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An immunological and chemical study of the alcohol-soluble proteins of cereals.

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The prolamines, or alcohol-soluble proteins, are the characteristic proteins of cereal grains. These proteins were isolated from wheat, *Triticum vulgare*, durum, *Triticum durum*, emmer, *Triticum dicoccum*, spelt, *Triticum spelta*, einkorn, *Triticum monococcum*, rye, *Secale cereale*, oats, *Avena sativa*, barley, *Hordeum vulgare*, corn, *Zea mays*, kafir, *Andropogon sorghum*, teosinte, *Euchlaena mexicana* Schrad., and sorghum, *Sorghum vulgare*, and subjected to chemical and immunological study.

The chemical study included the nitrogen distribution by the Van Slyke method, the free amino nitrogen, the free carboxyl groups, the true ammonia nitrogen, the cystine and tryptophane content, and the acid and alkali binding at various hydrogen ion concentrations and at different temperatures. This study showed certain similarities of chemical composition among the prolamines, as a class, as contrasted with the composition and behavior of such proteins as casein and fibrin. The chemical evidence suggested that the prolamines studied might be grouped into a "wheat group", which would include the proteins isolated from the genus *Triticum*, and a "corn group" including those isolated from maize, teosinte, kafir, and sorghum.

The genetic behavior of these groups has been extensively studied by plant breeders, although relatively more work has been done upon the wheat group. Sakamura,¹ Kihara,² and Sax³ have shown that *T. monococcum* is characterized by having 7 chromosomes, that *T. dicoccum* and *T. durum* have 14 chromosomes, and *T. vulgare* and *T. spelta* have 21 chromosomes. The fertility or

¹ Sakamura, *Bot. Mag.*, Tokyo, Vol. 32, 1918 (cited by Sax, *loc. cit.*).

² Kihara, *Bot. Mag.*, Tokyo, Vol. 33, 1919, Vol. 35, 1921 (cited by Sax, *loc. cit.*).

³ Sax, K., *Science*, 54, 413-15, 1921.

total or partial sterility of inter species crosses is in accordance with these chromosome numbers. In the "corn group" Collins⁴ has shown that teosinte will hybridize with *Zea mays*, indicating a genetic relationship.

Because of these observations it was believed that the immunological relationships of these proteins would prove of interest. Proteins of the "wheat group" were accordingly compared with proteins of the "corn group" and with each other, by means of the complement fixation test and by the anaphylaxis test, using the uterus strip method, the bronchospasm method, and the guinea pig reaction.

Complement fixation test:

Rabbits were immunized by repeated injections of gliadin, glutenin, and zein. Each of the three antisera obtained was tested against homologous protein and all the remaining proteins in amount of 0.01 mg., 0.1 mg. and 1.0 mg. The reactions brought out very clearly the relation of the proteins of the grasses to the proteins of wheat and corn. The prolamines from emmer, einkorn, spelt and durum are closely related to gliadin and glutenin from *T. vulgare*, while those from teosinte and kafir are closely related to zein from *Zea mays*. The former are more closely related to gliadin than they are to glutenin, while the protein from teosinte is more closely related to zein than is kafirin. No reactions were obtained between antisera for the corn group with proteins from the wheat group, or conversely.

Uterus strip method:

The results show the very close relationship of gliadin, and the proteins of emmer, einkorn, and spelt. A uterus strip that is sensitive to any one of these proteins is also sensitive to all of them. When the strip is desensitized to any one it is also desensitized to the others. A uterus strip sensitized to the protein of teosinte will contract and be completely desensitized to teosinte protein by exposing it to zein or to kafirin.

Bronchospasm method:

This is a new method described by Koessler and Lewis,⁵ which permits graphic tracings being made of the bronchospasm occurring in guinea pigs as a specific reaction to proteins to which the

⁴ Collins, G. N., *J. Heredity*, 12: 339-50, 1921.

⁵ Koessler and Lewis, in press.

guinea pigs are hypersensitive. The effects produced with the prolamines correspond completely to those observed by the uterus strip method.

Guinea pig anaphylaxis tests:

Experiments were made in which guinea pigs were sensitized with wheat gliadin or with zein, and then after two weeks given intro-peritoneal injections of the different prolamines. Although this method is not so delicate as the uterus strip and broncho-spasm methods, the results were quite the same, showing practical identity of proteins in the two groups. That is, guinea pigs sensitized with gliadin gave strong reactions when reinjected with the other four proteins of the wheat series, but none at all with proteins from the corn series. Animals recovering from these crossed reactions with the proteins of the wheat series, were protected against gliadin just as were the tissues in the uterine strip experiments.

Therefore we have data obtained by four different immunological methods all giving identical results, so that they may be looked upon as conclusive.

The detailed reports of these experiments will appear as separate publications. The chemical and physico-chemical data will appear in the Second Colloid Symposium Monograph to be issued by the Chemical Catalogue Company of New York City. The immunological data will probably appear in the Journal of Biological Chemistry.

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Further studies on the etiology of epidemic hiccough.

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In two previous epidemics I have isolated a streptococcus from infection atria in cases of epidemic hiccough which produced spasms of the diaphragm and other muscles in animals. This property was readily lost on ordinary aerobic cultivation, but by