1. A serological investigation of 2 inbreds.

It seems logical that there should be some degree of protein specificity differentiating inbred lines of corn. If such is the case, then different lines could be distinguished from each other serologically provided that our techniques are refined enough to detect the limited differences which are present.

Rabbits were the animals immunized. The inbred lines of corn, Hy and WF9, were used to induce antibody formation. 100 seeds of each inbred were soaked for 18 hrs. in distilled water at 0°C, and the embryos excised. These were ground into fine particles and extracted in Bloor's mixture for 14 hours. This solution was filtered and the precipitate extracted in a buffered salt solution for 30 hrs.

The rabbits used were bled and the sera checked against an aliquot of the protein solution of the two inbreds to check for acquired immunity for these particular proteins. All tests were negative.

Injections of the protein extracts were given intravenously in four doses. Dose sizes were 50 mg, 100 mg, and 200 mg of extracted protein in solution. The rabbits were injected with these doses at three day intervals. Eleven days after the last injection the sera were checked for antibody content. The titer was ca 1:5000. The rabbits were bled 13 days after the last injection; the sera obtained were filtered through a Seitz filter and stored at -2°C.

It was thought that the proteins common to both WF9 and Hy would induce, the formation of antibodies which could be removed by the heteroantigen leaving in the sera antibodies of a more specific nature. These antibodies would react with the homoantigen but not with the heteroantigen. If the protein components of the embryos of both inbred corn lines were similar then no reaction would be obtained using the homoantigen after the treatment of the antisera with the heteroantigen.

One cc. aliquots of antisera from the immunized rabbits were placed in tapered centrifuge tubes and .5 cc aliquots of heteroantigen were added. The tubes were shaken for 10 minutes and then centrifuged for 30 minutes at 1200 rpm. After this treatment all tubes had a considerable amount of precipitate. The controls, which consisted of antiserum alone, protein extract alone, protein extract plus water, and antiserum plus water were treated in the same fashion. No precipitate formed in the controls.

Subsequent treatments consisting of the addition of the heteroantigen to the antisera after centrifugation to remove precipitate of previous treatments failed in most instances to remove all of the antibodies capable of reacting with the heteroantigen, and leave antibodies specific for the homoantigen.

Some of the tests did give the results expected if there was a serological difference between the two inbreds, but the results were not consistent enough to prove the existence of such a serological difference.

The sera were also tested by means of the precipitin technique. Tubes in which the reactions were read were made from glass tubing having an inside diameter of 4 mm. and cut to a length of 5 cm. Approximately .2 cc. of serum was run into the bottom of a tube and the same amount of diluted protein extract introduced above the serum. The results of the precipitin reactions are given in table 1. In this table, the serum against which the extracts of the inbred lines of corn listed were run is designated as the antiserum. In recording the reactions, a heavy flocculent precipitate of considerable depth at the interface of the antiserum and the extract was read as a four-plus reaction, a reaction showing less depth was read as a three-plus reaction, a light but definite reaction was called a two-plus, and a very light reaction was read as a 1-plus reaction.

Table 1. Precipitin Reactions

| | | Dilution of Extract | | | | | |
|---|---|---|---|---|---|---|---|
| Hy antiserum | Extract | *1:5 | 1:10 | 1:20 | 1:40 | 1:80 | 1:160 |
| 1 | Hy | ++++ | +++ | ++ | + | + | + |
| 1 | WF9 | ++++ | +++ | ++ | + | + | + |
| WF9 antiserum | | | | | | | |
| 2 | WF9 | ++++ | +++ | + | + | + | + |
| 2 | Hy | ++++ | +++ | ++ | + | + | - |
| 3 | WF9 | ++++ | ++ | + | + | - | - |
| 3 | Hy | ++++ | +++ | + | + | - | - |

*Dilutions given in the table are to be multiplied by 100.

The data in table 1 indicate that if specific differences exist between these two inbred lines the precipitin technique is too crude to detect them.

Gerald Clary