4. The proteins of the Lubrol supernatant liquid were separated into four fractions, differences in solubility in strong solutions of magnesium sulphate, ammonium sulphate and water being utilized. The specific radioactivities of these fractions showed the same pattern of distribution, whatever the time interval after the injection of [^{14}C]phenylalanine. The final fraction containing these proteins least readily precipitated became labelled very quickly; 2 min. after injection it had a specific radioactivity as high as that of the proteins of the sodium perfluoro-octanoate pellet.

5. The present results indicate that a number of microsomal proteins which can easily be solubilized become labelled simultaneously.

The authors thank Mrs J. Tapley for technical assistance and Imperial Chemical Industries Ltd. for providing a sample of Lubrol W.

This investigation has been supported by grants to the Royal Cancer Hospital and the Chester Beatty Research Institute from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund and the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

REFERENCES

Allfrey, V., Daly, M. M. & Mirsky, A. E. (1953). *J. gen. Physiol.* **37**, 157.
Campbell, P. N., Greengard, O. & Kernot, B. A. (1958). *Biochem. J.* **68**, 18P.
Cohn, P. & Butler, J. A. V. (1957*a*). *Biochim. biophys. Acta*, **25**, 222.
Cohn, P. & Butler, J. A. V. (1957*b*). *Int. J. appl. Radiat. Isotopes*, **2**, 214.
Fiske, C. H. & Subbarow, Y. (1925). *J. biol. Chem.* **66**, 375.
Hultin, T. (1955). *Exp. Cell Res. Suppl.* **3**, 210.
Keller, E. B., Zamecnik, P. C. & Loftfield, R. B. (1954). *J. Histochem. Cytochem.* **2**, 378.
Littlefield, J. W., Keller, E. B., Gross, J. & Zamecnik, P. C. (1955). *J. biol. Chem.* **217**, 111.
Simkin, J. L. & Work, T. S. (1957). *Biochem. J.* **65**, 307.
Swanson, M. A. & Artom, C. (1950). *J. biol. Chem.* **187**, 281.

Preparation of some Deoxyribonucleic acid–Protein Complexes from Rat-Liver Homogenates

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(*Received* 24 *February* 1958)

Preparation of deoxyribonucleic acid from rat-liver homogenates by the action of various salts and phenol (Kirby, 1957) showed that deoxyribonucleic acid free from protein could be isolated when the salt was *p*-aminosalicylate but that about 1% of residual protein remained attached to the deoxyribonucleic acid when benzoate was used. These results suggested that some deoxyribonucleic acid may be bound to the protein through metal linkages, and it was thought that further information could be obtained by isolation and analysis of deoxyribonucleic acid produced by the action of different salts.

For this investigation fluoride and azide were chosen as typical inorganic salts with considerable power of complexing with metals. The action of diethyldithiocarbamate was compared with that of diethylacetate and the action of 2-nitroso-1-naphthol-4-sulphonate with 1-naphthol-4-sulphonate, since the first and third of this group have considerable chelating abilities. *cyclo*Hexanediamine-*NNN'N'*-tetra-acetate was examined because this agent was effective in releasing deoxyribonucleic acid from rat liver whereas a very similar chelating agent, ethylenediamine-*NNN'N'*-tetra-acetate, was not (Kirby, 1957). Finally the effect of benzoate at different concentrations was investigated to determine the effect of ionic concentration on the amount of residual protein attached to the deoxyribonucleic acid. It had been demonstrated previously that deoxyribonucleic acid extracted in the presence of 0·15M-salts contained more protein than when 0·3M-salts were used.

MATERIALS AND METHODS

Sodium salts were used in all cases and adjusted to pH 6·5–7·0 with NaOH or acetic acid with narrow-range indicator papers or a pH meter.

Isolation procedure. This was essentially as described previously. The animals were killed and their livers excised and dropped on to solid CO_2. The livers (usually 50–75 g.) were broken up in the appropriate salt solution (400–600 ml.) in a Waring Blendor for about 45 sec. The mixture was stirred and an equal volume of 90% phenol (w/w) was added immediately and stirring continued for 40 min. The conditions of centrifuging depended upon the viscosity of the aqueous layer, which was much more viscous when

more than about 5% of protein was present with the deoxyribonucleic acid (DNA). In these cases the mixture was centrifuged for 1 hr. at 0° at 700 *g*, the dark phenol layer removed by suction and the aqueous layer was centrifuged again in a Servall machine in polyethylene tubes for 1 hr. at 0° at 10 000 *g*. When the high viscosity precluded suction the aqueous solution was separated by careful pouring. When the salt concentration was less than 0·15 M the aqueous solution was made 4% with respect to sodium acetate before addition of an equal volume of 2-ethoxyethanol for precipitation.

DNA with only traces of protein which was precipitated for the first time with 2-ethoxyethanol was more difficult to collect on a rod or a spatula than DNA with more protein, and was better separated by centrifuging for about 1 min. at 500 *g*.

After further precipitations and treatment with ribonuclease the DNA–protein complex was separated from residual glycogen by extraction from 1·25 M-phosphate solution with 2-methoxyethanol, when the complex was extracted entirely into the organic layer and the glycogen remained partially insoluble and partially in the aqueous layer. After centrifuging and dialysis of the organic layer sodium acetate was added and the DNA material was precipitated by 2-ethoxyethanol. The white fibres were washed twice with ethanol–water (3:1), and allowed to stand for several hours over ethanol and then dried in a vacuum desiccator over $CaCl_2$.

Analyses. Determinations of N and P were carried out on materials containing 10–15% of moisture. $\epsilon_{(P)}$ values (Chargaff, 1955) were determined in 0·1 M-NaCl soln. When little protein was present the samples dissolved easily on shaking, but when much protein was present it was sometimes necessary to dissolve the product in water and then add an equal volume of 0·2 M-NaCl soln. Base compositions were estimated as described by Kirby (1957).

Amino acid determination. This was carried out essentially by the method used before (Kirby, 1957). The complex (15 mg.) was hydrolysed with 5·8 N-HCl, and the HCl evaporated, and the residue evaporated two or three times in the presence of acetic acid–water (1:4) and then dissolved in 0·1 ml. of acetic acid–water (1:4); 0·02 ml. was used for separation by paper chromatography and electrophoresis. A sample (7–10 mg.) of the product from the 0·07 and 0·14 M-benzoate preparation was used for hydrolysis, and the residue was dissolved in 0·2 ml. of acetic acid–water (1:4) and 0·01 ml. was used for each separation. Lysine and arginine were determined on Whatman no. 4 paper by single-way chromatography. After the separation colours were developed by spraying the papers with a solution of ninhydrin, and the colours were stabilized by spraying with a solution of cupric acetate monohydrate (150 mg., powdered in 6 ml. of water and 94 ml. of ethanol). The colours were eluted for 2 hr. with 80% methanol (80% 2-methoxyethanol has been found to be better than 80% methanol) and measured at 500 mμ.

Action of p-*aminosalicylate and phenol on the deoxyribonucleic acid prepared by the action of* 0·07 M-*benzoate.* DNA prepared by the action of 0·07 M-benzoate (54 mg.) was dissolved in water (100 ml.). To this solution sodium *p*-aminosalicylate (6 g.) and 90% phenol (w/w) were added and the mixture was stirred for 40 min. at room temperature. The mixture was centrifuged at 0° in an International centrifuge at 700 *g* for 45 min. The top layer was siphoned off and the DNA was precipitated with an equal volume of 2-ethoxyethanol. The fibrous precipitate was collected, and dissolved immediately in water (50 ml.); sodium acetate (2 g.) was added and the material was again precipitated with 2-ethoxyethanol (50 ml.). The insoluble product was removed, washed twice with 75% ethanol and allowed to stand for 16 hr. with absolute ethanol before pouring away the ethanol and drying the residue in a vacuum desiccator over $CaCl_2$. The yield was 22 mg.

Action of sodium benzoate and sodium azide on rat-liver homogenates. Rat liver (32 g.) was homogenized in a solution (250 ml.) of sodium benzoate (0·28 M) and sodium azide (0·03 M). An equal volume of 90% phenol was added and the mixture was stirred for 40 min. and then centrifuged for 1 hr. at 0° at 700 *g*. The lower phenol layer was removed and the top layer was centrifuged again. The clear supernatant was removed, and the DNA was precipitated with an equal volume of 2-ethoxyethanol and separated by centrifuging for 1 min. at 700 *g*. The DNA dissolved quickly in water and the separation procedure followed the method described previously (Kirby, 1957). The yield was 56 mg.

*Isolation of deoxyribonucleic acid from rat-liver homogenate by the action of ethylenediamine-*NNN′N′*-tetra-acetate, naphthalene-2-sulphonate and phenol.* Sodium naphthalene-2-sulphonate (17·5 g.) and tetrasodium ethylenediamine-*NNN′N′*-tetra-acetate (5 g.) were dissolved in water (500 ml.) and acetic acid (0·5 ml.) was added to bring the pH to 6·5. This solution (200 ml.) was used to homogenize 25 g. of rat liver and the mixture was stirred for 40 min. with 90% phenol (w/w) (200 ml.), after which DNA was isolated exactly as described previously. The yield was 41 mg.

Isolation of deoxyribonucleic acid–protein complex in the presence of free deoxyribonucleic acid. DNA (41·8 mg.) in which no protein was present was dissolved in water (250 ml.), sodium benzoate (5 g.) was added and this solution was used to homogenize 5 g. of rat liver. The homogenate was stirred with 250 ml. of 90% (w/w) phenol for 40 min. and the DNA was isolated exactly as described previously. The product had a lower viscosity than that usually isolated by the 0·14 M-benzoate procedure.

Hydroazinolysis. Anhydrous hydrazine was prepared by Braunitzer's (1955) method and the course of the experiments followed from his data. DNA (30 mg. each from 0·07 and 0·14 M-benzoate preparations) was used with 0·3 ml. of anhydrous hydrazine and *p*-nitrobenzaldehyde in ethyl acetate was used to remove the hydrazones. The final aqueous solutions were yellow. They were evaporated to dryness, the residue was dissolved in 0·1 ml. of acetic acid–water (4:1) and 0·02 ml. was used for chromatographic analysis, which was carried out as described by Kirby (1957). Only the DNA prepared by the action of 0·07 M-benzoate developed any colours with ninhydrin and the amino acids found were: leucine, alanine, glycine, aspartic acid, glutamic acid and serine.

RESULTS

Yields. The yield of the DNA–protein complexes isolated by the phenol procedure is usually 160–220 mg./100 g. of rat liver, irrespective of the amount of protein in the complex. Hence less

DNA is actually extracted by 0·07 and 0·14 M-benzoate solutions since about 50 and 30% respectively of the product is protein. There is no alteration in the base composition with varying amounts of protein.

Amino acid analyses. The analytical results of the various preparations are shown in Table 1. Apart from the amino acids reported in the table, cysteine, serine, threonine, glycine, alanine, proline, methionine and valine are present in the samples prepared by the action of 0·07 and 0·14 M-benzoate and 0·3 M-diethylacetate ions. Glycine is, of course, present in all hydrolysates owing to decomposition of the purines. Cysteine and proline are absent from the chromatograms of all other samples and so, presumably, are present in small quantities in the three preparations mentioned. Serine, threonine, alanine, methionine and valine are also present in preparations made with 0·21 M-benzoate, *cyclo*hexanediamine-*NNN'N*-tetra-acetate, 2-nitroso-1-naphthol-4-sulphonate and to a less extent in the azide and fluoride preparations.

Action of naphthalenesulphonic acids. Naphthalene-2-sulphonate was previously reported (Kirby, 1957) to be moderately active in releasing DNA, but only very little DNA could be isolated when the salt was used at 0·15 M concentration, although the solution gave a strong Dische (1930) reaction. 1-Naphthol-4-sulphonate (0·15 M) yielded 150 mg. of DNA/100 g. of rat liver but some ribonucleic acid (RNA) was present. Naphtholsulphonate was originally considered to be moderately effective since the DNA was not fibrous on precipitation. This, however, was due to coprecipitation of 1-naphthol-4-sulphonate; this was separated during the two-phase separation with 2-methoxyethanol when the DNA was extracted into the organic layer, whereas the naphtholsulphonate was largely insoluble. After dialysis of the organic layer the DNA precipitated in a fibrous manner. The presence of RNA may therefore be due to some inactivation of ribonuclease by 1-naphthol-4-sulphonate.

Since a solution of 2-nitroso-1-naphthol-4-sulphonate is saturated at a concentration of 0·03 M the comparative ability of naphthalene-2-sulphonate and 1-naphthol-4-sulphonate to release DNA under the conditions used at this concentration was examined. No DNA was released by naphthalene-2-sulphonate and only sufficient to produce a colour reaction by 1-naphthol-4-sulphonate. A good yield of DNA was produced by 2-nitroso-1-naphthol-4-sulphonate, although the final product contained some protein and was very faintly yellow.

Amino acids present after two treatments or combinations of salts. The analytical figures for the product of the reaction of *p*-aminosalicylate on the

Table 1. *Analytical figures for deoxyribonucleic acid–protein complexes*

Bases are expressed as moles/100 moles of total bases. Amino acids are expressed as a percentage of the DNA–protein complex isolated. $\epsilon_{(P)}$ values were estimated in 0·1 M-NaCl solution.

Salt	NaF	NaN_3	Sodium benzoate					Sodium diethyldithio-carbamate	Sodium diethyl-acetate	*cyclo*Hex-anediamine-tetra-acetate	2-Nitroso-1-naphthol-4-sulphonate
Concn. (M)	0·3	0·3	0·35	0·28	0·21	0·14	0·07	0·3	0·3	0·15	0·03
Guanine	22·0	21·2	21·3	21·0	21·8	22·1	21·6	21·0	22·6	20·6	20·8
Adenine	30·3	29·5	29·8	29·7	29·7	29·8	30·6	30·2	29·7	29·5	30·2
Cytosine	18·9	19·7	20·3	20·2	19·3	20·0	19·1	20·8	19·1	20·6	19·2
Thymine	28·8	29·6	28·6	29·1	29·3	28·1	28·7	28·0	28·6	29·3	29·8
N	11·8	12·2	11·75	10·7	12·3	11·75	13·0	12·1	12·65	12·0	13·3
P	7·4	7·8	7·2	6·65	6·65	6·0	5·2	7·7	6·4	7·1	7·14
N/P	1·59	1·57	1·64	1·61	1·85	1·95	2·5	1·58	1·98	1·7	1·87
$\epsilon_{(P)}$	—	6250	6620	7500	7650	8600	9250	6100	7600	7050	7100
Lysine	0·65	0·6	0·14	0·38	0·77	7·00	11·7	0·03	1·20	0·37	1·5
Arginine	0·21	0·22	0·10	0·2	0·28	3·00	5·6	0·05	0·55	0·22	0·7
Aspartic acid	0·11	0·13	0·11	0·21	0·19	5·00	10·5	0·10	0·82	0·65	1·0
Glutamic acid	0·13	0·15	0·09	0·2	0·30	4·3	7·0	0·05	0·75	0·45	0·45
Tyrosine	—	0·10	—	—	0·10	1·4	3·3	—	0·25	0·13	—
Phenylalanine	0·10	0·11	—	—	0·20	2·3	4·5	—	0·43	0·29	—
Leucine/isoleucine	0·83	0·57	0·13	0·17	0·57	12·6	16·5	—	2·9	0·72	2·00

DNA–protein complex produced by the action of 0·07 M-benzoate are shown in Table 2. The values indicate that about two-thirds of the residual protein was removed in this reaction.

The amino acid residues present after the treatments with ethylenediamine-*NNN′N′*-tetra-acetate combined with naphthalene-2-sulphonate and azide combined with benzoate are shown in Table 3.

Action of liver homogenate on protein-free deoxyribonucleic acid. Since it was possible that DNA might interact with cellular proteins during its liberation from the nuclei, an experiment was performed in which DNA free from protein was mixed with a rat-liver homogenate in 0·14 M-benzoate. The mixture was then extracted with phenol and the DNA isolated in the usual manner. If the added DNA absorbed no protein and the DNA from the liver homogenate had about 30 % of protein, which was the amount found when DNA was isolated from liver in the presence of 0·14 M-benzoate, then the total DNA isolated should have about 6 % of protein. Approximately half this value was found to be present and the results are shown in Table 4.

Table 2. *Analysis of some amino acids after reaction of the deoxyribonucleic acid–protein complex produced by* 0·07 M-*benzoate with* p-*aminosalicylate and phenol*

Amino acids are expressed as a percentage of total material.

Lysine	3·3
Arginine	2·9
Aspartic acid	2·9
Glutamic acid	2·5
Tyrosine	1·3

Table 3. *Amino acid analyses of deoxyribonucleic acid produced by actions of naphthalene-2-sulphonate combined with ethylenediaminetetra-acetate* (*a*) *and benzoate combined with azide* (*b*)

Amino acids are expressed as percentage of total weight.

	(*a*)	(*b*)
Lysine	0·23	0·33
Arginine	0·08	0·13
Aspartic acid	0·16	0·16
Glutamic acid	0·16	0·20
Leucine	0·30	—

Table 4. *Analyses of some amino acids present after extraction of rat liver with* 0·14 M-*benzoate and phenol in the presence of deoxyribonucleic acid*

Amino acids are expressed as a percentage of total material.

Lysine	0·60
Arginine	0·31
Aspartic acid	0·40
Glutamic acid	0·36
Phenylalanine	0·20

Physical measurements. DNA samples prepared by the actions of *p*-aminosalicylate, diethylacetate and 0·07 M-benzoate ions have been examined by Dr K. V. Shooter in a Spinco ultracentrifuge fitted with an ultraviolet-light-absorption optical system. For these experiments 0·005 % solutions of DNA in 0·2 M-NaCl were used. The sedimentation coefficient-distribution curves were calculated in the manner described by Shooter & Butler (1956).

The sedimentation coefficient–distribution curves for the DNA prepared with *p*-aminosalicylate covered a range from 10*S* to 60*S*, the average sedimentation coefficient being 24*S*. This distribution was very similar to those observed for DNA from other animal tissues (Shooter & Butler, 1956). DNA from rat liver prepared by the action of 0·3 M-diethylacetate and 0·07 M-benzoate ions had average sedimentation coefficients of 30*S* and 31*S* respectively. The distribution curves indicated that the preparation with about 7·5 % of protein had 5 % of particles with $S > 60$ and when 50 % of protein was present 20 % had $S > 60$. Butler, Phillips & Shooter (1957) have shown that aggregates of DNA cross-linked by protein can be broken down by treatment with chymotrypsin. Treatment of rat-liver DNA with this enzyme showed that the preparation with 7·5 % of protein had 13 % of these linkages broken, whereas 29 % were broken when the DNA contained 50 % of protein.

DISCUSSION

The DNA–protein complexes isolated by the use of various concentrations of sodium benzoate have the surprising property of being soluble in water and in 0·1 M-sodium chloride solutions even when 30–50 % of protein is present. Moreover, these complexes are sufficiently stable to be extractable by 2-methoxyethanol from phosphate buffer in exactly the same manner as free DNA. Cysteine is present and although Crampton, Stein & Moore (1957) and Davison (1957) found this amino acid in calf-thymus histone, Brunish, Farley & Luck (1951) did not find it in rat-liver nucleohistone. The inability of *p*-aminosalicylate to remove all the protein from a DNA preparation containing 50 % of protein is unexpected, although it is possible that the protein has been modified during the extraction with phenol. The viscosity of the solutions was much higher when proteins were present and hence a high viscosity alone cannot be a criterion of the purity of DNA.

The ability of the diethyldithiocarbamate ion to release DNA with considerably less protein than the DNA released by the diethylacetate ion, and the activity of 2-nitroso-1-naphthol-4-sulphonate compared with 1-naphthol-4-sulphonate at 0·03 M-concentration could be related in both cases to the

complexing powers of diethyldithiocarbamate and 2-nitroso-1-naphthol-4-sulphonate with metals.

Fluoride and azide produce DNA with similar amounts of residual amino acids and both these ions form complexes with metals. In this respect it is relevant that Felix, Jilke & Zahn (1956) prepared highly polymerized DNA from bull testes by treatment at 70° with saturated sodium chloride and 5 % sodium fluoride solution.

The DNA isolated after treatment with *cyclo*hexanediamine-*NNN′N′*-tetra-acetate has relatively high quantities of aspartic and glutamic acids. Although this compound is used in 0·15M-concentration it appears to be acting more like an aliphatic anion than a compound with chelating properties, and it is noteworthy that ethylenediamine-*NNN′N′*-tetra-acetate did not liberate DNA from liver in conjunction with phenol.

It seems reasonable to assume from these and the previous results that neither the ability of these salts to complex with metals nor the power to combine with proteins is alone sufficient to release DNA free from protein out of rat-liver cells, but that both capacities are essential. The amount of protein left then would be a measure of the ability of the anion both to complex with the metal(s) concerned and to interact with the nuclear proteins. By choosing a salt and a particular concentration, DNA with almost any amount of protein could be obtained.

That the combined abilities to complex with metals and to interact with proteins are essential is demonstrated by the liberation of DNA by the action (in conjunction with phenol) of naphthalene-2-sulphonate (0·15M) and ethylenediamine-*NNN′N′*-tetra-acetate (0·026M), neither of which separately released any quantity of DNA from rat liver. Azide (0·03M), however, made little difference when used in combination with benzoate (0·28M). There are two reasonable explanations of these results: either the chromosomes contain some DNA linked through a metal bond to the protein or the complexing agents prevent the DNA from acquiring metal ions and subsequently protein by a secondary reaction. It has been demonstrated previously that DNA could be removed from solution by collagen which had been treated with certain bivalent metal ions, but the reaction was slow and only 50 % of the DNA was absorbed during 14 days. But when DNA which was free from protein was homogenized with rat liver in the presence of 0·14M-benzoate the total DNA isolated had only about 3 % of amino acids, whereas DNA isolated directly in the presence of 0·14M-benzoate contained about 30 % of amino acids. It seems unlikely then that DNA combines with any protein released through breaking down the cells under the conditions used.

Moreover, calf-thymus DNA does not interact with bovine serum albumin to any extent unless the DNA has been heated first (Zubay & Doty, 1957), and mixtures of DNA and protein which did interact unspecifically would be expected to be insoluble because the cross-linking would be random. This is the case when calf-thymus DNA is mixed with histones (Bernstein, 1956). The protein moiety of such complexes may be expected to have a relatively high arginine content since the phosphate groups of the liberated DNA would be the most obvious points of attachment. In fact the DNA–protein complexes isolated from rat liver are soluble in water and have lower arginine contents than histones present in the nucleus (Kirby, 1957, Table 5), and the analytical values are more reasonably consistent than might have been expected from arbitrary mixtures of DNA and proteins.

It would be unwise to place too much reliance on the analytical values of the amino acid in view of the known limitations of the method used. Davison & Butler (1954), using the same method, found variations up to ± 10 %, and similar variations have been encountered here. However, the analyses do show that lysine and arginine are not the predominant amino acids in these proteins and indeed the amounts of glutamic and aspartic acids are frequently of the same order as the basic amino acids. The analytical values bear some similarities to those described by Davison (1957) for certain fractions of histones which have been separated on columns of carboxymethylcellulose. It is not surprising that different salts produce DNA–proteins with varying amounts of amino acids in view of the known heterogeneity of nucleohistones (Stedman & Stedman, 1950; Butler, Davison, James & Shooter, 1954; Grégoire & Limozin, 1954; Davison, James, Shooter & Butler, 1954; Davison & Butler, 1954; Daly & Mirsky, 1955; Crampton, Moore & Stein, 1955; Luck *et al.* 1956; Crampton, Stein & Moore, 1957; Davison, 1957).

It must be emphasized that these results apply only to rat-liver DNA and there is no reason to assume that phenol and *p*-aminosalicylate will produce DNA free from protein from all tissues and organisms. In fact this particular combination does not produce DNA free from protein from certain rat tumours or from wheat germ (results to be reported later), and Kay (1956) has reported that phage 1, which is active on *Salmonella typhi*, is inactivated and loses its DNA by reaction with phosphate, citrate and ethylenediamine-*NNN′N′*-tetra-acetate. Calcium and magnesium ions protect the phage against inactivation. It is quite possible therefore that a number of agents or combination of agents may have to be tried before a suitable system is found for other organisms.

Since DNA isolated from mammalian sources has no demonstrable biological activity it is necessary to compare samples by physical methods. The $\epsilon_{(P)}$ value (Chargaff, 1955) is an indication that DNA prepared by the phenol method has the same optical properties as samples prepared by other methods. The increase of $\epsilon_{(P)}$ with increasing amounts of protein may be due to scattering of the light, and this is almost certainly the case with DNA containing 30–50% of protein, where the solutions were opalescent.

Dr M. H. F. Wilkins and his co-workers have made a crystallographic examination by X-rays of DNA prepared from rat liver by the actions of *p*-aminosalicylate, *cyclo*hexanediamine-*NNN'N'*-tetra-acetate and diethylacetate ions. All the samples showed the 'A' pattern characteristic of the undenatured sodium salt of DNA (Wilkins, Seeds, Stokes & Wilson, 1953), and the best photographic pattern from the specimen prepared by the action of *cyclo*hexanediamine-*NNN'N'*-tetracarboxylate was not distinguishable from the pattern produced by calf-thymus DNA made by standard methods. Clearly phenol does not disintegrate the regular structure of the isolated DNA molecules and their ability to arrange themselves in a crystalline structure. Also the same crystalline structure is produced when the samples contain protein.

The sedimentation coefficient–distribution curves are further evidence that DNA prepared from rat liver by the phenol method is similar to other samples (Shooter & Butler, 1956). The rather remarkable feature was the relatively small difference in the weight-average sedimentation coefficient of the samples with 8 and 50% of protein. Since a relatively small proportion of the protein is affected by chymotrypsin it appears likely that most of this protein must be along the length of the DNA molecules and is not involved in cross-linking. This hypothesis would account for the observed solubility of the preparations.

SUMMARY

1. Deoxyribonucleic acid has been prepared from rat liver by the action of a number of salts in conjunction with phenol. The amount of residual protein attached to the deoxyribonucleic acid depends upon the salt used.

2. *p*-Aminosalicylate and diethyldithiocarbamate have been the only salts to produce a deoxyribonucleic acid nearly free from protein.

3. The ability to complex with metals, in addition to the power to interact with proteins, is required to liberate deoxyribonucleic acid with very little protein from rat liver. Neither naphthalene-2-sulphonate nor ethylenediamine-*NNN'N* tetra-acetate separately liberates any appreciable quantity of deoxyribonucleic acid but the two salts will do so when used together.

4. No protein was taken up on deoxyribonucleic acid which was mixed with a rat-liver homogenate in the presence of 0·14M-benzoate.

5. The relevance of these experiments to the nature of the deoxyribonucleic acid–protein bonding is discussed.

The author wishes to thank Dr K. V. Shooter for the sedimentation measurements, Dr M. H. F. Wilkins and co-workers for the X-ray measurements, Professor A. Haddow for his interest and Miss G. E. Adams for technical assistance.

This investigation has been supported by grants to the Royal Cancer Hospital and the Chester Beatty Research Institute from the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund and the National Cancer Institute of the National Institutes of Health, United States Public Health Service.

REFERENCES

Bernstein, M. (1956). *Proc. nat. Acad. Sci., Wash.*, **42**, 703.

Braunitzer, G. (1955). *Chem. Ber.* **88**, 2025.

Brunish, R., Farley, O. & Luck, J. T. (1951). *Nature, Lond.*, **168**, 82.

Butler, J. A. V., Davison, P. F., James, D. W. F. & Shooter, K. V. (1954). *Biochim. biophys. Acta*, **15**, 224.

Butler, J. A. V., Phillips, D. M. P. & Shooter, K. V. (1957). *Arch. Biochem. Biophys.* **71**, 423.

Chargaff, E. (1955). In *The Nucleic Acids*, vol. 1, p. 308. Ed. by Chargaff, E. & Davidson, J. N. New York: Academic Press Inc.

Crampton, C. F., Moore, S. & Stein, W. H. (1955). *J. biol. Chem.* **215**, 787.

Crampton, C. F., Stein, W. H. & Moore, S. (1957). *J. biol. Chem.* **225**, 363.

Daly, A. E. & Mirsky, A. E. (1955). *J. gen. Physiol.* **38**, 405.

Davison, P. F. (1957). *Biochem. J.* **66**, 703.

Davison, P. F. & Butler, J. A. V. (1954). *Biochem. biophys. Acta*, **15**, 439.

Davison, P. F., James, D. W. F., Shooter, K. V. & Butler, J. A. V. (1954). *Biochim. biophys. Acta*, **15**, 415.

Dische, Z. (1930). *Mikrochemie*, **8**, 4.

Felix, K., Jilke, I. & Zahn, R. K. (1956). *Hoppe-Seyl. Z.* **303**, 140.

Grégoire, J. & Limozin, M. (1954). *Bull. Soc. Chim. biol., Paris*, **36**, 15.

Kay, P. (1956). *Brit. J. exp. Path.* **37**, 560.

Kirby, K. S. (1957). *Biochem. J.* **66**, 495.

Luck, J. M., Cook, H. A., Eldredge, N. T., Halley, M. I., Kupke, D. W. & Rasmussen, P. S. (1956). *Arch. Biochem. Biophys.* **65**, 449.

Shooter, K. V. & Butler, J. A. V. (1956). *Trans. Faraday Soc.* **52**, 734.

Stedman, E. & Stedman, E. (1950). *Nature, Lond.*, **166**, 780.

Wilkins, M. H. F., Seeds, W. E., Stokes, A. R. & Wilson, H. R. (1953). *Nature, Lond.*, **172**, 759.

Zubay, G. & Doty, P. (1957). *Biochim. biophys. Acta*, **23**, 213.