

the solution, which seems to indicate that the iron present in the mineral is ferric. Some crystalline green arsenate (?) of copper was observed in the tube.

The results of the oxidation experiments detailed in this paper seem to bear out the relationship between arsenopyrite and marcasite indicated in the isomorphism of the two minerals, but the arsenic seems to be a disturbing element which fixes a limit, at an early stage of the oxidation, to further action of any given strength of the oxidant. The experiments with hydrochloric acid gas seem again to show the disturbing action of the arsenic present, which, at the higher temperature at least, is probably removed by heat alone, as is the case with the majority of diarsenides, leaving finally a compound readily acted on by the hydrochloric acid as shown by the large amount of sulphur removed in the three-hour's experiment at 325°. The results of this series of experiments are, therefore, not very significant as compared with those for pyrite and marcasite as given in Brown's paper. If, as indicated by the oxidation experiments, the arsenopyrite is rather to be compared with marcasite, in which Brown has shown that the iron is in the ferrous condition, we may look on arsenopyrite as a ferrous compound, but this conclusion seems to be negatived by the result of the single experiment of decomposing the mineral in a solution of copper sulphate under pressure. In this experiment it will be remembered, no considerable amount of ferrous iron was found. Further research in this latter direction must be made before definite statements as to the constitution of arsenopyrite can be formulated.

UNIVERSITY OF PENNSYLVANIA,
June, 1894.

THE PROTEIDS OF THE KIDNEY BEAN.¹

(PHASEOLUS VULGARIS.)

BY THOMAS B. OSBORNE.

THE only work on the proteids of the kidney bean having importance at the present time, is that of Ritthausen. This investigator in 1883, stated² that the extract of the white bean

¹ Reprinted from the report for 1893 of the Connecticut Agricultural Experiment Station, New Haven.

² *J. prakt. Chem.*, 103, 204.

which has a weak alkaline reaction, and is extremely difficult to filter, when made clear by subsidence, yields on adding acetic acid, a voluminous precipitate, amounting to about eleven per cent. of the seed, having the following composition:

| | |
|----------------|--------|
| Carbon | 51.48 |
| Hydrogen | 7.04 |
| Nitrogen | 14.40 |
| Sulphur | 0.42 |
| Oxygen | 26.66 |
| | 100.00 |

In 1884, Ritthausen¹ published the results of further study of this seed. He then stated that the proteid, obtained in his earlier experiments, was wholly insoluble in five per cent. sodium chloride solution, but partially dissolved in two per cent. brine, and by diluting was precipitated in a form entirely like the original substance. By treating the bean meal with alcohol he found that clarification of the extracts was greatly facilitated and he, therefore, used meal so treated in his subsequent experiments.

By extraction with two per cent. sodium chloride solution, and clearing the extract by subsidence, he obtained, in two cases, precipitates of proteid, amounting respectively to 13.2 and 11.45 per cent. of the meal. Another preparation, equal to 8.67 per cent., he obtained from an aqueous extract of the meal precipitated with hydrochloric acid, the precipitate being redissolved in potash water and the solution neutralized after filtering clear. By extracting the bean meal with dilute hydrochloric acid, he obtained 10.07 per cent. of proteid. Preparations made by precipitating aqueous solutions with hydrochloric acid and sodium chloride extracts by dilution and submitting the precipitates to analysis without re-solution and consequent purification, had a composition similar to that found for the earlier preparations.

The precipitate obtained from hydrochloric acid solutions, had a different composition, agreeing with that of the proteid extracted by dilute salt solution both from the older preparations and from the freshly formed precipitate produced by acids in the aqueous extract, as well as with that of the insoluble portion remaining after this extraction, and also with that of the

¹ *J. prakt. Chem.*, N. Folge, 29, 452.

precipitate produced by hydrochloric acid in the aqueous extract, redissolved in dilute potash water and precipitated by neutralization. An average of five quite closely agreeing analyses of the proteid thus obtained, is shown by the following figures:

| | |
|----------------|--------|
| Carbon | 52.55 |
| Hydrogen | 7.09 |
| Nitrogen | 16.18 |
| Sulphur..... | 0.43 |
| Oxygen | 23.75 |
| | 100.00 |

This proteid, Ritthausen remarked, "is so similar in composition to albumin, that one might be led to regard it as an albumin, low, indeed, in sulphur." In most respects the experiments of the writer now to be detailed, confirm these observations of Ritthausen, but in two particulars his statements have not been corroborated. The reaction of the aqueous extract, I have found in all cases, to be distinctly acid, even when the beans were tested within two minutes after crushing. Ritthausen's statement that his earlier preparations were wholly insoluble in five per cent. solution of sodium chloride, while a very considerable quantity was dissolved by two per cent. brine, is difficult to understand, unless as implied, though not directly so expressed, the presence of the proteid was detected by largely diluting the salt-water extracts. The writer has found that strong saline solutions, unless containing a large proportion of this proteid, are not precipitated by dilution, the salt being present in sufficient quantity to serve as a solvent for the diminishing percentage of the dissolved proteid.

I have been able to identify and obtain in a state of comparative purity, two distinct proteids, one, the most abundant, having quite the properties of a globulin, which I shall designate *phaseolin* and another, not so definite in character, that may be distinguished as *phaselin*.

PREPARATION OF PHASEOLIN.

An extract was prepared by treating 500 grams of freshly ground bean meal,¹ which had been previously exhausted with ether, with one liter of two per cent. sodium chloride solution.

¹ The "White Medium Field Bean," the seeds being about $\frac{7}{8}$ inch or one cm. in length, was used for this investigation.

The residue was strained out on coarse linen squeezed dry in a screw press, and again treated with a liter of the two per cent. salt solution. The resulting extract filtered very slowly and not wholly clear; about nine-tenths of it was finally obtained as a turbid filtrate. This was saturated with ammonium sulphate and the precipitate produced collected on a filter and separated as completely as possible from the solution. This precipitate was removed from the filter and treated with water. Much of the substance went into solution, but a considerable part remained undissolved. After twenty-four hours filtration, a very nearly clear liquid was obtained, amounting to about two-thirds of the solution. This was then dialyzed in a stream of river water for six days. When thus freed from chlorides, the contents of the dialyzer were transferred to a filter, but only a part of the separated proteid remained on the paper, the filtrate being milky. The precipitate collected was washed with water, alcohol and ether, and when dried over sulphuric acid, weighed sixteen grams. This preparation had the following composition:—1.

| PHASEOLIN, PREPARATION 1. | | | | |
|---------------------------|-------|-------|----------|-----------|
| | I. | II. | Average. | Ash-free. |
| Carbon..... | 51.73 | | 51.73 | 52.23 |
| Hydrogen... | 6.89 | | 6.89 | 6.95 |
| Nitrogen.... | 16.28 | 16.14 | 16.21 | 16.37 |
| Sulphur..... | 0.68 | 0.54 | 0.61 | 0.62 |
| Oxygen..... | | | | 23.83 |
| Ash..... | 0.96 | | 0.96 | |
| | | | | 100.00 |

As this preparation separated from a not perfectly clear solution, and therefore, presumably, was impure, a part of it was dissolved in one per cent. sodium chloride brine and precipitated by dilution. After standing a few hours to settle, the precipitate was filtered off and washed with distilled water, with alcohol and with ether. Its composition is as follows:—2.

| PHASEOLIN, PREPARATION 2. | | Ash-free. |
|---------------------------|-------|-----------|
| Carbon..... | 52.35 | 52.60 |
| Hydrogen..... | 6.63 | 6.69 |
| Nitrogen..... | 16.42 | 16.56 |
| Sulphur..... | 0.63 | 0.63 |
| Oxygen..... | | 23.52 |
| Ash..... | 0.82 | |
| | | 100.00 |

Another preparation was made by treating the fine ground beans, previously extracted both with ether and alcohol, with ten per cent. sodium chloride solution as long as any proteid was removed. The extract was allowed to stand over night and the very nearly clear, greenish-yellow liquid was decanted from the sediment and saturated with ammonium sulphate. The precipitated proteid was collected on a filter and dissolved in dilute salt solution. This liquid was filtered as clear as possible and then dialyzed. When freed from chlorides, the globulin which had separated was filtered off, and washed and dried in the usual manner. This preparation, 3, had the following composition:

PHASEOLIN, PREPARATION 3.

| | I. | II. | Average | Ash-free. |
|---------------|-------|-------|---------|-----------|
| Carbon..... | 52.19 | | 52.19 | 52.60 |
| Hydrogen..... | 6.67 | | 6.67 | 6.72 |
| Nitrogen..... | 16.01 | 16.06 | 16.04 | 16.17 |
| Sulphur..... | 0.63 | | 0.63 | 0.63 |
| Oxygen..... | | | | 23.88 |
| Ash..... | 0.79 | | 0.79 | |

100.00

As this preparation, 3, separated from a solution which could not be filtered perfectly clear, it is undoubtedly impure. In order to determine whether the preparations thus far obtained were mixtures of two or more globulins, the following were made by fractionally precipitating the extracts. The extraction was also carried out in such a way as to afford an approximate determination of the amount of phaseolin contained in the extracts.

One hundred grams of bean meal were treated with 500 cc. of one per cent. sodium chloride solution, strained through cloth, and allowed to stand so as to deposit most of the suspended matter. The solution was then decanted and 350 cc. of nearly clear extract obtained, being, therefore, approximately seven-tenths of the whole. This solution was then diluted with 1,050 cc. of distilled water and allowed to stand until the precipitate so produced had settled. The latter was collected on a filter, washed with alcohol and ether, and when air-dried was found to weigh 4.76 grams; preparation 4. The filtrate from 4 was diluted with about an equal bulk of water and carbon dioxide was

passed through it. After standing some time, a precipitate settled out, leaving the liquid nearly clear. This was decanted and the precipitate collected on a filter and treated like 4. This gave preparation 5, which weighed 3.76 grams. The filtrate from 5 was further diluted with a considerable quantity of water and carbon dioxide again passed through the solution. This gave preparation 6, weighing, air-dry, 1.12 grams. The filtrate from 6 was treated with acetic acid, and a further small precipitate, 7, obtained, weighing 0.7 gram. The four preparations together weighed 10.34 grams, and as they were obtained from seven-tenths of the total extract, were approximately equivalent to the proteid derived from seventy grams of air-dry bean meal or to 14.77 per cent. of the meal. It is evident that in preparing the globulin by dialysis in the manner just described, only a part was obtained. As preparation 4 was separated from an unfiltered extract, it was necessary to dissolve and reprecipitate it before submitting it to analysis. It was accordingly treated with one per cent. sodium chloride solution, but had become largely insoluble in brine. The insoluble matter was filtered off, the clear filtrate diluted, and carbon dioxide passed through it as long as globulin was precipitated.

The precipitate was filtered off, washed thoroughly with water, alcohol, and ether, and dried over sulphuric acid. Only one gram of proteid, preparation 4, was recovered.

PHASEOLIN, PREPARATION 4.

| | I. | II. | Average. | Ash-free. |
|----------------|-------|-------|----------|-----------|
| Nitrogen | 16.05 | 15.89 | 15.97 | 16.12 |
| Ash | 0.93 | | 0.93 | |

Analyses of the other preparations gave the following results:

PHASEOLIN, PREPARATION 5.

| | I. | II. | Average. | Ash-free. |
|----------------|-------|------|----------|-----------|
| Carbon..... | 52.14 | | 52.14 | 52.54 |
| Hydrogen | 6.78 | | 6.78 | 6.83 |
| Nitrogen | 16.35 | | 16.35 | 16.48 |
| Sulphur..... | 0.62 | 0.53 | 0.58 | 0.58 |
| Oxygen | | | | 23.57 |
| Ash | 0.77 | | 0.77 | |

 100.00

PHASEOLIN, PREPARATION 6.

| | | Ash-free. |
|----------------|-------|-----------|
| Nitrogen | 15.83 | 16.23 |
| Ash | 2.51 | |

PHASEOLIN, PREPARATION 7.

| | | Ash-free. |
|----------------|-------|-----------|
| Nitrogen | 16.70 | 16.87 |
| Ash | 1.04 | |

A larger quantity of meal was next extracted, and the extract fractionally precipitated in the following manner:

Five hundred grams of meal were treated with 1,500 cc. of one per cent. sodium chloride solution, and the extract filtered once through good filter paper. About two-thirds of the extract were thus obtained as a turbid solution. Three volumes of distilled water were added, and the large precipitate formed was designated A. Through the filtrate from A, a current of carbonic acid gas was passed, and the resulting precipitate filtered off and marked B. Precipitate A was treated with about fifty cc. of ten per cent. sodium chloride brine and filtered as well as possible. A considerable part of A had become insoluble, and the filtration was exceedingly slow. After two and one-half days, most of the solution had filtered very nearly clear. This was then diluted with about ten times its volume of water, and the precipitate thereby produced, filtered off, washed with alcohol and ether, and dried over sulphuric acid; preparation 8. The filtrate from 8 was again diluted with a large quantity of water and a second precipitate obtained in a like manner; preparation 9.

The filtrate from 9 gave a very small additional precipitate with carbon dioxide, weighing, when dry, only 0.2 gram. A portion of precipitate B was dried and marked preparation 10. Five grams of precipitate B were dissolved in one-half per cent. sodium chloride solution, previously heated to 70°, filtered hot from the considerable insoluble matter, an equal volume of water heated to 70° was added to the filtrate, and the solution very slowly cooled. The proteid separated in well-developed spheroids. When this precipitate was filtered off and washed with water, the substance began to dissolve, as the salts were washed out, in the same way as the globulins of the oat kernel, castor bean, and hemp-seed dissolve, when they are washed with water, after separating from warm salt solutions on cooling. Alcohol was then added to the contents of the funnel, and the substance washed with alcohol and ether, and dried. This preparation, 11, weighed two grams.

The insoluble proteid filtered from the solution which yielded 11, was dissolved in two-tenths per cent. potash water and after filtering, the solution was exactly neutralized with two-tenths per cent. hydrochloric acid. The precipitate, which was very readily soluble in dilute sodium chloride solution, after washing with water, alcohol and ether, and drying, weighed 0.65 gram; preparation 12.

Another portion of precipitate B weighing 3.35 grams, was treated with one-half per cent. sodium chloride brine, and heated to 70°. The insoluble matter was filtered off, washed with hot dilute salt solution, with water, alcohol and ether, and when dried formed preparation 13, weighing 1.16 gram. The dissolved proteid separated from the filtrate and washings of 13 on gradually cooling, and after washing with dilute alcohol, absolute alcohol, and ether, gave 1.54 grams of preparation 14. All were then dried at 110°, and analyzed with the following results:

PHASEOLIN, PREPARATION 8.

| | I. | II. | Average. | Ash-free. |
|---------------|-------|-------|----------|-----------|
| Carbon..... | 52.31 | | 52.31 | 52.72 |
| Hydrogen..... | 7.18 | | 7.18 | 7.24 |
| Nitrogen..... | 16.32 | 16.32 | 16.32 | 16.45 |
| Sulphur..... | 0.67 | 0.66 | 0.67 | 0.67 |
| Oxygen..... | | | | 22.92 |
| Ash..... | 0.80 | | 0.80 | |

100.00

PHASEOLIN, PREPARATION 9.

| | | | Ash-free. |
|-----------------|-------|--|-----------|
| Carbon..... | 51.89 | | 52.35 |
| Hydrogen..... | 6.83 | | 6.89 |
| Nitrogen..... | 16.37 | | 16.52 |
| Sulphur } | | | 24.24 |
| Oxygen } | | | |
| Ash..... | 0.88 | | |

100.00

PHASEOLIN, PREPARATION 10.

| | I. | II. | Average. | Ash-free. |
|---------------|-------|-------|----------|-----------|
| Carbon..... | 51.89 | 51.98 | 51.94 | 52.74 |
| Hydrogen..... | 6.84 | 6.64 | 6.74 | 6.84 |
| Nitrogen..... | 16.40 | | 16.40 | 16.65 |
| Sulphur..... | 0.63 | | 0.63 | 0.64 |
| Oxygen..... | | | | 23.13 |
| Ash..... | 1.52 | | 1.52 | |

100.00

PHASEOLIN, PREPARATION II.

| | | Ash-free. |
|-----------------|-------|-----------|
| Carbon | 52.34 | 52.49 |
| Hydrogen | 6.78 | 6.80 |
| Nitrogen | 16.80 | 16.85 |
| Sulphur } | | 23.86 |
| Oxygen } | | |
| Ash | 0.30 | |
| | | <hr/> |
| | | 100.00 |

PHASEOLIN, PREPARATION 12.

| | | Ash-free. |
|----------------|-------|-----------|
| Nitrogen | 16.54 | 16.67 |
| Ash | 0.81 | |

PHASEOLIN, PREPARATION 13.

| | | Ash-free. |
|-----------------|-------|-----------|
| Carbon | 52.76 | 53.06 |
| Hydrogen | 6.81 | 6.85 |
| Nitrogen | 16.59 | 16.68 |
| Sulphur } | | 23.41 |
| Oxygen } | | |
| Ash | 0.57 | |
| | | <hr/> |
| | | 100.00 |

PHASEOLIN, PREPARATION 14.

| | | Ash-free. |
|-----------------|-------|-----------|
| Carbon | 52.12 | 52.49 |
| Hydrogen | 6.68 | 6.73 |
| Nitrogen | 16.33 | 16.45 |
| Sulphur } | | 24.13 |
| Oxygen } | | |
| Ash | 0.72 | |
| | | <hr/> |
| | | 100.00 |

These results show that the greater part of the proteid extracted by sodium chloride solution, consists of a single globulin, amounting at least to fifteen per cent. of the seed. The greater part of the proteid remaining in the seed after extraction with salt solution, is presumably the same globulin, possibly inclosed in the tissue of the coarsely ground seed, so that it was not reached by the salt solution, or more likely, an insoluble or "albuminate" form of this globulin, as the following experiment indicates. After complete exhaustion with ten per cent. sodium chloride brine, of the meal from which preparation 3 was obtained, the residue was extracted with two-tenths per cent.

potash water, the extract allowed to stand over night and then decanted and precipitated with hydrochloric acid, added in very slight excess. The precipitate was washed by decantation, redissolved in potash water and filtered clear. This solution was then precipitated by hydrochloric acid, and as the proteid separated imperfectly, it was treated with alcohol and ether, and filtered off. After washing with dilute alcohol, absolute alcohol and ether, it was dried and analyzed; preparation 15.

PHASEOLIN, PREPARATION 15.

| | | Ash-free. |
|----------------|-------|-----------|
| Carbon | 51.57 | 52.47 |
| Hydrogen | 6.78 | 6.90 |
| Nitrogen | 15.71 | 16.00 |
| Sulphur) | | |
| Oxygen) | | 24.63 |
| Ash | 1.72 | |
| | | <hr/> |
| | | 100.00 |

Another preparation of the globulin was made by treating 100 grams of bean meal, previously extracted with ether, with 500 cc. of *distilled water*, straining through a cloth, allowing the extract to stand over night to deposit suspended matter and decanting the slightly turbid liquid, of which the 250 cc. thus obtained were diluted to 2,000 cc. and treated with a current of carbonic acid gas. On standing, the considerable precipitate settled out so that the solution could be filtered. The precipitate was washed with water, alcohol, and ether, and when dried over sulphuric acid weighed 5.5 grams. As the extract employed, measured one-half the volume of the water applied to the meal, the yield of globulin was in this case approximately eleven per cent. of the bean meal. Although precipitated from a somewhat turbid solution, and consequently not quite pure, this preparation was analyzed with the following results:

PHASEOLIN, PREPARATION 16.

| | I. | II. | Average. | Ash-free. |
|----------------|-------|-------|----------|-----------|
| Carbon | 52.48 | 52.49 | 52.49 | 53.22 |
| Hydrogen | 6.71 | 6.86 | 6.79 | 6.86 |
| Nitrogen | 16.32 | 16.18 | 16.25 | 16.48 |
| Sulphur | 0.45 | 0.48 | 0.47 | 0.48 |
| Oxygen | | | | 22.96 |
| Ash | 1.53 | 1.27 | 1.40 | |
| | | | | <hr/> |
| | | | | 100.00 |

It is evident from this analysis that the principal proteid extracted by water, is the same as that extracted by saline solutions, and as this substance is precipitated from the aqueous extract by dilution, it is undoubtedly dissolved by aid of the salts contained in the beans. It is not probable that the acid contained in the seed causes this solution, for in that case the proteid would not be precipitated by dilution, a considerable excess of acid being necessary to effect its precipitation. The meal after extracting with water, as just described, was treated with one per cent. sodium chloride solution, the extract filtered, largely diluted with water, and charged with carbon dioxide. Only a trifling precipitate resulted which, when prepared in the usual manner, weighed 0.52 gram. This preparation, 17, ash-free, contained 16.29 per cent. of nitrogen. These results show that nearly, if not quite as much of the globulin is extracted by water with help of the salts of the seed, as by use of stronger salt solutions.

[TO BE CONTINUED.]

A NEW AUTOMATIC PIPETTE.¹

BY EMIL GREINER.



WHEN taking a great number of samples in succession of the same volume of the same liquids, it is quite desirable to have a measuring instrument which will automatically take up the exact volume of liquid, without having to draw the same by mouth and watch for the mark, as is done when using the ordinary volumetric pipette. Therefore, I have devised an automatic pipette for such purposes, which is at present successfully employed in connection with the Babcock milk test. A glance at the accompanying cut will explain the practical value of this instrument, and as the pipettes can be made in all sizes required up to 50 cc., it can be adopted in many instances, where pipettes are used often and where accuracy is desired.

¹ Read before the New York Section, March 9, 1894.