IMMUNOCHEMICAL STUDIES OF THE PLANT PROTEINS:
PROTEINS OF THE WHEAT SEED AND OTHER
CEREALS. STUDY IX

R. P. Wodehouse

That wheat foods are active in causing asthma has become an
established fact and it has been shown that watery extracts made from
practically any of the wheat foods except those cooked at very high
temperatures (Goodale '16) will give positive skin reactions when
tested by means of the skin test (which is generally considered a test
for anaphylactic sensitization) to wheat asthmatics. This present
work was undertaken in order to find out which protein or proteins
of the wheat seed were responsible for the production of asthma by
isolating them individually in as pure a form as possible and testing
by means of the skin reaction.

The reserve proteins of wheat have been thoroughly studied by
Osborne and his co-workers (Osborne '10, '10.4, '07). By these in-
vestigators many different protein preparations were made from the
wheat seed and it was shown by very careful analyses of these that
there are five and probably only five distinct reserve proteins present
in the seed and these fall into the well-known protein classes as follows:

- Albumin of wheat = leucosin
- Globulin of wheat = wheat globulin
- Prolamine of wheat = gliadin
- Glutelin of wheat = glutenin
- Proteose of wheat = \begin{cases} 
\text{wheat natural proteose} \\
\text{wheat artificial proteose}
\end{cases}

These proteins can be distinguished by their elementary composi-
tion and by their amino-acid content which these investigators have
worked out (Osborne '10, Osborne and Clapp '06) but they are most
readily distinguished by their solubility characteristics which are used
to place them in the groups of plant proteins to which they belong.
For convenience they are briefly here summarized as follows:

1 Made possible through a gift by Mr. Charles F. Choat, Jr., Boston, to the
Peter Bent Brigham Hospital for the study of bronchial asthma.
caused the production of only a small precipitate, so the whole was poured into three volumes of a mixture of acetone and ether (80-20) and the precipitate so formed was centrifugalized out and washed in alcohol and ether and dried over sulphuric acid under diminished pressure. When desiccation was complete it formed a gray powder which was more or less insoluble according to the time of exposure to the alcohol baths.

Proteins Insoluble in Water or Neutral Aqueous Saline Solutions

Gliadin and glutenin occur mostly in the endosperm of the seed and are the proteins which make up gluten, the substance which gives what flour its capacity for making dough. In order to obtain the gluten the flour is mixed with water and kneaded into a stiff dough. This is then wrapped in muslin and kneaded under water until the starch is washed out. When it is mostly removed the dough may be taken from the muslin and the kneading continued under water until no further starch can be removed. In preparing the proteins for this work the gluten was next kneaded in several baths of 10 percent NaCl, then chopped fine and allowed to remain in a large volume of salt solution over night to complete the removal of the globulin, then (still chopped fine) it was allowed to remain in running water for some hours to remove the salt.

In order to dissolve out the gliadin it was now subjected to extraction with alcohol. This was done by boiling in 70 percent alcohol on the hot water bath for about an hour, using a reflux condenser to keep the alcohol from evaporating. It was then strained off through muslin and the extraction of the undissolved gluten was continued with a fresh bath of alcohol. In the meantime the first extract was filtered and the alcohol distilled off by heating in a retort on a boiling water bath. The alcohol recovered from this distillation was then diluted with water to make again 70 percent by the hydrometer test, and used for the next bath. This process was repeated five or six times or until nearly all the alcohol soluble protein was removed from the gluten. Care was taken during the evaporation of the gliadin solution in the still not to let too much alcohol evaporate. No attempts were made to see what percentage of alcohol remained or at what temperature it was boiling. Distillation was discontinued, however, while there was still enough alcohol left to lower the boiling point of the alcohol.

Leucosin is soluble in pure water or in water faintly acid or alkaline in reaction but is precipitated in an insoluble coagulum from a faintly acid solution by heat.

Wheat Globulin. In neutral media this is soluble only in saline solutions, the one generally used being 10 percent NaCl.

Gliadin is soluble in 50–80 percent alcohol but insoluble in water or absolute alcohol.

Glutenin is insoluble in water, alcohol or neutral salt solution but readily soluble in weak alkali, 1/5 percent being generally used.

Proteose is soluble in water and not precipitated by heat.

All of the proteins soluble in water or salt solutions may be precipitated by saturating their solutions with ammonium sulphate.

Whether these proteins are chemical individuals or whether we have to seek further separations is not definitely known. None has been prepared, as yet, in a crystalline form. Though some vegetable proteins have been crystallized, wheat globulin which, of the wheat proteins, approaches nearest to this condition, occurs only in more or less regular spheroids.

There is pretty good evidence to show that all of these proteins, including the “natural proteose,” exist preformed in the seed. Proteoses are generally regarded, however, as being the first product of hydrolysis of the higher proteins. Still Wells and Osborne (Wells and Osborne, '15) furnish good evidence that the “natural proteose” is not a product of hydrolysis but is a naturally existing protein of the seed.

The artificial proteose was prepared by hydrolysis from glutenin (as will be shown) and was used throughout the wheat experiments for comparison with the natural proteins in general and more especially to see if a proteose, known to be the product of hydrolysis, would give biological reactions similar to those of natural proteose.

Besides these individual proteins there were used in this work two “whole wheat” protein preparations made, one from raw wheat and the other from bread, by the author (Wodehouse, '16). Of course these two preparations do not, as their name might imply, contain all five of the wheat proteins in anything like equal proportions. On the contrary the solubilities, as indicated above, would prevent such preparations from containing more than a trace of glutenin and gliadin, and the bulk would be made up of the natural and decomposition proteoses together with leucosin and globulin where they were not precipitated by heat.
METHOD OF PREPARATION

Proteins Soluble in Aqueous Salt Solution

Since these proteins occur mainly in the embryo of the wheat grain and not very much in the endosperm (Osborne and Campbell, '00), ordinary white bread or pastry flour should not be used. The commercial "entire wheat" preparations give very good results except that the yield is small.

Leucosin, being soluble in pure water, can be extracted from the flour by simply soaking in cold water (with the addition of some preservative as thymol or toluol to prevent bacterial decomposition) for a few days, and decanting the supernatant fluid which then contains this protein together with some globulin, dissolved by virtue of the mineral salts contained in the grain, and proteose, together with sugars, etc. This method of preparing leucosin was discarded early in the work because it was found much easier to extract it together with the globulin and separate them afterwards. So the entire wheat flour was stirred in 10 percent salt solution (about 1,200 gm. to 3,500 cc.) and allowed to stand at room temperature for about three days (no preservative is necessary). The flour settles to the bottom and the supernatant solution (which is pinkish and syrupy) can be siphoned off. It is desirable to allow the flour to separate completely from the supernatant fluid so that further clearing will be unnecessary, for the viscosity of this solution renders it difficult to filter. Less than one half of the volume of the salt solution is recovered, the rest remaining entangled in the flour, so it is profitable to make a second extraction from the same flour by adding a volume of salt solution equal to that removed by decantation. This second extraction is almost as rich in protein as the first. Since the globulin is insoluble in water at neutral reaction it may now be separated out by dialyzing the whole solution in water until free from Cl (the other proteins may be separated from each other as will be subsequently shown), or all the proteins may be salted out together by saturation of the extract with ammonium sulphate, and separation effected by dialysis after again being dissolved.

In the preparation of natural proteose it was found desirable to follow the former method. When the salt extract is freed from NaCl by dialysis all of the globulin and possibly parts of some of the other proteins are thrown out of solution and can be removed by filtration.
If the filtrate be faintly acidulated and boiled the leucosin is coagulated and forms a flocculent precipitate which can be removed by filtration. The proteose, which now remains in solution, can best be obtained by reducing the volume by boiling on the water bath and then dialyzing against 95 percent alcohol when it appears in the form of a white precipitate which can be washed in alcohol and ether and dried over sulphuric acid giving a white powder. When prepared in this manner it is perfectly soluble in water or 0.01 M KOH, giving a clear solution.

The globulin precipitated by dialysis from 10 percent NaCl solution is largely insoluble when treated a second time with 10 percent NaCl. For this reason this method cannot be used advantageously for the preparation of globulin. It was found best to follow the method of first saturating the 10 percent NaCl extract with ammonium sulphate thereby throwing out of solution all of the proteins together. This precipitate can then be dissolved in 5 percent NaCl and the solution dialyzed free from Cl and SO₄ when the globulin is thrown out of solution in spheroids or imperfect crystals which can be separated from the solution by centrifugalizing. Globulin thus prepared can nearly all be redissolved in 5 percent NaCl and precipitated again by dialysis. In making the globulin preparations used in this work this was done twice in order to purify the preparations. It was then washed in water, 95 percent alcohol, absolute alcohol and ether and dried over sulphuric acid under diminished pressure. This preparation is completely soluble in 10 percent NaCl or weak alkali. It appears in more or less regular spheroids or imperfect crystals, so it can be considered to be reasonably pure.

Leucosin is very difficult to separate from proteose without coagulation, therefore no attempt was made to prepare it entirely free from proteose. The leucosin used in these experiments was prepared as follows: The NaCl extract, after the salt and globulin had been removed by dialysis was saturated with ammonium sulphate and the proteins thereby precipitated were filtered out and pressed as dry as possible between filter paper and redissolved in a small amount of water, in which they proved to be almost completely soluble. This solution was then dialyzed until free from the remaining ammonium sulphate, or until it failed to give a precipitate with barium chloride. This caused the production of a very small amount of an insoluble protein which was filtered out. The solution was then dialyzed against 95 percent alcohol until further reduced in volume. This
caused the production of only a small precipitate, so the whole was poured into three volumes of a mixture of acetone and ether (80–20) and the precipitate so formed was centrifuged out and washed in alcohol and ether and dried over sulphuric acid under diminished pressure. When desiccation was complete it formed a gray powder which was more or less insoluble according to the time of exposure to the alcohol baths.

PROTEINS INSOLUBLE IN WATER OR NEUTRAL AQUEOUS SALINE SOLUTIONS

Gliadin and glutenin occur mostly in the endosperm of the seed and are the proteins which make up gluten, the substance which gives what flour its capacity for making dough. In order to obtain the gluten the flour is mixed with water and kneaded into a stiff dough. This is then wrapped in muslin and kneaded under water until the starch is washed out. When it is mostly removed the dough may be taken from the muslin and the kneading continued under water until no further starch can be removed. In preparing the proteins for this work the gluten was next kneaded in several baths of 10 percent NaCl, then chopped fine and allowed to remain in a large volume of salt solution over night to complete the removal of the globulin, then (still chopped fine) it was allowed to remain in running water for some hours to remove the salt.

In order to dissolve out the gliadin it was now subjected to extraction with alcohol. This was done by boiling in 70 percent alcohol on the hot water bath for about an hour, using a reflux condenser to keep the alcohol from evaporating. It was then strained off through muslin and the extraction of the undissolved gluten was continued with a fresh bath of alcohol. In the meantime the first extract was filtered and the alcohol distilled off by heating in a retort on a boiling water bath. The alcohol recovered from this distillation was then diluted with water to make again 70 percent by the hydrometer test, and used for the next bath. This process was repeated five or six times or until nearly all the alcohol soluble protein was removed from the gluten. Care was taken during the evaporation of the gliadin solution in the still not to let too much alcohol evaporate. No attempts were made to see what percentage of alcohol remained or at what temperature it was boiling. Distillation was discontinued, however, while there was still enough alcohol left to lower the boiling
point of the solution sufficiently to cause it to boil vigorously on the hot water bath. In this way the risk of heating it to too high a temperature was avoided.

When this gliadin solution was allowed to cool a small part of the protein settled out in a gluey mass at the bottom, and part assumed the form of a fine suspension which would pass through filter paper and could not be removed by centrifugalizing. So it was warmed up enough to cause complete resolution and while still hot poured into the dialyzers and dialyzed against tap water for three days, using thymol as a preservative. At the end of this time the protein had all settled at the bottom. The supernatant fluid was discarded, the dialyzers torn open and the gliadin scraped off. At this stage the protein was light gray in color and resembled malleable rubber in consistency. It was thoroughly washed in distilled water, then cut up into fine pieces and digested successively with acetone and ether, absolute alcohol, ether, and dried over sulphuric acid under diminished pressure. When desiccation was complete it was ground in a mortar to a fine gray powder which could be used conveniently for making the tests in these experiments.

The residue from the gluten, remaining after the extraction of the gliadin, was now soaked in five or six volumes of 0.2 percent KOH to dissolve out the glutenin. This solution formed a thick opaque white fluid which could not be filtered; it was centrifugalized at high speed for about one hour; this precipitated a considerable amount of insoluble material. The supernatant fluid was poured off and very carefully neutralized by adding 1 percent HCl. This caused the production of a voluminous curly precipitate which reached a maximum at neutrality to litmus and would readily redissolve if made slightly more acid. This precipitated glutenin was removed by centrifugalization and the supernatant fluid, which was found to contain a large amount of protein, was evaporated down to about one fifth of its original volume on the water bath; the small amount of precipitate which had formed was discarded and the solution dialyzed free from KCl. Since no further precipitate was formed the dialyzer was transferred to 95 percent alcohol which reduced the volume still further and caused the appearance of a precipitate. Dialysis was continued in fresh baths of alcohol and finally absolute alcohol and when thus dehydrated the precipitate was removed, washed in absolute alcohol, ether and dried over sulphuric acid under diminished pressure. This gave a fine white powder which is called in these experiments "artificial proteose."
The precipitated glutenin was now dissolved in 0.2 percent KOH and centrifugalized to remove a small part that would not dissolve. It was then precipitated again by neutralization with HCl, washed in water, several baths of 70 percent alcohol to remove all traces of gliadin, absolute alcohol, ether, and dried over sulphuric acid, giving a fine white powder soluble in 0.01 M KOH.

The following table shows the reactions obtained from these wheat protein preparations. The tests, the results of which are here recorded, were all done under my observation by Dr. I. C. Walker (nos. 1–15 incl.), Dr. Turnbull (nos. 16–19 incl.), Dr. J. L. Goodale (nos. 20–22 incl.), Dr. Fritz B. Talbot (no. 23), and to them am I indebted for the use of their results.

Briefly described, the skin test, by means of which these results were obtained, is done by making small scarifications in the skin of the inner side of the forearm and applying separately to these the different proteins which are then moistened with 0.01 M KOH. A reaction is considered positive when an edematous swelling, which is usually surrounded by a red areola, makes its appearance about the scratch within a few minutes after the protein is applied. The intensity of the reaction is gauged by comparison with a control scratch upon which nothing but a drop of 0.01 M KOH has been put. For a more complete description of the test the reader is referred to the publications of the above mentioned investigators (Walker, this series no. V '17, Turnbull '16, Goodale '16, Talbot '16).

In recording these results ± is used to indicate a reaction scarcely stronger than the control and should probably very often be regarded as negative; + represents a quite definite reaction and the intensity of the reaction is represented in an arbitrary fashion by the number of plus signs, 4+ representing an edema about the size of a silver dollar. The size of the reactions alone, however, is a very inadequate comparison index of the anaphylactic activity of the proteins in question. For this reason the proteins were dissolved in 0.01 M KOH at a concentration of 1 percent and from this solution dilutions were made, in the same medium, in the series 1 : 100, 1 : 1,000, 1 : 10,000, etc., and wherever possible the tests were repeated using the dilutions instead of the dry proteins. Wherever this was done the lowest concentration to give a reaction is recorded in the table together with the size of the reaction.

In all, about seventy patients were tested, but only those are
| No. | Patient | Whole Wheat Proteins | Bread Proteins | Globulin | Gliadin | Glutenin | Leucosin | Nat. Proteose | Art. Proteose | Corn Protein | Oat Protein | Rice Protein | Barley Protein | Rye Protein | Wheat Pollen | Corn Pollen |
|-----|---------|----------------------|----------------|----------|---------|----------|----------|--------------|--------------|--------------|-------------|--------------|--------------|--------------|-------------|-------------|-------------|
| 1   | G.B.    | ±                    | ++            | ++       | ++      | 1:100    | 1:100    | ±            | 0            | 0            | 0           | 0            | 0            | 0            | 0           | 0           |
| 2   | J.M.    | ±                    | +             | 0        | ±       | 0        | 0        | ±            | 0            | 0            | 0           | 0            | 0            | 0            | 0           | 0           |
| 3   | C.K.    | ±                    | 0             | 0        | 0       | 0        | 0        | +            | 0            | 0            | 0           | 0            | 0            | 0            | 0           | 0           |
| 4   | C.N.E.  | ±                    | ++            | +        | +       | ±        | 0        | ±            | 0            | 0            | 0           | 0            | 0            | 0            | 0           | 0           |
| 5   | M.S.    | ++                   | +             | 0        | 0       | 0        | 0        | +            | 0            | 0            | 0           | 0            | 0            | 0            | 0           | 0           |
| 6   | F.A.    | +                    | ++            | +        | 3       | 3        | 3        | ±            | 0            | 0            | ±           | ±            | 0            | 0            | 0           | 0           |
| 7   | M.S.    | 0                    | 0             | 0        | 0       | 0        | 0        | +            | 0            | 0            | 0           | 0            | 0            | 0            | 0           | 0           |
| 8   | B.S.    | 0                    | 0             | 0        | 0       | 0        | 0        | +            | 0            | 0            | 0           | 0            | 0            | 0            | 0           | 0           |
| 9   | P.C.    | 0                    | 0             | 0        | 0       | 0        | 0        | +            | 0            | 0            | 0           | 0            | 0            | 0            | 0           | 0           |
| 10  | P.D.    | 0                    | 0             | 0        | 0       | 0        | 0        | +            | 0            | 0            | 0           | 0            | 0            | 0            | 0           | 0           |
| 11  | H.M.    | +                    | ++            | +        | +       | 1:100    | 1:100    | ±            | +            | 0            | ±           | +           | +           | ±           | +           | +           |
| 12  | F.G.    | +                    | 0             | ±        | ±       | 1:100    | 1:100    | ±            | 0            | 0            | ±           | 0           | 0           | 0           | 0           | 0           |
| 13  | G.B.    | ±                    | ±             | ±        | ±       | ±        | ±        | ±            | ±            | ±           | ±           | ±           | ±           | ±           | ±           | ±           |
| 14  | D.      | ++                   | +             | +        | +       | +        | ±        | 1:100        | 1:100        | 1:100        | ±           | 1:100       | ±           | ±           | ±           | ±           |
| 15  | H.      | ++                   | +             | +        | +       | +        | 0        | ±            | 0            | ±           | 0           | ±           | ±           | ±           | ±           | ±           |
| 16  | R.      | +                    | 0             | 0        | 0       | 0        | 0        | +            | 0            | 0            | 0           | ±           | +           | ±           | ±           | ±           |
| 17  | F.      | 0                    | 0             | 0        | 0       | 0        | 0        | +            | 0            | 0            | 0           | 0           | 0           | ±           | ±           | ±           |
| 18  | B.      | +                    | 0             | 0        | 0       | 0        | 0        | +            | 0            | 0            | 0           | ±           | +           | +           | +           | +           |
| 19  | S.      | +++                  | ++            | ++       | ++      | +        | +        | +            | 0            | +            | +           | +           | +           | +           | +           | +           |
| 20  | G.      | 4+                   | +             | ++       | ++      | 0        | +        | +            | 0            | 0            | ±           | +           | +           | ±           | ±           | ±           |
| 21  | Du.     | ++                   | +             | +        | +       | +        | +        | +            | 0            | 0            | 0           | ±           | ±           | ±           | ±           | ±           |
| 22  | De.     | +                    | ±             | 0        | 0       | 0        | 0        | +            | ±            | 0            | ±           | ±           | ±           | ±           | ±           | ±           |
| 23  | N.      | +                    | ±             | 0        | 0       | 0        | 0        | ±            | ±            | 0            | +           | +           | +           | +           | +           | +           |

*Not tested until after treatment when they were found to be negative.*
included which gave a positive reaction with one or more of the wheat proteins.

For the sake of comparison with the other cereals, corn, oat, rice, barley and rye proteins were tested, using for this purpose the preparations made by the author (Wodehouse, '16).\textsuperscript{2}

The writer is well aware of the incompleteness of the records shown in the following table but this is due to the fact that the investigation was carried out upon patients the number of which reacting to wheat proteins was limited. Then with patients it is not possible to repeat tests with the frequency and thoroughness that is possible when using animals for anaphylactic tests.

When this work was begun the writer was expectant of finding that some one of the proteins of wheat was entirely responsible for its anaphylactogenic properties or else that they all behaved in the same fashion and it was with the idea of isolating the “active principle” that the work was undertaken. That no such simple state of affairs exists can be seen from a glance at the table. On the contrary this work shows that, though all of the proteins are capable of calling forth anaphylactic symptoms, their method of action is so complex that with our present state of knowledge it baffles explanation. Several interesting obtentions to which attention is drawn in the following paragraphs should, however, be noticed.

In many cases where the whole wheat preparation gives a doubtful reaction or even in some where it gives a negative, some one or more of the individual proteins give a quite definite reaction and always one or more of the individual proteins are as active or, as is usually the case, more so than the whole wheat preparations.

Of the total number of patients with which any part of wheat gave a reaction

<table>
<thead>
<tr>
<th>Protein</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole wheat protein</td>
<td>reacted with 60</td>
</tr>
<tr>
<td>Bread protein</td>
<td>&quot;</td>
</tr>
<tr>
<td>Globulin</td>
<td>&quot;</td>
</tr>
<tr>
<td>Gliadin</td>
<td>&quot;</td>
</tr>
<tr>
<td>Glutenin</td>
<td>&quot;</td>
</tr>
<tr>
<td>Leucosin</td>
<td>&quot;</td>
</tr>
<tr>
<td>Natural proteose</td>
<td>&quot;</td>
</tr>
<tr>
<td>Artificial proteose</td>
<td>&quot;</td>
</tr>
</tbody>
</table>

\textsuperscript{2} These preparations were made by soaking the uncooked flour or meal of the cereals in water until a solution rich in protein was obtained. From this the protein material was precipitated by alcohol and the precipitate dried in alcohol and ether.
In computing these figures doubtful reactions were always counted as negative and this accounts for the small proportion (viz., 60 percent) of reactions obtained with whole wheat. Since it is composed of several different proteins this preparation would be expected to have proportionately more chances of producing reactions. On the other hand, however, being a mixture the active proteins would be diluted, to a large extent, by the inactive proteins and with cases in which the active proteins are few in number or weak in reaction this dilution might be sufficient to obscure their activity almost to, or even below, the limit of sensibility of the skin test, thus accounting for the large proportion of doubtful or negative reactions of the whole wheat while with the same cases at the same time the individual proteins reacted quite strongly.

In case No. 10 is seen a good example of this. Here "natural proteose" is the only one active but in the whole wheat preparation it does not call forth a response because its activity is obscured by the other four. With the case of No. 13 "natural proteose" and gliadin are the active parts but their activity is masked by the three other inactive parts. Except to Nos. 1 and 4 this explanation can be applied to all. However it is only tentative and a definite explanation must await further investigation.

An even more interesting result to be observed here is that the "natural proteose" is the most active, producing reactions with 72 percent of the cases while the "artificial proteose" only shows activity towards 36 percent. This shows that these two proteins are not immunologically alike and lends support to the contention of Wells and Osborne (Wells and Osborne, '15) that the "natural proteose" exists preformed in the seed and is not formed, as is the "artificial proteose" by reagents used in extraction and purification.

It is also to be seen that heating to a cooking temperature does not destroy the anaphylactogenic properties of wheat. However the heating employed in the cooking of bread somewhat reduces its activity in most cases. Nevertheless with some the reverse is true. In order to test this further a concentrated watery extract of flour was boiled for several hours. Another was heated in the autoclave at a temperature of 114° C. and a pressure of 15 pounds per square inch for one hour. When the coagula formed by the heat were filtered off and skin tests performed with the filtrates it was found that neither heating to a temperature of 114° C. nor prolonged boiling had reduced
their activity in the slightest degree. Nevertheless when the whole seeds of wheat and some of the other cereals were heated in a crucible until they became a light tan color all through, they were found to have completely lost their activity. This was also found to be true of the prepared cereal foods, which are said to be cooked at temperatures much exceeding any reached by an ordinary autoclave, such as “Puffed Wheat,” “Puffed Rice,” Kellogg’s “Toasted Wheat Biscuit,” “Shredded Wheat,” etc. When concentrated aqueous extracts made from these were tested by means of the skin test upon patients who were strongly sensitive to the corresponding cereals in the raw form no reaction whatsoever was obtained. In this connection it is interesting to note that in the preparation of the most active of the individual wheat proteins (viz., “natural proteose”) considerable boiling is employed. From this it is seen that only very high temperatures tend to diminish the anaphylactogenic activity of wheat and of some of the other cereals in relation to sensitization as revealed by the skin test.

In cases sensitized to the pollens of the Gramineae it has been pretty definitely shown that idiosyncrasy to pollen of one species of grass is almost always accompanied by sensitization to the pollens of all the grass family (Goodale, ’15). However, in cases allergic to the seed proteins of the Gramineae we see that this is not generally so, though sometimes it may be, especially with cases highly sensitized. This is entirely in keeping with Nuttall’s findings in the immunological relationships among the animal proteins. He says: “The more powerful the antiserum obtained the greater its sphere of action upon the bloods of related species. For instance, a weak anti-human serum produced no reaction with the blood of the Hapalidae, whereas a powerful serum did produce a reaction” (Nuttall, ’01). This is confirmed by Uhlenhuth (Uhlenhuth, ’01) in experiments upon the relationships between the ox, goat and sheep.

The question as to what extent subjects which are hyper-sensitive to the seed proteins of the Gramineae respond to the pollens of this family should be further investigated. It is interesting to note in passing that wheat pollen was entirely negative with the one case upon which it was tried although this case was extremely sensitive to the proteins of the seed. The same preparation of wheat pollen, however, gave good reactions with some grass hay-fever cases.

These tests were made by Dr. J. L. Goodale with the materials prepared by the author.
TOXICITY OF GALACTOSE AND MANNOSE FOR GREEN PLANTS

by addition to the nutrient solution or by addition of another sugar solution. For example, to obtain a solution containing 0.25 mol. galactose + 0.025 mol. saccharose, it was necessary to mix equal parts of 0.5 mol. galactose and 0.5 mol. saccharose. The volume of the medium in each tube was 25 cc. Sterilization was effected by autoclaving at fifteen pound pressure for fifteen minutes.

All the sugars used, with the exception of arabinose, were supplied by Dr. C. S. Hudson, in charge of the carbohydrate laboratory, U. S. Bureau of Chemistry, and are stated by him to be of very high purity. The arabinose used was a Merck reagent.

Character of the Injury. — The injurious action of galactose is made evident first in the roots. The primary root coming in contact with the agar may first become brown and in a few days death results. In other cases the tip of the root is killed and this stimulates the production of a large number of lateral roots, the tips of which, on coming in contact with the agar medium, are soon killed. A short primary root with many laterals results, the appearance of which is somewhat centipedal. Two plants in the same culture may, however, vary in the manner of injury, and the presence of certain sugars may alter the extent of the injury.

For the sake of clearness and definiteness, it seems desirable to describe the injury by a numerical system as well as by root lengths. Accordingly the following key is given: 0, no injury; i, primary root tip killed, laterals not injured; 2, the primary root tip may be killed, but the laterals may attain a length of a few centimeters and then growth is stopped or the roots are killed; 3, the primary root may penetrate the agar, but becomes brownish and five or six centimeters long; 4, the primary root may attain a length of a few centimeters, but becomes brown in color and the laterals do not grow beyond 0.5 cm.; 5, the primary root tip is killed and all laterals suffer likewise; 6, the primary root is entirely killed.

Antagonistic Action. — In the following experiment the galactose was supplied at a concentration of 0.025 mol. and the other sugars were used at the same concentration. In order to demonstrate conclusively that the total concentration was not responsible for any toxicity, a few cultures were made with the nontoxic sugars supplied at 0.05 mol. The experiment was begun on January 29, 1917, and concluded on February 13, 1917. The cultures were placed in the greenhouse and grown in the light. All cultures were made in triplicate.

SUMMARY

The five proteins globulin, gliadin, glutenin, leucosin and natural proteose were prepared from wheat according to the method of T. B. Osborne, and when they were compared in their anaphylactogenic properties with each other, with an artificial proteose prepared by hydrolysis from glutenin, with the whole wheat preparations and with the proteins of other cereals, it was found that (1) all are anaphylactogenic, but no two are immunologically exactly alike, (2) the natural proteose is the most active, (3) the natural proteose is different from the artificial proteose, (4) in any given case where whole wheat gives a reaction and in some where it does not some one or more of the individual proteins are sure to be found to be more active, (5) it does not necessarily follow that because a case is allergic to wheat it will be found to be also hypersensitive to the other cereals (though this is sometimes the case especially if sensitization is of a high order), (6) it probably does not follow that sensitization to the seed proteins of cereals necessitates sensitization to the pollens of the same species, though not enough experiments were done upon this to more than suggest that it is a problem that ought to be further investigated.

It is also shown that heating, except to very high temperatures, does not materially affect the anaphylactogenic properties of the wheat proteins.

MEDICAL CLINIC OF THE
PETER BRIGHAM HOSPITAL,
BOSTON, MASS.

LITERATURE CITED

imitate, but contamination or failure to germinate caused a loss of some of the cultures. The seeds were sterilized by immersion in a solution of calcium hypochlorite (calcium oxychloride, Baker) according to the method of Wilson (1915). The peas were treated for two hours and the wheat for five hours. The results are given in Table I.

**Table I**

Influence of Sugars on the Toxicity of Galactose

From the table it will be noted that the toxicity of galactose is prevented by glucose or saccharose, the former being slightly more effective than the latter since the primary root is not killed in the presence of glucose. None of the other sugars are effective in preventing the injurious action of galactose, although in the presence of levulose the primary root may continue its growth to a limited extent. Representative cultures are shown in Fig. 1.

All of the preceding experiments except those with levulose were repeated and similar results were obtained. In some earlier experiments (Knudson, 1916) it was noted that glucose does not antidote galactose if the concentration of the former is less than that of the latter. It was thought that some relation might be found between concentrations and antagonistic action. Accordingly the galactose was supplied in each case at a concentration of 0.0125 mol. solution, and the other sugars used at double this concentration.

**View This Item Online:** [https://www.biodiversitylibrary.org/item/181265](https://www.biodiversitylibrary.org/item/181265)

**DOI:** [https://doi.org/10.1002/j.1537-2197.1917.tb05465.x](https://doi.org/10.1002/j.1537-2197.1917.tb05465.x)

**Permalink:** [https://www.biodiversitylibrary.org/partpdf/314337](https://www.biodiversitylibrary.org/partpdf/314337)

**Holding Institution**
Smithsonian Libraries and Archives

**Sponsored by**
Biodiversity Heritage Library

**Copyright & Reuse**
Copyright Status: Not in copyright. The BHL knows of no copyright restrictions on this item.

This document was created from content at the Biodiversity Heritage Library, the world's largest open access digital library for biodiversity literature and archives. Visit BHL at [https://www.biodiversitylibrary.org](https://www.biodiversitylibrary.org).