as from sodium nitrate. This confirms the results obtained in Experiment A for 1894. The oat plants secured twice as much nitrogen from the six-tenths gram as from the three-tenth gram in case of the sodium nitrate and both dissolved leathers, showing that the nitrogen was fully utilized.

GENERAL CONCLUSIONS.

The above experiments, part of which cover two years, make clear that dissolved leather, when properly prepared, yields as available a source of nitrogen as the average animal matter used for fertilizing purposes.

The quantity of nitrogen obtained by the plants from sodium nitrate being represented as equal to 100, the quantity obtained from dissolved leather during two years has been shown to be equal to 70.[1]

In this connection I beg leave to add the results of the availability of the various sources of nitrogen as determined by P. Wagner. Sodium nitrate is taken as 100 in value, and the value of other sources are compared with it.

Sodium nitrate	100
Ammonium sulphate	90
Dried blood, ground horn, and green plants	70
Ground bone, ground fish, and flesh	60
Stable manure	45
Ground wool	30
Ground leather	20

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THE PROTEIDS OF THE POTATO.[2]

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SO far as we can ascertain, the only investigations of the proteids obtained from the tubers of the potato have been made by Rüling,[3] Ritthausen,[4] Zöller,[5] and Vines.[6]

[1] The Connecticut Experiment Station, in its recently issued report for 1895, confirmed these results.

[2] From the report of the Connecticut Agricultural Experiment Station for 1895. Communicated by the authors.

[3] *Ann. Chem.* (Liebig), 58, 306.

[4] Pflüger's Archiv, 21, 101.

[5] *Ber. d. chem. Ges.*, 13, 1064.

[6] *Journal of Physiology*. 3, 93.

Rüling contributes a partial analysis of the coagulum obtained by boiling the juice of the potato.

Ritthausen states that nearly the whole of the proteid of the potato is contained in the juice. He obtained two preparations from the juice by heating to 65° C., filtering off the coagulum and heating the filtrate to 76°. The two coagula were analyzed with results as stated beyond. He says, "these results do not contradict the assumption that the potato contains albumin, yet the content in sulphur is only one-half as great as in albumin of serum, egg and muscle.

Zöller extracted the pressed and washed potato pulp with ten per cent. sodium chloride brine and obtained a globulin, precipitable by saturating its solution with sodium chloride and when dissolved in ten per cent. sodium chloride brine coagulating on heating to 59° or 60°. From his result he concludes that the potato contains a globulin resembling myosin.

On investigating the juice of the potato Zöller obtained results which led him to conclude that the proteids therein dissolved are also globulins, but that further study was needed to explain their "peculiar deportment," especially, it is to be inferred, the fact of coagulation occurring at from 43° to 48°, and again, at 62°.

Vines states that prolonged treatment of the "crystalloids" of the potato with ten per cent. sodium chloride solution produces no apparent effect, but that they dissolve readily in a saturated solution of this salt, thus differing from all other protein crystals which he had observed.

Having had occasion to prepare a quantity of pure starch from the potato, we took advantage of the opportunity to examine the associated proteids.

After removing the skins, the tubers were crushed and squeezed in a drug press. The juice was strained through cloth and allowed to stand and deposit the greater part of the suspended matters. It was then saturated with ammonium sulphate and the precipitate so produced was filtered out. The potato pulp was washed with water and the washings after clearing were also saturated with ammonium sulphate. The

two precipitates thus obtained were united, dissolved in salt solution, filtered clear and dialyzed.

The washed pulp was then treated with ten per cent. sodium chloride solution; the proteid, thus extracted, was precipitated with ammonium sulphate, dissolved in salt solution, filtered clear, and also dialyzed. The globulin precipitated very slowly on dialysis, and after fourteen days was filtered out. The proteid obtained from the juice was much greater in amount than that from the salt extract of the pulp. The globulin, from both juice and salt extract, was then dissolved in salt solution, the solutions were united, filtered from a considerable quantity of insoluble globulin (rendered insoluble by long contact with water), and the solution again dialyzed. After freeing from chlorides, the contents of the dialyzer were filtered, the reprecipitated globulin was washed with water and alcohol and dried over sulphuric acid, giving preparation 1, weighing 7.34 grams.

The filtrate, from preparation 1, still contained proteid and was therefore saturated with sodium chloride, which completely precipitated the remaining globulin. This was then dissolved in dilute salt solution and dialyzed in water until free from chlorides and, as the proteid was not thus precipitated, the dialyzer was transferred to alcohol, which soon threw down all the proteid. This was filtered out, washed in water and absolute alcohol and dried, giving one-half gram of preparation 2.

The solutions, filtered from the globulin precipitated by the dialysis first described, were united and, in order to obtain the proteid in a solution of smaller volume, the liquid was saturated with ammonium sulphate, the precipitate produced was dissolved in a little water and the clear solution dialyzed, first in river water and then in distilled water. The globulin so precipitated was filtered out, washed with water and absolute alcohol and dried, yielding preparation 3, weighing 3.40 grams. The filtrate from this preparation was dialyzed into alcohol and the resulting precipitate filtered out, washed with absolute alcohol, and dried, forming preparation 4, which weighed 1.74 grams.

The filtrate from 4 was further dialyzed in alcohol and the proteid completely precipitated by adding absolute alcohol. This substance, after filtering out, washing with absolute alco-

hol and drying, weighed 0.53 gram and formed preparation 5.

These several preparations were analyzed after drying them at 110°, with the following results:

POTATO GLOBULIN. TUBERIN.

	Osborne and Campbell.					Ritthausen.	
	1.	2.	3.	4.	5.	I.	II.
Carbon	53.62	....	53.58	53.64	....	....	53.87
Hydrogen	6.80	....	6.91	6.83	....	....	7.30
Nitrogen	16.15	16.29	16.36	16.34	16.07	15.76	15.98
Sulphur	1.22	....	1.27	23.19	....	....	0.86
Oxygen	22.21	....	21.88				21.99
	100.00		100.00	100.00			100.00

The close agreement in composition among our five fractions is in itself, strong evidence that, besides this globulin, but little proteid is present in the potato. These five fractions practically include the whole of the proteid matter dissolved in the juice and salt extracts. The above figures given by Ritthausen for the composition of the proteid, obtained by coagulation of the juice at 65° and 76°, are also in close agreement with ours, excepting those for sulphur. The slightly lower nitrogen content of the coagulated globulin is to be expected, since proteids generally, if not always, yield some ammonia when coagulated by heat.

The potato globulin, when heated slowly in a double water-bath, shows a wide range of variation in its coagulation point depending on the conditions under which it is dissolved.

A solution of this globulin prepared by treating a portion of preparation 1 with ten per cent. sodium chloride solution and filtering out the insoluble matter, became turbid at 56° and a flocculent coagulum separated at 64°. After heating some time at 70° the coagulum was filtered out and the filtrate, when again tested, gave a turbidity at 72° and a flocculent coagulum at 76°.

Another preparation of this globulin was extracted, in the same way, with ten per cent. salt solution, and the dissolved proteid was filtered from the insoluble matter and precipitated by saturating the solution with sodium chloride. The precipitated globulin was washed with saturated salt solution and removed from the paper mixed with a considerable quantity of the concentrated brine. Distilled water was gradually added

until all of the proteid dissolved. The resulting solution was therefore almost completely saturated with the proteid. This solution, when slowly heated in the double water-bath to 44° C., and held at this temperature some minutes, became turbid and after a time flocculent, although the temperature remained perfectly constant. After raising the temperature to 50° it was filtered from the small coagulum which had formed and again heated, turbidity occurring at 50¼° and flocks separating at 51°. After heating some time at 56° the solution was filtered from the second small coagulum and again tested. Turbidity occurred at 58° and flocks separated at 59°, gradually increasing to a large coagulum at 66°, which was filtered out. The filtrate now became turbid at 63°, flocks forming at 66°, and increasing to a considerable coagulum at 70°. The temperature was raised to 80° and the coagulum, which was about the same in amount as that formed at 66°, was filtered out. The filtrate gave only a trace of coagulum on boiling. The two coagula first formed were very small compared with the last two.

This test was then repeated with the same solution diluted with an equal volume of water. This solution was heated for some time at 44°, but remained perfectly clear. The temperature was then very slowly increased and at 53° a turbidity formed which, however, was scarcely greater at 56°. Above this temperature the turbidity increased until flocks separated at 62°, and a large coagulum formed at 65°. The solution filtered at 66°, gave a turbidity at 66°, with flocks at 68°, which formed a large coagulum on gradually raising the temperature to 80°, the filtrate from which gave no more coagulum on boiling.

The test was again repeated by mixing four parts of the same solution with one of water, and the same results obtained as with the solution diluted with an equal volume of water. This shows that within wide limits the temperature of coagulation does not depend on the relative quantity of dissolved proteid, but that the very low coagulation point of the undiluted solution was probably due to the presence of nearly enough sodium chloride to cause precipitation of the globulin. It will be noticed that coagulation of the proteid, which began at 56°, was not completed until the temperature had reached at about 80°.

This does not necessarily show the presence of several proteids, for such gradual coagulation is characteristic of most plant globulins, many being only very slowly coagulated, even by long boiling.[1] The coagulum separated by heating solutions of this globulin to 75° C. is very soluble, on gently warming, in extremely dilute hydrochloric acid, even acid of 0.01 per cent. dissolving the substance readily at 40°–50° C. The coagulum dissolves quickly and completely in one-tenth per cent. caustic potash solution at 20° and in one per cent. sodium carbonate solution at 70° C. These solutions are precipitated by neutralization, but the substance thrown down is not soluble in salt solutions. The low heat-coagulation point obtained for the solution of the globulin precipitated with salt and dissolved in a minimum quantity of water is in accord with that given by Zöller for the proteid similarly obtained by him from the juice of the potato, and our observations explain to some extent the questions which he considered to require further investigation.

In order to determine more definitely whether other proteids were present with the globulin, a larger quantity of filtered potato juice, obtained from potatoes which had been washed carefully, but from which the skins had not been removed, was saturated with ammonium sulphate, the precipitate was dissolved in dilute salt solution, which was then filtered and saturated with sodium chloride. The globulin thus precipitated was filtered out, the filtrate was dialyzed for twenty-four hours in order to remove a considerable part of the salt, and was then saturated with ammonium sulphate. The small quantity of proteid thus precipitated was filtered out, dissolved in a little dilute salt solution and sodium chloride added to complete saturation. A considerable part of the dissolved proteid was thereby precipitated, which was filtered out and dissolved in dilute salt solution. The resulting liquid became turbid on heating to 58° C. and flocculent at 60°. The substance was evidently a part of the globulin which had escaped precipitation on the first saturation with salt, probably owing to the presence of some constituent of the juice.

The solution filtered from the salt saturation precipitate *last*

[1] Chittenden and Mendel, *Journal of Physiology*, 17, 52.

described, was diluted with two volumes of water and then saturated with ammonium sulphate. The proteid thus separated was filtered out, dissolved in water, and found to yield a turbidity at 52° and a flocculent coagulum at 58°, a coagulation point not essentially differing from that of the globulin.

The whole solution was then heated for some time at 70° C. in a water-bath, the coagulum which separated was filtered out, and the filtrate, after removing a small quantity of coagulum, which separated on heating to 75°, was boiled and found to remain clear. This solution was then saturated with ammonium sulphate and the very small precipitate produced was filtered out, dissolved in a small amount of water and tested with the following results: nitric acid added to the solution in the cold gave no precipitate; saturation with sodium chloride gave no precipitate even when acetic acid was added. The biuret test was without result owing to the strong brown color of the solution. This substance therefore failed to give the most characteristic reactions of the proteoses, yet it must be considered as a proteose since in its essential properties it agrees more closely with this class of proteids than with any other.

The experiments made by Zöller on the juice of the potato were repeated by us with the same results as described by him, except that we found the solution of the precipitate produced by saturation with salt, to yield a flocculent coagulum at 52°, while Zöller's solution coagulated at 46°–48°. It has already been shown that a solution of the globulin similarly prepared gave a coagulum at 44°, and on dilution with one volume, as well as with one-fourth volume of water, the same solution coagulated at 62°. It is thus evident that the temperature of coagulation is not to be depended upon as a means of identifying this proteid with certainty. The other reactions described by Zöller are those given by the potato globulin. From these results then it would appear that by saturating the juice of the potato with sodium chloride the greater part of the globulin is precipitated, but that a not inconsiderable part remains in solution. If this is separated by saturation with ammonium sulphate and the precipitate so produced is dissolved in water, a large part of this globulin can be precipitated by again saturating with salt. The

proteid still remaining in solution is nearly all coagulable and the solution on heating behaves exactly like a solution of the globulin.

CONCLUSION.

The proteids of the potato tuber consist of a globulin, for which we propose the name *Tuberin*, and a proteose, the latter occurring in very small amount. The properties of tuberin were found to be as follows:

It is precipitated by saturating its solutions with sodium chloride, sodium sulphate, magnesium sulphate, or ammonium sulphate. By acetic acid or nitric acid a precipitate is given readily soluble in an excess of acid even in the presence of salts. Potassium ferrocyanide gives no precipitate until acetic acid is added. Mercuric chloride gives no precipitate, but picric acid or tannic acid throw down the globulin. With the biuret, Millon's and the xanthoproteic tests the usual reactions are given.

Tuberin is soluble in very dilute saline solutions and therefore the juice of the potato contains the greater part of this proteid. By dialysis it is precipitated slowly and incompletely because of the difficulty of removing *all* soluble salts by this process. Like other easily soluble globulins it readily changes to the insoluble modifications, so that preparations made by dialysis are to a great extent insoluble in saline solutions. In contact with alcohol it very quickly loses its solubility.

When dissolved in ten per cent. sodium chloride solution tuberin shows a somewhat variable heat-coagulation point depending on the conditions under which it is tested. In general a flocculent coagulum is formed on heating to 60°–65° C. Coagulation is, however, not complete until the solutions have been heated for some time at 80° C. The composition of this globulin was found from an average of several accordant analyses to be:

TUBERIN.

Carbon	53.61
Hydrogen	6.85
Nitrogen	16.24
Sulphur	1.25
Oxygen	22.05
	100.00