IDENTIFICATION OF ALBUMIN 0.19 IN GRAIN PROTEINS OF CEREALS[1]

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ABSTRACT

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Proteins with antigenic determinants identical to those of albumin 0.19 from bread wheat (*Triticum aestivum*) have been found in the tetraploid wheat *T. durum* by means of an antiserum to the isolated 0.19 component. Accordingly, this component, which is an inhibitor of mammalian and insect α-amylases and which is absent from the electrophoretic pattern of *T. durum*, is not immunochemically specific for bread wheats as had been reported earlier. By means of rocket immunoelectrophoresis, the albumin component that is antigenically specific for bread wheats was shown not to be albumin 0.19. The specific component differed considerably in electrophoretic mobility from albumin 0.19 and had an isoelectric point of about pH 4.6, whereas albumin 0.19 had an isoelectric point of about pH 7.3. Albumin components antigenically identical to albumin 0.19 were found in wheat and rye, but not in barley. The presence of this albumin determinant in other species has been studied.

Italian scientists (1,2) found an albumin component that they called Mb 0.19 (according to its relative mobility) in the electrophoretic pattern of albumins from bread wheats (*Triticum aestivum*) but not in the pattern of albumins from durum wheats (*T. durum*). The 0.19 albumin had the same electrophoretic mobility, amino acid composition, and molecular weight (about 20,000) as albumin 13A of Feillet and Nimmo (3). Subsequently, 0.19 was shown to inhibit α-amylases of mammals (including humans) and insects (4,5).

Piazzi et al (4) reported an immunochemical method for the differentiation of flours from bread wheats and durum wheats based on the specificity of 0.19 for bread wheats. Their antiserum was prepared from an albumin fraction of bread wheat that included albumin proteins other than 0.19.

Our aim was to elucidate the immunochemical specificity of 0.19 for bread wheats and to study its presence in other species of wheat and related cereal grains. For this purpose, we used electrophoresis and isoelectric focusing and various immunochemical methods, such as double diffusion, immunoelectrophoresis, and rocket immunoelectrophoresis.

MATERIALS AND METHODS

Durum wheat (Charkovskaya 46), bread wheat (Saratovskaya 29), and samples of other species of *Triticum, Aegilops, Agropyron, Elytrigia, Hordeum,* and *Secale* were obtained from the collection of the N.I. Vavilov Institute of Plant Industry.

The antiserum against 0.19 was obtained as follows. The albumin fraction obtained after salt precipitation with $(NH_4)_2SO_4$ followed by gel filtration on Sephadex G-100 (6,7) was submitted to polyacrylamide gel electrophoresis in Tris-glycine buffer (pH 8.6; 0.6 g of Tris, 2.9 g of glycine diluted to 1 L). Disc electrophoresis was done in 5 × 75-mm tubes with 7.5% gels for 1.5 hr at 4 mA per tube (Fig. 1). The zone corresponding to albumin 0.19 was cut from the gel and

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the protein eluted from these zones by means of an electrophoretic procedure. The gel pieces were placed in the upper part of the electrophoretic device, which was connected with the lower part by a tube. Electrolyte was 0.05*M* acetic acid; current at 450 V effected the extraction of the protein from the gel pieces and the protein was collected by a bag of dialysis tubing attached to the bottom of the tube. At least one gel was fixed in 7% trichloroacetic acid or stained as a control (Fig. 1). The purity of 0.19 prepared in this way was determined by analytic electrophoresis.

Rabbits were injected with the protein according to the method used in our laboratory (8). A total of 4.5 mg of 0.19 were injected into each rabbit. In addition, antiserum was prepared against the whole albumin fraction of *T. aestivum* by the method of Piazzi et al (4). Double immunodiffusion and immunoelectrophoresis were done in 1% agar-agarose gels with a 1:5 ratio of agar to agarose. A barbital-acetate buffer was used (pH 8.6; 8.7 g of barbital, 6.48 g of sodium acetate, 1.89 g of NaOH, and 60 ml of 0.1*M* HCl made up to 1 L).

For quantitative estimation of albumins in the grain and for increasing

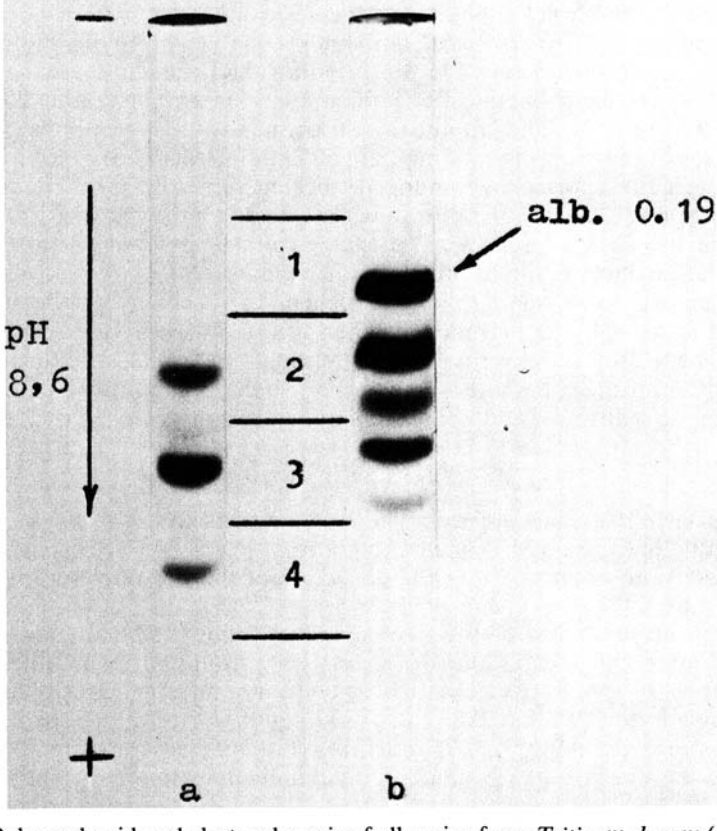

Fig. 1. Polyacrylamide gel electrophoresis of albumins from *Triticum durum* (a) and *T. aestivum* (b) (tris buffer, pH 8.6). Zones designated 1–4 were cut out of gels after electrophoresis.

sensitivity over the immunodiffusion method, the technique of rocket immunoelectrophoresis (9) was used with modification to improve its suitability for estimating cereal grain albumins. The antiserum was incorporated in the melted agarose (0.35 ml of antiserum per 5 ml of 1% agarose) at 55° C. The agarose was prepared in sodium barbital-citrate buffer, pH 8.6, ionic strength (I) 0.05; it was spread on 5 × 5-cm plates for immunoelectrophoresis. Soluble proteins of equal concentration (6–10 mg/ml) or derived from equal weights of

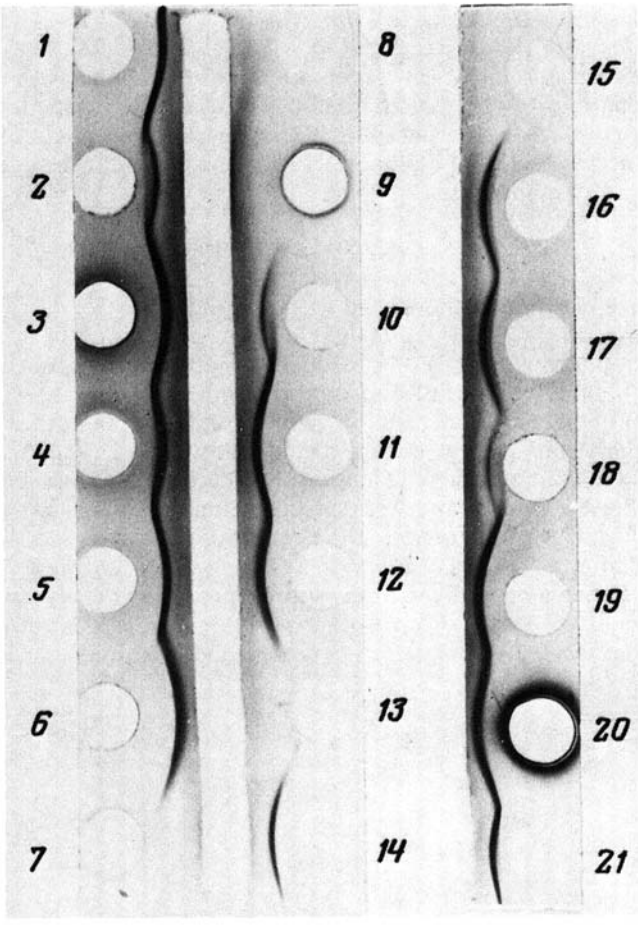

Fig. 2. Immunodiffusion analysis of soluble proteins and ethanol-soluble proteins (eth fr) with anti-0.19 immunoserum: 1, *Aegilops squarrosa;* 2, *Ae. speltoides;* 3, *Triticum durum;* 4, *T. aestivum;* 5, *T. aestivum* eth fr; 6, *T. urartu,* Armenia; 7, *T. boeoticum;* 8, *Elytrigia repens;* 9, *Hordeum spontaneum;* 10, *E. elongatum;* 11, *T. turgidum;* 12, *T. araraticum;* 13, *T. boeoticum* eth fr; 14, *T. urartu,* Iran; 15, *H. vulgare;* 16, *Ae. longissima;* 17, *T. urartu,* Turkey; 18, *E. trichophorum;* 19, *Secale segetale;* 20, *T. urartu* eth fr, Armenia; 21, *T. urartu,* Armenia.

flour were placed in holes 2.5 mm in diameter. The immunoelectrophoretic run was done at 4 V/cm for 4 hr and 40 min. Gels were stained with Coomassie Brilliant Blue R-250 and photographed through an orange filter.

Total soluble proteins were extracted from flour with 1*M* NaCl in phosphate buffer at pH 7.1, I 0.1, and 4° C. The ethanol-soluble fraction, which included all gliadins and some faster-moving components that were not gliadins, was extracted with 70% ethanol at 20° C after the proteins soluble in neutral buffer had been extracted thoroughly.

Electrophoresis of the ethanol-soluble fraction was done in 7.5% polyacrylamide gels for 2.25 hr at 4 mA. Gel length was 6 cm; the buffer was 0.013*M* acetic acid, pH 3.2 (8).

Isoelectric focusing of wheat grain albumins was done with the LKB-Multiphor apparatus (Sweden) with PAG-plate, pH 3.5–9.5. After focusing, the plates were fixed in 7% trichloroacetic acid and the isoelectric points of the bands were determined by means of a plot of pH as a function of the distance between the electrodes of the apparatus.

RESULTS AND DISCUSSION

The anti-0.19 immunoserum reacted with 0.19 of the soluble proteins of both *T. aestivum* and *T. durum* in immunodiffusion (Fig. 2) and immunoelectrophoresis (Fig. 3). The reaction was absent after absorption of the antiserum by soluble proteins of *T. durum*. The anti-0.19 immunoserum gave a positive immunoprecipitation reaction with soluble proteins of all species of wheat (*Triticum*) and *Aegilops* except the diploid wheats *T. boeoticum* and *T. monococcum* (Fig. 2); a positive reaction was obtained with soluble proteins of the diploid wheat *T. urartu* and weak precipitation line was obtained with the soluble proteins of *Ae. speltoides* and *T. zhukovskyi*.

Among 40 different species of *Elytrigia* and *Agropyron* that were studied, the antiserum to 0.19 reacted only with the soluble proteins of *E. intermedium, E. trichophorum, E. elongatum* (2n, 70), *E. caespitoseum, A. desertorum,* and *A. cristatum*. The anti-0.19 immunoserum gave good reaction with the soluble

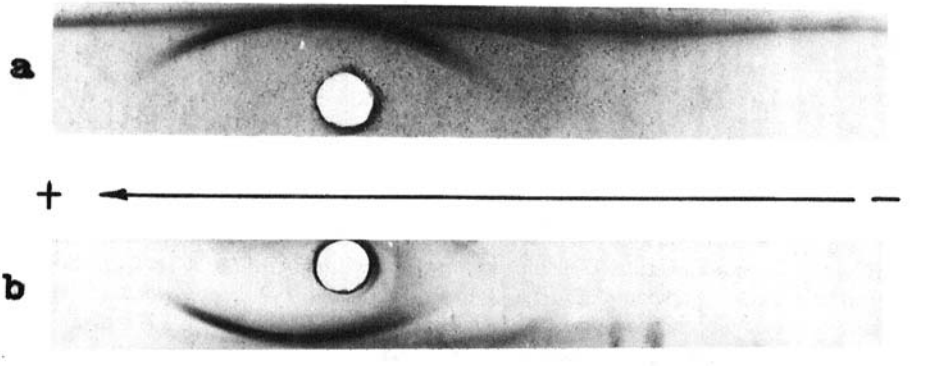

Fig. 3. Immunoelectrophoretic analysis of soluble proteins of *Triticum durum* (a) and *T. aestivum* (b) with antiserum against 0.19 prepared from *T. aestivum*.

proteins of all *Secale* spp., but did not react with those proteins of *Hordeum* spp. (Fig. 2). The presence of proteins with antigenic determinants equivalent to that of 0.19 in various genera and species seemingly correlates with the closeness of the relationships of those species and genera to *T. aestivum*. This can be illustrated by the examples of *Secale* and *Hordeum* in which the more closely related species of *Secale* reacted with the immunoserum, but the more distantly related species of *Hordeum* did not. The species of *Elytrigia* provide an especially good example of this insofar as the antiserum reacted only with the soluble proteins from species of this genus that can cross with *T. aestivum*.

In addition to reacting with those proteins soluble in neutral buffer from various species, the 0.19 antiserum reacted with ethanol-soluble proteins of *Triticum, Aegilops,* and *Secale*. No precipitation line was found for the ethanol-soluble fractions of *T. boeoticum* and *T. monococcum*.

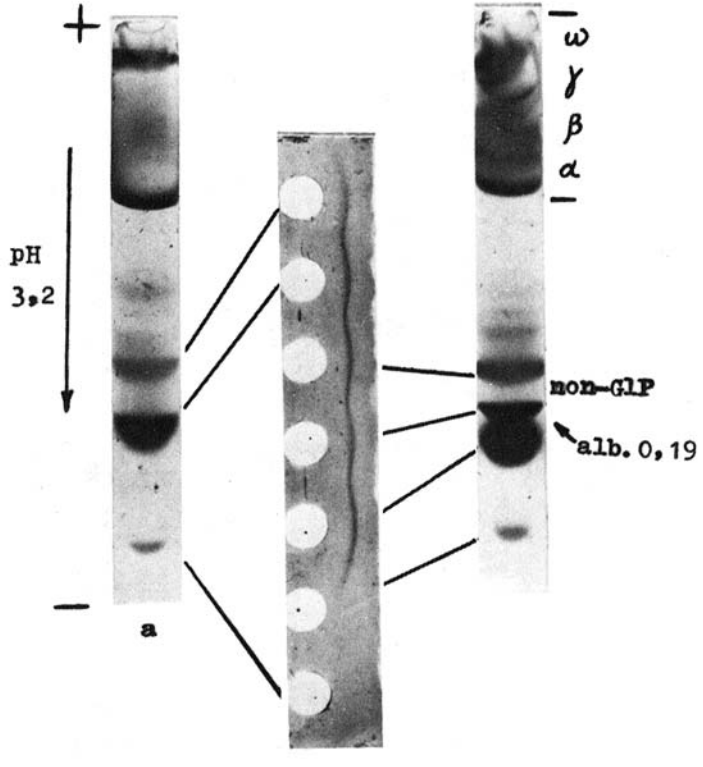

Fig. 4. Polyacrylamide gel electrophoresis of ethanol-soluble proteins of *Triticum durum* (a) and of *T. aestivum* (b) and immunodiffusion analysis of separate components of electrophoretic spectrum with antiserum against 0.19 from *T. aestivum*. Gliadin proteins in ethanol-soluble fraction are designated as α, β, γ, and ω, whereas nongliadin proteins are designated as non-GLP.

When the electrophoretic pattern of the ethanol-soluble proteins from *T. aestivum* was compared with that of 0.19, 0.19 was found to have the same electrophoretic mobility as one of the fast components that was not a gliadin protein (Fig. 4). The anti-0.19 immunoserum gave one identical precipitation line with the three components of this zone in the spectrum of *T. aestivum* and with the two components of *T. durum* from this zone (Fig. 4). Other components of the ethanol-soluble fraction did not give precipitation lines with the anti-0.19 immunoserum.

Rocket immunoelectrophoresis of soluble proteins with anti-0.19 immunoserum showed that the peak corresponding to 0.19 in the pattern was higher for the *T. durum* sample than for the *T. aestivum* sample when the proteins were prepared from the same weight of flour in each case (Fig. 5). This evidently resulted from higher protein content in the durum sample, because when equal concentrations of soluble proteins or of the ethanol-soluble fraction were taken, the heights of the peaks were the same. The 0.19 peak was absent from the proteins of *T. boeoticum* and *T. monococcum*. The equivalent peak in *T. urartu* was approximately the same as that for *T. aestivum* and *T. durum*. The equivalent peaks of *E. elongatum, E. trichophorum, T. zhukovskyi,* and *Ae. speltoides* were lower than those of *T. durum* and *T. aestivum,* indicating lower concentrations of the 0.19 equivalent protein in these species in comparison with

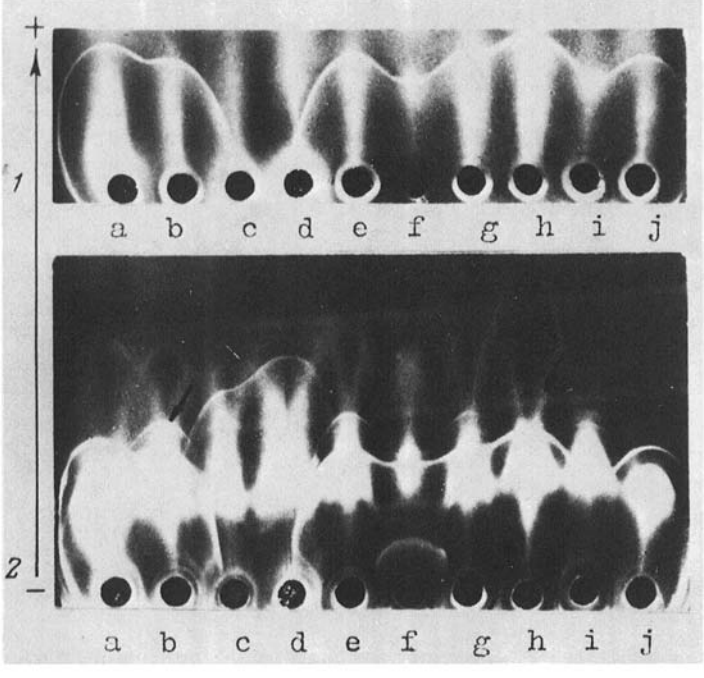

Fig. 5. Rocket immunoelectrophoresis of soluble proteins: a, *Triticum durum;* b, *T. aestivum;* c, *T. boeoticum;* d, *T. monococcum;* e, *T. aestivum;* f, *T. zhukovskyi;* g, *Aegilops longissima;* h, *Ae. squarrosa;* i, *Ae. speltoides;* j, *T. urartu.* 1, Antisera against 0.19 from *T. aestivum;* 2, antisera against albumin fraction of *T. aestivum.*

T. durum and *T. aestivum*. These data provide evidence that rocket immunoelectrophoresis is a suitable method for the quantitative determination of albumins in mixtures of cereal grain proteins. Furthermore, rocket immunoelectrophoresis extends the applicability of the immunochemical method, permitting simultaneous identification and quantitation of a number of components in the precipitation spectrum.

By means of rocket immunoelectrophoresis with antiserum prepared according to Piazzi et al (4), an albumin component was discovered that was specific for *T. aestivum*. This specific component most likely accounts for the finding of Piazzi et al (4) that they could differentiate *T. durum* flour from *T. aestivum* flour by their immunochemical method. This specific albumin component is not 0.19; its peak in rocket immunoelectrophoresis was higher and weaker (marked by arrow in Fig. 5) than the peak of albumin 0.19.

The component that was specific for *T. aestivum* was found also in the albumins of *Aegilops squarrosa* and *A. longissima*. It was absent from *A. speltoides* and all diploid species of *Triticum*. The amount of this component in the wheat albumin mixture was low and was not discovered by routine immunodiffusion and immunoelectrophoresis methods with rabbit antiserum. Piazzi et al (4) discovered the specific albumin component when they worked with goat antialbumin immunoserum.

To find out the location of the *T. aestivum*-specific albumin in the electrophoretic spectrum of *T. aestivum,* proteins from separate zones (as marked in Fig. 1) of the electrophoretic patterns of *T. aestivum* and *T. durum*

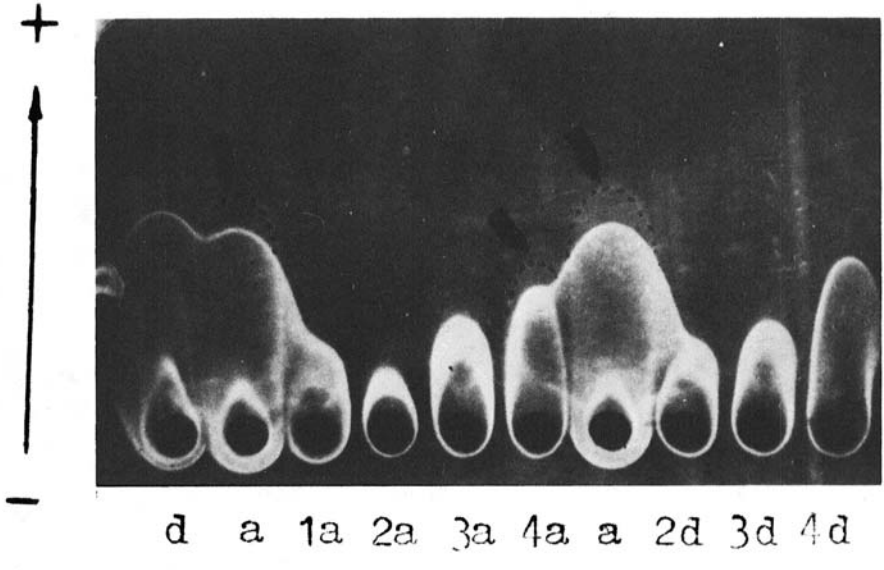

Fig. 6. Rocket immunoelectrophoresis of albumins from *Triticum durum* (d) and *T. aestivum* (a) and of proteins from separate zones of albumin electrophoretic spectrum of *T. aestivum* (1a–4a) and of *T. durum* (2d–4d). Zones 1–4 as in Fig. 1. Antiserum against albumin fraction of *T. aestivum*.

were studied immunochemically. Figure 6 shows that the albumin component specific for bread wheats was present only in the fourth zone of the albumin electrophoretic pattern of *T. aestivum*. This component was not found in any of the zones of the electrophoretic spectrum of the albumins from *T. durum*. In this way, the component specific for bread wheat was found to have a relatively high electrophoretic mobility. Figure 6 shows that all zones of the patterns of *T. durum* and *T. aestivum* contain proteins that are antigenically identical to 0.19. Accordingly, antiserum against 0.19 gives immunoprecipitation reactions with soluble proteins of *T. durum* and other species (Fig. 2 and 3). By means of rocket immunoelectrophoresis, we were able to determine the content of albumin proteins antigenically identical to 0.19 in various grains. Sodini et al (2) have determined that 0.19 has an isoelectric point at pH 7.3. This was substantiated in the present study; Fig. 7 shows that 0.19 focused around pH 7.3. The albumin components of electrophoretic zones 4a and 4d focused around pH 4.5–4.8 (Fig. 7). The isoelectric focusing pattern of zone 4 from *T. durum* showed one line, whereas that of zone 4 from *T. aestivum* showed two lines. The component of this pair, marked by the arrow in Fig. 7, seems likely to be the component specific for bread wheat because it did not appear in the *T. durum* pattern.

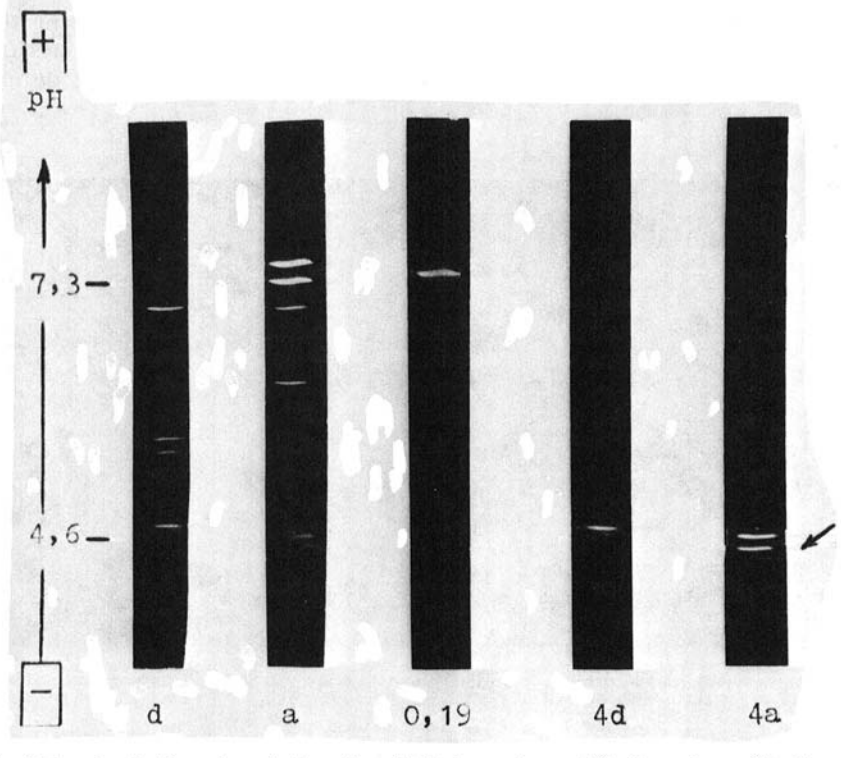

Fig. 7. Isoelectric focusing of albumins of *Triticum durum* (d), *T. aestivum* (a), albumin 0.19, zone 4 of *T. durum* (4d), and zone 4 of *T. aestivum* (4a). Arrow indicates component specific for *T. aestivum*.

In our laboratory, a reliable method for the serologic differentiation of bread and durum wheat flours has been developed (9) based on an antiserum prepared against the ethanol-soluble proteins of *Ae. squarrosa,* the species that contributed the D genome to bread wheats. This antiserum to the D genome was easy to prepare in contrast with workups involving the total albumin mixture from bread wheats, and was highly active.

The data described above show that albumin 0.19 as an antigen is not specific for bread wheats. It has been discovered immunochemically in durum proteins, although it shows up in a different portion of the electrophoretic spectrum as compared with the electrophoretic spectrum of bread wheats (10). The emphasis here is on the immunochemical character of 0.19—no component with exactly the same electrophoretic mobility is found in durum wheats. In our laboratory, we also have observed the opposite situation in which components with identical electrophoretic mobilities were not identical on an immunochemical basis. (These components also showed different numbers of bands when compared by isoelectric focusing.)

The observations regarding 0.19 may be explained by the existence of multiple forms of α-amylase inhibitors as previously described by Silano et al (11,12). They note that these α-amylase inhibitors exist in forms with different molecular weights of 11,000, 22,000, 44,000, and 60,000 and that the higher molecular weight inhibitors may arise from combinations of lower molecular weight subunits. Perhaps a limited number of ancestral genes control the latter. Antiserum against 0.19 seems likely to be able to give an identical immunochemical reaction with the different molecular forms of these proteins.

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