when the cell has been slightly injured or treated with mild acid. It is entirely possible that what is seen in detail in the electron micrographs is identical with the needle-forms recognized in the optical microscope.

The interesting question in connection with these preliminary results is whether the groups of virus particles exist as such in the intact plant cells, or whether they form subsequent to the crushing of the cells. If the latter is true, they can be considered aggregates of the individual virus particles. But if further work should show that they exist in the intact cells, then the interesting question will arise as to whether or not the virus bundles represent the geometrical growth pattern of the virus.

References

1. BAWDEN, F. C. *Plant viruses and virus diseases.* Waltham, Mass: Chronica Botanica, 1943.
2. CROOK, E. M., and SHEFFIELD, F. M. L. *Brit. J. exp. Path.*, 1946, **27**, 328.
3. OSTER, GERALD, and STANLEY, W. M. *Brit. J. exp. Path.*, 1946, **27**, 261.
4. SIGURGEIRSSON, T., and STANLEY, W. M. *Phytopath.*, 1947, **37**, 26.
5. STANLEY, W. M. *Chronica Botanica*, 1943, **7**, 291.
6. TAKAHASHI, WILLIAM N., and RAWLINS, T. E. *Phytopath.*, 1948, **38**, 279.

Infrared Spectra and the Amide Linkage in a Native Globular Protein

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The presence of absorption characteristic of amides in the 6-μ region of the spectrum has been observed in proteins by several investigators (*1*, *6*, *8*) and has frequently been cited as evidence against the "cyclol 6" structure postulated by Wrinch (*9*) for native protein molecules. The results reported, however, have been for insoluble, fibrous proteins or for samples in the form of a dried film. In view of the pronounced sensitivity of proteins to drying, as well as to deposition in a film, it has always been possible to cast doubt on this evidence for the amide linkage, at least insofar as native globular proteins are concerned, on the ground that the molecule had been denatured during the experiment.

A protein which is relatively stable to the drying process is serum albumin. It has also been observed recently that native serum albumin produces a modification in the visible spectra of many compounds (*3*) and that this ability is lost on denaturation (*5*). It has seemed appropriate, therefore, to examine the infrared absorption of a dried film of crystallized bovine serum albumin and then to test the sample for possible denaturation by seeing whether it is still capable of causing characteristic spectrophotometric shifts.

The infrared absorption spectrum of bovine serum albumin in the 6-μ region is illustrated in Fig. 1, together with the synthetic, water-soluble, polypeptide, polylysine

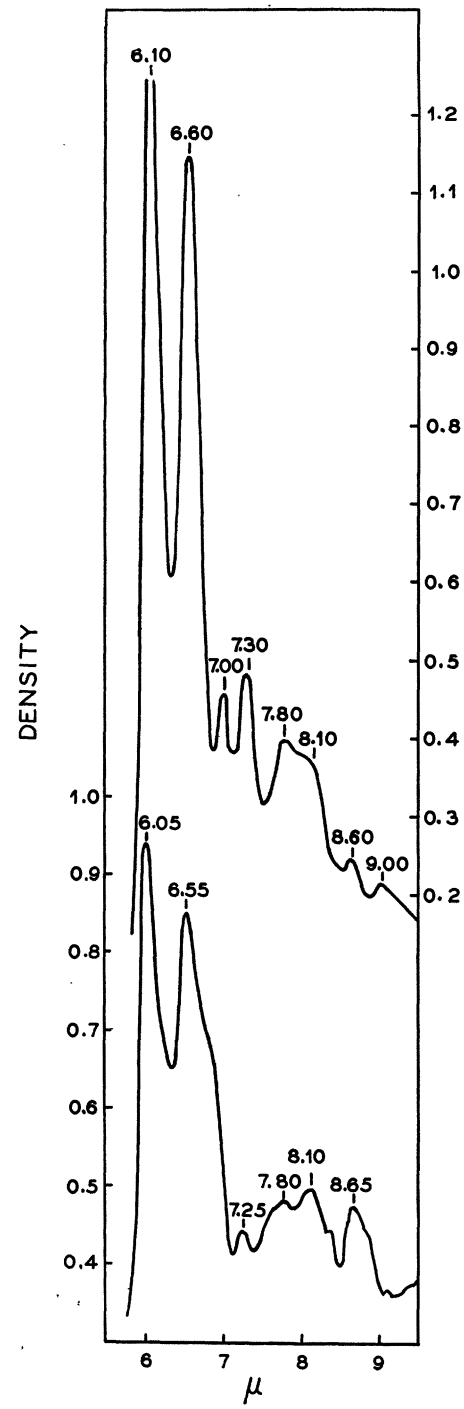

FIG. 1. Infrared absorption spectra of films of bovine serum albumin (top) and polylysine (bottom).

(*2*)[1] for comparison. The C=O peaks at 6.05 and 6.10 μ are essentially identical in position and characteristic of the simple amides in the solid state (*4*, *7*). Thus there can be no doubt that amide carbonyl groups are present

[1] We are very much indebted to E. Katchalski for his generous gift of a substantial sample of polylysine hydroiodide.

in the film of bovine albumin used to obtain the spectrum illustrated.

That this same sample of bovine serum albumin was still in the native form is demonstrated by the data in Table 1. The protein film, dissolved in phosphate buffer at pH 7.6, depressed the optical absorption of methyl orange, as is characteristic of native albumin. Furthermore, the degree of depression, in comparison with that obtained with the control, untreated sample, was roughly in proportion to the quantity of protein. Clearly then, no significant fraction of the albumin was denatured by deposition as a film, drying, or infrared radiation.

TABLE 1

TEST OF NATIVE CHARACTER OF DRIED PROTEIN FILM BY EXAMINATION OF ITS EFFECT ON SPECTRUM OF METHYL ORANGE

Wave length (A)	Depression of optical density by protein	
	From dried film*	Untreated†
4,600	0.063	0.099
4,700	0.097	0.138
4,800	0.117	0.165
4,900	0.134	0.175
5,000	0.123	0.167

* Contained 8 mg of protein.
† Contained 12 mg of protein.

In view of these experiments, there would seem to be little room left for doubt that native, globular proteins contain an amide-type carbonyl group.

References

1. DARMON, S. E., and SUTHERLAND, G. B. B. M. *J. Amer. chem. Soc.*, 1947, **69**, 2074.
2. KATCHALSKI, E., GROSSFELD, I., and FRANKEL, M. *J. Amer. chem. Soc.*, 1947, **69**, 2564.
3. KLOTZ, I. M. *J. Amer. chem. Soc.*, 1946, **68**, 2299.
4. KLOTZ, I. M., and GRUEN, D. M. *J. Phys. colloid Chem.*, 1948, **52**, 961.
5. KLOTZ, I. M., TRIWUSH, H., and WALKER, F. M. *J. Amer. chem. Soc.*, 1948, **70**, 2935.
6. LENORMANT, H. *Compt. rend.*, 1945, **221**, 58.
7. RICHARDS, R. E., and THOMPSON, H. W. *J. chem. Soc.*, 1947, 1248.
8. STAIR, R., and COBLENTZ, W. W. *J. Res. Nat. Bur. Stand.*, 1935, **15**, 295.
9. WRINCH, D. M. *Proc. roy. Soc., Lond.*, 1937, **A161**, 505.

The Effect of Choline-Deficiency on Uterine Activity of Rats

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Peet and Sampson (*7*) reported recently that two post-partum albino rats, one nursing young and one non-lactating, maintained for 39 days on a choline-deficient diet, were found at autopsy to lack completely contractility of the uterus and to have partial atrophy of the uterus and ovaries. Two controls, both nursing young, which received respectively 30 mg and 200 mg of choline every second day, showed normal uterine activity. A yellow, oily condition of the hair on the rats receiving the choline supplements was also noted.

The work reported here was undertaken on a larger group of animals to confirm these observations if possible.

Nine Carworth Farms adult virgin female rats of the Wistar strain were used unmated and nine similar rats were mated. Two of the mated rats died during pregnancy and seven delivered litters, six of these nursing from 7 to 10 young apiece. All virgin rats were placed on the experimental diets at the same time, and each mother was given the experimental diet following delivery of her litter. The diets were distributed among the animals as follows: 3 virgin females on Purina Dog Chow; 3 virgin females, 3 nursing females, and 1 post-partum nonlactating female on the choline-deficient diet; and 3 virgin females and 3 nursing females on the choline-deficient diet plus a choline supplement.

TABLE 1

EFFECTS OF CHOLINE-DEFICIENCY AND LACTATION ON BODY WEIGHT AND ON OVARIAN AND UTERINE WEIGHTS

Rats & diet	No. in group	Average body weight change	Average ovarian weight	Average uterine weight
Nonlactating virgin rats				
Chow controls	3	+38 gm	68 mg	476 mg
Diet controls	3	+37 gm	66 mg	445 mg
Choline-deficient ..	3	+39 gm	59 mg	421 mg
Nonlactating postpartum rat				
Choline-deficient ..	1*	+56 gm	64 mg	438 mg
Nursing rats				
Diet controls	3*	−29 gm	74 mg	244 mg
Choline-deficient ..	3*	−40 gm	69 mg	291 mg

* One animal from each group was removed from the yeast-casein supplement during the last 14 days of the experiment.

The choline-deficient diet developed by Hegsted and associates (*6*) was used in this work as it was in the work by Peet and Sampson.

The 30 mg choline supplement given every second day was chosen for all control animals in preference to the 200 mg since that amount more nearly approximates physiological levels of the substance (*1, 2, 4, 8*). The additional supplement (*7*) of 1 gm dried brewer's yeast and 5 gm extracted casein was provided every second day to all rats on the basal diet except 3, which were deprived of it completely for the last 14 days of the experimental period in an effort to intensify the choline-deficiency.

Vaginal smears were taken daily and rats were weighed at weekly intervals. All rats were killed by cerebral concussion on the 39th day with the exception of the animals on the Dog Chow diet, which were killed at later intervals when control uteri were desired. At the time of experimentation the ovaries and uteri were rapidly excised, cleaned of excess fat, and weighed.