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First Steps toward a Genetic Chemistry

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The development of a genetic chemistry—a chemistry of the genetic substance—will undoubtedly be a long and exacting and inspiring task. As genetics is a central theme in the science of biology, so we may expect genetic chemistry to become, in time, an integrating core for cellular biochemistry.

The goals of a genetic chemistry, to the extent that we are presently able to define such goals, will be to achieve an understanding of the physical nature of the hereditary units and of the variations these units undergo during the various phases of the life of the cell, to learn how these units are reproduced from generation to generation, to learn how they are modified by extrinsic or intrinsic factors, and to learn in precise chemical terms how they influence the manifold activities of the cells in which they reside.

We are most certainly a long way from these goals, and the paths to their achievement are by no means obvious. Yet I should like to summarize the evidence which leads us to believe, with near certainty, that we have taken a first and essential step toward the creation of a genetic chemistry—that we have, at least, identified one of the principal constituents of the gene. As we review this evidence, I believe it will be possible at the same time to provide a comprehensive summary of the current status of genetic chemistry and of the at least more immediate prospects for further advance.

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When I was preparing this article, I thought back to what was said about my subject—that is, about the physical nature of the gene—in the course in elementary genetics which I had some years ago. To the best that I could recall, nothing was said about it. I attributed this curious result to the passage of years and to an overcrowded memory, so I thought it might be of interest to see how this subject is treated in a current textbook. I examined the book which is presently in use in the basic genetics course at Iowa State College, and after a fairly assiduous search I managed to find one hint that the gene is composed of something more substantial than ectoplasm. In the introductory chapter the statement appeared, "We shall discover that hereditary factors are complex protein molecules which we call genes." Fortunately this thesis was not developed further in the text.

As many know, there is a small but vociferous group who would like to modify that quotation and replace the word *protein* by the term *nucleic acid*—in particular, by *deoxyribose nucleic acid*, or DNA. Now so much has been written and uttered and speculated about DNA in the past few years that I am sure the concept that DNA has something to do with genetics is hardly new to you. What I shall do is to attempt to summarize, a bit critically, the evidence which has been accumulated, very largely within the past decade, that links DNA with the hereditary factors—that is, the genes. In so doing, I should like particularly to distinguish that which we believe we know, and that which, in truth, we merely suspect.

The evidence which links the chemistry of the gene with the chemistry of DNA is at present necessarily circumstantial. Such must be the case until we become sufficiently advanced in our understanding that we are able to demonstrate the action of a gene *in vitro*. But circumstantial evidence can be of varied degrees of credibility. The value of circumstantial evidence is, roughly, inversely proportional to the number of alternative explanations that one can advance for the observed phenomenon. The following eight lines of evidence, which are listed in a rough order of increasing significance, are, I believe, important in establishing a correlation that links DNA and the hereditary factors: (i) the location of DNA; (ii) the specific diploid content of DNA; (iii) the metabolic stability of DNA; (iv) the complementary structure of DNA; (v) the similarity of the ultraviolet absorption spectrum of DNA and the ultraviolet action spectra for mutation; (vi) the nature of the transforming factor; (vii) the role of DNA in bacterial virus infection; (viii) the parallel transfer of genes and DNA in bacterial mating.

The first five of these lines of evidence are the more indirect, and, essentially, they serve merely to present DNA as a likely candidate for a genetic role. The last three are more explicit. In essence they tell us that, in those instances where we observe hereditary factors to enter a cell, the substance DNA enters in a parallel manner—and, indeed, in two instances, only DNA is observed to enter. I should now like to discuss these links in somewhat more detail.

Location

The earliest circumstantial evidence for our thesis was simply that of location. One finds DNA in the structures where one might expect to find genes—50 to 60 percent of the dry mass of a sperm head is DNA (*1*). Within cells, DNA, with a few rare exceptions, is confined to the chromosomes, and within the chromosomes it is localized to bands (*2*), the same bands to which genetic analysis had earlier assigned the hereditary factors.

The principal value of this line of evidence, which is qualitative and indirect, was heuristic. It suggested that it just

Table 1. DNA complement (haploid) of various organisms.

Organism	DNA complement (g)
T2 Bacteriophage	0.0002×10^{-12} per particle
Escherichia coli	0.01×10^{-12} per cell
Sponge	0.06×10^{-12} per cell
Coelenterate	0.3×10^{-12} per cell
Echinoderm	0.9×10^{-12} per cell
Teleosts	$0.5–1.5 \times 10^{-12}$ per cell
Birds	$1–2.0 \times 10^{-12}$ per cell
Turtle	2.5×10^{-12} per cell
Man	2.9×10^{-12} per cell
Rat	3.0×10^{-12} per cell
Cow	3.2×10^{-12} per cell
Frog	7.5×10^{-12} per cell
Dipnoi	50×10^{-12} per cell
Amphiuma	84×10^{-12} per cell
Lillium	53×10^{-12} per cell

might be worth while to know a great deal more about DNA. And historically this evidence played a large role in the rescue of the nucleic acids from some 70 years of neglect.

Specific Diploid Content

It was a logical extension of this morphological implication to inquire whether DNA could satisfy the quantitative requirements of a specifically genetic substance. On morphological grounds, it is believed that all cells of an adult organism have a full diploid complement of chromosomes, with the exception, of course, of the gametes, which have a haploid complement. And careful measurements (*3*) have shown that, if appropriate corrections for polyploidy are made, the DNA content per cell is uniform among all the various types of tissues with the exception of the gametes, which have one-half the normal amount. Again, in polyploid cells the DNA content appears to be discretely 2 or 4 or 8 times the diploid value (*4*). Thus for a particular species, the DNA content accurately parallels the chromosome complement. Indeed, the constancy of DNA content has provided the biochemist with an excellent reference point to use in comparisons of the compositions of various tissues (*5*).

Another type of quantitative correlation that was attempted in this manner did not prove quite so successful. It might have been pleasing if one could have correlated the diploid DNA content with biological complexity. One might have expected that, in the course of evolution, the development of more complex organisms was associated with a greater and more varied DNA content. To some extent this is true, but as Table 1 indicates, the exceptions are such as to make the whole hypothesis at present rather suspect (*6*).

While the constancy of the diploid content of DNA was a pleasing confirmation of our hypothesis, this result raised certain questions. This constancy obviously could not be true of a growing tissue. The DNA content of the cell must double at some stage. This thought has led to several experiments which indicate that the duration of DNA synthesis is relatively brief and that synthesis generally occurs in interphase or in early prophase (*7*). Furthermore, the constancy of over-all DNA content raises anew the question of the physical basis of cellular differentiation. Many people of course believe differentiation to be a matter of cytoplasmic origin rather than of nuclear basis. Frankly, this view affronts my sense of cellular integration. The constancy of DNA content does seem to imply that differentiation is not a matter of the loss of certain genes or of excess replication of others. But it does not indicate that all the genes are functional. And, indeed, there are several lines of evidence which suggest that not all the DNA of mature cells is in the same physical state. Studies by enzymic extraction of nuclei (*8*), by salt extraction of tissue (*9*), by fractionation of isolated DNA (*10*), and studies of methyl-green binding in nuclei (*11*) suggest that the associations between DNA and other cellular constituents are not uniform within a cell and are physiologically variable. I would suggest that investigation of these leads with more refined techniques could prove to be of great value.

Metabolic Stability

The third item of indirect evidence associating DNA with the gene is the matter of the metabolic stability of DNA. This is certainly not a sufficient proof, nor is it really even necessary, but in a way it is satisfying to believe that the physical basis of the gene—the factor that is passed on from cell generation to cell generation—is physically conserved in the process. Despite several apparently misleading reports, it now appears to be generally agreed that DNA is metabolically inert—once an atom is incorporated into a DNA molecule, it remains there for the life of the cell (*12*). The only evidence with which I am familiar which appears contrary to this conclusion is that of Zamenhof on the incorporation of bromouracil into DNA in bacteria in stationary phase (*13*). This may be a special situation.

The metabolic stability of DNA in turn permits one to exclude certain hypotheses concerning its function. However DNA may function, it is clearly not, for instance, a precursor of cytoplasmic ribose nucleic acid (RNA), nor does it seem to form linkages with other substances that would labilize its components. These are negative conclusions, but they help to delimit the problem.

Structure

As we continue our list of associations and properties which suggest that DNA is a highly logical candidate for the gene, we might observe that the very structure of DNA is at least compatible with this notion. Indeed, Hershey (*14*) has said, "If one wished to invent a genetic material, one would almost certainly choose a DNA constituted along the lines proposed by Watson and Crick."

The generally accepted structure for DNA is the two-ply helix first proposed by Watson and Crick (*15*) and shown in Fig. 1. Each chain is a string of nucleotides, of which there are four or five kinds in a given chain. The nucleotides each contain a phosphate residue, a deoxyribose, and one of the purine or pyrimidine bases shown in Figs. 2 and 3. The two chains of the helix are complementary in that specification of one enables one, almost, to specify the other. Ade-

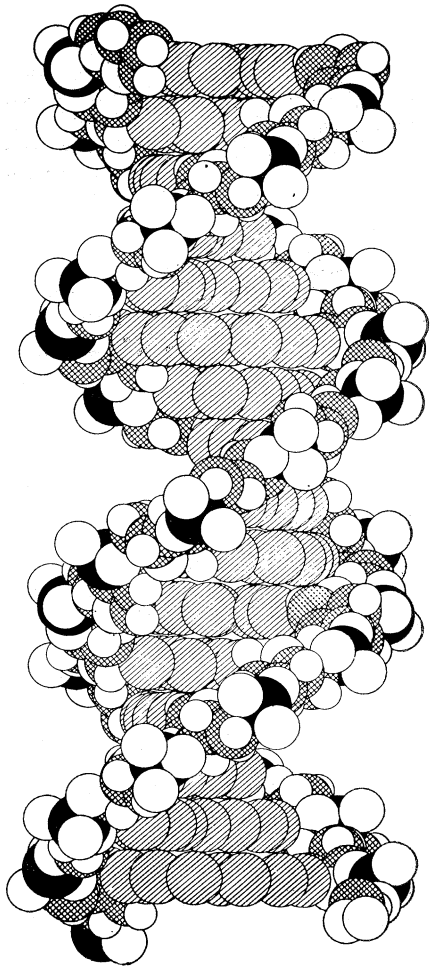

Fig. 1. Molecular model of the two-strand helix of DNA. [Courtesy of L. D. Hamilton]

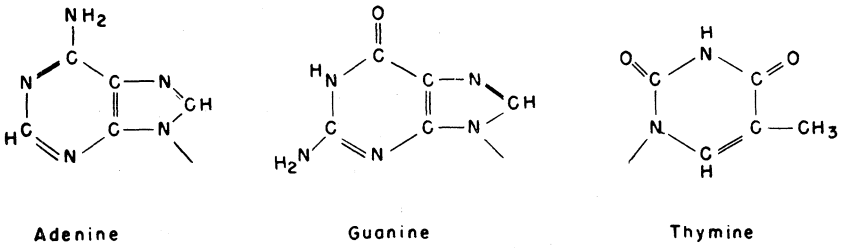

Fig. 2. Three heterocyclic groups found in all DNA preparations.

nine is always opposite thymine and guanine is opposite cytosine (Fig. 4). Then the basic information, which if it is a gene, DNA must carry, is postulated to reside in the sequence of nucleotides along the chain. This information is thus present twice—a nice bit of insurance against the disaster which would ensue upon the loss of a gene. I might interject that there is no experimental evidence at all to support this postulate that the information is carried as a nucleotide sequence—we simply lack any credible alternative hypothesis.

As a further bit of fancy, it has been suggested that when the DNA is to be duplicated, the chains in some manner separate, perhaps only a small segment at a time, and each chain serves as a template upon which to build up its complement, resulting in two chains like the original. Various modes of accomplishing this feat have been proposed which would result in various distributions of the original parental material among the daughters (*16*).

I would like to comment briefly on the Watson-Crick structure and the associated proposals. We may ask, does this structure apply to all DNA and at all times? The evidence that originally led to its formulation was the x-ray diffraction patterns of DNA fibers obtained by Wilkins and by Franklin (*17*). Such data, which arises from only the ordered regions of the fibers, provides information about only the ordered regions. Hence it can logically be said to apply only to that fraction of the fiber which is ordered. Estimates of this fraction range from 5 to 50 percent.

More conclusive evidence for the generality of a two-fibered structure is now available from the physical-chemical studies of Thomas (*18*) and of Schumaker, Richards, and Schachman (*19*). Both groups conclude from studies of the changes in light-scattering or in viscosity during enzymatic degradation of DNA that the great bulk—more than 90 percent—of their preparations must have been in the form of double chains.

In addition, the generally found near molar equality of adenine and thymine and of guanine and cytosine (*20*) is very neatly explained by this structure.

However, all the x-ray and physical studies with which I am familiar have been made on DNA from mature cells, such as thymus or DNA from cells with a migratory mission, sperm or phage. I think it would be premature to suggest that DNA is at all times in the life of the cell in this form. Indeed, there are sufficient reports of DNA fractions (obtained by varied modes of fractionation) which do not have complementary base ratios (*21*), that I think there may be small but important exceptions hidden behind an over-all complementarity.

This matter of complementarity also deserves a closer look. In its simplest form, it would limit the composition of DNA to four kinds of nucleotides. In fact, many, if not most, DNA's studied have five types (*22*). Most of the natural variants involve a perturbation upon the five position of cytosine.

In general, when such variants occur, the molar proportion of cytosine plus the substituted cytosine equals the molar proportion of guanine. However, the modified cytosine does not appear to be randomly introduced in exchange for cytosine. Studies of the dinucleotides obtained by the action of pancreatic deoxyribonuclease on thymus DNA (*23*) indicate a considerable difference in the distribution of cytosine and 5-methylcytosine (Table 2). It is hard to envision how such a distribution can be explained by a replication scheme guided by simple complementarity.

A more conclusive argument against a simple complementarity mechanism of replication arises from the following data. In T2 bacteriophage there is no cytosine—it is replaced by 5-hydroxymethylcytosine (HMC). Furthermore, about 77 percent of this 5-hydroxymethylcytosine is substituted by glucose. Now the DNA of T2 bacteriophage has been shown by Levinthal (*16*) to consist of one large piece, comprising about 40 percent of the DNA and a number of considerably smaller pieces. Brown and Martin have shown (*24*) that it is possible to fractionate DNA from T2 bacteriophage chromatographically into two fractions, and one of these corresponds to Levinthal's large piece. In addition, they have shown (*25*) that the 5-hydroxymethylcytosine lacking glucose is confined to the large piece—that is, only about 60 percent of the large piece is glucose-substituted, while all of the glucose of the small pieces is so substituted. Since these components of T2 DNA are all replicated in the same cell at the same time, the limited glucose substitution of the large piece cannot reasonably be ascribed to some metabolic limitation. Hence it would appear (to put the matter mechanistically) that when the replication of the large piece takes place, the machinery knows when to put in glucose-substituted 5-hydroxy-

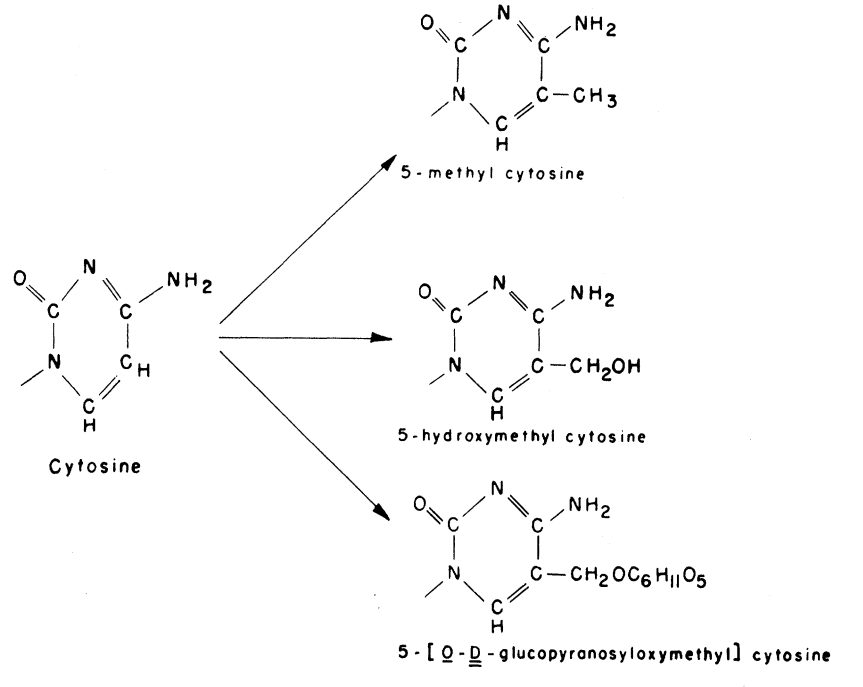

Fig. 3. Cytosine and the various substituted cytosine rings found in some DNA preparations.

methylcytosine or when to insert unsubstituted 5-hydroxymethylcytosine, for the glucose content remains unchanged through many generations.

To me, then, it appears that these perturbations of the nucleotides are significant and that replication of DNA involves a more precise mechanism than of simple complementarity.

Ultraviolet Spectra

The next piece of indirect evidence that I have cited for the association of DNA with the gene is the correlation of the ultraviolet action spectra for production of mutations in various organisms with the general shape of the ultraviolet absorption spectra of nucleic acids (*26*). To go beyond this simple correlation is certainly hazardous. We do not know what, physically, constitutes a mutation. Nor are we as yet very clear about what happens to DNA after the absorption of an ultraviolet photon.

As I indicated in the previous section, we suspect that the information content of a DNA molecule is contained in the sequence of its nucleotides. We know painfully little concerning such sequences. Most physical measurements suggest that DNA preparations as generally obtained consist of double chains varying in molecular weight from a few millions to several tens of millions—that is, of chains varying from a few thousand to tens of thousands of nucleotides. All DNA preparations to date have undoubtedly consisted of a mixture of a great many such molecules. One calf thymus nucleus would contain several hundred thousand of such molecules, presumably of many, many species. Hence any information obtained about sequence from such a preparation could only be of a statistical nature. A limited amount of information is available (*23*), such as is shown in Table 3, which lists the molar proportions of the various dinucleotides isolated from a deoxyribonuclease digest of calf thymus DNA.

It is possible in this way to specify the nucleotide sequence about some 10 percent of the phosphate links. I am confident that it will be possible to increase this fraction to 30 or 40 percent within a few years.

To proceed much further in sequence studies, which we expect will surely be necessary for the further understanding of mutation, we must be able to provide homogeneous preparations of DNA, as the protein chemists can now do with, say, insulin or ribonuclease. There seem to be two approaches open to this problem. One is to attempt to fractionate the components of these mixtures. Considerable effort is being expended upon such fractionation, and some success (*10, 27*) has been obtained (Fig. 5). The actual basis of these empiric fractionations is, however, still not understood.

The other approach would be to seek a biological system in which there were only a few—or ideally, one—type of DNA molecule. The viruses are the obvious choice for such an approach, and among these, the smaller the better. We have recently isolated a bacterial virus with a DNA content of about 2 million per particle—some 3000 nucleotide pairs. We do not as yet know whether the DNA is in one piece, but in any event, this virus should provide the closest approach yet to a homogeneous DNA, and sequence data upon its DNA may begin to acquire more than statistical significance.

If we believe that mutation consists of a change in nucleotide sequence, then we might inquire whether it could be possible to induce specific types of genetic alteration by the introduction of specific kinds of changes in nucleotide composition. Thus one can substitute 5-bromouracil for thymine and, as Litman and Pardee, and Zamenhof *et al.*, have shown (*28*), this is a highly mutagenic device. Whether the mutations induced are actually the product of specific tampering with the thymine residues or

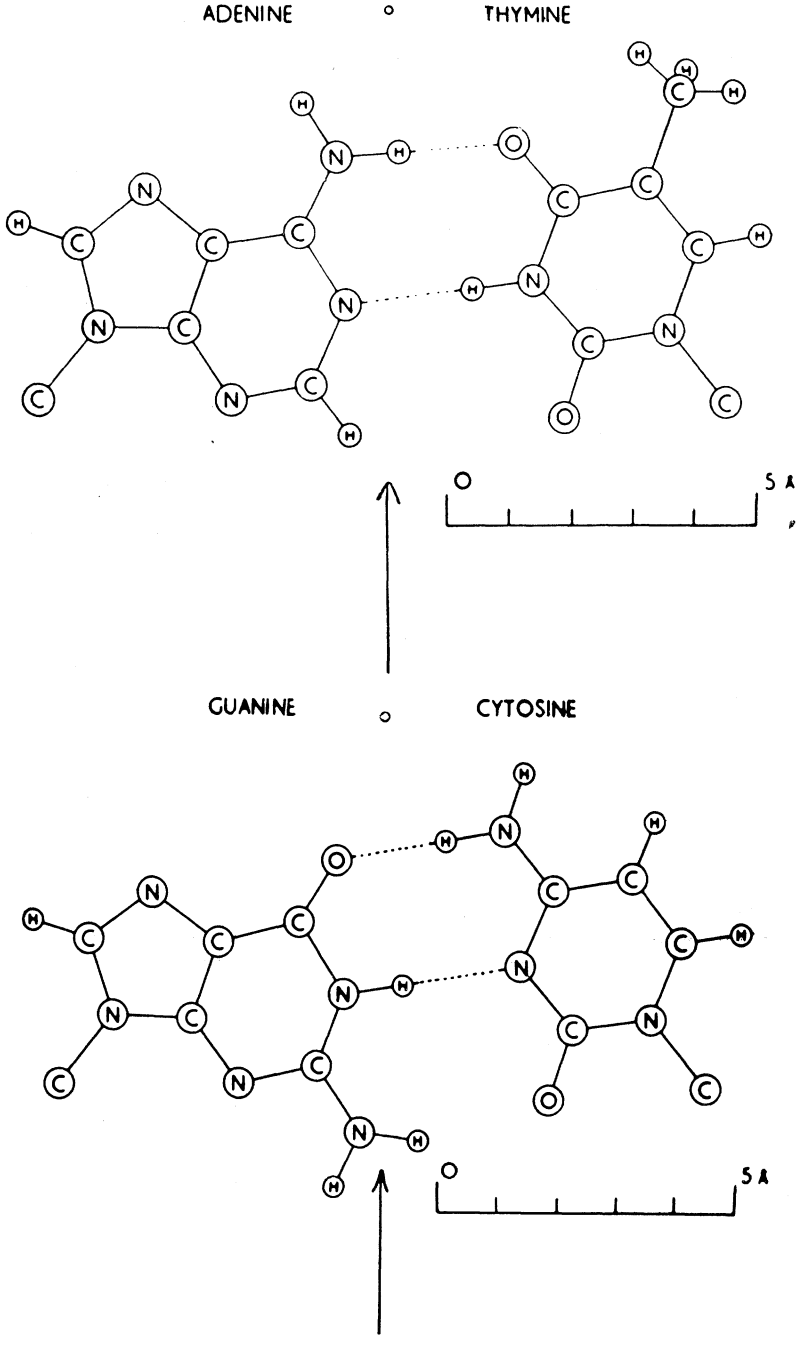

Fig. 4. The complementary base pairs of DNA.

Table 2. Comparison of the distribution of deoxycytidylic acid (C) and 5-methyldeoxycytidylic acid (M) among the dinucleotides obtained from calf thymus DNA by digestion with pancreatic deoxyribonuclease.

C-Dinucleotide	Mole fraction of digest	M-Dinucleotide	Mole fraction of digest
C-p-C-p-	1.11	M-p-M-p-	0
C-p-T-p-	0.78	M-p-T-p-	0
T-p-C-p-	2.34	T-p-M-p-	0
C-p-A-p-	0	M-p-A-p-	0
A-p-C-p-	3.22	A-p-M-p-	0
C-p-G-p-	0.75	M-p-G-p-	0
G-p-C-p-	0.12	G-p-M-p-	1.03

whether they are merely a general result of perhaps upsetting the pace of DNA synthesis remains to be determined.

We have some reason to believe that the effects of ultraviolet radiation will be to some extent localized to the cytosine residues (*29*). Such irradiation then may, under favorable circumstances, provide a specific kind of altered nucleotide sequence. Further understanding of genetic chemistry will almost certainly lead to more specific means of producing genetic modifications.

Transformation

As we now consider, briefly, the more explicit lines of evidence it will be wise to keep in mind that these all are, at present, demonstrable only with microorganisms and so their generality is thus unproved, even if often assumed.

The now classic tale of the transforming factor must be well known. Exposure of cells of strains of certain types of microorganisms to pure DNA from related donor strains which have distinguishing immunological or biochemical characteristics can result in the permanent acquisition by the first strain of the phenotypic character of the donor strain. Furthermore, the DNA of the transformed strain then has the power to pass the acquired characteristic on to still other strains (*30*). Studies involving the simultaneous transfer of two or more characteristics have shown the possibility of linkage which will again persist in the DNA of the transformed strain. This linkage must be thought to reside in the molecules of the DNA preparation (*31*).

That the DNA accomplishes this transformation by actual entry into the transformed cell has been shown by the elegant tracer studies of Goodgal and Herriot (*32*), and indeed they have obtained the pleasing result that on the average, per specific transformation, an amount of DNA equivalent to the DNA complement of one cell must enter the transformed cell.

Physically these DNA preparations with transforming power are no different than those prepared from varied sources. Indeed, the availability of a functional DNA has been of the greatest value, for assay of its biological activity has enabled one to assess the effects of various types of preparatory procedures, storage conditions and so on, on DNA.

When examined more closely, the phenomenon of transformation raises a great many questions, as has been thoughtfully discussed in several papers by Hotchkiss and by Harriet Ephrussi-Taylor (*33*). In solution, a DNA molecule is a greatly extended coil, which pervades a volume some 5000 to 10,000 A in diameter. How does such a molecule get into a bacterial cell, and indeed what is its physical state within the cell? Hotchkiss has shown (*34*) that it is only at certain stages of the mitotic cycle that the cells are ready to receive such molecules, but we do not understand their mode of entry.

And after the foreign DNA enters the cell, what accommodation is made with the indigenous DNA? Most likely some type of recombination process occurs. The occurrence of allogenic transformations which result in cells with new characters, distinct from those of either recipient or donor strains, suggests this very strongly. If transformation is by recombination, then how large a DNA piece must be exchanged between recipient and donor? It is conceivable that a few nucleotides at the right locus might be adequate.

I mention these matters merely to indicate that while the ability of DNA preparations to produce hereditable metabolic modifications is a striking phenomenon, our ignorance of the accompanying intracellular phenomena gravely limits the conclusions to be drawn.

Table 3. Molar proportions of dinucleotides in thymus DNA digest. M, 5-methyldeoxycytidylic acid; C, deoxycytidylic acid; T, thymidylic acid; A, deoxyadenylic acid; G, deoxyguanylic acid. $XY = X$-p-Y-p, where Y refers to the nucleoside carrying the phosphate monoester group in each case.

X/Y	M	C	T	A	G
M	0		0	0	0
C		1.11	0.78	0	0.75
T	0	2.34	1.38	0.10	0.16
A	0	3.22	1.36	0.46	0.97
G	1.03	0.12	2.61	0.20	0.82

Viral Infection

The role of nucleic acid in viral infection is best established for the T2 bacteriophage. Hershey's careful experiments have shown that, after attachment of the virus to the cell membrane, the DNA of the virus particle penetrates the cell wall while at least 97 percent of the protein remains external to the cell and appears to play little if any role in the subsequent development of the phage (*35*). If, as is commonly done, we chose to disregard the ambiguous 3 percent of protein, then we must conclude that the inherited properties of the virus are passed from generation to generation through the nucleic acid. Indeed, many varied pieces of information are most conveniently explained if we simply state that the action of the virus is to intro-

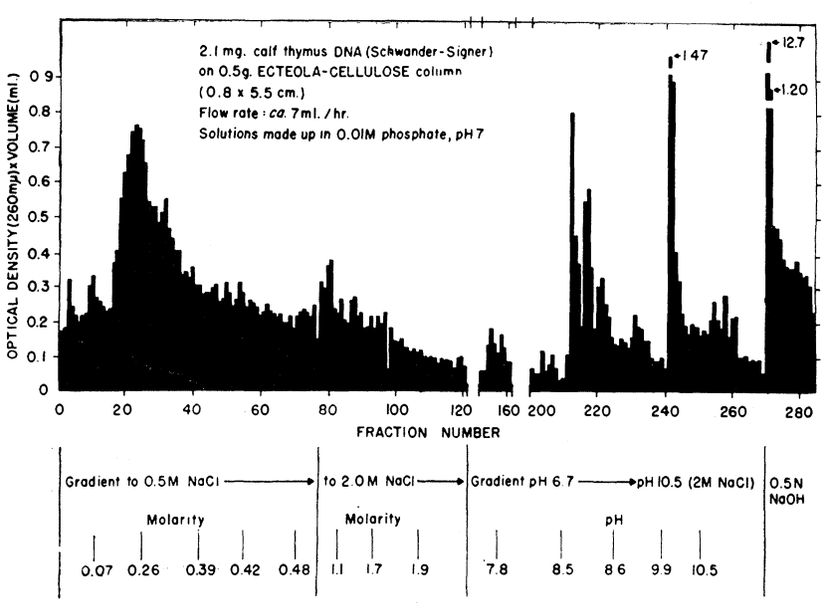

Fig. 5. Fractionation of thymus DNA on a substituted cellulose column (*27*).

duce its own genome, which, anthropomorphically put, destroys the bacterial genome and takes over the cellular control, directing it, of course, specifically to the synthesis of new virus. This concept neatly explains such observations as the ability of phage-infected thymineless mutants to synthesize thymine (*36*), the appearance of the new base, 5-hydroxymethylcytosine, and so forth.

We should note that with some of the other bacterial viruses the situation may be less simple. These viruses may be more dependent on the function of some of the bacterial genes and indeed there exists presumptive evidence for the recombination of the DNA of some viruses with the host DNA (*37*). Doubtless there are many degrees of molecular parasitism.

Bacterial Mating

That bacterial mating is on such a time scale that it can be conveniently interrupted and the transfer of genetic information placed on an accurate time scale has been brilliantly demonstrated by Jacob and Wollman in France and Skaar and Garen in this country (*38*). Thus, by mixing two genetically marked strains of crossable bacteria and by disruption of the union at various times after mixing by the action of a blender, it has been possible to show that gene A enters from the donor cell to the recipient at 12 minutes, gene B at 15 minutes, and so forth. And the order of entry of genes is just that order which has been deduced for the order of genes along the chromosome from mapping experiments. Similar results have been obtained by Skaar and Garen using a virus-susceptible donor, and employing phage to disrupt the mating.

That, as the genes enter, DNA enters, can be shown nicely by use of phosphorus-32 labeled donors, and this has been done. The amount of phosphorus-32 transferred, as shown both physically and biologically, is found to correlate in a general way with the fraction of the genome which has entered the recipient cell.

Conclusion

This concludes the most essential evidence. If now we will concede the point —if we acknowledge that these links between DNA and the hereditary factors are good and sound and enduring, we may face ahead and ask how shall we proceed to create a genetic chemistry—and the answer is, of course, on many fronts. Particularly, I think, we may ask what differentiates the DNA of different alleles. This is a structural question and hopefully will soon be susceptible to attack, and this in turn may lead to the linkage of structure with function. Also we may note that DNA is most certainly not to be found alone in the cell, and we may ask with what is it associated—and how these associations change during the cycle of mitosis, and we may hope that these associations may provide hints to help us discover how the information built into the DNA is passed on in duplication and is passed out to guide the metabolism of the cell.

To those who may have been chafing because I may seem to have equated DNA with the gene, let me hasten to say that I am confident that further experimentation will emphasize the interplay of protein and of ribosenucleic acid with DNA in the expression of the hereditary characters. And for those for whom the term *hereditary factor* itself has become fuzzy, let me hope that the development of a genetic chemistry will help to illuminate both the gene and the problem of its definition.

I believe this hard-won recognition of the role of DNA has brought us into a new era in genetics and in biochemistry. The gene, once a formal abstraction, has begun to condense, to assume form and structure and defined reactivity.

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