

spectrum which are responsible for the blue colour which it exhibits. A five-centimetre column of the acetone extract exhibits a purplish-blue colour which is spectroscopically revealed as due to a conspicuous absorption-band covering the yellow region of the spectrum, a less conspicuous diffuse band in the red and a general weakening of the green region of the spectrum. The blue region in the spectrum is transmitted with fair strength. These features are recognisable in the spectrophotometer record of the absorption by a centimetre column of the acetone extract reproduced as Fig. 3. The spectral behaviour of the flower thus definitely belongs to the class Florachrome A.

#### 5. *Tecoma stans*

This is a large shrub belonging to the botanical class *Bignoniaceae*. It is a very hardy quick grower attaining a height of about 10 feet. The foliage is handsome, consisting of

graceful pinnate leaves. The shrub is commonly planted for screening compound walls or as hedging. The flowers are golden-yellow in colour, large, funnel-shaped and widely expanded and appear as clusters in terminal branches. The colour is readily extracted from them by immersion in acetone. Examined *in vivo*, the flowers exhibit a practically complete extinction of the shorter wavelengths in the spectrum upto about 510 m $\mu$ , while the rest of the spectrum appears in full strength. The acetone extract exhibits the same spectroscopic behaviour. Figure 4 reproduces a spectrophotometer record of the diluted extract. It shows three bands in the blue-violet region of the spectrum, indicating that the pigment may be identified as a carotenoid.

The records reproduced above were made in the Instruments Section of the Indian Institute of Science, to the authorities of which institution, the thanks of the author are due.

## MICROSCOPIC SCREENING OF RICE GRAINS FOR PROTEIN CHARACTERISTICS

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**A** MICRO-DYE-BINDING capacity (DBC) technique was earlier described for analyzing proteins in single grains of rice.<sup>1</sup> Since rice is mostly consumed in the milled or polished form, and since polishing results in varying extent of losses of the outer layers of endosperm, a study of proteins of the caryopsis *in situ* would be of interest. Several histological and ultrastructure studies have been conducted in the rice grain to obtain morphological and biochemical information on the developing grain.<sup>2-7</sup> In our programme aiming at the development of rice strains with superior quantity, quality and distribution of proteins, a rapid test that would enable the screening of thousands of strains of wild and cultivated rices as well as segregating material from crosses between diverse strains and varieties obtained in induced mutation programmes became necessary. For this purpose, a system of microscopic screening of transverse sections of the grain was developed. This technique is discussed in this paper in relation to the D.B.C. method for single grains, reported earlier.

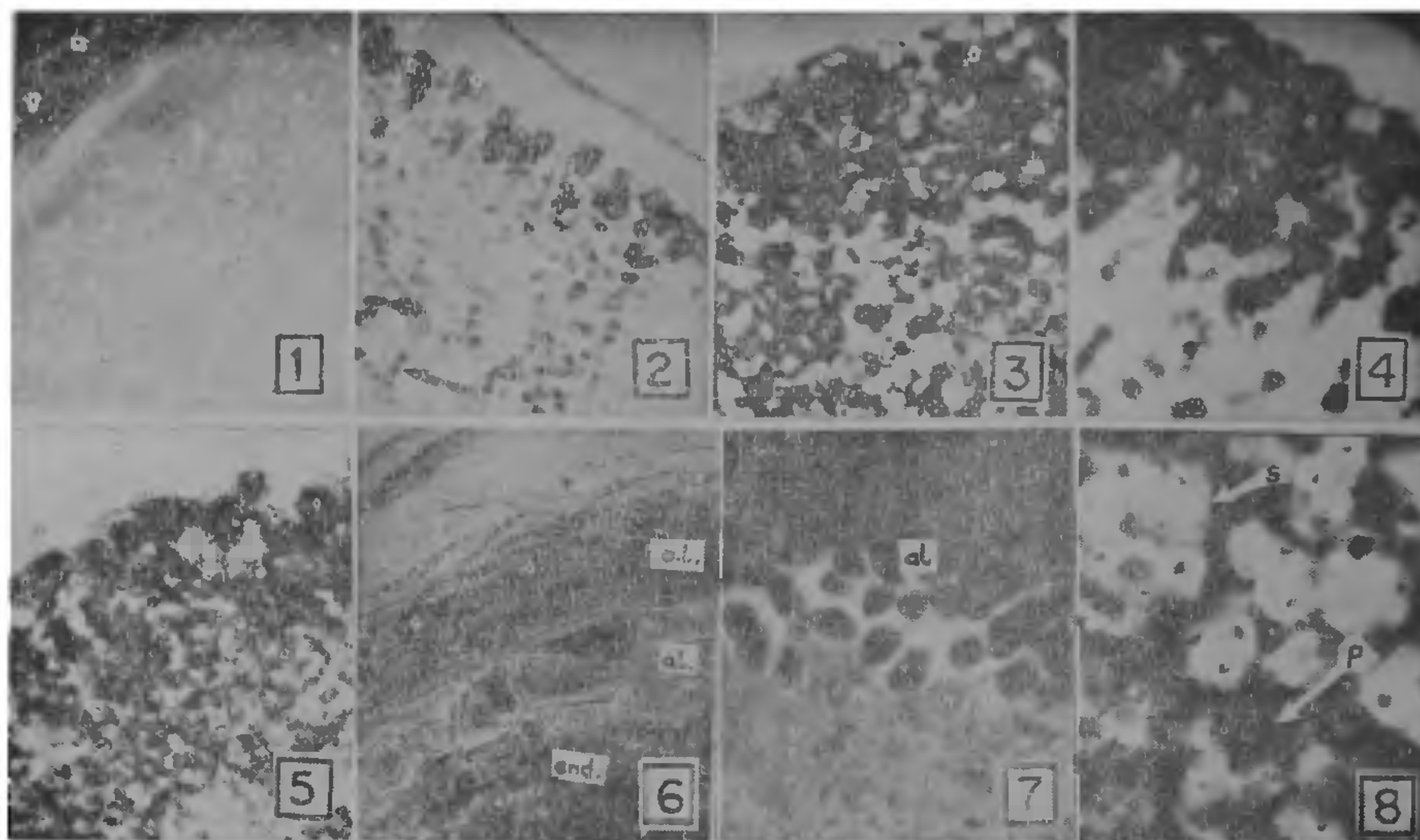
Rice grains, of uniform maturity, were fixed in Corneys fixative for 48 hours and stored in

70% alcohol. Protein specific dyes such as Orange G, Bromophenol blue, Sakaguchi's reagent, Alloxan, Xylidine-ponceau, Ninhydrin, Amido black and Nigrosine were used. Among these Bromophenol blue was found useful for routine studies because of both ease of preparation and satisfactory resolution. The hand sections were cut with a 'Weck' blade and stained for two minutes in 1% aqueous Bromophenol blue solution; dehydrated with ascending grades of alcohol/water, clove oil and xylene and finally mounted in Euperal. One technician was able to screen 15-20 strains in 7 hours.

The proteins of rice grain occur in the form of (a) well-formed irregular bodies, 3-20  $\mu$  in size, surrounded by amyloplasts (Fig. 8)<sup>2,8</sup> and (b) a membranous network composed of very small dots (Fig. 2).<sup>2,4</sup> These structural differences are genotypically determined.<sup>9</sup> A variety might exhibit either of the two types of configuration or both with varying intensities (Figs. 3 and 5). In the material studied by us, the following four major types of protein distribution were recognised:

- Type 1. Peripheral and diffused network.
- Type 2. Single layer of well-formed bodies,





FIGS. 1-8. Transverse sections of various rice strains. Fig. 1. Protein pattern : 1-0 type. (Peripheral diffused.) Fig. 2. Protein pattern 2-1. Dense peripheral protein bodies and dispersed membranous characterization (arrow). Fig. 3. Protein pattern 3-2. Deep-seated membranous proteins with few disrupted bodies on periphery. Fig. 4. Protein pattern 4-0. Deep distributed dense protein bodies. Fig. 5. 4-3 pattern. The idealmost characterization having body proteins and dispersed proteins with equal intensities. Fig. 6. A magnified view of the outer tissues of a mature rice caryopsis. Two layers of aleurone (*al.*) and several compressed layers of outer tissues (*o.l.*) are seen. Fig. 7. A strain of rice showing five layers of aleurone (*al.*) zone. Fig. 8. Protein bodies (*P*) interspaced between the compound starch amyloplasts (*S*).

THE RELATIONSHIP BETWEEN THE MICROSCOPE SECTION SCORE(Y) AND D.B.C. VALUES(X)

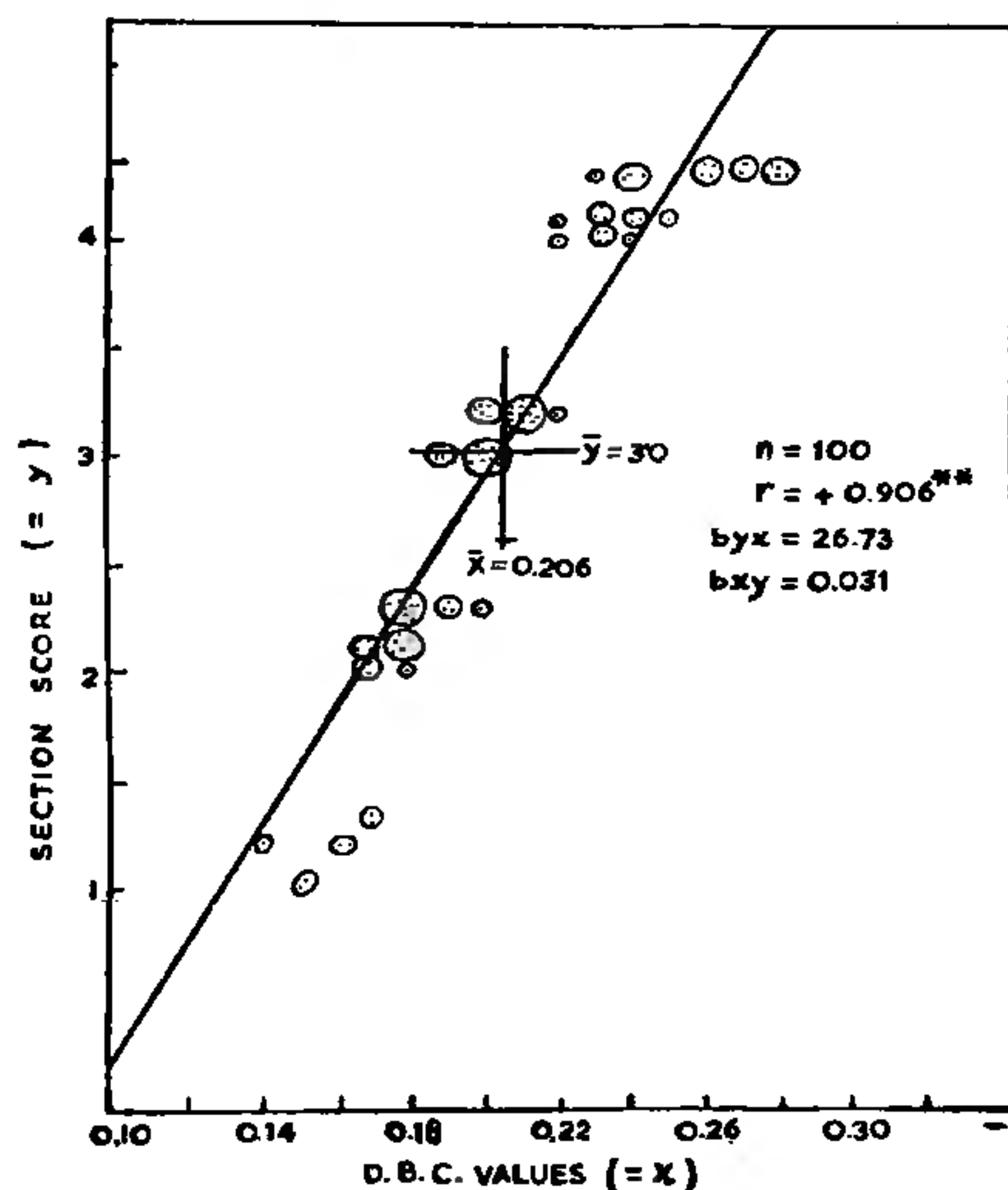


FIG. 9

Type 3. Deep diffused network.

Type 4. Deep multilayered protein bodies.

It was observed that some genotypes have mixed profiles (Figs. 3 and 5), e.g., type 2-3 would have a single layer of well-formed bodies with a network of diffused proteins in the inner layers and Type 4-3 will have many layers of dense protein bodies intermixed with a deep diffused network.

The authenticity of these microscopic observations, and thereby the practicability of using the above-mentioned scale, was confirmed with the single grain D.B.C. technique. A remarkably high correlation coefficient (+ 0.906\*\*) was obtained between the score readings and the D.B.C. absorption values (Fig. 9). Thus, the visual scoring of protein can be successfully quantified.

Among the cereal proteins, rice protein has the most desirable aminogram.<sup>9,10</sup> When comparisons of the amino-acid content expressed as percentage of protein of brown rice, milled rice, germ, polishing and bran are made, it is found that the bran and germ aminograms are better than those of brown and polished rice.



This qualitative difference is more marked in the case of lysine, threonine and tryptophan.<sup>11,12</sup> This observation suggests that milling results in not only quantitative but qualitative losses as well. Hence, deep-seated and diffused protein bodies belonging to the type 4·3 pattern would be desirable for retaining the nutritive value of milled rice. The rapid technique described in this paper would be of use in screening world collections and mutagen-treated populations for this trait.

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## ENHANCED VIRAL HAEMAGGLUTINATION WITH TRYPSINISED ERYTHROCYTES

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MORTON AND PICKLES<sup>1</sup> observed that treatment of erythrocytes with trypsin resulted in marked increase in agglutinability of M, N, S and A, B, O and P receptors by their corresponding antisera. Trypsin treatment of erythrocytes was also found of great help in the demonstration of Rh incomplete antibodies. On the other hand, marked reduction or abolition of viral haemagglutination with trypsin-treated erythrocytes has been reported for influenza,<sup>2</sup> ECHO<sup>3</sup> and reo type 1 and 2 viruses.<sup>4</sup> We are not aware of any report in literature describing enhancement of agglutinability of trypsinized red cell by any virus. The present report describes the increased haemagglutination titres of arboviruses against trypsinized cells.

The experiments were carried out on the erythrocytes of ducks, roosters, sheep, guinea-pigs, human blood group 'O' subjects, rabbits and frogs (*Rana tigrina*). The blood was

collected in Alsever's solution and stored at 4° C. for at least 24 hours to avoid non-specific agglutination and were used within 5 days of collection. The red cells were washed thrice with isotonic saline. The packed cells were then divided into two aliquots. To one aliquot 0·1% trypsin (Difco 1 : 250) was added in proportion of 1 : 5. The trypsin solution was prepared in phosphate buffered saline pH 7·2 and was Seitz-filtered for sterilization. To another portion of packed erythrocytes, phosphate buffered saline was similarly added for control. Both the test and control erythrocytes were then incubated at 37° C. for 1 hour. After incubation the cells were immediately washed thrice with isotonic saline. The erythrocytes were then suspended in 0·4% concentration in virus-adjusting diluent of pH 6·6 for KFD virus and pH 6·4 for JBE virus antigens. The two arboviruses used were Kyasanur Forest Disease (KFD) and