would seem to indicate conclusively that steroid sulphatase and sulphatase C are different enzymes. The results in Table 3 show that from a quantitative point of view the amount of steroid sulphatase present in rat liver is negligible compared with the amounts of arylsulphatases present, but on the other hand the steroid sulphatase activity is as great as, or greater than, the ability to synthesize DHAS. The livers of male rats have a considerably higher steroid sulphatase activity than do the livers of female animals. This sex difference also occurs with sulphatase C, as shown in Table 3, and as has been previously noted by Dodgson, Spencer & Thomas (1953). It is of interest to compare these figures for the sulphatase activity of rat liver with the ability to produce SO_4^{2-} ion from cysteine, via cysteinesulphinic acid. According to Awapara (1955) rat liver can form approximately $3 \mu\text{M-SO}_4^{2-}$ ion/g./hr. through this pathway. If this be the case then it is obvious from the values in Table 3 that the rate of production of SO_4^{2-} ion by sulphatase activity could be much greater than its rate of production from cysteine. No information is available on the actual activities *in vivo*.

From a practical point of view mammalian steroid sulphatase is probably of little interest. Although it has been used to hydrolyse urinary steroid sulphates (Langecker, 1956), its use for this purpose must be limited by the specificity of the enzyme, as with the steroid sulphatase of *P. vulgata*. Further, the specific activity of the available preparations of mammalian steroid sulphatase is very much less than that of the molluscan enzyme (in the assay described above the ox enzyme is used in a concentration of 2–3 mg./ml. whereas the molluscan enzyme is used in a concentration of only about 0.1–0.2 mg./ml.). For many purposes therefore the molluscan enzyme would be more suitable, as for instance the recovery of steroids is less satisfactory in the presence of large amounts of protein. The further purification of the mammalian enzyme is made difficult by its insoluble nature.

SUMMARY

1. The presence of a steroid sulphatase in ox and rat liver has been confirmed. The enzyme is localized in the microsomes and has not been obtained in a soluble form.

2. Steroid sulphatase hydrolyses the 3β -sulphates of the 5α - and Δ^5 -series of steroids.

3. The optimum substrate concentration is 0.2 mM-dehydroepiandrosterone sulphate and the optimum pH is 7.8 in 2-amino-2-hydroxymethylpropane-1:3-diol buffers.

4. Steroid sulphatase is distinct from arylsulphatase C although the two enzymes are closely associated.

5. Quantitatively the amount of steroid sulphatase in rat liver is negligible compared with the amounts of arylsulphatase present. The enzyme is present in amounts comparable with those of the system synthesizing steroid sulphate.

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Histones from Normal and Malignant Cells

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The functions of the histones in the cell nucleus have been the subject of considerable speculation, prompted by the reports of the tissue specificity of the histones (Stedman & Stedman, 1950a). Although a difference between the basic protein in

the sperm and somatic tissues of the salmon and fowl has been amply demonstrated (Stedman & Stedman, 1950a; Daly, Mirsky & Ris, 1950), differences between various somatic tissues have not been so well defined. Cruft, Mauritzen & Stedman (1954) reported that the histones from tumours had a lower isoelectric point than those

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from normal tissues, as a result of which they precipitated at pH 7.

In the experiments reported below, the histones from normal and ascites-tumour cells of a pure line of mice have been compared. A preliminary report of this work was given at an informal discussion of the Faraday Society, 12 October 1956.

EXPERIMENTAL AND RESULTS

Tissues. For the isolation of cell nuclei, the livers, kidneys and spleens of 20–40 pure-line mice (C₅Hb) were used in each experiment. They were stored in solid carbon dioxide for up to 1 week before treatment. Ehrlich and Landschütz ascites cells were transplanted into the same line of mice and the cells were harvested after 5–7 days. By using ascites cells the danger of including necrotic tissue in the preparation was avoided. Although the ascitic fluid occasionally contained a considerable number of erythrocytes, the percentage of nucleated non-tumour cells was low (about 10%). A few preparations from Walker carcinoma tissue of the rat were studied.

Isolation of cell nuclei. The cell nuclei were isolated from the tissues in dilute acetic acid (Stedman & Stedman, 1950*b*) or 0.005 M-citric acid. In both procedures the tissues were initially homogenized for 30 sec. in a Waring Blendor and the homogenate was filtered through 120-mesh metal gauze. The nuclei were separated from the cell debris by centrifuging, and the course of the purification was followed by light microscopy, the orcein-fast green stain (Kurnick & Ris, 1948) being used. Samples of several preparations were fixed in buffered osmic acid, embedded in butyl methacrylate and sectioned for examination in the electron microscope (Davison & Mercer, 1956).

Some preparations of nuclei were dried by successive washes in alcohol, acetone and ether.

Extraction of the histones. To extract the histones, the nuclei were ground in a ball mill at 0° with 0.25 N-HCl for 3–16 hr. The debris was centrifuged down, and the supernatant was dialysed against water or 0.5 N-acetic acid, at 0°. The extracts were freeze-dried or preserved frozen at –30°.

A list of the preparations from mouse tissues is given in Table 1. The proportion of the nuclear nitrogen recovered in the histone extracts is indicated. When the acid extracts were dialysed to pH 6.5 a precipitate formed, as described

by Cruft *et al.* (1954): the fraction insoluble at pH 6.5 will be referred to as the 'neutral precipitate', and the percentage of the total acid extract which this comprised is also listed in Table 1. The proteins in the supernatant at pH 6.5, which in every case studied formed the greater proportion of the extracted protein, will be referred to as the 'neutral supernatant'. It was found that the freeze-dried proteins became less easy to dissolve on keeping, and it was usually necessary to add 0.5 N-acetic acid to achieve a clear solution. Some of the preparations could not be dissolved completely, despite the use of acid, and these were centrifuged before examination. Later preparations were preserved in 0.5 N-acetic acid at –30°. No quantitative analyses were made on samples which could not be completely redissolved in dilute acetic acid.

Electrophoresis. The proteins were dissolved to give a 0.7–1.0% solution, and the solutions were dialysed overnight against the appropriate Miller & Golder (1950) buffers at 0°. Electrophoresis was performed in a Perkin-Elmer apparatus.

In every case studied the neutral supernatant fractions migrated to the cathode at all pH values up to 8. Most of the diagrams resembled those from calf-thymus histone, i.e. two peaks below pH 6 and two or three above (Figs. 1, 2). The Walker carcinoma histone of the rat also moved to the cathode at pH 8.76 (Fig. 3). No experiments were performed above pH 9 since the solutions became cloudy and commenced to precipitate.

Although these histone extracts appeared similar to the calf-thymus histones the migrating peaks showed a distinct trailing which was probably indicative of some non-histone proteins in the preparations.

Chromatography. The histones were chromatographed on carboxymethylcellulose columns (Peterson & Sober, 1956). The adsorbent was buffered at pH 6.5 and the proteins were eluted with a NaCl concentration gradient (Davison, 1957). The distribution of the proteins in the effluent fractions was determined by the absorption of the solutions at 2780 Å or by the reaction with ninhydrin (Moore & Stein, 1954).

Except for the liver no. 2 and the kidney no. 1 preparations, all the neutral supernatant solutions listed in Table 1 gave elution diagrams (measured by the ninhydrin reagent) containing four major peaks more or less clearly resolved. A small unretarded peak could also be resolved if the application of the concentration gradient was delayed. In different preparations the relative sizes of the peaks varied

Table 1. *Mouse-histone preparations studied*

Tissue	Nuclei isolation medium	Nuclear nitrogen recovered in histone extract (%)	Histone extract precipitated at pH 6.5 (%)
Ehrlich ascites tumour	1 Acetic acid	15	—
	2 Citric acid	10	27
	3 Citric acid	24	25
	4 Acetic acid	—	—
Landschütz ascites tumour	1 Acetic acid	27	23
	2 Acetic acid	33	11
Liver	1 Citric acid	21	—
	2 Acetic acid	34	8
Spleen	1 Citric acid	26	—
	2 Acetic acid	—	—
Kidney 1	Acetic acid	29	20

(Fig. 4). A chromatogram of a Landschütz ascites-tumour extract was very similar (Davison, 1957). No characteristic features were detected in which the tumour-protein preparations differed from those from normal tissues.

The liver and kidney preparations mentioned above showed only three peaks, and the diagrams appeared to resemble closely those from calf-thymus histones (Davison, 1957). This suggests that the calf-thymus histones and the pure mouse histones have very similar chromatographic

Fig. 1. Ascending (a) and descending (b) diagrams of Ehrlich ascites-histone no. 2 during electrophoresis at pH 4.60 (ionic strength 0.2, 18 mA, 105 v, 2 hr.).

Fig. 2. Ascending diagrams of (a) calf-thymus histone and (b) mouse Ehrlich ascites-histone no. 3, during electrophoresis at pH 4.38. (Ionic strength 0.2, (a) 15 mA, 80 v, 3 hr.; (b) 15 mA, 72 v, 2 hr.)

Fig. 3. Ascending diagram of Walker carcinoma histone of the rat, under electrophoresis at pH 8.76. (Ionic strength 0.2, 15 mA, 80 v, 140 min.)

Fig. 4. Elution diagrams at pH 6.5 of (a) Ehrlich ascites no. 4 supernatant, (b) mouse-liver no. 1 supernatant, and (c) mouse-spleen no. 2 supernatant.

properties, and the added complexity in most of the diagrams is due to the presence of non-basic proteins in the extracts. The fact that the electrophoresis studies showed the major part of all the neutral supernatants to migrate like histones is not belied by the large size of the chromatographic peaks which precede the two characteristic histone peaks emerging last (e.g. Fig. 4a). The leading components may be complexes between histones and other proteins, and in any case the ninhydrin colour yield of a protein is not proportional to the weight of the protein.

Analysis: comparison of neutral precipitate and supernatant. The Ehrlich ascites-histone no. 3 was dialysed to pH 6.5 and the precipitate and supernatant proteins were separated. They were hydrolysed in sealed tubes with 6N-HCl at 110° for 24 hr. The amino acid compositions of the hydrolysates were determined by the fluorodinitrobenzene method of Levy (1954), modified by employing the coupling conditions suggested by Fraenkel-Conrat & Singer (1956). The chromatography was also modified at the recommendation of Dr D. M. P. Phillips by replacing the Whatman no. 1 by no. 7 paper, and by using *tert.*-amyl alcohol saturated with buffer, pH 6 (Blackburn & Lowther, 1951), in place of xylene-ammonia as the first solvent. The latter was inconvenient in a laboratory where ninhydrin was being used.

The amino acid compositions of the neutral precipitate and supernatant fractions are given in Table 2. Each analysis is the mean of four determinations. Arginine and histidine were not measured. The proportions are not corrected for any destruction of the amino acids during hydrolysis.

The substantially higher proportion of the dicarboxylic acids in the precipitate was not unexpected, but the high lysine content suggested that some histone might be present in the precipitate as well as in the supernatant.

Neutral precipitate. The neutral precipitate from both tumour and normal tissue extracts could be redissolved almost completely in 0.5N-acetic acid. The pH of this solution could be adjusted to 4 before reprecipitation commenced. Exploratory experiments showed that this solution contained two fractions which could be separated by chromatography. The neutral precipitate from the Landschütz ascites-extract no. 2 was shaken in 0.5N-acetic acid and the fraction which remained insoluble was centrifuged off (F_1). The supernatant solution was dialysed to pH 3 and chromatographed on a carboxymethylcellulose column buffered at the same pH. The absorption of the effluent solutions at 2780 Å was measured and the elution diagram was plotted (Fig. 5). The first peak was due to low-molecular-weight material. The fractions corresponding to the two major peaks (respectively F_2 and F_3) were grouped, the pH was adjusted to 10 with NaOH, and 3 vol. of acetone were added. After standing overnight at 0° the precipitates which had formed were centrifuged down and hydrolysed. The amino acid composition of each fraction was determined by the fluorodinitrobenzene method. The results are shown in Table 3. F_1 , F_2 and F_3 were present in the approximate ratios of 2:1:6, by wt. It is doubtful, however, if the whole of F_2 was precipitated in the alkaline conditions employed.

An attempt was made to precipitate the non-histone component of the neutral precipitate by using 0.34M- HgSO_4 in 0.88M- H_2SO_4 (Mirsky & Pollister, 1946). Although a precipitate containing more dicarboxylic residue than

basic residues was obtained, it represented only a very small fraction of the total protein. This would suggest that some of the acid material is of relatively low molecular weight.

Table 2. *Amino acid composition of neutral precipitate and supernatant from acid extract of ascites-tumour cells*

Composition is expressed in molar ratios (no. of residues/100 residues). Arginine and histidine were not determined.

	Supernatant	Precipitate
Alanine	12.5	10.3
Aspartic acid	17.9	25.6
Glutamic acid		
Cystine	0.2	0.3
Glycine	9.6	8.4
Leucine	15.0	14.5
Isoleucine		
Lysine	14.4	10.7
Phenylalanine	2.8	3.0
Proline	4.7	3.9
Serine	6.8	8.1
Threonine	6.8	6.4
Tyrosine	2.4	2.6
Valine	7.0	7.1

Fig. 5. Elution diagram of Landschütz ascites 'neutral precipitate' chromatographed at pH 3.1.

Table 3. *Amino acid composition of fractions from 'neutral precipitate'*

Fractions were obtained from Landschütz ascites-tumour extract. Composition is expressed in molar ratios (no. of residues/100 residues).

	F_1	F_2	F_3
Alanine	13.3	9.75	12.4
Arginine	Low	0.35	9.4
Aspartic acid	8.1	12.6	5.8
Glutamic acid	11.7	11.6	9.8
Glycine	11.2	11.1	9.5
Histidine	1.6	2.4	1.5
Leucine	15.6	15.5	13.8
Isoleucine			
Lysine	11.0	9.3	10.5
Phenylalanine	2.5	3.3	2.3
Proline	3.0	2.4	3.9
Serine	6.6	9.0	7.4
Threonine	7.8	5.9	6.9
Tyrosine	1.5	2.0	1.3
Valine	6.1	5.6	5.7

DISCUSSION

From these experiments it is apparent that ascites-tumour cells contain histones which are similar in electrophoretic and chromatographic behaviour to those from normal mouse tissues. The electrophoretic behaviour of the Walker carcinoma histones of the rat was also unexceptional, and resembled that of the histone from calf thymus. Thus the results obtained by this comparative study do not agree with the report of Cruft *et al.* (1954), who found the whole of the malignant histones which they extracted to be qualitatively distinguished from the normal by their low isoelectric points.

Apart from the first two preparations of tumour nuclei, no consistent difference was noted between the proportions of normal or tumour nuclei which could be extracted with acid. This is in agreement with the preliminary report published by Allfrey, Mirsky & Stern (1955), who found the histone content of normal and malignant leucocytes to be the same. However, Stedman & Stedman (1947) and Hamer (1951) have reported that tumour cells have a low histone content. This discrepancy may be due to the fact that these latter authors used more dilute acid for the extraction of the histones.

Although it is not claimed that the basic and acid proteins isolated from the 'neutral precipitate' are homogeneous, it is felt that the resolution into an acid and a basic fraction suggests that the precipitate may result from the aggregation of the histones in the extract with contaminating acid proteins. This would be expected to occur as the pH of the solution was taken above the isoelectric point of the acid proteins. The small proportion of the neutral precipitate which could not be re-dissolved has a composition characteristic of neither the acid nor the basic components; it may be some contaminant which has become denatured.

The contamination of the acid extracts with non-basic proteins is not surprising. Davison & Mercer (1956) have shown that nuclei isolated in aqueous media have protein (presumably cytoplasmic) adhering to the outside of the nuclear membrane. Adsorption within the nucleus is also possible since the membranes of isolated nuclei are permeable to protein (Anderson, 1953). It is possible that the malignant transformation is accompanied by the replacement of a normal by an abnormal histone, but the latter will apparently not be revealed by a study of the gross characteristics of the histones. It will require a more complete resolution of the individual components of the histone mixture than has yet been achieved.

SUMMARY

1. Nuclei have been isolated from mouse-ascites cells and normal tissues. From normal- and malignant-cell nuclei similar proportions of protein could be extracted by acid.

2. From most of these acid extracts a proportion of protein precipitated on dialysis to pH 6.5.

3. The proteins soluble at pH 6.5 were examined by chromatography and under electrophoresis. The greater proportion of them was histone.

4. The proteins insoluble at pH 6.5 were separated by chromatography into an acidic and a basic fraction.

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