The possibility of activity of the enzyme with Gm-1-P as substrate was investigated by preparing an equilibrium mixture of Gm-6-P and Gm-1-P with rabbit muscle phosphoglucomutase[10]. After heating to destroy the mutase, the solution was added to an acetylating system (Fig. 3). The reaction was then allowed to proceed to completion, and the amount of acetylated amino sugar at various times was determined in aliquots of the reaction mixture before acid hydrolysis (analysis for Ac-gm-6-P), and after acid hydrolysis (analysis for Ac-gm-6-P plus any Ac-gm-1-P which may have been present). Inasmuch as there was no increase produced by acid hydrolysis in the amount of acetylated product, and since the acetylating reaction went at least 90% of the way to completion calculated from the Gm-6-P added, there was no evidence for any acetylation of Gm-1-P. Since the analytical method used to determine Ac-gm-6-P is specific for acetylhexosamines in which carbon atom 1 is not combined in glycosidic linkage, Ac-gm-1-P would be expected to give a positive test only after acid hydrolysis to N-acetyl-glucosamine.

The enzyme which acetylates Gm-6-P does not require Mg^{++} or inorganic phosphate ion. It is not inhibited by fluoride ion. The Michaelis constant for Gm-6-P appears to be about $5 \cdot 10^{-4}$ *M*. The enzyme appears to be saturated at rather low concentrations of acetyl-CoA. The further purification of this enzyme, its properties, and its role in glucosamine metabolism are being studied.

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Fractionation of deoxyribonucleoprotein

CHARGAFF[1] *et al.* recently achieved a fractionation of DNA into fractions of differing base composition by extracting denatured nucleoprotein with sodium chloride solutions of increasing concentration. They suggested that the basis of this fractionation might be the existence of bonds of varying stability between the protein and the DNA which were broken progressively with increasing salt concentration. In a previous communication[2], we showed that nucleoprotein fractions containing nucleic acids having differing base compositions may be obtained by successive extractions with salt solutions of constant strength. It was shown also that the proportions of protein to DNA varied in the nucleoprotein fractions obtained in this way.

A partial analysis has now been made of the proteins present in the calf thymus nucleoprotein fractions obtained by successively extracting chloroform-denatured nucleoprotein with 0.6 *M* sodium chloride solutions. It has been found that the ratios of the basic amino-acids, arginine and lysine, differ considerably in the different fractions (Table I).

In these experiments the saline extracts of chloroform-treated nucleoprotein were obtained as previously described[2]. Normal hydrochloric acid was then added to each extract to a final concentration of *N*/5 HCl. The solutions were allowed to stand several hours and then centrifuged. The resulting supernatant solutions were adjusted to pH 11 with ammonium hydroxide and the histones precipitated by addition of two volumes of acetone. The histones were redissolved in water, dialysed and finally freeze dried. All operations were carried out below 5° C.

It has been shown that relatively small amounts of protein remain with the nucleic acid after one extraction with a strong acid[3]. However, there is no reason to suppose that the strong acid preferentially extracts one histone component (although weak acids do)[3]. The amino-acid compositions of these residual amounts have not so far been determined, but they are unlikely to change the general character of the results given here, which are thought to be of sufficient interest to justify publication.

TABLE I

COMPOSITION OF CALF THYMUS HISTONE FRACTIONS

Fraction	*Molarity of extracting sodium chloride*	*N P of salt extract*	*Histone % nitrogen*	*Valine*	*Proline / Valine*	*Arginine / Valine*	*Lysine / Valine*
1	0.6	16.1	17.5	1.0	2.0	0.6	4.7
2	0.6	5.9	17.2	1.0	2.0	0.7	5.5
3	0.6	3.7	17.7	1.0	1.2	2.0	3.2
4	0.6	3.4	16.8	1.0	0.6	1.5	1.7
5	0.6	3.3	17.5	1.0	0.7	1.4	1.9
6	1.7	3.7	18.2	1.0	0.7	1.7	2.0

The protein fractions were hydrolysed for 24 hours by refluxing in 18% HCl. Residual hydrochloric acid was removed from the hydrolysate by extraction with diethylhexylamine[4]. The amino acids were resolved on paper using a descending solvent of ammonia in secondary butanol[5], and developed with ninhydrin. The yellow proline-ninhydrin complex was eluted in 50% methanol and estimated by measurement of optical density at 350 mμ. The ninhydrin compounds of lysine, arginine and valine were coupled with a copper salt, the resulting pink spots eluted in methanol and their absorption estimated at 504 mμ[7]. Hydrolysates were chromatographed in triplicate alongside solutions obtained by extrapolation.

STEDMAN AND STEDMAN[8] obtained two distinct histones from calf thymus which they showed by the intensity of the spots in paper chromatography to vary considerably in the ratio of lysine to arginine. Using different methods, DAVISON AND BUTLER[9] have also extracted two histones from calf thymus nucleoprotein, which have differing amino-acid compositions. It was shown that one component owed its basic properties to a high percentage of lysine, while the other contained an excess of arginine. The lysine-rich and the arginine-rich components of thymus histone were found to contain approximately equal quantities of valine. We have therefore expressed in Table I the amino-acid compositions of the present fractions in the form of molar ratios, with valine as unity. It has been found that the successive fractions show a decreasing content of proline, while lysine which is very high in the first two fractions is also smaller in the later fractions. The proportion of arginine present increases with successive fractions.

It is evident from these data that the two distinct histone components[8,9] are unequally distributed among the present histone fractions. It would appear that the nucleoprotein contains some molecules in which nucleic acid with a high guanine and cytosine content is linked to a histone containing a high proportion of lysine, while other nucleoprotein molecules contain DNA, rich in adenine and thymine and linked to histone rich in arginine.

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