small offspring were born in the same litters after heterospermic insemination, the average difference in birth weight between them was greater than when they were conceived in different litters after homospermic insemination. The enhancement effect is presumed to be due to in utero competition between fetuses resulting in a disproportionately large gain in weight by those having a slight advantage over their litter-mates.

The influence of litter size on fetal size is manifested through a local effect and a general effect[24]. The local effect refers to an influence on the growth of a fetus determined by the presence of other individuals within the same horn. General effect refers to an influence of other individuals in the uterus; it is independent of their distribution between the horns. Thus, an increase in the number within the same horn could have both local and general effects on the fetus, whereas an increase in the number in the other horn could have only a general effect.

McLaren and Michie[25] presented evidence which shows the inadequacy of the classical explanation of the effect of litter size on fetal weight, viz. that there is only a limited pool of nutrient in the maternal blood for which the fetuses compete, so that the more fetuses there are, the less nutrient there is for each. In species in which the young are born at a relatively advanced stage of development, however, competition for nutrients may still play a part, particularly in the later stages of pregnancy. In this respect, the results of Healy et al.[26] were explained by the theory that the chief factor regulating fetal growth is the pressure at which maternal blood is supplied to the placenta. In the rabbit, the number of maternal arterial vessels which penetrate into the maternal placenta varies from 4 to 12 with an average of 8, whereas the number of maternal venous vessels varies from 1 to 6 with an average of 3[27]. This may explain the marked inter- and intra-litter variability in fetal weight[28].

Zusammenfassung. Intrauterine Embryonenüberfrachtung wurde experimentell durch Übertragung von Neuseeländer-Spenderkaninchen in Neuseeländer- und Chinchilla-Empfängerkaninchen, von Chinchilla-Spenderkaninchen in Neuseeländer-Empfängerkaninchen erreicht. Die Empfängertiere wurden 9 Tage nach Begattung zur Prüfung der Implantation und 29 Tage danach zur Beurteilung des fötalen Entwicklungszustandes eröffnet bzw. abgetötet. Chinchilla-Kaninchen zeigten erhöhte Implantationskapazität und verringertes fötales Überleben gegenüber den Neuseeländern.

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Extraction and Analysis of Histone Protein from the Roots of Three Plant Species

Introduction. There is little doubt that histone is an important, although possibly non-permanent, component of chromosomes. Staining techniques[1] and extraction procedures[2] support its presence in the nucleus. Several recent investigations have implicated histone in cell metabolism, and particularly in the regulation of gene action[3], indicating that it plays more than a purely structural role in chromosome organization. Whereas methods for the extraction of histone have been developed for animal cells, its location in plant cells has been revealed primarily by cytochemical methods, and although histone has been characterized in a number of animal species[4], less information of a comparable nature is available for plants.

Consequently, and because of their advantages for chromosomal studies, three plant species, *Allium cepa*, *Pisum sativum*, and *Vicia faba*, have been examined for their histone content. In attempting to determine whether methods used for the isolation of whole histone from animal cells were applicable to plant cells, several techniques were employed. Procedures involving extraction with various concentrations of NaCl or dilute alkali were used to isolate nucleoprotein. Following removal of nucleoprotein, histone was separated from the complex by 0.2 *N* HCl, by chloroform (producing a chloroform-protein gel), or by saturating an aqueous solution of nucleoprotein with NaCl. One of the several methods originally used with calf thymus was found to be best suited for extraction of histone from plant cells[5]. The procedures were mild, rapid, and relatively straightforward.

Materials and methods. Histone extractions were made from seedling roots of *Allium cepa* var. Southport White Globe[6], *Pisum sativum* var. Alaska[6], and *Vicia faba* var. Seville Longpod. Root tips averaging 5 mm in length were excised, weighed, and placed in distilled water at 4°C. (It is essential that extraction steps be performed at temperatures under 5°C.) They were ground in 90 ml distilled water for 30 sec in a blendor at low speed, and disruption of the cells was completed in a glass homogenizer. The method of Crampton et al.[5] was followed in all extractions summarized in Figure 1. For the initial step homogenized roots of pea and onion were treated

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with 0.6 *M* barium acetate, whereas the bean roots were treated with 2.9 *M* NaCl since barium acetate caused the formation of a black homogenate, possibly through tissue destruction. The acetone and ether-washed extract was dried and acid hydrolyzed according to the method of HNILICA and BUSCH[7] for subsequent amino acid analysis. (Approximately 3 mg of histone was obtained per g of tissue.) Since most, if not all, of the tryptophan is destroyed by acid hydrolysis, an alkaline method was used to test for its presence[8]. Calf thymus hydrolysates were prepared from commercially obtained histone extract.

A second method was followed for the extraction of histone from *Vicia faba* roots. This involved the procedures utilized by SPORN and DINGMAN for chicken nuclei[9]. According to their method, histone is defined as protein-soluble in 2 *M* NaCl at pH 1 and, after precipitation in 25% trichloroacetic acid containing 1 *M* NaCl, is soluble in water. (For consistency in analysis of histone and comparisons among the several species, however, all the data reported in the Figures are derived from the method of CRAMPTON et al.[5], as described above.)

Confirmation of the extract as histone was established in several other ways, including comparison with free amino acids. A portion of non-hydrolyzed protein was dissolved in 0.2 *N* HCl and its absorbance measured with a Bausch and Lomb Recording Spectrophotometer (Spectronic 505). Another sample of non-hydrolyzed protein was heated with Millon's reagent, since protamine yields a negative test. Free amino acids were extracted with cold 80% ethanol by a modification of HUNT's method[10].

One-dimensional chromatograms were executed using Whatman No. 1 paper equilibrated with the appropriate solvents. The latter included a mixture of butanol (50 ml), glacial acetic acid (12 ml), and water (50 ml), which separates into two layers. The lower layer was used as the stationary phase and the upper one as the mobile phase. The mobile phase was allowed to travel over the paper for 14 h, the chromatograms were dried, and the solvent run twice more. A phenol-water mixture (100:20) was also used. The stationary phase included this mixture and a separate one of 0.3% ammonium hydroxide. A single 24 h solvent run provided satisfactory separations with phenol-water. Identification of methionine was accomplished with a solvent consisting of 30 ml methanol, 55 ml butanol, 15 ml water, and 1 ml citrate buffer at pH 5. This solvent descended the paper during a 48 h period, the bottom edge of the chromatogram having been serrated to allow an even flow of solvent off the end.

Amino acids were located by dipping the dried chromatograms in ninhydrin solution and drying. For quantitative determinations ninhydrin-treated papers were dried for 22 min at 60°C, and 2 cm squares of the colored spots were cut out for 30 min elution in 3 ml of 71% ethanol. A similar square was cut from a colorless area of the chromatogram to serve as the standard. The amount of light absorbed by the eluate was measured at 575 mμ; the higher the absorbance value, the higher the concentration of amino acid in the eluate.

Identifications of amino acids in an extract were confirmed through comparisons with Rf values of standards, and by overspotting. In the latter instances, a known mixture of amino acids was spotted on paper over a sample of the histone hydrolysate prior to addition of the mobile solvent phase. Comparison of absorbance values of amino acid contents were made between this spot and a sample of the histone hydrolysate as well as with a sample of the amino acid mixture. The result of several such experiments using four amino acid mixtures was the tracing of each amino acid to a specific location (Rf) and its elimination from other positions.

Observations. Of several solvent systems tested two, butanol and phenol, were found to separate all the amino acids characteristic of histone hydrolysates (Figures 2 and 3). Rf values were obtained for 17 amino acids, and 14 of these were confirmed by the overspotting technique. The remaining three, cystine, methionine, and proline, were present only as traces. Histone has been characterized by the absence of tryptophan and the presence of small amounts of the sulfur-containing amino acids methionine and cystine[11]. No tryptophan was found in any plant extract after alkaline hydrolysis and chromatography in 50 butanol:12 acetic acid:50 H_2O, or in a mixture of 9 butanol:1 acetic acid:2.5 water, which clearly separates tryptophan from other amino acids. In agreement with the observations of other investigators[5,12,13], lysine, alanine, and leucine-isoleucine appeared to be in higher concentrations than the other amino acids.

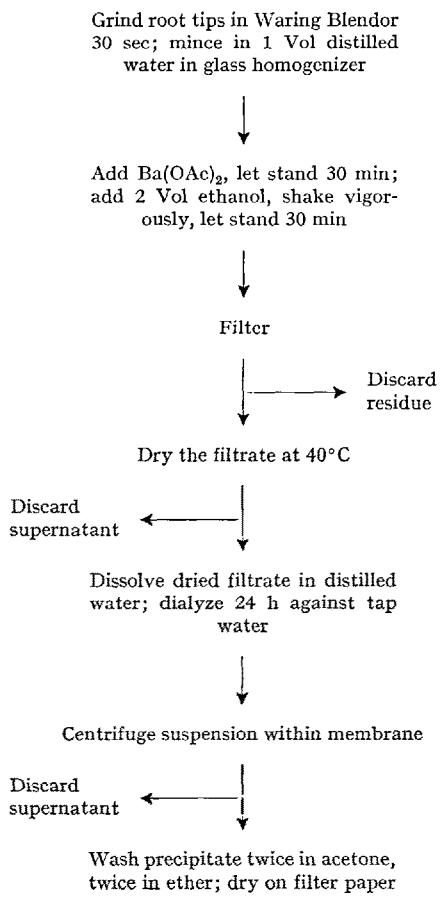

Fig. 1. Method for extracting histone from plant tissue.

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A positive reaction with Millon's reagent was recorded for all hydrolysates. Although this is essentially a test for tyrosine and provides a positive reaction with some non-histone proteins, the result eliminates the possibility that the protein extracted is a protamine. The UV-absorption spectra of histones are generally like those of tyrosine, and the amount of absorbance of histones has been correlated with their tyrosine contents[14,15]. Spectrophotometric curves of the extracted protein peaked at about 275 mμ for all species examined (Figures 4 and 5). Although the absorption peaks of tyrosine and tryptophan are quite similar[16], the absence of tryptophan in any of the extracts strongly suggests that the peak of absorption was due to the presence of tyrosine. Furthermore, the curves

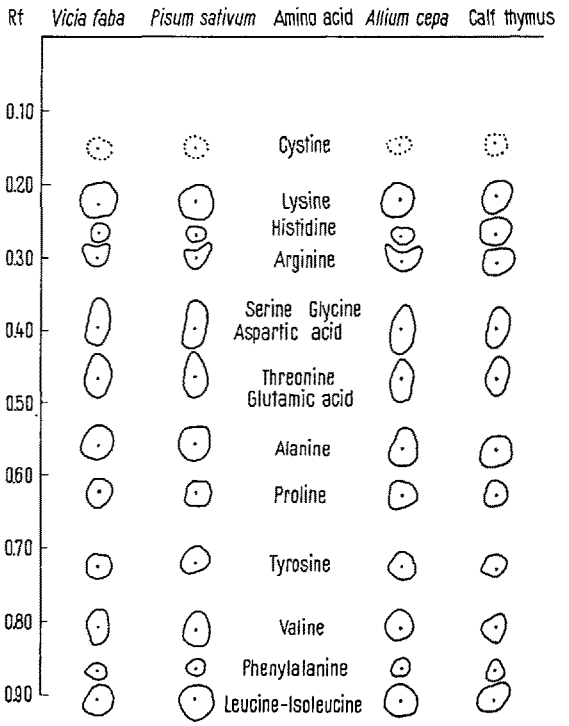

Fig. 2. Comparison of histone hydrolysates from three plant species and calf thymus, as traced and reduced from original chromatograms in butanol solvent (following the method of CRAMPTON et al.[5]).

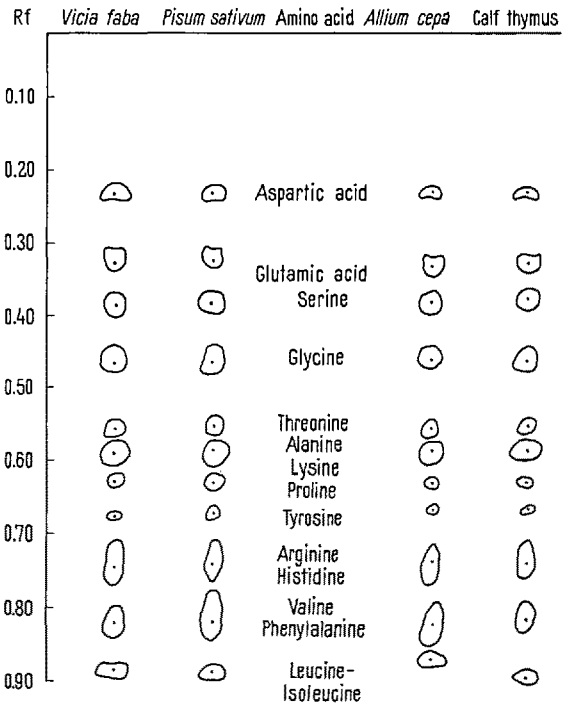

Fig. 3. Comparison of histone hydrolysates from three plant species and calf thymus, as traced and reduced from original chromatograms in phenol solvent (following the method of CRAMPTON et al.[5]).

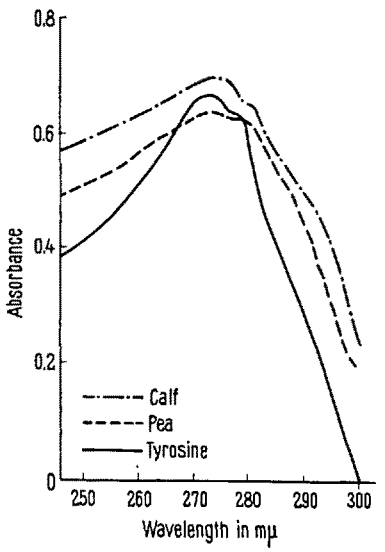

Fig. 4. Comparison of the absorption spectra of L-tyrosine with histone of calf thymus and *P. sativum*.

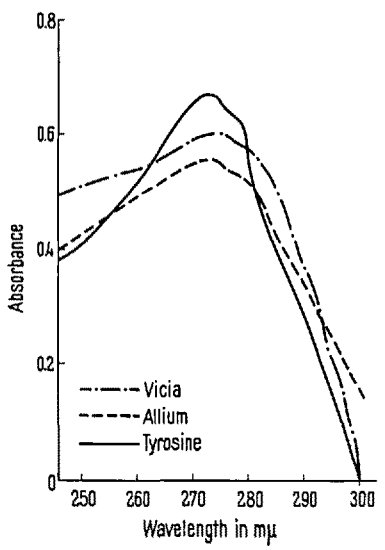

Fig. 5. Comparison of the absorption spectra of L-tyrosine with histone of *V. faba* and *A. cepa*.

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indicate that little if any nucleic acid (which absorbs at 260 mμ) 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 studies[17]. 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 DINGMAN[9] 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 μmoles of known quantities of amino acid. With such curves the absorbance values of arginine and lysine obtained from histone hydrolysates were converted to μmoles of amino acid present. Ratios of lysine-arginine, as based on μmoles 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 extraction[11]. Furthermore, some *nucleolar* basic proteins, which in the pea have lower levels of lysine and arginine than histones[18], 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 extracts[22].

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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PRAEMIA

Eidgenössische Technische Hochschule, Zürich

Ruzicka-Preis für Chemie des Jahres 1965

Im Jahre 1957 konnte mit Schenkungen der schweizerischen chemischen Industrie ein Fonds errichtet werden, aus dem alljährlich ein junger Forscher schweizerischer Nationalität für eine hervorragende veröffentlichte, in der Schweiz oder im Ausland ausgeführte Arbeit auf dem Gebiet der allgemeinen Chemie mit dem Ruzicka-Preis für Chemie ausgezeichnet werden kann. Gemäss Art. 5 des Fondsstatuts dürfen die Kandidaten dem Fondskuratorium von dritter Seite vorgeschlagen werden. Die Vorgeschlagenen dürfen höchstens 45 Jahre alt sein. Der Preis wird auf den Antrag des Fondskuratoriums durch den Schweiz. Schulrat erteilt und jeweils im September überreicht. Nominationen sind unter Angabe der auszuzeichnenden Arbeit bis *spätestens Samstag, den 29. Mai 1965* an den Präsidenten des Schweiz. Schulrates, ETH, Leonhardstr. 33, 8006 Zürich (Schweiz), erbeten.