypothesis that the primary genetic material is D.N.A. So far as I am aware, we do not know any facts which contradict this hypothesis.

Further evidence for the role of nucleic acids in genetic continuity comes from the study of viruses. Whether viruses are to be regarded as "living" or not is a matter for academic debate, and we can leave it on one side for the moment. But at least there is a close similarity between genes and viruses, and, indeed, when one considers the results of transduction experiments in *Salmonella* using a bacterial virus (Zinder and Lederberg, 1952), the difference between the two appears negligible. In the phages or bacterial viruses the evidence is very strong that genetic continuity resides in the D.N.A. In these materials infection of the host cells is accomplished through injection of phage D.N.A.; the protein coat remains outside and at most only very small amounts of protein enter the host.

Although quite a number of viruses other than bacteriophages are known which contain D.N.A. and protein—that is, are deoxyribonucleoproteins—many others, including some of the most thoroughly investigated plant viruses, are ribonucleoproteins. Particularly striking observations have been made which show that it is the ribonucleic acid (R.N.A.) component which is responsible for genetic continuity in tobacco mosaic virus. In this case the virus particle itself is a hollow cylinder of protein material, the R.N.A. being embedded in the hollow central portion. Both Fraenkel-Conrat (1956) and Gierer and Schramm (1956) have separated the R.N.A. from the protein of tobacco mosaic virus and shown that the infectivity is carried by the R.N.A. and not by the protein, although recombination of the R.N.A. and protein gives a more infective material than the protein-free R.N.A. More striking still are Fraenkel-Conrat's observations that if the R.N.A. of one strain of virus is combined with the protein

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component of another the nucleoprotein so produced is infective, but on infection it is the nucleoprotein—that is, virus—corresponding to the R.N.A. component that is exclusively produced. Somewhat similar observations have been made with other R.N.A.-containing viruses.

The evidence available, then, shows clearly that nucleic acids can and do act as genes or carriers of genetic information, and it is at least highly probable that they do so in all forms of living matter and in the viruses. In living cells the primary carriers of the information—the hereditary nuclear genes—are almost certainly the D.N.A.s. These D.N.A. molecules are not part of the dynamic pool of metabolites in cells. Studies with radioisotopic tracers have shown that they do not lose their isotopic constituents once they are incorporated and the precursors are incorporated only when new D.N.A. is being synthesized, normally at the time of cell division. The D.N.A. has indeed an astonishing degree of metabolic inertness and is in this sense quite unlike any other type of organic molecule in the cell. R.N.A. can clearly provide genetic continuity in the viruses, but in living cells it seems to occupy a lower place than D.N.A., being in some way controlled by the latter just as the R.N.A. itself controls protein synthesis, and being further subject to much more rapid synthesis and turnover.

Interaction of Nucleic Acids and Protein

The relationships between D.N.A., R.N.A., and protein, and the way in which control of the one by the other is exercised, are matters to which one could devote not one, but several lectures, and I shall confine myself here to giving a very simple—and, no doubt, oversimplified—picture which seems to me to be in accord with the bulk of our present knowledge, and which I accept as a working hypothesis. It is given by the following scheme:

A somewhat more elaborate and more comprehensive picture of cellular economy due to Potter (1957, 1958) is given below. It emphasizes the interaction of genes and environment through "feed-back" mechanisms which form part of the overall control of growth and normal functioning of the living cell.

These facts and views on the genetic importance of the nucleic acids have an obvious relevance to the cancer problem, and from them stem two of the main current hypotheses on which approaches to cancer and virus chemotherapy are based. These are the deletion hypothesis, which has been clearly expounded in publications by Potter (1957, 1958), and the attractive virus hypothesis discussed by Stanley (1958). According to the former, one must think of normal cells undergoing mutations leading to the loss of the negative feed-back systems which keep in check the specific enzymes needed for cell division and the synthesis of D.N.A., the deletion of some specific D.N.A. molecules necessary for surface protein production, and of katabolic enzyme formation (through deletion of R.N.A. enzyme-forming systems).

The mutations triggering off the process might be a result of inherent random errors in nucleic acid replication, errors brought about by action of carcinogens or radiation damage, or loss of certain enzyme systems which fail to replicate fast enough during cell division, a situation which might arise through competition with a viral infection or by combination with carcinogens such as azo-dyes. Any or all of these may be involved in the different types of cancer. The virus theory would seek to explain all types of cancer (other than the present generally accepted virus cancers) by the intervention of viruses present in cells, but which are inactive or non-virulent until stimulated or modified either by external conditions, by carcinogens or radiation, or presumably also by random mutation. It seems to me, at least, that there is really less difference between these hypotheses than might at first sight appear. Both depend on mutation in nucleic acids and differ essentially in how the cancer process starts; perhaps a good deal depends, too, on one's definition of a virus. One thing seems clear—the two hypotheses lead to essentially similar approaches to cancer chemotherapy. Everything comes back finally to nucleic acid.

Having now cleared the ground by indicating in a general way the facts about genetic control by nucleic acids, we must consider what these materials are, and how they can act as carriers of information. Here there is available a moderate amount of fact and a very large amount of speculation. Much of the speculation is reasonable, but there has been a tendency at times to forget that it is speculation and that much more experimental work is needed before we can feel sure about the details of nucleic acid function.

Chemical Structure of Nucleic Acids

It is now just under eight years since the general theory of the chemical structure of the nucleic acids was announced by Brown and Todd (1952, 1955), based on an exhaustive study of the chemistry of the simple nucleosides and nucleotides and the hydrolytic behaviour of the two types of nucleic acid (D.N.A. and R.N.A.) found in nature. According to the theory, which is now firmly established, both types are polydiesters of phosphoric acid, built up by polycondensation of many individual nucleotide units to give linear polynucleotides with a regular recurring 3':5'-internucleotidic linkage. The ribopolynucleotides can be represented diagrammatically as I and the deoxyribopolynucleotides as II.

The individual nucleosides are N- β -glycosides of heterocyclic bases of the purine or pyrimidine series, the

sugar being D-ribofuranose in the case of the R.N.A.s, and 2-deoxy-D-ribofuranose in the D.N.A.s. Typical examples are adenosine (adenine 9- β -ribofuranoside) and thymidine (thymine 3- β -2'-deoxy-D-ribofuranoside). It seems that most R.N.A.s contain only four simple nucleosides (adenosine, guanosine, uridine, and cytidine), and the very interesting discovery in recent years that some preparations of R.N.A.s from various natural sources contain traces of other "unnatural" nucleosides is probably an indication that very small amounts of other nucleic acids with a composition quite different

from the common ones do occur alongside them in some cells. The D.N.A.s yield four main nucleotides (deoxyadenosine, deoxyguanosine, deoxycytidine, and thymidine), together with two less frequent components (5-methyldeoxycytidine and 5-hydroxymethyldeoxycytidine), and in this series, too, trace constituents occur in some natural materials. Variable estimates have been given for the molecular weights of the natural materials; although they may be subject to error, values of about 2 million to 15 million are frequently quoted. Whatever the accuracy of these determinations may be, it is clear that we are dealing here with very long polynucleotide chains, and that, even with as few as four monomeric units, a fantastic number of individual nucleic acids is possible, each differing from the other in the sequence of nucleoside residues.

Self-replicating Systems

Following on the chemical work in nucleic acid structure, it was possible to interpret the x-ray diagrams of D.N.A. obtained by Wilkins (1956) and others, and this led to the realization that D.N.A. normally exists as a double molecule in which two polynucleotide chains combine to form a double helical structure with the sugar and phosphate on the outside and the bases in the

core of the helix lying at right angles to the main axis (Fig. 1). Watson and Crick (1953) were now able to make their brilliant contribution by suggesting the reason for the existence of the D.N.A. double helix, and at the same time to reveal how D.N.A. could form

FIG. 1.—Diagrammatic representation of the D.N.A. double helix. The ribbon-like helices are made up of the sugar residues linked by phosphate groups, and the horizontal rods represent the paired bases.

a self-replicating system. They drew attention to the fact that analyses of a large number of D.N.A. samples from different sources showed that, taking the four main bases present in the nucleosides, the ratio of adenine to thymine was 1:1 and of guanine to cytosine 1:1, although this was far from true for the ratios of other pairs—for example, adenine to guanine. They suggested that this was due to specific hydrogen bonding between these pairs of bases, and that it was this specific bonding between the bases which held the double helix together (Fig. 2).

FIG. 2.—Specific pairing of adenine with thymine and of guanine with cytosine.

Although it cannot be said that the Watson-Crick theory has been completely verified, I think that all evidence obtained since its promulgation is so much in its favour that it can be regarded as correct in its essentials. Particularly striking in this connexion are the enzymic syntheses of D.N.A. by Adler *et al.* (1958); Bessman *et al.* (1958a, 1958b); Schachman *et al.* (1958). The observation by Kernberg and his colleagues that an existing D.N.A. molecule will grow in the presence of the appropriate enzyme and a mixture of the four nucleoside triphosphates, but will add only one or two residues when one nucleoside triphosphate alone is used, is precisely what one would

expect on the Watson-Crick theory of specific base pairing and is hard to explain otherwise.

The replication theory of Watson and Crick can be simply explained in the following way. If we describe the four main deoxynucleosides by the initial letters of their trivial names—that is, A, G, T, and C—and we assume that in the double helix of D.N.A. the pairing of A with T and of G with C is obligatory, then the expression (III) could represent part of a D.N.A. molecule, the sequence in each polynucleotide chain being determined by the other.

If we imagine the two strands of the helix to separate as in IV and to be in a medium where D.N.A. synthesis is occurring, each of the separated chains will build upon itself the complementary chain giving, as in V, two double helices of D.N.A., each identical with the original (III). This furnishes a beautiful illustration of the way in which D.N.A. may provide genetic continuity in a cell, for, if on division even one strand of D.N.A. is passed on, the progeny must continue to reproduce D.N.A. of the original parental pattern.

It is generally believed that nuclear D.N.A. acts as a kind of template for the synthesis of R.N.A.—and also for chromosomal protein—and it seems that the R.N.A. produced also forms a self-replicating system. True, isolated R.N.A. does not seem to exist normally in a double helical form, but the same base-pairing rules should apply in R.N.A. as in D.N.A.—that is, adenine with uracil and guanine with cytosine—and even in D.N.A. it is the single strands which are active in the replication process. The stable double helical form, although appropriate to the inert D.N.A., would hardly be expected in the very active R.N.A. It will be recalled in this connexion that polyribonucleotides synthesized by means of the Ochoa enzyme (polynucleotide phosphorylase) (Grunberg-Manago and Ochoa, 1955) can form two-stranded and even three-stranded helical structures under certain conditions, although under others they exist as single-stranded random coils.

These views about the manner in which nucleic acids can be regarded as self-replicating systems, even if they are oversimplified in some respects, do provide an explanation of mutations. Any change which will destroy the base-pairing pattern, even at one point in the molecule, would be enough to alter the hereditary characteristics—that is, to bring about mutation. Equally, interference with the base-pairing or rupture of the polynucleotide chain might in theory provide a means of destroying a virus or rendering it harmless. And it is on this knowledge, incomplete though it is, that any rational approach to the chemotherapy of virus disease, and also, I believe, of cancer, must ultimately be based at the present time. Although I believe that the virus and cancer problems are very closely related, it is convenient to consider them briefly in turn.

It is through immunology that the major contributions to the control of virus diseases have been made, and I have no doubt that vaccine therapy will continue to be

of great importance. But at the same time our increasing knowledge of the nucleic acids and the real nature of viruses may well lead to a refinement of immunological methods resulting in more effective agents with very high specificity in their action. However, I do not propose to discuss the immunological aspects of virus and tumour therapy, and will confine myself to the approaches related to chemotherapy in the usually understood sense of the term.

Therapeutic Lines of Attack on Virus Diseases

Treatment of virus infections with a large variety of chemical agents has been tried in the past, but relatively little success has been recorded. Apart from some positive effects with antibiotics, the significance of which in our present context I find difficult to assess, most of the meagre positive results seem to have come from the use of substances related to pyrimidine and purine, in which there would seem to be a possibility of incorporation of the drug molecules into the virus polynucleotide, thereby destroying its replication properties, or of interfering—possibly by competitive inhibition—with the synthesis of the natural nucleosides or nucleotides. As an example, I would recall to your attention experiments carried out by Matthews and Smith (1955) and Matthews (1957), who achieved some measure of protection against the cucumber virus by prior spraying of the plants with 8-azaguanine. This effect was plausibly believed to be due to incorporation of the latter substance into the R.N.A. of the rapidly proliferating virus.

In the same way, there is evidence that other purine and pyrimidine antagonists—for example, 4-azauracil, 4-azathymine, 5-bromouracil, 5-fluorouracil—can be incorporated into nucleic acids *in vivo*. Of course, it is not necessary that such agents must be incorporated into nucleic acid in order to be active, nor, strictly speaking, can we say that where they are incorporated it is the production of the abnormal nucleic

acid which is the direct cause of the observed action on the virus. The chemotherapeutic agents may—and in, for example, the case of 5-fluorouracil and 6-mercaptopyrimidine do—act by being antimetabolites for the synthesis of a precursor (thymine in the fluorouracil case) of the nucleic acids. Incorporation might then, in some cases at least, be secondary in importance, but it is an attractive and wholly reasonable aim to seek to destroy a virus by making it incorporate into its nucleic acid component some unnatural bases or nucleosides which would necessarily upset its replication.

From this standpoint, I would suggest that instead of the simple purine and pyrimidine analogues which have so far been used it might be desirable to try the effect of small synthetic oligonucleotides containing in them abnormal nucleotides—including those modified in the sugar or phosphate residues as well as those with unnatural base components. Evidence from the investigations of Ochoa and others on polynucleotide

phosphorylase seem to show that the purified enzyme needs an oligo- or poly-nucleotide primer. This suggests that oligonucleotides might well serve as much better starting-points for R.N.A. synthesis in the cell than a single base or nucleoside, and that such compounds might therefore be better as therapeutic agents.

Mutations might well be brought about or total inactivation of a virus achieved by modification of the

6-MERCAPTOPURINE

GUANINE

8-AZAGUANINE

virus nucleic acid by chemical reagents which attack and modify individual groups in the nucleotide units. Thus Schuster and Schramm (1958) and Gierer and Mundry (1958a, 1958b) showed that nitrous acid, which deaminates adenosine, guanosine, and cytosine residues, could, according to the conditions, mutate or totally inactivate the R.N.A. of tobacco mosaic virus—and it appears that the protein coat of the virus is permeable to nitrous acid, since the effects can be produced on intact virus particles. The demonstration of mutation by this means is particularly interesting, since radiation or other chemical agents usually produce only total inactivation when they attack the virus. Nitrous acid is not a very discriminating reagent and is not, therefore, likely to have any practical value, but it is interesting that the protein does not protect the infective nucleic acid from attack by small molecules. Against larger molecules there may be protection, but, as the viral R.N.A. seems to separate from the protein coat in the initial stages of infection and multiply in the nucleus, the possibility of chemical attack on the nucleus, and presumably unprotected, R.N.A. remains.

Another possible chemical line of attack might be to use synthetic polymers with the object of blocking the virus polynucleotide chains by attachment either through covalent or hydrogen bonds, so as to prevent polynucleotide synthesis on the virus template. Little work has been done on this approach as yet, but the interesting experiments of Allfrey and Mirsky (1958), in which he was able to show that replacement of a large proportion of the D.N.A. in cell nuclei by a number of anionic polyelectrolytes, such as polyethylene sulphonate, heparin, and chondroitin sulphate, allowed the nuclei to continue much of their normal biochemical activity, suggest that possible therapeutic agents of this type are worthy of serious consideration.

The various types of approach to a chemotherapy of virus diseases are all under examination in greater or less degree, and are thus not to be regarded as novel. But, although I believe they are likely to form in one

guise or another the basis for further developments, we need a good deal more information about the viruses and their nucleic acids before these developments can be made on a truly rational basis. It is important, therefore, to consider at least the major gaps in knowledge which are baffling the chemists. In the first place, of course, the methods discussed rest on preventing the multiplication of viral nucleic acids. But they are applicable to any nucleic acid and not specific to viral nucleic acid, so that success really depends on an assumption that synthesis of viral nucleic acid can be stopped and the virus inactivated before serious damage is done to the nucleic acids of the host cell.

Now it is by no means impossible that this could be done, although it has to be remembered that there are certain cells in any living organism which are rapidly dividing and in which vigorous nucleic acid synthesis is going on; there is an obvious danger that these may be seriously damaged as well as the virus. If we are to avoid this danger, we must find out in more detail the peculiarity in the nucleotide sequence or composition of the virus polynucleotides—for some special feature they must surely have to explain their capacity to impose themselves so successfully on the economy of the host cells. It is difficult to see how we can reach the desired specificity in a chemotherapeutic agent (other than by pure chance) unless this kind of information is first obtained.

Cancer Chemotherapy

The same kinds of considerations apply to the problem of cancer chemotherapy. It is in one sense a problem more complex and in another simpler than that of the orthodox virus diseases. It is simpler only in the sense that in the early stages it may be more localized and that destruction of complete cells rather than one constituent in them would usually be a satisfactory solution. But, of course, the great difficulty of administering an agent to kill rapidly proliferating tumour cells without killing other rapidly proliferating cells—for example, in bone marrow—remains. This difficulty has gravely hampered investigations in this field, since if the agent is designed to interfere with nucleic acid synthesis or replication, then either a very high specificity is required or some means must be found of localizing the effect of the drug. Of course, if we recall the "deletion hypothesis" of Potter, mentioned earlier, in which the loss of the feed-back system of control is regarded as the primary feature of a tumour cell, it should be possible to attack the tumour cell at the level of its enzyme proteins rather than at the nucleic acid level and still achieve success.

Methods based on this general theory have been, and are being, applied, and promising results are being obtained. Thus the work of Bergel *et al.* (1957) on xanthine oxidase in tumour therapy is of very great interest, as well as other efforts to work with enzyme systems in which there seems to be at least a quantitative difference between normal and tumour cells; similarly, work on abnormal amino-acids—for example, fluorophenylalanine—aimed at interference with protein synthesis has given some interesting results. But from the nucleic acid viewpoint—since ultimately the problem is bound up with the activity of one or more nucleic acids of abnormal type (without going into the question of their origin)—broadly speaking, methods similar to those mentioned above in connexion with virus diseases are the ones most likely to be followed. They have indeed been followed consciously or unconsciously, as

can be seen from the work on 6-mercaptopurine or 5-fluorouracil, and on the so-called mutagenic agents, to name only a few agents of promise. In the tumour problem, as with the viruses, however, we lack vital knowledge about the nucleic acids of the cell.

What are the significant differences between the nucleic acids of the tumour cell and those of the normal cell? Differences there must be, but I am sure they are not likely to be determined on the basis of gross analysis of cellular nucleic acid preparations as we now know them. For surely there must be many different nucleic acids in the nucleus of an animal cell, and the abnormality of the tumour cell is not *a priori* likely to be noticed in an analysis of the mixture for purine and pyrimidine bases. The vital difference may lie only in base sequence of a few of the nucleic acids present, although there is another possibility which should perhaps be borne in mind. In the last year or two there have been a number of discoveries of very small amounts of abnormal bases and nucleosides in hydrolysates of many natural nucleic acids. Since these preparations were doubtless heterogeneous (it is doubtful whether a homogeneous nucleic acid has yet been prepared), it seems to me that instead of these abnormal nucleosides being present as very widely spaced units in a long polynucleotide chain they may well represent residues from nucleic acids of quite unusual structure which are present as very minor components of the nucleic acid preparations examined. If this is so, is it possible that these minor components are important in the economy of the cell? Such questions—and they are important questions—we cannot answer until we can separate and study the nucleic acids as homogeneous individuals.

Conclusion

To sum up, then, it seems to me that the future progress of chemotherapeutic work based on nucleic acids in the tumour and virus field, if it is to be something other than a hit-and-miss procedure, depends on real physical and chemical advances being made in two main directions. Firstly, methods must be found—and they do not exist to-day—for separating into their individual components the nucleic acids of cells and of preparing pure individual nucleic acids: this may first be attained with the nucleic acids of some of the plant viruses. Secondly, we must perfect experimental methods which will allow us to study the structure of these pure nucleic acids in detail and clarify their relationship with one another. Given these advances, we would not have an immediate solution to the problems we have been discussing, but without them I find it hard to be hopeful about quick results in virus chemotherapy. For we are dealing here with a branch of chemotherapy which demands agents of a remarkable specificity which has so far been observed only in immunological studies.

It is this need for a very high degree of specificity, coupled with the need for complete and irreversible inactivation of certain polynucleotides, that makes the problem of virus and tumour disease so intractable. But the progress so far made in their treatment with chemical agents suggests that, with the further development of basic knowledge of nucleic acid structure and function, the combined work of the chemist and biologist will in the future yield a real solution of these vital problems.

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CONTROL OF ADRENAL SECRETION OF ELECTROLYTE-ACTIVE STEROIDS* ADRENAL STIMULATION BY CROSS-CIRCULATION EXPERIMENTS IN CONSCIOUS SHEEP

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PART II

In the light of the evidence that local changes of adrenal ionic environment were not sufficient to account for the changes of electrolyte-active steroid secretion which occur during change of Na⁺ balance, two possibilities existed: (1) Other non-hormonal changes at the adrenal level were operative and, in combination with the ionic factors outlined above, were sufficient to cause alterations of adrenal secretion seen during change of sodium balance. (2) That a hormonal stimulus was acting upon the adrenal from a distance. In view of the fact that the transplanted adrenal gland is almost certainly denervated, the stimulus would be humoral, not neural.

It will be recognized readily, in relation to the many physiological factors which change in Na⁺ depletion, that the way is open to induce many combinations of change at the adrenal level. Apart from infusions of

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