TABLE II

INHIBITION OF LIVER GLUCOSE-6-PHOSPHATASE BY 1,5-SORBITAN-6-P

Enzyme action was followed by the liberation of free sugar[9]. 1,5-Sorbitan is non-reducing.

| *Substrate* | *Substrate concentration moles/liter* | *1,5-Sorbitan-6-P concentration moles/liter* | *Per cent inhibition* |
|---|---|---|---|
| Glucose-6-P | 0.0143 | 0.0214 | 11 |
| Galactose-6-P | 0.0143 | 0.0214 | 45 |
| Allose-6-P | 0.0143 | 0.0214 | 30 |
| L-Sorbose-1-P | 0.00625 | 0.0188 | 50 |

and may reflect competitive inhibition by the glucose ester formed through isomerase action. Neither fructose-1,6-di-phosphate nor 6-phosphogluconate were attacked at appreciable rates.

As judged from the fact that the lowest $K_m$ value is exhibited with glucose-6-phosphate as substrate, this ester appears to be the primary substrate and when present in a mixture of esters, as may occur under physiological conditions, it may be presumed to be preferentially dephosphorylated.

ROBERT K. CRANE

*Department of Biological Chemistry, Washington University School of Medicine, Saint Louis, Missouri (U.S.A.)*

[1] C. DE DUVE, *Ciba Foundation Colloquia on Endocrinology*, 6 (1953) 22.
[2] H. BEAUFAYS AND C. DE DUVE, *Bull. soc. chim. biol.*, (in press).
[3] R. K. CRANE AND A. SOLS, *J. Biol. Chem.*, 210 (1954) 597.
[4] M. SWANSON, *J. Biol. Chem.*, 184 (1950) 647.
[5] H. G. HERS, J. BERTHET, L. BERTHET AND C. DE DUVE, *Bull. soc. chim. biol.*, 33 (1951) 21.
[6] R. K. CRANE AND A. SOLS, in *Methods in Enzymology*, S. P. COLOWICK AND N. O. KAPLAN, Editors, Academic Press, Vol. I (1955).
[7] W. C. SCHNEIDER AND G. H. HOGEBOOM, *J. Biol. Chem.*, 183 (1950) 123.
[8] C. H. FISKE AND Y. SUBBAROW, *J. Biol. Chem.*, 66 (1925) 375.
[9] M. SOMOGYI, *J. Biol. Chem.*, 160 (1945) 69; 195 (1952) 19.
[10] J. H. ROE, J. H. EPSTEIN AND N. P. GOLDSTEIN, *J. Biol. Chem.*, 178 (1949) 839.

Received May 2nd, 1955

## Lupin seed proteins

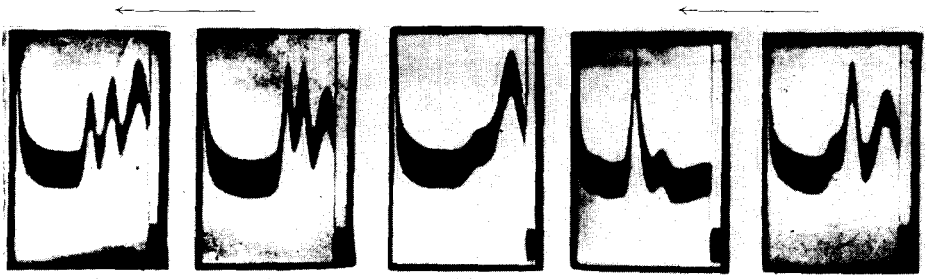
### II. A PHYSICO-CHEMICAL STUDY OF THE PROTEINS FROM YELLOW LUPIN SEED (*LUPINUS LUTEUS*)

In part I[1] the physico-chemical properties of the globulins from blue lupin seed (*Lupinus angustifolius*) were described. The proteins from yellow lupin seed (*Lupinus luteus*) were examined similarly. The present communication describes the results of this examination.

The proteins of defatted and decorticated yellow lupin seed meal were extracted with 10% sodium chloride and then precipitated by adding ammonium sulphate to 85% saturation. Measurements on a Spinco electrically-driven ultracentrifuge revealed (Fig. 1(a)) three major components of sedimentation constants 11.6, 7.4 and 2.0 (designated as the $s_{11.6}$, $s_{7.4}$ and $s_{2.0}$ components) for this preparation in buffer of ionic strength ($I$) = 0.31 and pH = 7.0. This preparation was very yellow in colour and it was therefore considered necessary, for reasons outlined previously[1], to attempt to remove the colouring matter from the meal before the proteins were extracted. Complete removal of these impurities was effected by repeated extraction of the meal with cold water using a Waring blendor.

The proteins of purified lupin seed meal were again extracted with 10% sodium chloride and precipitated by 85% saturation with ammonium sulphate. Fig. 1(b) shows sedimentation diagrams of this preparation in buffer $I$ = 0.31, pH = 7.0. Here again three components ($s_{11.6}$, $s_{7.4}$ and $s_{2.0}$) were observed. When Fig. 1(b) is compared with Fig. 1(a) a decrease in concentration of the $s_{2.0}$ component is noticed. Hence some of the $s_{2.0}$ component was removed by the water extraction used for the purification of the meal. The proteins extracted in the water wash were precipitated

by adding ammonium sulphate to 85 % saturation and when examined in the ultracentrifuge (Fig. 1(c)) did show a major $s_{1.9}$ component and a small concentration only of the $s_{7.4}$ component.

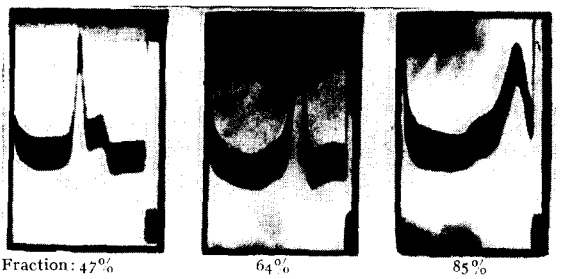


(a) (b) (c) (d) (e)

Fig. 1. Sedimentation diagrams of yellow lupin seed protein.

The proteins of purified lupin seed meal were fractionated according to the procedure described by DANIELSSON[2] for the fractionation of pea globulins. The sedimentation diagrams obtained in buffer $I = 0.31$, pH = 7.0 for the first fraction, *i.e.*, the fraction precipitated by dialysis against buffer $I = 0.25$, pH = 4.7, are shown in Fig. 1(d) and for the second fraction obtained by dialysis against distilled water in Fig. 1(e). Whereas a major $s_{11.6}$ component and small concentration of the $s_{7.4}$ component were found for the first fraction, the second fraction showed two major $s_{7.4}$ and $s_{2.0}$ components. These two components are both precipitated by water dialysis and therefore represent two globulins.

In the next attempt to fractionate the three globulins of yellow lupin seed, successive amounts of saturated ammonium sulphate were added to a 10 % sodium chloride purified lupin seed extract. The fractions obtained at 47 %, 64 % and 85 % saturation with ammonium sulphate, when examined in the ultracentrifuge (Fig. 2), showed respectively major $s_{11.6}$, $s_{7.4}$ and $s_{2.0}$ components.



Fraction: 47% 64% 85%

Fig. 2. Sedimentation diagrams of yellow lupin seed protein fractions.

The 47 % fraction, containing a major $s_{11.6}$ component in phosphate buffer $I = 0.31$, pH = 7.0, was dialysed overnight against borate buffer $I = 0.1$, pH = 8.8 and was thereby almost quantitatively converted into a component of sedimentation constant 7.2. When this solution was dialysed back to $I = 0.31$, pH = 7.0, a major $s_{11.6}$ component formed again. Hence the $s_{11.6}$ component forms a reversible dissociation-association system. The $s_{7.4}$ component on the other hand was stable with respect to these changes and therefore is not the same as the dissociated $s_{7.2}$ component.

When the proteins of yellow and blue lupin seed are compared, it is found that both contain a globulin ($s_{11.6}$ for the yellow and $s_{11.6}$ for the blue), which can be reversibly dissociated into a $s_{7.15}$ component in the case of the blue lupin and $s_{7.20}$ for the yellow. The second globulin ($s_{7.40}$ for the yellow and $s_{7.79}$ for the blue) in both cases was stable to changes of pH and ionic strength. Whereas blue lupin seed contains a low molecular weight fraction ($s_{1.6}$), which could easily be extracted with water and thus represents an albumin, the yellow lupin contains a $s_{2.0}$ component which is composite of an albumin and globulin fraction.

This communication is published with the permission of the South African Council for Scientific and Industrial Research.

F. J. JOUBERT

*National Chemical Research Laboratory,*
*South African Council for Scientific and Industrial Research, Pretoria (South Africa)*

[1] F. J. JOUBERT, *Biochim. Biophys. Acta*, 16 (1955) 370.
[2] C.-E. DANIELSSON, *Biochem. J.*, 44 (1949) 387.

Received March 29th, 1955