THE CHEMISTRY OF THE NUCLEIC ACIDS AND NUCLEOPROTEINS

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This contribution makes no attempt to be a complete compendium of the literature but is designed as a critical survey of the present position in a few main sections of this field. Considerable difficulties and delays have been encountered in obtaining copies of certain journals and any omissions on this score are regretted.

NUCLEOPROTEINS AND NUCLEIC ACIDS

Isolation of nucleoproteins.—Many methods for isolating nucleoproteins involve a stage, either during the extraction or more generally in the precipitation process, which is relatively drastic and may produce an alteration in the chemical and physical properties of the nucleoprotein; thus the majority of preparations involve precipitation of the extracted material with hydrochloric or acetic acids. The extraction processes vary considerably and employ water (1 to 13), dilute alkaline solution (14 to 17), sodium chloride solution (18, 19, 20), or buffer solutions of pH values ranging from 4 to 11 (21 to 26), followed in each case by precipitation with acid. It has been suggested (27) that nucleoproteins prepared thus are of variable composition, the precipitated nucleic acid carrying with it varying quantities of loosely bound protein, and these methods are now considered unsatisfactory (28) in view of the possible rupture of the nucleic acid-protein bond during the acid precipitation. A more controlled extraction of liver nucleoprotein is that in which the tissue was treated with a solution containing $0.03M$ sodium bicarbonate and $0.5M$ potassium chloride (29, 30) and the nucleoprotein precipitated by adjusting the solution to pH 4.2. Even these conditions may, however, be too drastic, and more recently attention has been concentrated on modifications of the original mild methods (1 to 11, 15), coupled with precipitation of the nucleoprotein with saturated ammonium sulphate (12, 31) or calcium chloride (6, 7, 32) solutions. Thus an early method (32) has been modified (33) whereby fresh pulped calf thymus is extracted with water at 5° C. for twenty-four to thirty-six hours, and after clarification of the extract the nucleoprotein is pre-

cipitated either by the addition of an equal volume of 2 per cent sodium chloride solution or of 0.2 per cent calcium chloride solution. A satisfactory, gentle procedure, apparently of fairly general application for the isolation of desoxypentose nucleoproteins (34 to 39), depends on the rather remarkable changes in solubility of the nucleoproteins in sodium chloride solutions of different strengths. They dissolve in 1*M* sodium chloride, forming viscous opalescent solutions, but are insoluble in 0.14*M* sodium chloride, although soluble in 0.02*M* or less or in pure water. No complete explanation of these phenomena has so far been put forward. The minced tissues, after being washed with 0.14*M* sodium chloride solution to remove cytoplasmic material, are extracted with 1*M* sodium chloride (2*M* in certain cases) and after clarification of the extract the nucleoprotein is precipitated in a distinctly fibrous form by dilution with sufficient water to bring the sodium chloride concentration to 0.14*M*. The nucleoprotein may also be precipitated from the concentrated salt solution by the addition of alcohol (13), as has been used for the preparation of nucleoproteins during the isolation of nucleic acids (40, 41); this method is, however, more likely than the former process to bring about denaturation of the protein. Both the desoxypentose nucleoprotein and the pentose nucleic acid have been isolated from the same tissue sample (42) by precipitating the aqueous extract of minced rat liver with 0.4 per cent calcium chloride, and extracting the desoxypentose nucleoprotein from the solid with 10 per cent or 1*M* sodium chloride. The pentose nucleic acid was extracted from the residue with boiling 10 per cent sodium chloride, a process which was not considered to change the composition of the pentose nucleoprotein (43).

The isolation of nucleoproteins from cells possessing a resistant wall has been satisfactorily accomplished (11, 28) by disintegration of the cell by means of intense audible sonic vibrations (44), the nucleoproteins being then extracted from the cellular debris with water and precipitated with 0.1*N* hydrochloric acid (11) or ammonium sulphate (28).

Development of the technique of differential centrifugation has provided an important method whereby the macromolecular nucleoproteins, generally in association with lipoid material, may be isolated from various tissues with a minimum of chemical action. This has permitted the preparation of the active nucleoprotein fraction of the Rous sarcoma I (45) and chicken tumour I (46, 47), and of various fractions from chick embryo (48, 49) and mammalian cells and tissues (50, 51, 52). The method is also used extensively for the isolation of the virus nucleoproteins (53, 54, 55).

Isolation of nucleic acids from nucleoproteins.—Decomposition of a nucleoprotein into its constituent nucleic acid and protein is most easily brought about by alkaline hydrolysis (56), followed by removal of the protein with colloidal iron and precipitation of the nucleic acid in acid solution. An obvious objection, however, is that the alkalinity also causes some degradation of the nucleic acid. This degradation has no doubt often been considerable (57), but in some cases (see under viruses) this is apparently the only method which has so far been available; an alternative successful procedure is hydrolysis of the crude nucleoprotein with pepsin (15, 58, 59). A remarkably simple preparation of the desoxypentose nucleic acids from the nucleoproteins of calf thymus and rat liver is that in which the nucleoprotein solution in water or 1*M* sodium chloride is saturated with sodium chloride, the deposit of protein removed, and the nucleic acid, in a highly polymerised state, precipitated by the addition of alcohol (8, 42, 60, 61).

Complete separation of the protein and nucleic acid of nucleoproteins may be effected by decomposing the nucleoprotein in 0.5 per cent sodium carbonate solution at 50° C. for one to two hours and shaking the solution, neutralised to pH 7, with chloroform containing a small amount of a foam-preventing agent such as amyl alcohol; the protein concentrates at the interface forming a chloroform-protein gel which is easily separated by centrifugation (11). This method has yielded successfully the nucleic acids from the nucleoprotein of *Streptococcus pyogenes* (11) and from a fraction isolated from type III pneumococci (62), and the desoxypentose nucleic acid of thymus (34).

Procedures used in special cases but yet to be tested in wider fields are the heat-denaturation of tobacco mosaic virus nucleoprotein (63), the electrophoresis of tuberculin nucleoprotein (13), and dialysis of thymus nucleoprotein dissolved in 1*M* sodium chloride against 1*M* sodium chloride, which results in diffusion of the protein (34).

Direct isolation of nucleic acid.—It has repeatedly been stated that the methods usually adopted for the direct extraction of nucleic acids are unsatisfactory (64, 65, 66), and in spite of the fact that nucleic acids are labile towards alkali, the majority of the procedures employ sodium hydroxide. By use of this reagent nucleic acids have been obtained, for example, from yeast (58, 65, 66), pancreas (67) and

various animal tissues (68, 69), onion bulbs (70), malignant tissue (71, 72, 73), and bacteria (74 to 77). Extraction with sodium chloride solution yielded nucleic acid from yeast (18) and liver (41); in the latter case a nucleoprotein was probably isolated and subsequently converted into the nucleic acid during purification (see below).

In general, it would seem to be desirable to prepare nucleic acids by isolation and subsequent decomposition of the nucleoprotein.

Separation of pentose and desoxypentose nucleic acids.—It was early realised (78) that a tissue could yield both pentose and desoxypentose nucleic acids depending on the method of isolation, but the significance of the nature of the nucleic acid in this connection seems to have been lost sight of in subsequent work. The apparently contradictory statements that sodium chloride solution extracts a pentose nucleic acid from liver (41) and a desoxypentose nucleoprotein from thymus gland (34) emphasise this point. The fresh minced thymus glands were first washed with 0.14*M* sodium chloride solution and the desoxypentose nucleoprotein then extracted with a one molar solution. The dried liver powder on the other hand, was extracted directly with 10 per cent sodium chloride, and the "crude nucleic acid" purified first by precipitation with barium acetate and then with glacial acetic acid; both procedures have previously been stated to precipitate pentose nucleic acids, but not desoxypentose nucleic acids, and also to remove any protein impurity (40). For the isolation of either a pentose nucleic acid or a desoxypentose nucleic acid, it would therefore seem that two procedures are possible, (*a*) fractionation of the nucleic acids using barium acetate and glacial acetic acid as the precipitant, or (*b*) fractionation of the nucleoproteins by means of sodium chloride. The first method has been described in the case of the nucleic acids of pancreas, the pentose nucleic acid being precipitated with acetic acid and the desoxypentose nucleic acid with alcohol (79); the second method, as has been mentioned, was used for the nucleic acids of rat liver (42).

Properties of Nucleoproteins

The nucleoprotein isolated by mild methods (33, 34) from calf thymus has a molecular weight of the order of 2×10^6 (80) and its solutions in 1*M* sodium chloride show the high viscosity and marked streaming birefringence generally associated with highly asymmetric macromolecules. Once the nucleoprotein has been dissolved in water or in 0.02*M* sodium chloride solution, changes occur which result in the nucleoprotein being less fibrous when reprecipitated in 0.14*M* salt (34), and its solutions in 1*M* sodium chloride are less viscous and show less streaming birefringence than those of the original nucleoprotein. These changes are apparently permanent and cannot be attributed to fractionation of the nucleoprotein. Viscosity measurements (33), however, are only in partial agreement with these observations; nucleoprotein extracted with water from calf thymus showed a much lower viscosity in dilute buffer (0.005*M* potassium acid phosphate plus 0.005*M* dibasic sodium phosphate) than in the same buffer containing 5 per cent sodium chloride, which observation would suggest that the viscosity change is reversible. A more detailed comparison of the two apparently conflicting observations cannot be made owing to the lack of experimental data recorded (34).

Nature of the linkage between nucleic acid and protein.—Until comparatively recently it was not infrequently assumed that the bonds between nucleic acid and protein are invariably electrovalent. This form of linkage may have been produced artificially by the relatively drastic methods of preparation of the materials examined. That supposition is supported by the results of a direct comparison of the nucleoproteins of streptococci (28), the nucleoproteins having been precipitated from the aqueous extracts by acid on the one hand and by ammonium sulphate on the other; in the latter case it is to be emphasized that the nucleoprotein had not come into contact with either acid or alkali at any stage. The examination of the nucleoproteins, which contained a pentose nucleic acid (11), was made by four methods, (*a*) deproteinisation by shaking with chloroform, (*b*) fractionation by ammonium sulphate, (*c*) precipitation with neutral calcium chloride solution, and (*d*) measurement of electrophoretic mobility. The natural nucleoproteins, unlike their acid-precipitated congeners, were completely soluble on the acid side of their isoelectric point. The most interesting results were observed in the precipitation by neutral calcium chloride; both the acid-treated nucleoprotein and an artificial protein nucleate included in the investigation formed an immediate precipitate, whereas no precipitation occurred with the natural nucleoprotein. These data imply either that the phosphoric acid groups of the natural nucleoprotein are bonded in such a manner as not to be available for reaction with calcium ions, or alternatively that the calcium salt of the natural nucleoprotein is soluble. Whatever the true explanation, it is clear that acid precipitation of the pentose nucleoproteins of streptococci does change their properties.

The nucleoproteins of cell nuclei (34), when extracted by mild methods, give evidence that the bond between the desoxypentose nucleic acid and the protein is electrovalent. Thus dialysis of a solution of the nucleoprotein of trout sperm in 1*M* sodium chloride against 1*M* salt solution resulted in the diffusion of the protein through the membrane, leaving the highly polymerised desoxypentose nucleic acid behind. Furthermore, extraction of the nucleoprotein solution with a chloroform-octyl alcohol mixture caused accumulation of the protein at the interface whilst the nucleic acid remained in solution (34). The desoxypentose nucleic acid and protein components of the nucleoprotein of tuberculin were separated by electrophoresis (13, 81) suggesting the presence of an electrovalent bond, but it should be pointed out that an acid precipitation was employed in the preparation. There are indications that the bonds in the pentose nucleoprotein of haemolytic streptococci are stronger than a dissociable salt linkage (25). The generalisation has been put forward (82) that the bonds between pentose nucleic acid and protein are covalent whereas those between desoxypentose nucleic acid and protein are electrovalent, but the evidence so far obtained with carefully prepared nucleoproteins is as yet insufficient to warrant this generalisation, which is certainly not in agreement with the data obtained from the virus nucleoproteins (see below).

The problem of the nucleic acid-protein bonds in nucleoproteins is complicated by experiments which suggest that there is a difference in properties when the cell nuclei of normal rat liver are isolated at pH 6.0 to 6.2 and at pH 3.8 to 4.0 (39). In the former case the nucleoprotein was easily extracted with 5 per cent sodium chloride solution, whereas in the latter little or no extraction occurred. Denaturation of the protein, which might conceivably have caused insolubility of the nucleoprotein in the sodium chloride solution, was not responsible for the firmly bound state, because, as was pointed out, this is found also in the nuclei of chicken erythrocytes prepared at pH 6.8 to 7.0, a treatment considered unlikely to cause denaturation. Further, denaturation of the protein is generally regarded as liberating the firmly bound nucleic acid from the protein, as for example in tobacco mosaic virus (63).

Electrophoretic and other studies.—The interaction of nucleic acids with various proteins to form complexes or protein nucleates has been known for some time (see for example 83, 84), but it is only recently that these complexes have been studied systematically

and their properties compared with those of nucleoproteins isolated from tissues. The method of investigation generally employed is that of determining the electrophoretic mobility, and it has been observed that some interaction occurs between thymus desoxypentose nucleic acid and seralbumen (85), although it could not be decided from the data obtained whether several firmly bound nucleoprotein compounds or only loose complexes were formed; the latter appeared more probable since it was observed that the electrolytic environment played an important part in determining the electrophoretic behaviour. Electrophoresis of mixtures of ovalbumen and yeast ribonucleic acid (86) gave similar results, clear evidence of complex formation being obtained in the isoelectric region of the ovalbumen, although in more alkaline solutions the two constituents migrated independently. Addition of the nucleic acid to ovalbumen in solutions more acid than the isoelectric point brought about partial precipitation of a complex.

It has not always been appreciated that resemblance of electrophoretic behaviour does not necessarily imply complete chemical similarity. This point is borne out by the electrophoretic comparison at pH 7 of (*a*) a nucleoprotein solution which is not precipitated by the addition of calcium chloride, and (*b*) a solution of the same nucleoprotein which had been treated with acid and could be precipitated by calcium chloride (28). The mobilities of the two specimens were very similar, that of the natural nucleoprotein being only slightly higher than that of the acid-treated sample, but no definite component split off during the electrophoresis of the latter material. It was concluded that both specimens were "nucleoprotein," but stress was laid on the excellence of the calcium chloride precipitation test for "showing changes that take place in nucleoproteins." Liver nucleoprotein has also been observed to migrate as a single component, although in this case the bond between nucleic acid and protein is such as to permit both components to be separated by dialysis (34). In contradistinction to these observations, the nucleoprotein of tuberculin (81) splits off a highly mobile component, largely nucleic acid, in solutions more alkaline than pH 5, although at pH 5 and pH 2.2 the nucleoprotein fraction traveled as a single component. This was held to show that in these solutions either the electrical properties of the nucleic acid and protein components were identical below pH 5, or a dissociation occurred at pH 5, releasing the nucleic acid. The latter explanation was preferred (81) and it was suggested that an association could occur between the imino group of histidine in the protein and a sec-

ondary phosphoryl group of nucleic acid, both of which dissociate in the region of pH 5 to 6. It was concluded (87), largely on the basis of an electrophoretic examination of the nucleoprotein of calf thymus between pH 3 and 9, that the nucleoproteins are definite compounds with a constant ratio of nucleic acid to protein, but the preceding observations suggest to the writers that this conclusion is not entirely justified. The electrophoretic evidence would only point to such a conclusion if the natural nucleoprotein and the artificial protein nucleate, prepared by mixing the unaltered nucleic acid and protein of the original nucleoprotein, were found to behave differently when examined electrophoretically, the latter splitting into two fractions at a certain pH value.

Addition of various albumens to solutions of sodium desoxypentose nucleate of thymus reduced the viscosity of the latter to values between that of the original nucleic acid solution and that of the protein (88), and at the same time diminished the streaming birefringence of the sodium nucleate solutions. The ability of the proteins to lower viscosity was dependent on their being in the native state since heat-denatured proteins were considerably less effective. These phenomena were at first attributed (88) to a polymerisation of the sodium nucleate although alternative explanations (89), such as complex formation between the protein and the nucleic acid, are feasible, and subsequently (90) it was considered that aggregation of the nucleate particles, leading to a more globular form, would account for the loss of streaming birefringence and structural viscosity.

Examination of solutions containing horse serum albumen and sodium thymus desoxypentose nucleate (90) showed that the osmotic pressures of the mixtures were in nearly every case almost identical with the value for the protein component, a result which was interpreted as suggesting that the presence of the protein causes aggregation of the nucleate ions and adsorption of the sodium ions onto the resulting micelle, thus rendering them osmotically inactive. Some preliminary experiments have been recorded on the adsorption of nucleate ions onto a protein monolayer (34) but no details were given.

Virus nucleoproteins.—The isolation and properties of virus nucleoproteins have been described elsewhere (53, 54, 55, 91, 92, 93) and this review is confined to the nature of the bonds between nucleic acid and protein, and to the distribution of the nucleic acid in the virus. All viruses so far isolated contain pentose nucleic acids, except those of psittacosis (94), vaccinia (95), rabbit papilloma (96), influenza A (PR8 strain) (97, 98), influenza B (Lee strain) (98, 99), and swine influenza (98, 100) which contain nucleic acids of the desoxypentose type. The nature of the bond between nucleic acid and protein is not known but seems to be relatively strong, except in the case of tobacco ring spot virus, of which part of the nucleic acid at least appeared to be less firmly bound (54), and of equine encephalomyelitis virus (96, 101), in which the linkage was reported to be weaker than in rabbit papilloma virus. The conditions necessary for decomposition of the viruses are, however, not the same. Thus, for example, only 62 per cent of the nucleic acid was liberated from bushy stunt virus (102) by treatment with 5 per cent sodium hydroxide for two hours at 4° C., whereas 68 to 78 per cent of the nucleic acid was liberated from tobacco mosaic virus when the 5 per cent sodium hydroxide was neutralised immediately after it had been added to the virus (103); the yield of this nucleic acid increased to 90 per cent if the virus was in contact with the alkali for two hours. Both the rabbit papilloma (96) and vaccinia (95) viruses were only decomposed by heating with 5 per cent sodium hydroxide for thirty minutes. An alternative procedure for decomposing tobacco mosaic virus is by heat-denaturation (63); this method is of importance as the nucleic acid isolated is very probably in, or almost in, its native condition.

The proteins of two strains of tobacco mosaic virus were electrophoretically homogeneous and identical (104), and a mixture of the nucleic acid–free proteins formed a single boundary although a mixture of the intact viruses gave a double boundary. From this it was concluded that both virus proteins were the same and that the difference in the two strains was due to the nucleic acid, as had been suggested previously (105) on the basis of the nucleic acid contents of the two strains. This interpretation, however, is faulty, since it has been shown that two different proteins can give a single boundary in an electrophoresis apparatus (106); furthermore, amino acid analysis showed that the difference in two strains of tobacco mosaic virus involves differences in the protein (107). This, however, does not exclude the possibility that differences may also occur in the nucleic acids. In agreement with this conclusion, the electrophoretic mobilities of these mutants of tobacco mosaic virus were not paralleled by the nucleic acid contents as determined by ultraviolet absorption spectra (108).

X-ray analysis (109, 110, 111) of tobacco mosaic virus did not indicate a concentration of the comparatively dense nucleic acid in any

particular part of the nucleoprotein, and it was suggested (104) that the virus may conceivably consist of either a long protein chain with nucleic acid side groups, or a regular arrangement of alternate nucleic acid and protein residues. The latter suggestion was preferred (104) on the basis of the improbably large molecular weight of the protein in the former scheme. In contrast with this conclusion, comparison of the molecular size of the tobacco mosaic virus protein and the nucleic acid obtained from it (63) led to the view that the nucleic acid exists as a thread-like macromolecule, of which the length is that of the intact virus. From measurements of absorption of polarised ultraviolet light by tobacco mosaic virus particles, oriented by streaming through a quartz capillary, it was considered probable that the pentose nucleic acid is arranged in an ordered manner and that the planes of the purine and probably pyrimidine rings are parallel to one another and perpendicular to the long axis of the molecule (112).

Structure of Nucleic Acids and the Tetranucleotide Hypothesis

Originally the term "tetranucleotide" indicated the occurrence of the four appropriate nucleotides or the corresponding nitrogenous derivatives in equimolecular proportions in the decomposition products of a nucleic acid, and was thus used to describe certain of the then known nucleic acids at a period when these were regarded as having a molecule so simply composed. Now that the complex nature of nucleic acids as polynucleotides is established, the term is still applicable in this sense to such polynucleotides as conform to the tetranucleotide ratio in their nucleotide contents.

Later, as a development arising from the recognition of this complex character, the name has been used to denote a unit, consisting of one molecule of each of the four nucleotides, which by recurrent combination with itself forms the polynucleotide; it seems to be implied that the mode of this union is uniform throughout the polynucleotide. Finally, it has been postulated that in each of these units the four nucleotides are always combined in a fixed manner in an unchangeable sequence. As a logical outcome of these later hypotheses there has arisen the conception of certain polynucleotides as "polytetranucleotides."

The term "tetranucleotide" has thus a graded series of implications ranging from a statistical expression of analytical results to a definition of an exact chemical structure, and in order to avoid confusion the sense in which the name is being used must be defined; the terms "statistical" and "structural" tetranucleotide are suggested for this purpose. It is opportune to review and assess the evidence on which these interpretations are based, all the more so because the importance of polynucleotides in the biological fields makes it essential that the possibilities of resemblance or divergence between polynucleotides from different sources should be clearly recognised so that conjectures involving nucleic acids may be based only on accepted chemical knowledge. The question of the existence of structural tetranucleotides has been discussed briefly (89, 113).

Present information allows polynucleotides to be classified in two main groups depending on the nature of the component sugars as pentose or desoxypentose. The existence of hybrid polynucleotides containing both types of sugar has been postulated (114), but it has been pointed out that in the absence of experimental evidence the suggestion is at present purely speculative (115). The literature also records the isolation of materials which on decomposition yielded the fragments of both types of acid, but it is probably correct to regard these as mixtures, in so far as their nucleic acid components are concerned, until the reverse has been proved. Where examination has been adequate, polynucleotides are characterised up-to-date by the presence of the nitrogenous radicals guanine, adenine, and cytosine, together with uracil in the pentose polynucleotides and thymine in the desoxypentose polynucleotides. Tuberculinic acid A (74, 116, 117, 118) seems so far to be unique in containing 5-methylcytosine (65, 119), but the presence there of this pyrimidine does suggest the need for close identification of pyrimidine components, perhaps more thorough than hitherto.

Origins of the tetranucleotide hypothesis and the newer conception of molecular sizes.—Although Miescher formed a picture of "nuclein" as a multi-basic, phosphorus-containing acid of molecular weight so large as to prevent dialysis, those properties were seldom stressed during the earlier part of the present century (60, 120, 121), but instead the development of the subject followed increasingly problems of detailed structure. This concentration of effort led to the wide assumption, chiefly from five causes (89), that the molecules of both types of nucleic acid, typified by the desoxypentose acid of thymus and the pentose acid of yeast, consisted of simple tetranucleotides. Recent data have shown that nucleic acids are considerably larger than tetranucleotides (for summaries and references, see 89, 122); the sizes

vary with the type and source of the acid and with the method of isolation and purification, but the general assumption of polynucleotide character for all nucleic acids seems to be justified. Nevertheless, the extent to which information obtained on isolated samples of polynucleotides can be applied to consideration of them in their native states is more obscure. Some methods employed in isolation may bring about considerable alteration in properties (59, 123), and although the suggestion (124) is probably correct that polynucleotides of lower size are formed by transverse breakage of larger molecules, the doubt remains that alterations of the polynucleotide may also be caused by processes of de- and repolymerisation, analogous to those observed in the case of thymus desoxypentose nucleic acid (61, 88, 125). Thus, measurements (126) of the effect of pH changes on the viscosities of solutions of a sodium desoxypentose nucleate of high molecular weight from thymus suggested that in neutral solution this salt acts as a linear polymer which is rapidly depolymerised to a considerable extent as the pH is altered from neutrality. The effect was complete at pH 2.6 and 11.6, and was reversed when these solutions were neutralised, the nucleic acid material then repolymerising slowly. Sedimentation velocity and diffusion measurements showed that both the degraded and the repolymerised polynucleotides have wider distributions of molecular weights than the original substance and that some of the molecules in the repolymerised acid are much larger than those present in the original solution. The sensitivity of these polynucleotide molecules to their concentration in solution, to the presence of added salts, and to changes of pH emphasises the difficulties inherent in studies of the structures of the acids in their natural condition.

The recognition of the complex character of nucleic acids undoubtedly opened afresh the whole question of their structure, but it is surprising to observe how readily there has occurred a mental superposition of the newer knowledge of molecular sizes onto the older ideas of the simple tetranucleotides, bringing with it the concept of the polytetranucleotide. Had the sizes of nucleic acid molecules been realised at an earlier date, it is doubtful whether the hypothesis of the structural tetranucleotide would have gained such a firm hold as is apparently the case. The relevant data are discussed below.

Desoxypentose polynucleotides.—Examination (124, 127) of the molecular sizes of samples of the polynucleotides from thymus prepared by different methods showed that treatment with alkali or enzymic preparations, or the action of heat, causes diminution as compared with that of material obtained by a milder procedure (60), in which only neutral solutions and temperatures near 0° C. were used.

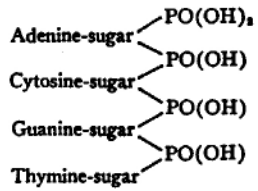
Enzymic degradation appears to proceed in two stages, possibly by two distinct enzymes, although the demarcation is indistinct and it is not clear whether the preparations causing the more deep-seated fission are not merely the more active. The α-nucleic acid of thymus, the large polynucleotide of which the soluble salts show gel-forming properties, is converted by the nucleogelase of commercial pancreatin into the β-acid which no longer forms gels but is still precipitable by acids from solutions of its salts (120, 121). On the other hand, laboratory-made extracts of fresh or dried pancreas contain an enzyme, thymopolynucleotidase, which causes a more fundamental hydrolysis of the α-acid, either previously isolated or while still present in the minced thymus gland (128, 129, 130); presumably it attacks the β-acid similarly. An enzyme causing similar effects has been recorded as present in a variety of animal and plant tissues and named thymonucleodepolymerase (131, 132).

The results of thymopolynucleotidase action were a fall in viscosity and rise in conductivity of the solution and the liberation of one acid equivalent (titrated to pH 9) for each four atoms of phosphorus present; the products were oligonucleotides corresponding to 3.9 nucleotides in size (129, 130). Oligonucleotides are amorphous powders and differ from the α- and β-acids in being soluble in hydrochloric acid and in having sodium and magnesium salts which dissolve in their own weight of water to form solutions which are viscous but do not gel. They differ from nucleotides in being precipitated, as are the higher polynucleotides, by molybdate in acid solution. Estimations of guanine and adenine indicated that both purines were present in the proportions of one molecule for each four atoms of phosphorus.

Treatment of the α-acid, free or in the thymus, with hot alkali produced a similar series of changes and resulted in mixtures of oligonucleotides and material resembling the β-acid; the size of the latter when prepared in this way was 8.5 to 10.5 (from the gland) or 18 to 19 (from the α-acid) nucleotides, whereas the former corresponded to 3.3 to 3.6 and 3.2 to 4.0 nucleotides respectively. The materials prepared directly from the gland still contained at least 5 to 6 per cent of pentose-containing substances. Drastic alkaline extraction and purification of the desoxypentose nucleic acid of beef spleen (133) yielded material considered to be closely similar to the oligonucleotides. This product and the corresponding deaminated acid and thymic acid were

water-soluble penta-basic acids with approximately the correct analytical compositions and weights for molecules composed of four nucleotides (133, 134).

It has been postulated (113, 129, 133) that the oligonucleotides (I) are true "structural tetranucleotides" from which the polynucleotides are formed by polymerisation and that the tetranucleotide has the structure given to it by Levene (135). Definite positions were assigned (113) to the nucleotides of adenine and thymine, the relative orientation of the others being undetermined, on the basis of the following experiments. A mixture of mononucleotidase and oligonucleotidase (diesterase) from intestinal mucosa rapidly split off 25 per cent of the total phosphorus from the oligonucleotides. Further dephosphorylation occurred, and when this had reached 50 per cent the phosphorus-free fraction contained only one molecular proportion of nucleoside, consisting of a mixture of about equal parts of two nucleosides, those of adenine and thymine. Neither the guanine nucleoside nor mononucleotides were present in the solution.



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If such postulates could be substantiated, an important advance would have been made. Whatever may come to light in the future, there is at present in the writers' opinion no evidence to justify the recognition of the existence of the "structural tetranucleotide," as will be seen from the following discussion.

First, only 65 to 70 per cent of the organically combined phosphorus was actually isolated as oligonucleotides in the alkaline or enzymic preparation, and there was thus ample possibility of the formation of other products. Second, the nature of the linkage attacked by thymopolynucleotidase is unknown; the enzyme is highly specific and does not attack yeast ribonucleic acid or a variety of representative potential substrates (128, 132). Third, the nature of the linkages attacked by alkali is unknown, and it is not clear whether these are the same as those split by the enzyme and thus whether the oligonucleotides

obtained by the two methods are the same or different. Fourth, all measurements made on the oligonucleotides, as on all polynucleotide material, give average results only. In a mixture of oligonucleotides, therefore, each molecule need not have the same composition or weight as its neighbours; it is stated (113) that fractional dialysis has so far failed to effect separation into fractions of lower and higher molecular weight, but it is doubtful whether the sensitivity of such a method would permit the recognition of small differences. A massing of any particular nucleotide in the polynucleotide chain is therefore not excluded. Fifth, even if the oligonucleotides were tetranucleotides, there is no reason why their nucleotides should be arranged uniformly in each molecule, and in the writers' opinion the experimental results could be explained in other ways which leave open the question of a regular sequence. There does not seem to be evidence that the adenine and thymine nucleosides both arise from the same oligonucleotide; the same statistical result would have ensued had each nucleoside been removed in its entirety from two different molecules. Furthermore, consideration and experience of enzyme experiments in this field suggest that their use in the interpretation of complex structures demands full information as to the constitutions of all the products and the specificity of the enzymes. In a fission of (I) to give the observed result the oligonucleotidase of intestinal mucosa must attack at approximately equal rates a 3- and a 5-phosphoester linkage. Knowledge whether the enzyme specificity discriminates between these is necessary for the interpretation of the results, but is unknown, since the positions of the phosphoryl groups in the desoxypentose nucleotides have not yet been determined; these were prepared by the action of the enzymes of intestinal mucosa on thymus desoxypentose nucleic acid (136, 137, 138). Sixth, Levene's structure (135) for a tetranucleotide cannot be accepted with certainty on the basis of the existing data. The proposal of (I) as the structure of desoxypentose nucleic acid (135, 139) was partly dependent on a supposed parallelism between that acid and yeast ribonucleic acid as regards the general form of their internucleotide linkages. The structure formerly proposed (140) for yeast ribonucleic acid has now been shown (141) to be at variance with the experimental facts in this respect (see below), and without drawing unwarranted parallels between these two acids it may eventuate that ideas on the internucleotide linkages of desoxypentose nucleic acids may also need revision.

On a wider issue the question arises whether desoxypentose nu-

cleic acids from different sources are one polynucleotide, uniform throughout nature, or whether there are a number of chemically distinct individuals. It seems frequently to be either assumed or implied that the former is the case (see however 142). The writers are not aware of facts which favour either alternative, but attention is drawn to the considerable possibilities of variation in structure of polynucleotides which could arise from divergences of molecular size, relative orientation and proportions of nucleotides, and nature of the sugar components.

Several instances are recorded (e.g., 68, 143) in which hydrolysis of nucleic acids from different sources yielded the nitrogenous bases in amounts differing considerably from those expected on the basis of the tetranucleotide ratio. Such results, however, need to be confirmed or refuted through improved technique, or other methods, and it may be that a beginning has been made (82) in the use of the diphenylamine colour reaction (144, 145) to determine the ratio of total purine to total pyrimidine nucleotides. The method does not distinguish between individual purine or pyrimidine nucleotides. The interpretation of even this simple estimation may be complicated, since it is stated on the one hand to be positive specifically for the sugar of the purine nucleotides (82, 146) and on the other to be given by all the sugar of a desoxypentose nucleic acid (147, 148); these divergences may depend on the exact conditions since the glycosidic linkages vary in stability. According to a recent report (82) all the desoxypentose nucleic acids so far examined, but from sources as yet unnamed, agree in having equimolecular proportions of total purine and total pyrimidine nucleotides.

It seems to be widely accepted that the sugar (thyminose) is *d*-2-desoxyribofuranose in all nucleotides. This may be the case, but it should be realised that the assumption of the furanose structure is based solely on the demonstration of its presence in the thymine nucleoside (149, 150, 151), and that the identification of the sugar itself applies only to the guanine nucleoside of the acid from thymus (152, 153, 154); the sugars of the other nucleosides (135, 155) and of nucleic acids from other sources do not seem to have been prepared. A detailed study of the identification and estimation of the sugars in several nucleic acids (147) by the Dische carbazole colour reaction assumes that the sugar is all desoxyribose, although authentic samples of desoxyribose and its guanine nucleoside gave inferior colours. Not infrequently the classification of a nucleic acid as "desoxyribose" nucleic acid rests merely on a positive result in the Feulgen (156), Dische diphenylamine (81, 144), Thomas (157), or Kiliani (158) colour reactions; these are commonly regarded as specific for thyminose, whereas in reality they demonstrate the presence of a desoxy-sugar, and according to the experimental conditions (see above) may refer only to the purine nucleotides.

Yeast ribonucleic acid.—This is the only pentose nucleic acid which has been considered seriously from the standpoint of a "structural tetranucleotide." The constitutions (159) and syntheses (89) of the component nucleotides have been reviewed; and since purines and pyrimidines are present in approximately the tetranucleotide ratio (160 to 165), the acid is a "statistical tetranucleotide." The original conclusion (56) that the sugar radicals are *d*(—)-ribose has been confirmed (166, 167, 168), at any rate as regards some, by identification as the ribonic phenylhydrazide and benziminazole derivative. Small quantities of *l*-lyxose benziminazole were also isolated, together with some *d*-arabobenziminazole; the formation of the latter has been observed as the result of epimerisation during the alkaline oxidation of ribose (169), but this may not explain its presence in products formed from the nucleic acid (168), and the possible presence of sugars other than ribose in nucleic acids should remain open for the present. In this connection it may be noted that the colours developed by ribose, arabinose, and lyxose in the Dische carbazole reaction for pentoses were so similar in intensity that these sugars could not be distinguished (147). Not infrequently a nucleic acid is classified as a "ribose" nucleic acid (e.g., 170, 171) solely by a negative colour reaction for desoxypentose and a positive result in one of the methods for detection or estimation of pentoses, which depend on the formation of furfural. Theories of the biogenesis of ribose have been reviewed (89).

As with the desoxypentose nucleic acids, alkaline and enzymic fission reduce the molecular size of yeast ribopolynucleotide without dephosphorylation. Milder conditions of hydrolysis than those causing complete fission into nucleotides converted an already partially degraded acid into a product considered to be a structural tetranucleotide (134). This and the corresponding deaminated material were water-soluble, approximately penta-basic acids of the correct analytical compositions and molecular weights of 1177 and 1121 (calculated 1304 and 1307). It has been pointed out (141) that these materials cannot have been true tetranucleotides; the deaminated tetranucleotide should have been hexa-basic since the hydroxyl of xanthine titrates

over the range pH 6 to 8 and its presence was demonstrated in deaminated yeast ribonucleic acid by electrometric titration. Moreover the low molecular size of the product was not confirmed (172), and it was concluded (141) that these fission products were really mixtures of small polynucleotides and that there is no evidence on these grounds for the existence of a "structural tetranucleotide."

At one time it seemed possible that a study of the enzyme ribonuclease (ribonucleinase, ribonucleodepolymerase) (see 122, 173) might provide evidence of a "structural tetranucleotide." Further data, however, show that the enzyme causes deeper fission; the average size of the dialysable products of the action of preparations of heat-stable pancreatic enzymes corresponded to that of a dinucleotide (128), and nucleotides were isolated from the products of digestion by crystalline ribonuclease (174). Nevertheless, the exact effect of heat-stable pancreatic enzymes is not clear; enzymes isolated from whole pancreas provide small and variable yields of the four nucleotides, whereas the claims that depolymerisation took place without formation of appreciable quantities of nucleotides arose from experiments with enzyme preparations made from commercial pancreatin. The possible parallel with somewhat analogous observations in the fission of thymus desoxypentose polynucleotide with pancreatic enzymes is worthy of note.

The results, on which exact positions of certain nucleotides relative to each other in a structural tetranucleotide were defined from enzyme experiments (175) or aqueous pyridine hydrolyses (176), seem to the writers to indicate merely that certain linkages in the polynucleotide are more labile than others. It is thus clear that there is at present no evidence for the "structural tetranucleotide" as a unit of yeast ribonucleic acid.

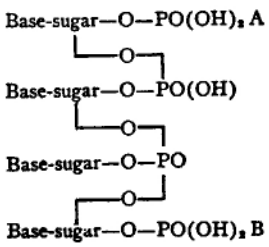
It is generally agreed that the action of the heat-stable pancreas enzymes renders about half of the phosphorus of the acid non-precipitable by the uranyl-trichloracetic acid reagent (177) although still organically combined; the remainder resembles the original acid in being precipitated by the reagent and also, although less readily (178), by hydrochloric acid. It should not be automatically inferred that the precipitable material is unchanged acid. It has been pointed out (122) that yeast ribonucleic acid must contain at least two different types of linkage, one labile, the other resistant, to the action of ribonuclease, and several alternatives are possible, in that the liberated nucleotides may not come from every nucleic acid molecule, but may result from

transverse fission of the polynucleotide chain, or may be lopped from a main polynucleotide trunk bearing nucleotides as branches (141) (see below). In addition, hints are not lacking that all samples of the acid are not identical in structure; lability to alkaline hydrolysis is a variable property (179, 180), ribonuclease activity alters considerably with the sample of nucleic acid and is also sensitive to the age of the substrate solution (181), and only certain preparations of the acid give guanine-uridylic acid on hydrolysis (176, 182, 183).

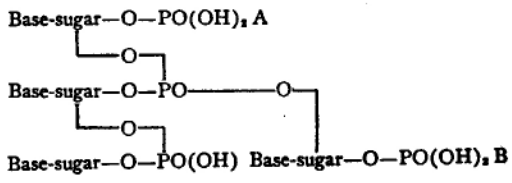
New possibilities have been brought to light for the internucleotide linkages of yeast ribonucleic acid. In view of the complete agreement that the polynucleotide exhibits four phosphoryl dissociations and since hydrolysis results in the appearance of secondary phosphoryl groups, it might be assumed that the polynucleotide is a polymer of the structural tetranucleotide proposed by Levene (140) and that in consequence each of its phosphoryl groups, except the terminal one, should exhibit one primary dissociation. This view seems to be incompatible with the experimental facts (141). Samples of the acid, and of the deaminated acid prepared by a method which avoided any fall in molecular weight, were titrated electrometrically between pH 2 and 12. Comparison of the experimental curves with those constructed theoretically showed that for each four atoms of phosphorus there were three primary and one secondary phosphoryl dissociations. A hypothetical structural tetranucleotide forms a useful standard for the assessment of results (89), and if these observations are considered on the basis of a polytetranucleotide from this standpoint only, each individual unit of the polymer would contain one triply esterified phosphoryl group; the shape of the titration curves agrees best, but not absolutely, with a formulation in which this is the group of uridylic acid. Two dissociations would be primary and associated with doubly esterified phosphoryls, and one phosphoryl group would be singly linked to a sugar radical and would exhibit both primary and secondary dissociations. Possible formulae in agreement with the facts, including the presence of titratable, and hence unsubstituted, purine and pyrimidine hydroxyls, are II and III; polymerisation would occur either through the group A or B, the other member of the pair remaining singly linked.

Without necessarily assuming a uniform tetranucleotide, it was considered that structural relationships of this nature may occur in the polynucleotide, and three points are noteworthy. First, the literature shows a marked tendency for the phosphorus analyses to be low. In a

polymer of nucleotides all united successively through their phosphoryl groups, a deficiency of phosphorus could only occur by removal of the singly linked terminal phosphoryl, but the extent of the deficiency was often greater than could be accounted for in this way. In a polymer



II



III

of II or III it would be possible for up to 25 per cent of the total phosphorus to be absent without upsetting the main polynucleotide structure. Second, a structure can be visualised in which nucleotides form side chains and could be removed by the action of ribonuclease, leaving a molecule sufficiently large to resemble the original acid in its failure to dialyse and in its precipitation reactions. Third, mixtures of enzyme preparations containing a phosphomonoesterase and a phosphodiesterase produced only 75 per cent dephosphorylation of yeast ribonucleic acid (184), and these mixed enzymes caused only 75 per cent dephosphorylation after pretreatment of the acid with a boiled extract of pancreatin (185); these results are in agreement with the presence of one triply linked phosphoryl for each four atoms of phosphorus.

Russell's viper venom, containing a phosphodiesterase (186, 187) but only very weak nonspecific phosphomonoesterase activity, dephosphorylated yeast ribonucleic acid ultimately to 50 to 55 per cent (188). If during their action the enzymes were inactivated with cyanide ions, dephosphorylation continued slowly in the alkaline medium or more rapidly in 1 per cent sodium hydroxide, thus demonstrating the existence in the acid of alkali-labile phosphoryl linkages. These labile groups were assigned tentatively to the hydroxyls at C_2 of the sugar radicals, and a working hypothesis was suggested that the polynucleotides of intermediate size which do not suffer loss in molecular weight when deaminated, and hence are free from phospho-amide groups (113, 182, 183), may be composed of nucleotides joined mutually through phosphoryl at C_2 and C_3 of the pentose. Union of such polynucleotides through phospho-amide groups, e.g., guanine-uridylic acid, might form a large polynucleotide in its native state, and the ultimate presence or absence of alkali-labile phospho-amide groups in a given sample of yeast ribonucleic acid might depend on their survival or destruction in the alkali at present used to extract the acid from yeast.

Other pentose polynucleotides.—Cold alkali converted the higher polynucleotides of tobacco mosaic virus into particles of molecular weight of 15,000, and it was specifically stated that there is at present no evidence that a unit smaller than this but larger than a nucleotide exists as a fundamental unit of the virus acid (63). Alkaline fission yielded an insoluble trisodium guanylate apparently identical with that from yeast ribonucleic acid, and a uracil nucleotide isomeric with uridylic acid; the purine content was 20 per cent lower than that required by the tetranucleotide ratio (103).

A pentose nucleic acid (41), believed to occur in the cytoplasm, was isolated from sheep liver; part of the sugar was identified as *d*-ribose and the amounts of total purine and easily hydrolysable phosphorus were consistent with the tetranucleotide ratio.

The pentose polynucleotide of pancreas (26, 189, 190) contains the four nucleotides (67, 191, 192) in the ratio, it has been proposed, of one molecule of adenylic acid and each pyrimidine nucleotide to varying proportions of guanylic acid, ranging from two to four molecules (40, 43, 79, 193). The pentose nucleic acid of rat liver appears to resemble pancreas pentose nucleic acid in its high nitrogen: phosphorus ratio, which exceeds that required by a tetranucleotide (42).

No conclusions can be drawn as to the structure of pentose nucleic acids which have been characterised only by detection of some or all of the nitrogenous components, or by the demonstration of the pentose nature of the sugar (for example, 47, 76, 170, 171, 194 to 197) or by the isolation of incompletely identified nucleotides (198).

In view of the wider possibilities of isomerism of nucleic acids recognised now as compared with formerly, claims that two acids are identical, as for example yeast ribonucleic acid and triticonucleic acid of wheat germ (58), require confirmation.

Terminology of nucleic acids.—Some discussion has taken place on this subject. On the one hand (199, 200), it was proposed that the name chromonucleic acid should be used instead of thymus nucleic acid as the biological term for the substance described chemically as

desoxyribose nucleic acid, and that plasmonucleic acid should be substituted for yeast nucleic acid as the biological equivalent of the chemical term ribose nucleic acid. On the other hand (159, 201), the necessity was stressed in the light of present information of defining a nucleic acid by referring both to its origin and its type (pentose or desoxypentose). This would avoid confusion until such time as it can be stated with certainty either that two (or more) individual nucleic acids exist and that all examples of each are identical in chemical constitution, or that two (or more) types of nucleic acid exist and that each type comprises a number of examples of related but different constitutions; closer specification of the type would be permissible when justified on chemical grounds. The suggestion (123) has also been made that in the characterisation of different nucleic acids the treatment in the course of isolation, such as the degree of tissue autolysis, reagents used, etc., as well as the chemical and physical properties, should be described.

Location of Nucleic Acids in Cells

In an extensive survey of the nucleic acids of embryonic and adult tissues (202), the dried powders, freed from acid-soluble and lipoid phosphorus compounds, were extracted with 10 per cent sodium chloride and the total nucleic acids precipitated with lanthanum acetate. Analyses for phosphorus, pentose, and desoxypentose confirmed the belief (202) that pentose nucleic acids are of general occurrence in animal cells, and indicated that rapidly growing tissues are characterised by high concentrations of both types of acid.

In the light of recent reviews (82, 122, 203), the remainder of this section is restricted to surveys of the criticism levelled at the widely accepted theory of the part played by nucleic acids in the nucleus and to the progress made in elucidating the mode of occurrence of cytoplasmic nucleic acids.

Nucleic acids of the nucleus.—For some time it has been considered as established that desoxypentose nucleic acids are located principally in the nucleus and that pentose nucleic acids characterise the cytoplasm. This concept is largely founded on the cytological application of ultraviolet photomicrography to demonstrate the presence of purine and pyrimidine rings and on the use of Feulgen staining to detect desoxypentose nucleic acids (for representative papers, see 204 to 209, and for reviews, see 82, 203, 210, 211). The hypothesis that nucleic acids play an essential part in the reproductive cycle of the cell and in

the genic perpetuation of inherited characteristics (212) is largely dependent on results achieved by means of these techniques, and hence the specificity of the Feulgen reaction in denoting the site of desoxypentose nucleic acids is a vital factor. Question of its validity might necessitate reconsideration of a large mass of conjecture and experimental conclusions. Criticism has been made and refuted (213 to 216), and it has been pointed out that the reaction is specific for desoxypentose only in the absence of lipoids (96). Serious doubts have now been thrown on its reliability as an indicator both of the total amount of desoxypentose polynucleotide material (113) and also of the location of such substances in the cell (113, 217).

Cellular nuclei contain, besides nucleic acid and histone, an acidic protein, chromosomin (217), postulated as the characteristic material of chromatin. It may be identical with, or related to, a new protein of cellular nuclei, reported earlier by other workers (57). Chromosomin has the property of taking up the basic, water-soluble dye produced by the interaction of decolorised magenta and hydrolysed desoxypentose nucleic acid, and hence the appearance of this dye in the nucleus of the cell does not, it was maintained, necessarily indicate the presence there of the desoxypentose nucleic acid. Furthermore, it was possible to stain chromosomes directly with the "developed nucleal stain" (218). It was suggested that desoxypentose nucleic acid is present in the nuclear sap, rather than in the chromosomes, and may form the mitotic spindle, and that chromosomin is "the chemical basis of inheritance" (219). Histone, on the other hand, was regarded as filling the role of regulating mitosis (220). Previously, desoxypentose nucleic acids were regarded as essential to the duplication of the chromosomes (211), and although no concrete proposals were made, there was a tentative assumption that the genes consist of desoxypentose nucleic acids combined with protein (210).

These postulates as to the part played by chromosomin and the location of desoxypentose nucleic acids outside the chromosomes have been adversely criticised (221, 222). Enzymic destruction of nucleic acid in the nucleus rendered the chromosomes not stainable by Feulgen's method, whereas hydrolysis of the protein component by trypsin did not alter the selective ultraviolet absorption of the nucleus in the region characteristic of nucleic acids. The parts of the chromosomes which can be stained by the Feulgen technique contained a high proportion of phosphorus, in accord with the presence there of nucleic acid, and isolated chromosome threads were found to contain 40 per

cent of desoxypentose nucleic acid (for references see 221, 222). Analyses of different tissues (220), which contain varying quantities of nuclear sap but show little variation in the amounts of nucleic acid, were quoted in opposition to the hypothesis that the nucleic acid is present in the nuclear sap. The fact that mitotic spindle fibres are positively birefringent with respect to their length, whereas desoxypentose nucleic acid is negatively birefringent, was held to be in apparent opposition to the suggestion that the spindle consists of a gel of desoxypentose nucleic acids (222). It was also stated (223) that the assumption of chromosomin as an integral constituent of the nucleus in no way affects the views which, chiefly with the aid of ultraviolet absorption measurements, have been advanced in regard to the protein and nucleic acid metabolism and the distribution of those substances in the nucleus. Replies to these criticisms have been made (219, 224). In view of this controversy, many may hesitate to acknowledge as yet definite functions or dominant roles for these cellular constituents.

Nucleic acids of the cytoplasm.—Comparatively little attention has been paid to the organisation of cytoplasmic nucleic acids until recent years. By high-speed centrifugation, fractions consisting of submicroscopic particles were obtained from chicken tumour I agent (46) and normal chicken embryo (48); these were concentrated separately from the nuclear material and toward the centripetal end. The particles from the tumour agent had high tumour-producing activity whereas those from normal embryo were inactive, and both contained lipoid together with a pentose nucleoprotein. From the tumour agent, a pentose nucleic acid was isolated, having a broad absorption band with a maximum at rather shorter wave-length than 2600 Å; this was regarded as indicating the presence of a high proportion of guanine, and guanine nucleotide was isolated after alkaline hydrolysis (47, 50, 225). These observations constitute a marked advance in the study of the chicken tumour and in knowledge of the location of pentose nucleic acids in cytoplasm. Similar particles (microsomes), obtained from various tissues, had a constant composition irrespective of the source (51, 52), consisting of phospholipids and pentose nucleoproteins associated in definite proportions. Nucleoproteins have been found in conjunction with lipoid material by other workers (for summary, see above and 203). The part played by microsomes in the structure of the cytoplasm has been discussed (52) but no fully substantiated proposal has yet been put forward. They have been regarded as fragmented mitochondria (50) and as independent cellular structures associated with

differentiation of the cell, and from which, for instance, the secretory granules of the pancreas are derived.

An interesting aspect of the nucleic acids of bacterial cells has been revived recently. For some time it has been known that Gram-positive pneumococci can become Gram-negative, and this change was brought about by extracting the cells in neutral solution (20) and by an enzyme, apparently identical with the pancreas enzyme which acts upon yeast ribonucleic acid (226). The material released into the solution during the former process contained pentose nucleic acid and a nucleoprotein (20, 227). A similar change has now been effected in yeast cells and Gram-positive bacteria by extraction with a solution of a bile salt (228), and it was also possible to restore the Gram-positive reaction by replacing the responsible material, an essential component of which appears to be the magnesium salt of a pentose nucleic acid; other salts of nucleic acid could not be plated back in this way. In agreement with the previous workers, the stainable material could be progressively extracted, that part on the surface of the cell being removed first.

Induced Transformation of Pneumococcal Types

Among micro-organisms, the most striking example of the reproducible and controllable induction of inheritable and specific alterations in cell structure and function is the transformation of specific types of pneumococcus. This type of change has been brought about both *in vivo* and *in vitro,* and analogous transformations have been carried out in the field of viruses (for references, see 62). Avery, MacLeod & McCarty (62) have now isolated from type III pneumococci a biologically active fraction which in exceedingly minute amounts is capable under appropriate conditions of inducing the transformation of unencapsulated R variants of pneumococcus type II into fully encapsulated S cells of type III. Other variants are not transformed in this way. Examination of the active extract indicated, within the limits of the methods employed, that protein, unbound lipoid, and serologically active polysaccharide were absent, and that it consisted principally, if not solely, of a sodium salt, in homogeneous viscous form, of a desoxypentose nucleic acid of molecular weight of the order of 500,000. It is possible, as the authors suggest, that the biological activity of the material is not an inherent property of the nucleic acid but is due to minute amounts of some other substance so intimately associated with it as to escape detection. If, however, as

the evidence strongly suggests, the transforming principle is a sodium salt of a desoxypentose nucleic acid, this type of polynucleotide must be regarded not merely as structurally important but as functionally active in determining the biochemical activities and specific characteristics of pneumococcal cells. This would appear to be the first occasion on which specific transformation has been experimentally induced *in vitro* by a chemically defined substance, and its implications are of the greatest importance in the fields of genetics, virology, and cancer research.

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