Phytochemistry, 1964, Vol. 3, pp. 417 to 419. Pergamon Press Ltd. Printed in England

STUDIES ON THE PROTEINS OF SEEDS OF THE LEGUMINOSEAE—I.

ALBUMINS

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(*Received* 6 *November* 1963)

Abstract—The albumins of seventeen species of the Leguminoseae have been electrophorized on polyacrylamide gels. The potential usefulness of this technique as a tool in taxonomic studies is discussed.

INTRODUCTION

It is generally accepted that the enzymically active proteins of leguminous seeds form part of the water-soluble protein fraction (albumins).[1] In view of this it is surprising that the albumins have not been examined in detail; Osborne[2] extracted a single albumin component, which he called legumelin, from pea, and Spragg,[3] using the Tiselius apparatus, separated the albumin fraction of pea into several components. The albumins of pea have been separated into five or six fractions using DEAE-cullulose.[4]

In the present work the albumins extracted from various legumes have been separated by polyacrylamide gel electrophoresis; the possibility of using the protein patterns obtained in this way as a tool in taxonomic studies is discussed.

RESULTS AND DISCUSSION

Figure 1 shows a photograph of the albumins of *Pisum arvense* separated on polyacrylamide gel and also the absorption peaks obtained by scanning the gel with a densitometer. Below the photograph is a chart of the R_p values of bands which were seen by eye. The albumin fraction from the seeds of this species was resolved into twenty-three bands. The major bands shown in the photograph are matched by the major peaks in the trace but neither method detects all the bands that can be seen with certainty by eye. The disadvantage of presenting the results as R_p charts arises when comparing charts of different gels. The R_p value gives no indication of the quality of the band (i.e. whether diffuse, its colour and width), although these factors are most important when comparing gels. The disadvantage of the photographs is that not all bands shown on the R_p chart appear on the print (cf. Fig. 1 R_p 0·7–1·0), although in certain cases photographs show bands not visible to the eye; this appears to be related to the relative intensity of the bands compared with intensity of the background staining. The densitometer trace shows certain minor shoulders not observed by visual inspection of the gels, and the significance of these peaks is under investigation.

The protein patterns obtained by this method are reproducible. Duplicate values of the albumins of *Pisum arvense* were compared with the mean R_p values calculated from eight similar gels; the greatest variation was 0·02 R_p units, most of the values lying within 0·01 of the

[1] C. D. Danielson, *Ann. Rev. Plant Physiol.* 7, 215 (1956).
[2] T. B. Osborne, *The Vegetable Proteins*, 2nd ed., Longmans, Green and Co., London (1924).
[3] S. Spragg, Ph.D. Thesis, Bristol University (1955).
[4] J. E. Varner and G. Schidlovsky, *Plant Physiol.* 38, 139 (1963).

mean value, that is within the accuracy of measurement ($\pm 0{\cdot}25$ mm). Though changes may take place during the standard extraction procedure, the same material extracted at different times gave the same pattern, within the accuracy of the method. There is however a limit to the number of bands detectable in any albumin preparation. Attempts to increase this number by putting more protein (300 μg) per column led to an increase in background colour which obliterated faint bands and merged those lying closely together.

If the albumin fraction contains the enzymically active proteins the results shown in Fig. 1 indicate that there are either twenty-three proteins belonging to this class in the albumin fraction of *Pisum arvense* or, more likely, that each band represents more than a single component and that some proteins are present in such small amounts that they are not detected.

Examination of a large number of gels (see Figs. 1–4) shows that the protein pattern on any one gel can be split into three regions; a region near the origin with R_p between 0·0 and 0·15, with very narrow and clearly defined bands; a second region with R_p 0·15–0·55 with wide conspicuous bands; and a third region, the remainder of the gel, in which the bands are wide and poorly defined. A conspicuous narrow band has been detected at R_p 1·0 in all extracts of plant material. This kind of zonation is not confined to the Leguminoseae having been also observed in water extracts of *Neurospora crassa* mycelium, salt extracts of red beetroot and in the albumin fraction of wheat embryo. The significance of this zonation is not yet fully understood.

Albumin preparations from the seeds of *Cytisus scoparius* collected from two different localities (Pwllolfa, North Wales, and Dungeness, Kent) had the same R_p values, i.e. in this instance the protein pattern did not vary with the source of the material.

Examination of Figs. 2–4 shows that so far as the investigation has proceeded the protein patterns obtained from different species within a genus resemble one another more closely than do those of species belonging to different genera. In some instances seeds of species of genera which are grouped together on morphological and cytological grounds have a similar protein pattern; for example, *Cytisus*, *Spartium* and *Lupinus* (Fig. 2) which belong to the sub-tribe Genistinae, *Rhynchosia* and *Phaseolus* (Fig. 3) to the sub-tribe Phaseolinae, and *Vicia* and *Pisum* (Fig. 4) to the sub-tribe Viciinae. All of these genera belong to the sub-family Papilionoideae, whereas seeds of *Caesalpinia gilleseii* (Fig. 3), which belongs to the sub-family Caesalpinoideae, have a protein pattern which is distinctly different. In view of the complexity of the taxonomy of the Pisum taxa used and the uncertainty of identification (see Materials) no attempt has been made to compare the protein patterns obtained within this group.

Previous attempts to use proteins as taxonomic characters have, in the main, depended upon serological techniques. Boyden[5] has discussed the use of serological techniques and concluded that they may provide useful taxonomic data. Gell, Hawkes and Wright[6] used these methods with *Solanum* species and separated the thirty-eight species studied into groups which were in fairly close agreement with orthodox taxonomic classification and with cytogenetic relationships. The method was not useful, however, with the South American species studied. This paper also gives a general discussion of the use of serological techniques in plant taxonomy which need not be reiterated here. Further examples of the use of serological techniques are discussed by Davis and Heywood.[7]

[5] A. Boyden, *Systematic Zool.* **12**, 1 (1963).
[6] P. G. H. Gell, J. G. Hawkes and S. T. C. Wright, *Proc. Roy. Soc.* **B151**, 364 (1960).
[7] P. H. Davis and V. H. Heywood, *Principles of Angiosperm Taxonomy*, Oliver & Boyd, London and Edinburgh (1963).

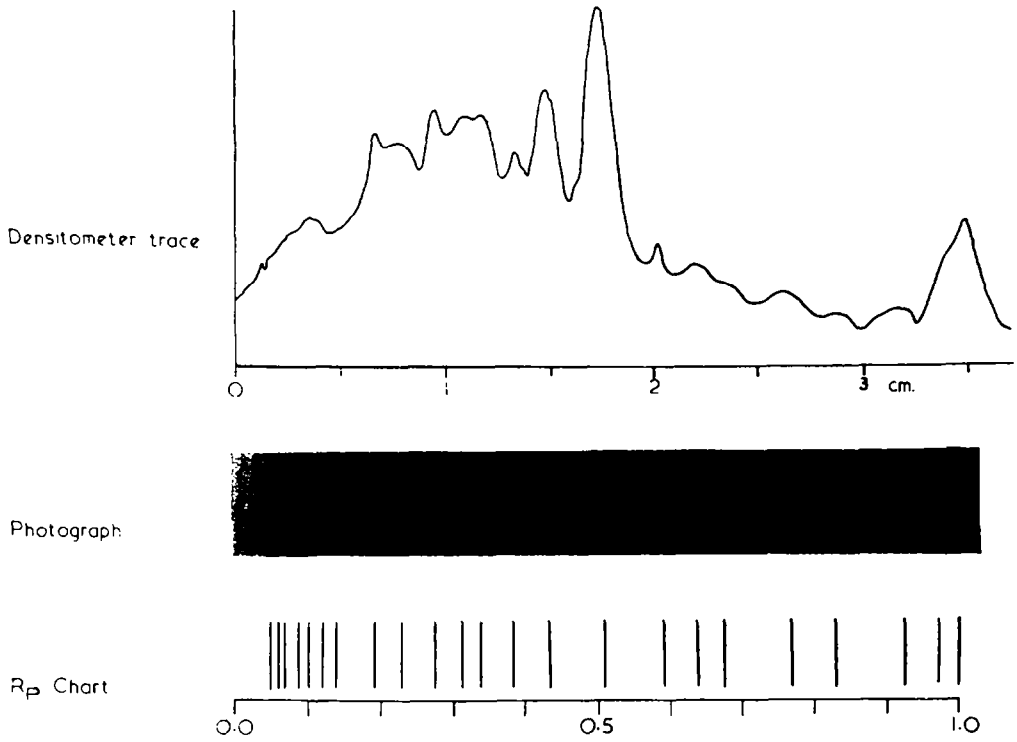

FIG. 1. ALBUMINS OF *Pisum arvense*.

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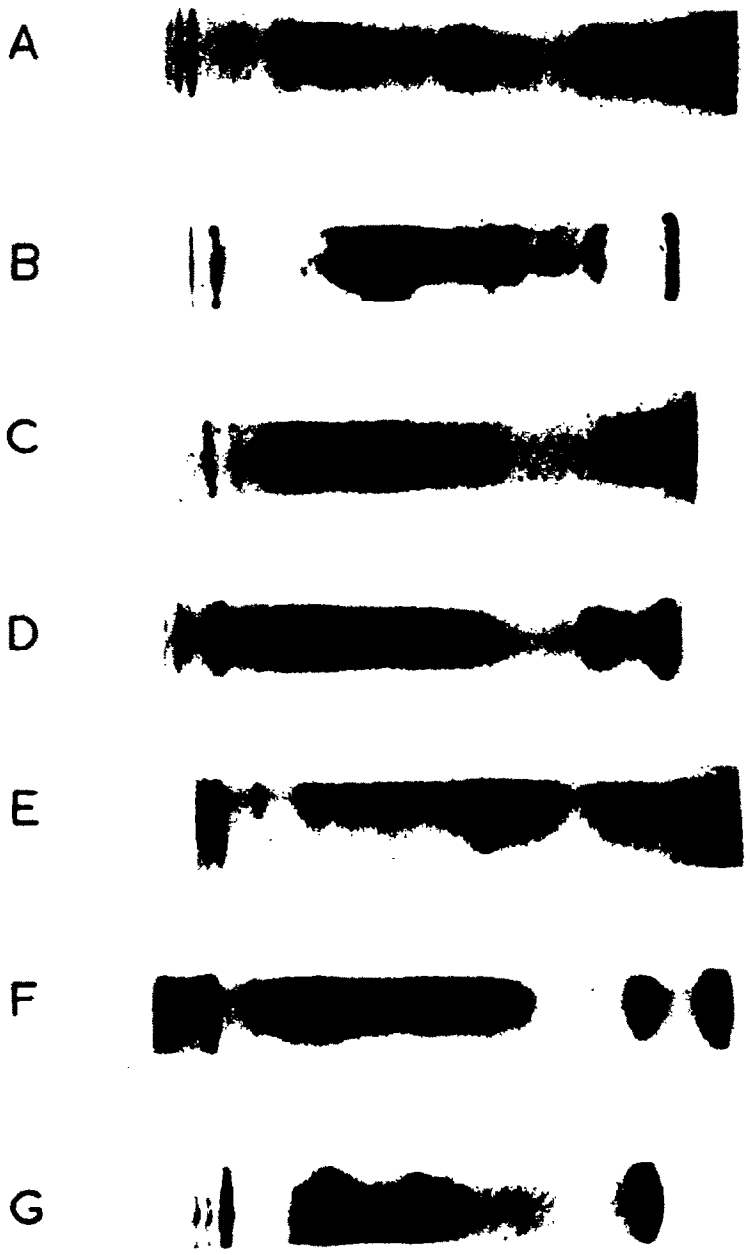

FIG. 2. ALBUMINS OF A, *Lupinus mutabilis*; B, *L. douglasii*; C, *L. albus*; D, *L. polyphyllus*; E, *L. micranthus*; F, *Cytisus scoparious*; G, *Spartium junceum*.

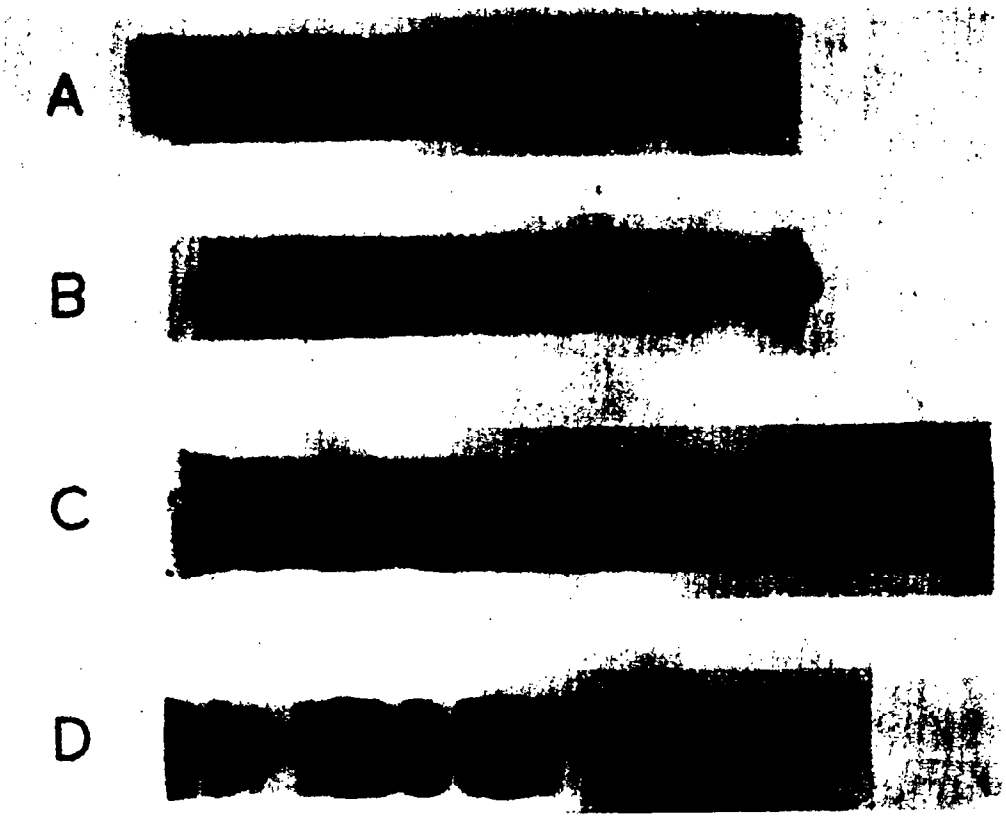

FIG. 3. ALBUMINS OF A, *Phaseolus vulgaris*; B, *P. coccineus*; C, *Rhynchosia phaseoloides*; D, *Caesalpinia gilliesii*.

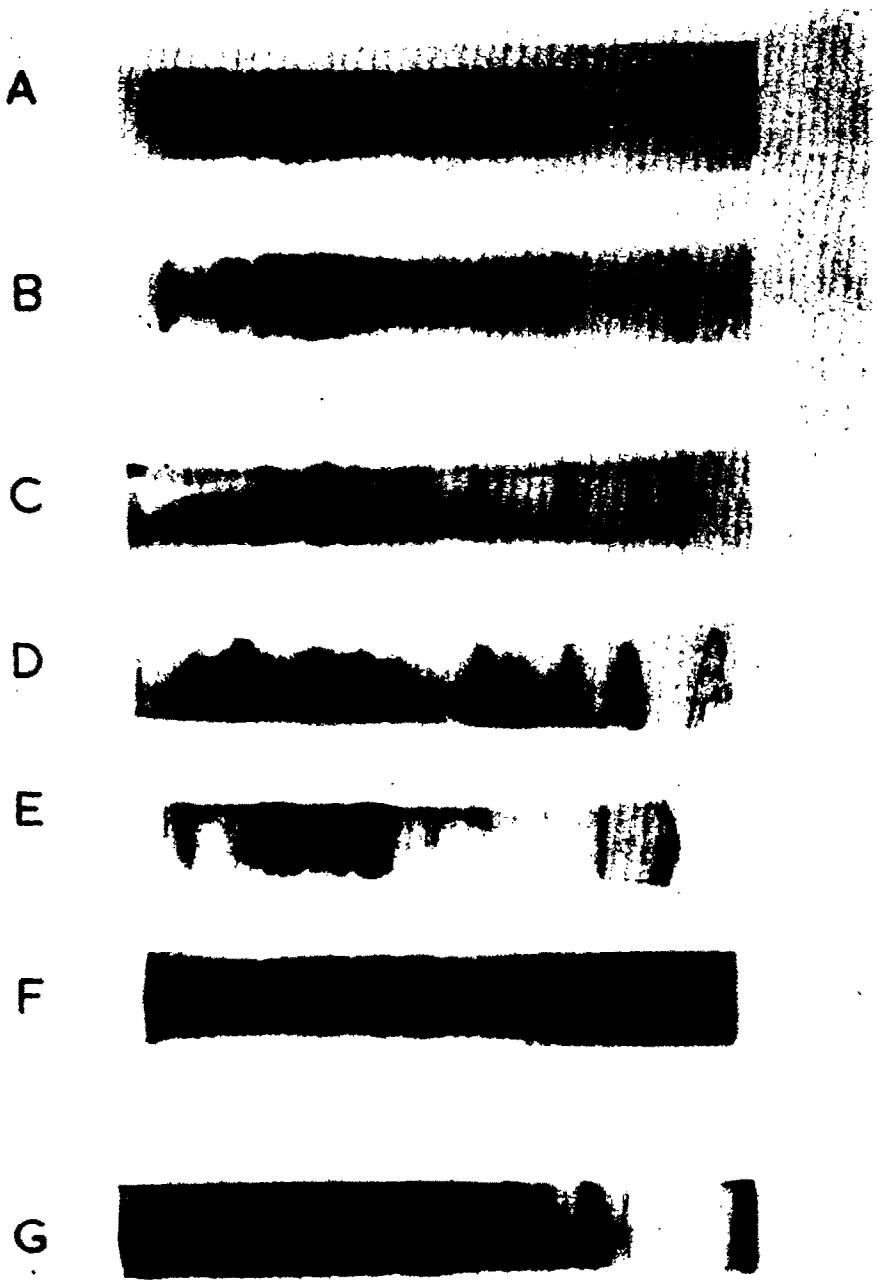

FIG. 4. ALBUMINS OF A, *Vicia sepium*; B, *V. sativa*; C, *V. pisiformis*; D, *V. dumetorum*; E, *Pisum jomardii*; F, *P. sativum*; G, *P. arvense*.

The results of the present investigation showing similarities in the protein pattern of seeds of species from closely related genera are encouraging. If, as a result of the extension of these investigations it can be shown that there is a close correlation between protein pattern and some other feature, or with taxonomic position, the technique will provide a useful source of new data. It would be most valuable, however, in cases where evidence from the protein pattern deviated from that expected on the basis of existing classifications, since it might point to the necessity for a taxonomic reinvestigation.

MATERIALS

The seeds used in this investigation were supplied by the courtesy of Mr. J. K. Hulme, Director of the Botanic Garden, University of Liverpool, and, except for *Cytisus scoparius* subsp. *prostratus*, which was supplied by Mr. I. Gill, Department of Genetics, University of Liverpool, no attempt was made to check their identification.

The material of Pisum employed was received as *P. arvense*, *P. sativum* and *P. jomardii*. *P. sativum* is not known from the wild and *P. arvense* has been recorded as native in Georgia but is frequently classified with *P. sativum* within the subspecies *sativum*. *P. jomardii* is treated by some authors as a subspecies, again of *P. arvense*—subsp. *asiaticum*.

EXPERIMENTAL

Seeds with testas removed were ground within a glass mortar and pestle. Large seeded, cultivated forms were ground in a 9-in. cross-beater mill and the flour dredged through a 0·2-mm sieve. Seed meals (1 part) were extracted with 5% (w/v) K_2SO_4 (5 parts) with continuous stirring for half an hour. The slurry was centrifuged at 1000 *g* for 5 min and the supernatant decanted into $\frac{18}{32}$-in. Visking dialysis tubing and dialysed for 20 hr against running tap water. The supernatant obtained from this dialysis was centrifuged, freeze-dried and then subjected to electrophoresis.

Electrophoresis in Polyacrylamide Gels with pH Discontinuities (Disc Electrophoresis)

The method used was that of Davis and Orstein,[8] except that samples (200 μg) were applied to the columns in a mixture of 25% (w/w) sucrose (1 part) and large pore solution (1 part).[9]

Presentation of Results

Gels were photographed in water and also scanned by using transmitted light by a 'Chromoscan' recording densitometer. R_p values were obtained in an analogous way to the R_f value in chromatography. Electrophoretograms of plant material examined in this work contained a fast-moving band (see Fig. 1) and the R_p value of any given band is the ratio of its distance from the origin to the distance from the origin of this fast-moving band.

Acknowledgements—We would like to thank Dr. V. H. Heywood for helpful discussions and a critical appraisal of the manuscript. Also Mr. J. Bloor for production of the photographs. D. J. F. acknowledges financial assistance from the D.S.I.R.

[8] L. Orstein and B. J. Davis, *Disc Electrophoresis*, Preprint by Distillation Products Industries (Eastman Kodak Co.), Rochester, N.Y. (1961).
[9] D. J. Fox, D. A. Thurman and D. Boulter, *Biochem. J.* 87, 29P (1963).