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.

HUBERT BRADFORD VICKERY
GEORGE W. PUCHER
HAROLD E. CLARK
CONNECTICUT AGRICULTURAL EXPERIMENT STATION

[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.

in eleven birds. The prospective birds were kept under close observation so that the exact time of laying could be recorded and then preparations were made for opening the body cavity as soon as possible. These preparations, including the anesthesia, required from 10 to 20 minutes. Four birds were found to have ovulated before the ovary could be exposed.

Of the eleven hens in which ovulation occurred under observation, only two had the infundibulum enclosing the ovum at the time of bursting of the theca. In two others, the ova were picked up immediately, indicating that the infundibulum and the follicle must have been very closely associated at the time of ovulation. In three cases, although the bird remained alive and apparently normal, the released ova were not picked up by the infundibulum. In the four other cases the infundibulum began to engulf the ovum within periods of 4 to 10 minutes after the rupture of the follicular membrane. In four birds, where the ovary was exposed within 14, 15, 18 and 20 minutes after laying the previous egg, ovulation had already occurred when the abdominal cavity was opened.

At the time of ovulation the blood vessels in the cephalic end of the oviduct were greatly congested. This portion of the oviduct was also very active. There was some indication of unusual enlargement of the blood vessels of the graffian follicle. Slightly in advance of the rupture of the follicular membrane the blood supply appeared to be reduced and there was a perceptible increase in the width of the stigma. The ovum to be released varied widely as to its position in the ovary.

The act of rupturing the follicular membrane was instantaneous. The released ovum was very much flattened and practically assumed the shape of the cavity into which it fell. In the first ovulation observed the ovum was so devoid of shape that the observers gained the impression that the vitelline membrane had been ruptured. The action of the infundibulum in engulfing the ovum seemed to be entirely random. It would partially engulf the ovum and then recede. The wave-like advances and recessions of the infundibulum sometimes occurred several times. The enclosing of the entire ovum may require as much as 30 minutes. When entirely enclosed the pressure exerted on the ovum by the musculature of the oviduct was very evident. In the infundibulum, where the progress of the egg was more rapid than elsewhere, the shape of the ovum was much distorted, sometimes being much elongated and at other times assuming dumb-bell shape.

Our observations would indicate that it is not necessary for the egg to be ovulated directly into the infundibulum. In fact most of the ovulations seen were not of this type. This study demonstrates that it is possible for the infundibulum to pick up the ovum after it has been released in the cavity about the ovary. In no case did the infundibulum appear to be exerting any pressure upon the follicle previous to ovulation. It is believed that the few cases where the infundibulum was around the ovum when released were purely chance situations, since the infundibulum is very active at this time and may be in contact with any part of the ovary. At times the infundibulum was seen to enclose partially the immature follicles.

In addition to making observations on the act of ovulation we were able also to secure practically complete time records of the passage of the egg down the oviduct for five birds. The positions of the egg were marked at 15-minute intervals by means of a shallow stitch taken in the wall of the oviduct immediately behind the egg. After the egg had traversed the length of the oviduct the organ was removed and records were made of the distances between stitches. Our observations do not fully agree with the statement made by previous workers as to the time required for egg formation in each portion of the oviduct. Earlier workers have depended entirely upon autopsies for their data. The average results from the five birds studied would indicate that the following were the periods spent by the egg in the various parts of the oviduct: Infundibulum—18 minutes; albumin secreting section—2 hours and 54 minutes; isthmus—1 hour and 14 minutes; uterus and vagina—20 hours and 40 minutes.

D. C. WARREN
H. M. SCOTT

KANSAS AGRICULTURAL EXPERIMENT STATION

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