| Protein. | Source. | $(\alpha)_D^{20°}$. |
|---|---|---|
| Edestin | Hemp-seed | —41.3° |
| Globulin | Flaxseed | —43.53° |
| Globulin | Squash-seed | —38.73° |
| Excelsin | Brazil nut | —42.94° |
| Amandin | Almonds | —56.44° |
| Corylin | Filbert | —43.09° |
| Globulin | English walnut | —45.21° |
| Globulin | Black walnut | —44.43° |
| Phaseolin | Kidney bean | —41.46° |
| Legumin | Horse bean | —44.09° |
| Zein | Maize | —28.00° |
| Gliadin | Wheat | —92.28° |

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[FROM THE LABORATORY OF THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION.]

# THE GLOBULIN OF THE ENGLISH WALNUT, THE AMERICAN BLACK WALNUT AND THE BUTTERNUT.

BY THOMAS B. OSBORNE AND ISAAC F. HARRIS.

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PROTEINS, whose chemical identity is probable, have thus far been found only in seeds which are botanically closely related to each other.

Thus the chemical identity of gliadin from wheat or rye, of legumin from vetches, horse beans, lentils or peas, of vicilin from the three latter seeds, of phaseolin from the kidney or adzuki beans and of legumelin from the seeds of numerous legumes appears to be highly probable.

The strict chemical identity of carbon compounds of such high molecular weight can, of course, not be positively asserted, since the possibility of isomeric or homologous compounds of very similar properties is great. However, a rigid comparison of these supposedly identical proteins has as yet shown no difference whatever between them. Some proteins, which we had formerly regarded as identical, have recently been found to differ in the proportions of their various decomposition products, and it is certain that their molecules have a different structure. All these proteins were found in seeds which were *not* botanically closely related and should therefore, by analogy, *not* contain the same proteins. So marked is this difference in the protein constituents of the different seeds that the question may be asked: Is not this chemical difference in the food of the growing embryo an important factor in determining the nature of the developing organism?

Now that wide differences in the structure of the molecules of the different proteins are more fully recognized, it seems probable that the course of the chemical reactions, leading to the development of the growing embryo, will be largely influenced by the nature of the protein food presented to it at the beginning of its existence. Whether similar differences in the protein food of the animal embryo exists is not so definitely determined, but the few facts on record point strongly that way, for Panormoff's investigations indicate that hen's, dove's and crow's eggs contain distinctly different albumins.

The closest resemblance between proteins from unrelated seeds which we have as yet encountered is presented by corylin from the filbert (*corylus*) and the globulin from the English walnut (*Juglans regia*), the only difference, as yet discovered, being a slightly smaller yield of ammonia by the latter, when decomposed with hydrochloric acid, and a slight difference in the precipitation limits with ammonium sulphate.

In order to see if the same relations would be shown by the globulins from the botanically, closely related American black walnuts and butternuts (*Juglans nigra* and *J. cinera*) we have examined these nuts with the following results.

THE GLOBULIN OF THE BLACK WALNUT (*Juglans nigra*).

The nuts were freed from their shells, crushed, the greater part of the oil pressed out and the remainder extracted with ether. The residual meal was then extracted with 10 per cent. sodium chloride solution, the extract filtered clear and dialyzed. The globulin was deposited in spheroids which were filtered out, washed thoroughly with water and then with alcohol, and dried over sulphuric acid. Dried at 110°, this preparation had the following composition:

BLACK WALNUT GLOBULIN, PREPARATION I.

| | |
|---|---|
| Carbon | 50.95 |
| Hydrogen | 7.10 |
| Nitrogen | 18.84 |
| Sulphur | 0.78 |
| Oxygen | 22.33 |
| | 100.00 |
| Ash | 0.38 |

This preparation was then dissolved in 10 per cent. sodium chloride solution, a large quantity of the insoluble form of the globulin filtered out and the clear solution dialyzed for several days. The globulin, which again precipitated in spheroids, was filtered out, washed with water and with alcohol and dried over sulphuric acid. The 32 grams thus secured, which formed about one-half of the original material, had, as the following figures show, essentially the same composition, when dried at 110°, as the preparation from which it was derived.

BLACK WALNUT GLOBULIN, PREPARATION 2.

| | I. | II. |
|---|---|---|
| Carbon | 51.06 | 51.07 |
| Hydrogen | 6.87 | 6.84 |
| Nitrogen | 18.96 | 18.96 |
| Sulphur | 0.77 | .... |
| Oxygen | 22.34 | .... |
| | 100.00 | |
| Ash | 0.33 | |

This preparation was almost wholly soluble in 10 per cent. sodium chloride solution and showed the same reactions as those given in an earlier paper for the globulin of the English walnut.[1] A solution containing about 5 per cent. of the globulin and 10 per cent. of the sodium chloride remained clear until heated to 99°, when it became turbid and, after continued heating, yielded a considerable coagulum. Solutions of the globulin from the English walnut yielded a trace of coagulum at lower temperatures, which was unquestionably due to a trace of another protein, adhering to the preparation in minute quantity. Saturation of the solution with sodium chloride gave a very slight precipitate and with magnesium sulphate a very considerable one, in this respect exactly resembling the globulin of the English walnut. The other reactions were also, in all respects, the same.

When decomposed by boiling with strong hydrochloric acid, the proportion of nitrogen in the different forms of binding were as follows:

| N as $NH_3$. | Basic N. | Non-basic N. | N in MgO precipitate. | Total. |
|---|---|---|---|---|
| 1.71 | 5.61 | 11.45 | 0.19 | 18.96 |
| 1.86 | 5.85 | 10.92 | 0.33 | .... |
| 1.83 | 5.85 | 11.04 | 0.24 | .... |

The specific rotation of this globulin was determined as follows:

[1] This Journal, 18, 609 (1896).

| Solvent. | Amount per cc. Gram. | Observed rotation. | Length of tube. | Specific rotation. $(\alpha)_D^{20°}=$ |
|---|---|---|---|---|
| 10 % NaCl | 0.0272 | 1.22° | 1 dm. | —44.85° |
| ...... | 0.0273 | 1.20° | 1 dm. | —44.0° |

The precipitation limits with ammonium sulphate were determined by making a 2.7 per cent. solution of the globulin in one-tenth saturated ammonium sulphate solution and then adding different quantities of a saturated solution of ammonium sulphate to 2 cc. of the globulin solution previously diluted with enough one-tenth saturated to make a final volume of 10 cc.

The mixture became turbid when the amount of sulphate was equal to that in 2.8 cc. of a saturated solution. When sulphate equal to 4.6 cc. was added, only a very minute quantity of protein remained unprecipitated, which was doubtless a trace of some other adherent protein. With less than 4.6 cc. the amount in solution was greater. The precipitation limits are therefore 2.8 cc. and 4.6 cc.

THE GLOBULIN OF BUTTER NUT (*Juglans cinerea*).

The preparation of this globulin, which we examined, was very kindly given to us by Dr. A. L. Dean, of Yale University, for which we here wish to express our thanks. Dr. Dean extracted the crushed meats of the nut with petroleum ether and exhausted the residual meal with 10 per cent. sodium chloride solution. The clear, filtered extract was dialyzed in running water for seven days when the globulin, which separated in spheroids, was filtered out and washed with water, with alcohol and with ether. As the quantity of the globulin was not sufficient for further purification by reprecipitation, it was examined in the condition in which it was received.

Dried at 110° it had the following composition:

BUTTER-NUT GLOBULIN, PREPARATION I.

| | | |
|---|---|---|
| Carbon | 50.85 | 50.91 |
| Hydrogen | 6.79 | 6.88 |
| Nitrogen | 18.62 | 18.59 |
| Sulphur | 0.80 | .... |
| Oxygen | 22.94 | .... |
| | 100.00 | |
| Ash | 3.54 | 3.67 |

This preparation was almost completely soluble in 10 per cent. sodium chloride solution and showed throughout the same reactions as those given by the globulins of the English and black walnut.

When decomposed by boiling with strong hydrochloric acid, the proportion of nitrogen in the different groups of nitrogenous decomposition products was as follows:

| N as $NH_3$. | Basic N. | Non-basic N. | N in MgO precipitate. | Total. |
|---|---|---|---|---|
| 1.83 | 5.77 | 10.87 | 0.14 | 18.61 |

The specific rotation of this globulin was found as follows:

| Solvent. | Amount per cc. | Observed rotation. | Length of tube. | Specific rotation. $(\alpha)_D^{20^\circ} =$ |
|---|---|---|---|---|
| 10 % NaCl | 0.0212 | 1.97° | 2 dm. | —46.9° |
| 10 % NaCl | 0.0223 | 0.98° | 1 dm. | —43.9° |

The precipitation limits of a 4 per cent. solution of the globulin, dissolved in one-tenth saturated ammonium sulphate, were determined in the same manner as for the black walnut globulin and found to be 3.1 cc. and 5.5 cc., the amount remaining in solution with 5.5 cc. being more than in the case of the black walnut, at the higher limit, which was doubtless due to the lack of sufficient purification of this preparation, and possibly also to a difference in acidity between the preparations examined.

## SUMMARY.

In the following table the results of our examination of the globulin from the seeds of the three species of *Juglans* are brought together for comparison with those of the globulin of the filbert, *Corylus*.

### COMPOSITION.

| | *J. regia.* | *J. nigra.* | *J. cinerea.* | *Corylus.* |
|---|---|---|---|---|
| Carbon | 50.80 | 51.07 | 50.88 | 50.72 |
| Hydrogen | 6.84 | 6.86 | 6.84 | 6.86 |
| Nitrogen | 18.96 | 18.96 | 18.62 | 19.02 |
| Sulphur | 0.80 | 0.77 | 0.80 | 0.83 |
| Oxygen | 22.51 | 22.33 | 22.86 | 22.57 |
| | 100.00 | 100.00 | 100.00 | 100.00 |

### PERCENTAGE OF NITROGEN IN THE GROUPS OF NITROGENOUS DECOMPOSITION PRODUCTS.

| | N as $NH_3$. | Basic N. | Non-basic N. | N in MgO precipitate. |
|---|---|---|---|---|
| *J. regia* | 1.84 | 6.08 | 10.93 | 0.11 |
| *J. nigra* | 1.80 | 5.77 | 11.14 | 0.25 |
| *J. cinerea* | 1.83 | 5.77 | 10.87 | 0.14 |
| *Corylus* | 2.20 | 5.75 | 10.70 | 0.16 |

### SPECIFIC ROTATION $(\alpha)_D^{20^\circ}$.

| | |
|---|---|
| *J. regia* | —45.21° |
| *J. nigra* | —44.42° |
| *J. cinerea* | —45.40° |
| *Corylus* | —43.09° |

### PRECIPITATION LIMITS WITH AMMONIUM SULPHATE.

| | | |
|---|---|---|
| *J. regia* | 2.8 cc. | —4.6 cc. |
| *J. nigra* | 2.8 cc. | —4.6 cc. |
| *J. cinerea* | 3.1 cc. | —5.5 cc. |
| *Corylus* | 3.7 cc. | —5.3 cc. |

The only positive difference shown by the preparations from these four seeds is the greater quantity of N yielded as ammonia by the globulin from the filbert (*Corylus*). This difference exceeds the limits of experimental error by too great an amount to be overlooked and may be taken as evidence that the globulin of *Corylus* differs in structure from that of *Juglans.* A positive difference between the corylin and the globulin from *J. regia* and *J. nigra* is also shown by the precipitation limits with ammonium sulphate. This difference, however, is not very great and, in view of our ignorance of the extent to which these limits may be altered by slight differences in the conditions of experimentation, cannot be considered as conclusive evidence, though it strongly indicates a difference between the globulins.

The precipitation limits of the globulin from *J. cinerea* are higher than those found for *J. regia* and *J. nigra,* but in view of the very close agreement between these preparations in all other respects we are inclined to attribute this difference to the greater purity of the preparations from the latter seeds.

In consequence of these facts, it seems desirable to give a name to the globulin of Juglans, retaining the designation corylin for the globulin of *corylus.* We propose therefore to call the principal protein which we have obtained from the nuts of the three species of the former genus Juglansin.

The botanical relations which are shown by the proteins of seeds deserve further careful study, as it is not improbable that the natural relations of some plants may be thus discovered.

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[FROM THE LABORATORY OF THE CONNECTICUT AGRICULTURAL EXPERIMENT STATION.]

## THE TRYPTOPHANE REACTION OF VARIOUS PROTEINS.

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AS LONG ago as 1831, Tiedemann and Gmelin recognized among the decomposition products of protein bodies a substance whose solution was colored a deep violet-red with chlorine or bromine. The nature of this substance remained wholly unknown until Hopkins and Cole recently succeeded in isolating it in a state of purity and recognizing it as most probably indol-amino-propionic