SUMMARY

1. Evidence is presented for the presence of pyridoxamine phosphate oxidase and a pyridoxal phosphate phosphatase in aqueous extracts of *Escherichia coli*.

2. Spectrophotometric methods have been developed for the precise measurement of substrate and product concentrations in both enzyme systems. Specific activities of the order of 10^{-2} μ -mole/30 min./mg. of protein/ml. of reaction mixture are reported.

3. Pyridoxamine phosphate oxidase was inhibited by dialysis, and further inhibition was caused by pyruvate, α -oxoglutarate and oxaloacetate at a concentration of 0.02 mM. Inhibition of activity by mepacrine suggests that a flavin coenzyme is involved.

4. Pyridoxal phosphate phosphatase was also found to be highly active in mammalian 'alkaline'-phosphatase preparations. The *Escherichia coli* extracts used exhibited high 'acid'-phosphatase and low 'alkaline'-phosphatase activities when disodium phenyl phosphate was used as substrate. No dephosphorylation of pyridoxamine phosphate could be detected.

5. The presence of glucose in *Escherichia coli* growth medium increased the relative amount of phosphatase compared with oxidase activity.

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The Sedimentation Characteristics of Deoxyribonucleic Acid from Human Tissues

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Polli & Shooter (1958) have presented the data obtained from sedimentation experiments with preparations of deoxyribonucleic acid from normal human spleen and leucocytes and from leucocytes

of patients with chronic myeloid and lymphatic leukaemia. It was found that the average sedimentation coefficients at infinite dilution for preparations from leucocytes of lymphatic leukaemia were higher than those observed for preparations from normal leucocytes. Different preparations

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from these two last-named sources gave reproducible results, whereas the average sedimentation coefficients at infinite dilution for the preparations from leucocytes of myeloid leukaemia exhibited a much greater scatter, ranging from 27s, the value observed for deoxyribonucleic acid from normal leucocytes, to 22.8s. Another group of 23 preparations has now been studied in an attempt to confirm and define more precisely these differences in sedimentation characteristics.

Since it has been shown that the presence of small amounts of residual protein in preparations of deoxyribonucleic acid from calf thymus can markedly affect the distribution curves of sedimentation coefficients (Butler, Phillips & Shooter, 1957), the amount and composition of the residual protein in some of the present preparations have been determined. Attempts have also been made to disrupt any protein-linked aggregates which might be present in the preparations, by treating them with proteolytic enzymes.

EXPERIMENTAL

Blood specimens from leukaemic patients were collected by exchange transfusion and the leucocytes isolated in the manner described by Polli (1951). The sample of normal leucocytes was obtained from the pooled blood of four donors. Placentae were obtained during Caesarean operations and were immediately frozen in solid CO_2 : in all four cases studied the tissue can be regarded as normal. The spleen samples were also frozen quickly in solid CO_2 after removal from the patient. Deoxyribonucleic acid (DNA) was prepared from these cells and tissues by the detergent method of Kay, Simmons & Dounce (1952) as modified by Chargaff (1955). Fibrous tissue was removed from the homogenates of placenta and spleen samples by filtration through gauze during the first three stages of washing with 0.14M-NaCl.

The samples of DNA were dissolved in 0.2M-NaCl-0.025M- KH_2PO_4 -0.013M-NaOH, pH 7.5. The sedimentation experiments were performed in a Spinco model E ultracentrifuge fitted with the u.v.-light-absorption optical system. For six of the samples experiments were performed at four concentrations in the range 0.008-0.001% of DNA and curves giving the distribution of sedimentation coefficients were calculated in the manner described by Shooter & Butler (1956). The concentration dependence of the sedimentation coefficients was the same for all six samples, the average sedimentation coefficient at infinite dilution being 1.0-1.2s greater than the average sedimentation coefficient observed at a concentration 0.001-0.0015% of DNA. Experiments with the remaining samples were therefore performed at only one concentration in the range 0.001-0.0015%. The average sedimentation coefficients quoted in the tables are the values at the 50% concentration point on the distribution curves. These values are about 10% less than the weight-average sedimentation coefficients (Butler, Laurence, Robins & Shooter, 1959).

To determine the effect of proteolytic enzymes on the preparations trypsin (Armour: crystalline), and chymo-

trypsin (L. Light and Co. Ltd.), ficin and papain (Sigma Chemical Co.: crystalline) were added to solutions of DNA in 0.2M-NaCl pH 7.5, and the sedimentation characteristics were determined after storing for 3-16 days at 4° and then for a further 3-6 hr. at room temperature. In each case a weight of enzyme was added equal to the weight of DNA present in the solution.

The amino acid composition of the residual protein in the preparations was determined in the manner described by Phillips & Johns (1959). The protein content of the preparations quoted in Table 1 represents the sum of the amounts of amino acids which have been determined; no allowance has been made for tryptophan, which is destroyed during the acid hydrolysis, or for glycine, since most of this amino acid observed in the chromatograms is the product of the degradation of adenine and guanine.

RESULTS

The information obtained from the analysis of the residual protein present in eight of the DNA preparations is given in Table 1. It may be noted that the residual proteins in these preparations contain greater amounts of aspartic acid and glutamic acid than of the basic amino acids lysine and arginine. In this respect the analyses resemble those reported for the residual protein in preparations of DNA from calf thymus (Butler *et al.* 1957) and from mouse and rat spleen and liver (Kirby, 1959). The amount and composition of the protein in the preparations from human cells and tissues vary from sample to sample and there is no obvious correlation with the source of the DNA, i.e. there is no evidence that the residual protein represents some part of the nucleoprotein complex characteristic of a particular cell or tissue. Solutions of all the eight preparations of DNA mentioned in Table 1 have been treated with ficin, papain, trypsin and chymotrypsin and in all cases it has been found that the distribution curves of sedimentation coefficients of control and enzyme-treated solutions did not differ by more than ± 0.5 s, a variation well within experimental error. With some of the preparations of DNA from calf thymus investigated by Butler *et al.* (1957) it was found that treatment with proteolytic enzyme did not affect the average sedimentation coefficient, but the presence of high-molecular-weight, poly-disperse aggregates of DNA cross-linked by protein could be deduced from the observed increase in the amount of u.v.-absorbing material sedimenting in the range 10-50s, which was produced by the enzyme treatment. No evidence of this type of effect was found with any of the preparations of DNA used in the present work.

The average sedimentation coefficients for all the preparations investigated are presented in Table 2. In all cases the distribution curves covered a range from 10 to 50s and no significant differences in the shapes of the curves were observed.

Table 1. *Amino acid composition of the residual protein of preparations of deoxyribonucleic acid*

Results are expressed as moles of amino acids/100 moles of all amino acids found. Arginine and histidine were not assayed in samples E, F and G. Cystine, methionine and hydroxyproline were not seen in any hydrolysate: glycine, tryptophan and amide were not determined.

DNA preparation	Leucocytes of myeloid leukaemia				Leucocytes of lymphatic leukaemia		Spleen	Placenta
	E	F	G	D	B	C		
Alanine	6.3	6.7	6.3	8.1	7.2	6.9	6.3	8.0
Aspartic acid	25.8	9.7	10.7	10.5	11.5	25.5	20.6	10.4
Glutamic acid		9.7	10.7	16.1	13.6			16
Valine	26.4	29	28	6.5	27.4	8.3	26.2	6.4
Leucines				17.7		15.9		17
Phenylalanine	9.4	12	12.5	7.3	6.5	5.5	7.5	7.2
Tyrosine	0	0	0	+	0	0	0	+
Serine	14.5	14.3	12	8.1	9.3	10.3	8.7	8
Threonine	6.9	10	8.2	5.6	4.6	4.8	11.2	5.6
Proline	6.9	8	10.7	3.2	5.8	4.8	6.9	3.2
Lysine	3.8	0	0	5.6	3.6	10.3	6.9	5.6
Arginine	—	—	—	8.9	5.1	5.5	3.7	8
Histidine	—	—	—	2.4	5.1	2.1	1.9	4
Total protein (as % of DNA)	0.52	0.29	0.67	0.85	0.63	0.99	0.75	0.99
Average sedimentation coefficient (s)	26.8	27	25.6	23.6	27.6	30	26	26

Table 2. *Average sedimentation coefficients for solutions of deoxyribonucleic acid*

Sedimentation experiments were performed with solutions of DNA in 0.2M-NaCl, pH 7.5, at concentrations in the range 0.001–0.0015%. Figures in parentheses are taken from Polli & Shooter (1958) and have been corrected to a DNA concentration of 0.0015%. Preparations which have been analysed for residual protein content are indicated by the same letter as was used in Table 1.

Source	Clinical condition	Average sedimentation coefficient (s)
Placenta	Normal	26.0 A, 25.0, 27.0, 26.2
Leucocytes	Normal	26.0 (26.0, 25.8)
	Lymphatic leukaemia	27.6 B, 30.0 C, 28.3, (29.8, 28.5, 27.4)
	Myeloid leukaemia (untreated patients)	23.1, 23.6 D, 27.0, (21.8, 21.8)
	Myeloid leukaemia:	
	Before treatment with busulphan	(26.0)
	After treatment with busulphan	(23.5)
	Myeloid leukaemia:	
	First blood sample	26.8 E
	Second blood sample (7 days after first sample)	27.0 F
	Third blood sample (after treatment with busulphan; 10 days after second sample)	25.6 G
Spleen	Myeloid leukaemia treated with busulphan	26.2
	Acute leukaemia	24.6, 24.0, 25.4
	Normal	(25.2, 25.8)
	Banti's disease	23.0
	Banti's disease, two preparations from the same tissue	23.1, 22.2
	Hereditary spherocytosis	25.0
	Myeloid leukaemia	26.0 H
	Brill-Simmers disease	(28.8)

DISCUSSION

It is evident from the results given in Table 2 that the preparations of DNA that have been studied exhibit quite marked differences in sedimentation characteristics. The major problem is to determine whether these differences reflect real variations in the degree of polymerization of the DNA (cf. Butler *et al.* 1959) or whether they arise from cross-linking of the DNA particles to different

extents by the residual protein in the preparations. The attempts that have been made to degrade such aggregates which might be present in our preparations, by treatment with four different proteolytic enzymes, have all proved fruitless. It may also be noted (Table 1) that there is no correlation between total amount of residual protein and the average sedimentation coefficient. It would therefore seem, on the basis of these negative results, that the DNA preparations do not

contain detectable amounts ($< 5\%$) of aggregates cross-linked by protein and hence that the protein molecules or polypeptides must be bound along the lengths of single DNA particles. The amount of protein present is so small that if it is bound in this way it would have an insignificant effect on the sedimentation characteristics of the DNA. We may assume therefore that the differences observed reflect real differences in the degree of polymerization of the DNA.

The average sedimentation coefficients observed for the new preparations of DNA from leucocytes of lymphatic leukaemia and from normal leucocytes agree well with those reported by Polli & Shooter (1958). Preparations of DNA from placenta give distribution curves of sedimentation coefficients very similar to those found for DNA from normal leucocytes. Taking the results for the preparations from placenta and normal leucocytes as characteristic of normal tissues and cells, it is found that the average sedimentation coefficients for the preparations from the leucocytes of lymphatic leukaemia are significantly higher than normal ($P < 0.01$). With the molecular-weight determinations of Butler *et al.* (1959) as a basis it can be calculated that DNA from leucocytes of lymphatic leukaemia has a mean molecular weight about 30% higher than normal.

In agreement with the results reported by Polli & Shooter (1958) it has been found that the average sedimentation coefficients for different preparations of DNA from leucocytes of myeloid leukaemia vary considerably. Four of the preparations gave results indistinguishable from the normal and three had lower average sedimentation coefficients. A preparation of DNA from the spleen of a patient with myeloid leukaemia also gave a result similar to the normal. In the one set of preparations available no evidence of any change in the characteristics of DNA obtained from leucocytes of one patient was observed in 7 days (Table 2). After treatment with Myleran (busulphan) the DNA obtained from the leucocytes of the same patient showed a slight decrease of the average coefficient. One further preparation from leucocytes of a patient treated with busulphan has been studied but this did not differ from the normal. No further clinical or histological evidence can be cited which could account for the variations found with this group of preparations from leucocytes of myeloid leukaemia.

The three preparations from the leucocytes of patients with acute leukaemia are very similar, the average sedimentation coefficients being lower than normal. This is too small a group to decide whether the results are characteristic of the disease.

The data obtained for the three preparations of DNA from spleen are of some interest since these

diseases would not be classified as malignant. The average sedimentation coefficients for the two preparations from spleen associated with Banti's disease are considerably lower than normal, the decrease in mean molecular weight being about 30%. The preparation from the spleen of the patient with hereditary spherocytosis is close to normal.

The results of the present work confirm the observations of Polli & Shooter (1958) and demonstrate that the physical properties of preparations of DNA from diseased human tissues and cells may be very different from preparations from normal sources, the mean molecular weights of the preparations varying by as much as $\pm 30\%$. We cannot be sure, however, that the DNA prepared is identical with the DNA in the living cell; degradation may occur during preparation, particularly if there is an abnormally high concentration of deoxyribonuclease in the diseased cells. To find out more about the origin and significance of the variations observed requires detailed studies of the DNA synthetic and degradative mechanisms of the cells.

SUMMARY

1. Distribution curves of sedimentation coefficients at a concentration 0.001–0.0015% have been determined for a group of preparations of deoxyribonucleic acid from normal and diseased human tissues and cells.

2. Preparations from normal sources gave average sedimentation coefficients of 25–27s; all preparations from leucocytes of lymphatic leukaemia were found to have higher than normal sedimentation coefficients, whereas in preparations from leucocytes of myeloid leukaemia the results were the same as, or less than, normal.

3. Sedimentation coefficients lower than normal have been observed with preparations from spleens associated with Banti's disease.

4. Eight of the preparations have been analysed to determine the amount and composition of the residual protein. There appears to be no correlation between these results and the source of the preparation of deoxyribonucleic acid or its sedimentation characteristics.

5. The variation in sedimentation behaviour observed between different samples does not appear to be due to the presence of varying amounts of protein cross-linked aggregates, since treatment with any of the enzymes trypsin, chymotrypsin, ficin or papain has no detectable effect.

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Metabolism of Polycyclic Compounds

18. THE SECRETION OF METABOLITES OF NAPHTHALENE, 1:2-DIHYDRONAPHTHALENE AND 1:2-EPOXY-1:2:3:4-TETRAHYDRONAPHTHALENE IN RAT BILE*

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A metabolite of sulphobromophthalein has been detected in the bile of rats (Combes, 1959) and of human subjects (Grodsky, Carbone & Fanska, 1959), which gives rise to glycine and glutamic acid on acid hydrolysis. Grodsky *et al.* (1959) suggested that the metabolite is a glutathione conjugate. In a study of the metabolism of naphthalene, 1:2-dihydronaphthalene and 1:2-epoxy-1:2:3:4-tetrahydronaphthalene by rat-liver slices (Booth, Boyland & Sims, 1960*b*; Booth, Boyland, Sato & Sims, 1960*a*), it was found that these compounds are converted into glutathione conjugates, which yield the corresponding cysteine conjugates on incubation with rat-kidney homogenates. The present work shows that the bile of rats treated with either naphthalene, 1:2-dihydronaphthalene or 1:2-epoxy-1:2:3:4-tetrahydronaphthalene contain conjugates of glutathione, cysteinyl glycine, cysteine and *N*-acetylcysteine in addition to other metabolites previously reported as urinary excretion products.

EXPERIMENTAL

Materials. 1:2-Dihydronaphthalene and 1:2-epoxy-1:2:3:4-tetrahydronaphthalene were prepared as described by Boyland & Sims (1960). *S*-(1-Naphthyl)glutathione,

S-(1-naphthyl)-L-cysteine and 1-naphthylmercapturic acid were prepared by the methods of Booth *et al.* (1960*b*). A product which appeared to be mainly *S*-(1-naphthyl)-L-cysteinylglycine was obtained by boiling *S*-(1-naphthyl)-glutathione (100 mg.) with water (150 ml.) under reflux for 48 hr. The solid obtained on evaporation of the water was extracted with saturated aq. NaHCO₃ and the extract acidified with acetic acid to yield a light-brown powder (25 mg.). On paper chromatograms developed with solvent 1 described below, the solid yielded one large and two small spots, the latter being indistinguishable from *S*-(1-naphthyl)glutathione and *S*-(1-naphthyl)-L-cysteine. The large spot was eluted from chromatograms and treated by the methods described below to yield compounds indistinguishable from *S*-(1-naphthyl)-L-cysteine and glycine after hydrolysis with HBr and from alanine and glycine after hydrogenolysis with Raney nickel followed by acid hydrolysis. No other amino acids were detected. A similar result was obtained when *S*-(1-naphthyl)glutathione was heated with *n*-H₂SO₄ at 100° for 1 hr.

S-(1:2:3:4-Tetrahydro-2-hydroxy-1-naphthyl)glutathione was prepared by the method of Booth *et al.* (1960*a*) and *S*-(1:2:3:4-tetrahydro-2-hydroxy-1-naphthyl)-L-cysteine and *N*-acetyl-*S*-(1:2:3:4-tetrahydro-2-hydroxy-1-naphthyl)-L-cysteine by the methods of Boyland & Sims (1960). A preparation which appeared to be a mixture of the stereoisomeric forms of *S*-(1:2:3:4-tetrahydro-2-hydroxy-1-naphthyl)-L-cysteinylglycine was obtained as a gum when L-cysteinylglycine was treated with 1:2-epoxy-1:2:3:4-tetrahydronaphthalene under the conditions used by Booth *et al.* (1960*a*) for the reaction of the epoxide with glutathione. The gum formed one spot on paper chromatograms, and

* Part 17: Booth, Boyland, Sato & Sims (1960*a*).