peared ill, and 4 of the 6 had definite pulmonary lesions at autopsy on the fourth day. None of the mice of the Rockefeller Institute strain showed pulmonary involvement.

In the fifth passage, death of all 6 Swiss mice and of 2 of 6 of the Rockefeller Institute strain occurred in 48 hours. The surviving mice were killed. All showed extensive pulmonary consolidation involving most of the left lung and cardiac lobe, and usually part of the right upper and middle lobes. A Berkefeld V filtrate of a 10 per cent. lung suspension was then inoculated intranasally into 6 Swiss mice. This filtrate was found to be bacteriologically sterile in both aerobic and anaerobic cultures. Death occurred in these mice in from 48 to 72 hours, all animals showing marked pulmonary consolidation.

Subsequently, the inoculation of a 5 per cent. lung suspension, or Berkefeld V filtrates of from 5 to 10 per cent. suspensions of lung, caused death in Swiss mice in about 48 hours. Mice of the Rockefeller Institute strain survived somewhat longer. In practically all instances, direct cultures of the heart's blood and of the cut lung surface have been free from bacteria. Cultures of the emulsified tissue in blood broth not infrequently reveal various Gram-negative bacilli, but they are usually few in number. These bacteria do not appear to be related to the disease process, since they are inconstantly observed. Furthermore, subsequent bacteria-free emulsions or filtrates have been fully active.

This strain of infective agent (S. S.) has been passed through 11 series of mice. A suspension of the lungs from mice of the eighth serial passage inoculated intranasally into a ferret produced a characteristic febrile reaction. At the same time, different dilutions of the mouse lung suspension were made, and a small amount of each dilution was inoculated intranasally into 4 mice. By the seventh day all animals receiving a 1:1600 dilution, or more, had succumbed with typical pathology.

More recently, an unfiltered sterile lung suspension of a ferret, the tenth passage animal of the original Puerto Rico passage strain (P. R. 5) was transferred to mice. In the first mice, killed on the fourth day, only mild pulmonary lesions were seen. In the second passage, however, all died on the fourth day with typical extensive involvement. The mice of the third passage, which received a bacteria-free 10 per cent. suspension of lung, died in 48 hours. These results are of special interest, since earlier attempts to transmit infection to mice with filtrates containing this strain were unsuccessful.

The lesions in the mouse's lung tend to involve the entire lobe, spreading peripherally. The surface is smooth and bluish gray or reddish blue. When the trachea is cut, a copious foamy liquid exudes. The cut surface of the lung is rather viscid, but firm. Stained films usually reveal mononuclear cells, but no bacteria. Microscopically, there is thickening of the alveolar walls and a moderate amount of edema in the alveolar spaces. The degree of hyperemia is variable. There is a perivascular small round cell infiltration. The cellular reaction, unlike acute bacterial infections, is predominantly of the mononuclear cells. The number of polymorphonuclear leukocytes varies. In many of the cells degenerative changes are seen in the nuclei and protoplasm.

The results of the experiments, both in ferrets and mice, indicate that the agent producing the disease in these animals is a filterable virus. It has been possible to produce the infection with filtrates, which, in aerobic and anaerobic cultures, are bacteriologically sterile. The pulmonary lesions are bacteria-free. Furthermore, the microscopic pathology of the involved lung resembles that of pulmonary lesions produced by other virus infections, rather than that of bacterial infections.

In the current issue of *The Lancet,* Andrewes, Laidlaw and Smith[7] report their success in transmitting to mice the viruses derived from both swine influenza and human influenza. The results in the present study are apparently in complete agreement with theirs. They have been able, in addition, by the use of specific antiserum from hyperimmune animals (horse and ferret), to neutralize the action of the respective viruses in mice.

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GLUTAMINE IN THE TOMATO PLANT[1]

ALTHOUGH the amide glutamine is probably widely distributed in nature,[2] very few direct attempts have been made, since its first isolation by Schulze and Bosshard,[3] to study the function of this substance in the metabolism of the plant. The lower homologue, asparagine, on the other hand, has received a great deal of attention. The hypothesis has been advanced by Prianischnikow[4] that asparagine is synthesized in the plant in response to an accumulation of ammonia from any cause. The reaction is regarded as a detoxification of the ammonia by conversion into a

[7] C. H. Andrewes, P. P. Laidlaw and W. Smith, *Lancet,* 2: 859, 1934.

[1] A part of the expense of this investigation was shared by the Connecticut Agricultural Experiment Station and the Carnegie Institution of Washington. Dr. Clark is the holder of a National Research Council fellowship, 1933–34.

[2] A. Stieger, *Ztschr. f. physiol. Chem.,* 86: 245, 1913.

[3] E. Schulze and E. Bosshard, *Ber.,* 16: 312, 1883.

[4] D. Prianischnikow, *Biochem. Ztschr.,* 150: 407, 1924.

neutral compound that can be translocated and stored in readiness for subsequent syntheses in which nitrogen is required. Prianischnikow[5] has pointed out, however, that certain species, especially the oil seeds, store glutamine instead and, in fact, regards glutamine and asparagine as physiologically equivalent to each other.

The culture experiments on which this generalization was founded were carried out with young seedlings. Support for the view that asparagine fulfils an analogous function in the *mature* plant has been obtained from the experiments of Mothes[6] with a number of species, and of Vickery, Pucher, Wakeman and Leavenworth[7] on the leaf of the tobacco plant. The present experiments show that glutamine is formed in considerable amounts in tomato plants when these are grown in solutions that provide ammonia as the sole source of nitrogen; the inference may therefore be drawn that this amide can likewise be employed by a mature plant for the purpose of detoxifying ammonia.

Tomato plants, 36 days old, of the Marglobe variety, were transferred to sand cultures and grown for 41 days with continuous renewal of culture solution. Two different solutions were employed; both were based on Tottingham's solution, but one included calcium nitrate as a source of nitrogen, the other ammonium sulfate. Both solutions were adjusted to pH 6.7 and to a total osmotic pressure of approximately one atmosphere, of which 0.3 was supplied by the nitrogen-containing salt.

At the termination of the experiment, the tops of 11 plants from each culture were dried in a rapid stream of air heated to 80° C., and 11 plants from each were extracted with boiling water, as described by Vickery and Pucher.[8] Determinations of the ammonia in the dried tissue, and in the hot-water extract therefrom, drew attention to the presence of a substance that was decomposed with the production of considerable quantities of ammonia during the process of preparing the water extract. The dried tissue was therefore investigated for glutamine.

The ammonia nitrogen was determined by distillation of 0.2, or 0.4 gm of the dry powder, in the presence of 10 cc of a 12.5 per cent. suspension of light magnesium oxide and 25 cc of water, for 15 minutes at 40° *in vacuo*. The distillate was Nesslerized and read either in a colorimeter or a Pulfrich photometer against suitable standards.

[5] D. Prianischnikow, *Ber. deut. botan. Ges.*, 40: 242, 1922.

[6] K. Mothes, *Planta*, 1: 472, 1926.

[7] H. B. Vickery, G. W. Pucher, A. J. Wakeman and C. S. Leavenworth, *Carnegie Institution of Washington, Pub.* 445, 1933.

[8] H. B. Vickery and G. W. Pucher, SCIENCE, 73: 397, 1931.

The glutamine amide nitrogen was determined according to a slight modification of the method of Chibnall and Westall,[9] in which a 0.2 gm sample of the tissue was suspended in 10 cc of phosphate-borate buffer at pH 7.0 and heated in a boiling water bath for 2 hours to hydrolyze the amide. The suspension was then cooled, 2 cc of normal sodium hydroxide, 10 cc of magnesium oxide suspension, and 20 cc of water were added, and the mixture was distilled at 40° as before. The increase in ammonia brought about by the hydrolysis was taken to represent the glutamine amide nitrogen. Data in support of the accuracy of this method to determine glutamine will be submitted in a later joint publication from this laboratory and that of Professor Chibnall.

The results of the analyses are expressed in Table 1 in milligrams of nitrogen per 11 plants. It is strikingly clear that glutamine had accumulated in the plants grown on ammonia, particularly in the stems, to a far greater extent than in those grown on nitrate, notwithstanding the fact that the latter plants were much larger and more fully developed. The glutamine amide nitrogen made up over 15 per cent. of the soluble nitrogen in the stems of the plants grown on ammonia, the corresponding figure for the stems of the plants grown on nitrate being only 1.9 per cent.

TABLE 1

MILLIGRAMS OF NITROGEN AS AMMONIA, AND AS THE AMIDE NITROGEN OF GLUTAMINE, IN 11 TOMATO PLANTS

	Ammonia	Glutamine amide	Total soluble
Leaves, ammonia culture.....	101	63	913
Stems, " "	120	213	1,390
Leaves, nitrate culture.....	41	25	1,943
Stems, " "	52	70	3,654

Considerable interest attaches to the fact that glutamine accumulated mostly in the stems. The question of the location in the plant at which amide synthesis actually took place is clearly raised. If the synthesis occurred in the roots, does the high concentration in the stem represent an elaborated form of nitrogen undergoing translocation to the leaves and growing points, there to be used for further syntheses? On the other hand, if the glutamine was synthesized in the leaves from ammonia transported from the roots, is the high concentration in the stem an evidence of storage? Problems such as these still await experimental attack.

The identity of the amide in the tomato plant tissue

[9] A. C. Chibnall and R. G. Westall, *Biochem. Jour.*, 26: 122, 1932.

was established by direct isolation. To this end, 5,794 gm of tops of tomato plants, the greater part of which had been grown in sand culture supplied with ammonium sulfate as a source of nitrogen, were treated with ether to plasmolyze the cells, according to the method of Chibnall,[10] and the vacuole content was expressed at the hydraulic press. The press residue was then ground to a pulp and pressed again. The extract measured approximately 5 liters and was treated with an excess of basic lead acetate. The filtrate from the precipitate produced contained 726 mg of amide nitrogen (hydrolysis with 2 *N* sulfuric acid for 4 hours at boiling temperature).

The procedure of Schulze and Bosshard for the isolation of glutamine was then followed. This consists in precipitating the amides with mercuric nitrate from neutral solution. The precipitate is decomposed with hydrogen sulfide and the resulting solution, after being freed from hydrogen sulfide by distillation *in vacuo* for a short time, is neutralized with ammonia and concentrated at a low temperature to crystallization. The crude crystals so obtained were treated with norit in warm concentrated aqueous solution, and crystallization was brought about by the addition of alcohol. The glutamine then separated in tiny colorless needles, which weighed 4.78 gm. The preparation contained 18.94 per cent. nitrogen (theory 19.18 per cent.), 9.60 per cent. amide nitrogen (theory 9.59 per cent.) and yielded gas equivalent to 18.13 per cent. of nitrogen in the Van Slyke amino nitrogen apparatus. It is characteristic of glutamine to yield practically all its nitrogen under these conditions. The substance decomposed at 180° to 181°. The copper compound, prepared by boiling an aqueous solution of a sample for a few moments with an excess of cupric hydroxide, contained 15.56 per cent. of nitrogen (theory 15.84) and 17.94 per cent. of copper (theory 17.97 per cent.). The yield of recrystallized glutamine accounted for 63 per cent. of the total amide nitrogen in the filtrate from the basic lead acetate precipitate.

The mother liquor from the crude glutamine crystallization, on evaporation and treatment with alcohol, yielded several successive crops of crystals from which 0.44 gm of asparagine (water of crystallization 11.93 per cent., theory 12.00 per cent.; nitrogen 18.42 per cent.; theory 18.66 per cent.) was isolated; this is the equivalent of 5.6 per cent. of the original amide nitrogen. The mother liquors were collected and boiled with hydrochloric acid; glutamic acid hydrochloride weighing 0.53 gm, of melting point 198° to 199° and unchanged on admixture with authentic material, was then isolated. This, calculated as glutamine, accounts for a further 5.5 per cent. of the amide nitrogen of the extract. In all, 74 per cent. of the amide nitrogen was accounted for as crystalline products of demonstrated purity.

It is clear from these experiments that the marked increase in amide nitrogen brought about by culturing tomato plants in a solution that provides ammonia as the sole source of nitrogen is obviously due to an accumulation of glutamine in the tissue; asparagine, although present, plays an entirely subordinate rôle from the quantitative point of view.

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[10] A. C. Chibnall, *Jour. Biol. Chem.*, 55: 333, 1923.

OVULATION IN THE DOMESTIC HEN[1]

INFORMATION regarding the act of ovulation in the domestic hen seems to be largely of a speculative nature. We were able in this study to observe the act of ovulation and to secure detailed records of the progress of the egg through the oviduct. Patterson[2] quoted Coste's[3] statement that the infundibulum actually clasped the follicle before ovulation and that the pressure exerted was probably a causative factor in ovulation. Patterson stated that he was able to confirm Coste's findings but supplied no details regarding his observations.

We first made a number of autopsies upon birds for which the laying had been carefully timed. By the autopsies it was possible to determine relatively accurately the time relationship between ovulation and the expulsion of the previous egg. The results indicated that ovulation usually occurred within an hour after laying.

In order to be able to witness the act of ovulation, we anesthetized birds as soon as possible after laying. Ether has usually been found unsatisfactory as an anesthetic for birds because of the retention of it in the air sacs. Nembutal (Pentobarbital sodium, 1 gr. per cc) proved effective for observations covering periods of several hours when injected into the circulation by way of the vena humeri profunda. From ½ to ¾ cc, depending upon the size of the bird, was used for the initial injection. It was found necessary to repeat the injections at intervals from one half to two hours. Occasionally a bird would die immediately following an injection, but otherwise the results with this anesthetic were very satisfactory.

All reported observations were made upon Single Comb White Leghorn hens. Ovulation was observed

[1] Contribution No. 82 from the Department of Poultry Husbandry.

[2] J. Thomas Patterson, "Studies on the Early Development of the Hen's Egg. I. History of the Early Cleavage and of the Accessory Cleavage," *Jour. Morph.*, 21: 101–134, 1910.

[3] M. Coste, "Histoire du developpment des corps organises," Tome 1, Paris, 1874.