

indicate that little if any nucleic acid (which absorbs at 260 $m\mu$) is attached to the histones.

In order to eliminate the possibility that free amino acids might have contaminated the protein extracts, separate chromatographic analysis was performed using the methods described above. In *V. faba* 16 different spots, including tryptophan, were found with the butanol solvent as compared with only 12 from histone extracts. In *P. sativum* a large amount of homoserine was detected, confirming earlier studies¹⁷. No traces of these amino acids were found in the histone extracts of either species. Moreover, during the histone extraction procedure a solution containing histones was dialyzed in a cellophane membrane, the membrane pores being small enough to prevent the passage of histone but not of free amino acids. Finally, no deviation in the amino acid composition of histone was observed in over 20 extractions. Therefore it is unlikely that there was any contamination from free amino acids.

Extraction of protein from the roots of *Vicia faba* after the method described by SPORN and DINGMAN⁹ served further to identify the extract as histone. When chromatographic analysis was performed on the hydrolyzed protein, its amino acid composition proved identical to that shown in Figures 2 and 3, which derive from the method of CRAMPTON et al.⁵. Taken together, these results confirm the identification of the extracts as histone-type protein.

On examination of the profiles in Figures 2 and 3 it is evident that there are no significant gross qualitative differences among the amino acid compositions of the plant species and calf thymus. On the other hand, there may be at least one difference between the plant histone and that of animals, for example, calf thymus. Arginine and lysine from *A. cepa* histone were separated by the butanol solvent and their absorbance values recorded by the methods described. Standard curves were established for each and demonstrated a linear relation between absorbance value and μ moles of known quantities of amino acid. With such curves the absorbance values of arginine and lysine obtained from histone hydrolysates were converted to μ moles of amino acid present. Ratios of lysine-arginine, as based on μ moles of amino acid, were thereby calculated for six separate extracts. The mean value from these figures was 1.86 ± 0.08 . This is higher than the value of 1.70 obtained by JOHNS et al.¹³ for calf thymus, and significantly higher than the range of 1.51 to 1.61 obtained by CRAMPTON et al.¹² for other animal tissues.

Conclusions. Although our methods were the same as those of CRAMPTON et al.¹² the difference in lysine-arginine ratios may be due to the possibility that the more tightly bound arginine-rich fraction of histone was not sufficiently removed from the DNA during extraction¹¹. Furthermore, some *nucleolar* basic proteins, which in the pea have

lower levels of lysine and arginine than histones¹⁸, may be present as contaminants in the extracts. Such proteins, or at least a certain fraction of them, may be precursors to ribosomal structural protein. In this connection, the total percentage of basic amino acids in ribosomes is about the same as that in histones^{19,20}, but ribosomes are richer in acidic amino acids. Since the protein extracted from the species reported here was obtained from whole cells rather than isolated nuclei, contamination by ribosomal protein may have occurred. On the other hand, treatment with NaCl in certain of the extraction procedures makes this unlikely. Our data are supported by those of other workers in two respects: amino acid content of histones and lysine-arginine ratios. Recent information on plant cells has indicated lower arginine content in wheat germ, tobacco, and pea embryo histones as compared with thymus histone^{18,21}.

Of major significance, then, are the findings that (1) a method developed for the extraction of nuclear protein from roots of *A. cepa*, *P. sativum*, and *V. faba* provides protein extracts that meet several tests for the identification of histone protein, (2) the amino acid compositions of the histones from the three plant species are identical to each other and to calf thymus histone, and (3) the only significant difference between plant histone and animal histone is that the lysine-arginine ratio is higher in the plant extracts²².

Résumé. Des essais réitérés ont montré aux auteurs que la protéine extraite des racines d'*Allium cepa*, de *Vicia faba* et de *Pisum sativum* est identifiable à l'histone. L'analyse de l'amine acide des hydrolysates des histones ne révèle pas de différences entre les trois espèces végétales citées et le thymus de veau. Dans les végétaux, cependant, le rapport lysine-arginine est plus élevé.

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¹⁸ E. W. JOHNS, in *The Nucleohistones* (Ed.: J. BONNER and P. Ts'o, 1964), p. 52.

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²⁰ C. F. CRAMPTON and M. L. PETERMANN, *J. biol. Chem.* 234, 2642 (1959).

²¹ J. A. V. BUTLER, in *The Nucleohistones* (Ed.: J. BONNER and P. Ts'o, 1964), p. 36.

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PRAEMIA

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