# THESIS

On

# THE INFLUENCE OF DIFFERENT METHODS OF INCUBATION ON THE DEVELOPMENT AND CHEMICAL COMPOSITION OF THE CHICK TO THE STAGE OF PIPPING THE SHELL

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## Foreword.

In this thesis are emboided a portion of the data obtained on a problem pursued co-operatively by the Departments of Poultry Husbandry and Chemistry. The problem was started previous to my beginning the work and some of the data included here were obtained by other workers in the departments mentioned. I have used such of the data as was necessary to make clear the conclusions to be drawn from the results I obtained. It is understood that this thesis is an incomplete report of the experiment, and, since incomplete, not to be published except in combination with the other data obtained from the experiment.

# THE INFLUENCE OF DIFFERENT METHODS OF INCUBATION ON THE DEVELOPMENT AND CHEMICAL COMPOSITION OF THE CHICK TO THE STAGE OF PIPPING THE SHELL.

## Part I.

### Review of Literature.

The hen is the original incubator. When in the wild state, she laid two or three dozen eggs a year, hatching and rearing her broods at a season when food was plentiful and weather conditions favorable. Now, centuries of domestication have so perverted the natural instinct that hens of some breeds can not be depended upon to hatch and rear their own chickens. It is said that, where artificial incubation has been practiced for centuries, the native hens very seldom show any signs of broodiness.

What first led to artificial incubation is now unknown, but it can be readily understood that poultry and eggs could not possibly be as plentiful where natural incubation was the rule as where large numbers of chicks could be hatched and reared artificially. Demand for poultry products may have led to this means of supply.

### Historical.

Strong (64) says that Herodotus mentions the use of artificial incubation in Egypt as early as 450 B. C. The method was much the same as the one in present vogue in that country, except that the heat was then supplied from above the eggs, and shallow troughs of water distributed about the hatching chamber to supply moisture.

De Reaumur (53) also states that, in his time, heat was furnished above the egg chamber during the winter season. In summer, the fires were built in the lower story and the eggs placed above.

Wright (75) and others give the following method as now used in Egypt. The secrets of the trade have, for generations, been handed down from father to son in certain families, and these people run the hatching ovens; either owning and operating their own ovens or being hired by owners.

The egg ovens are located in towns or villages which are agricultural centers. The eggs are usually bought outright, and the chicks distributed through dealers to the village women who rear them. The ovens vary in size and capacity, each being capable of hatching 200,000 to 600,000 chicks in a season of three months. One old man and a boy are known to have put out 200,000 to 250,000 chicks in a season.

The ovens are flat-roofed buildings constructed of sun-dried bricks, the ground space being perhaps 60 x 100 feet. Vestibules protect against sudden changes of temperature produced by opening of doors. These vestibules open into a passage which extends lengthwise through the building. Doors on each side of the passage lead into the hatching chambers. The chambers are of two stories, the lower being four feet, and the upper, eight feet high. They are separated by a brick platform which has a hole in the center large enough to admit the body of the attendant. Directly above this hole is an opening in the top of the chamber to allow smoke and odors to pass off.

The eggs are placed on old matting spread over the platform, and are covered with bran. Small fires of straw or dried dung are built on the floor of the lower story along the walls. The fires are few or many, according to the temperature of the eggs. No thermometers are used, the operator judging the temperature by touching the eggs. Two or three times a day the attendant stands in the opening leading to the second story and turns the eggs, moving them about in the bran. The

infertile and dead eggs are tested out the sixth and tenth days. The door of the hatching chamber is closed and calked when the attendant is not inside.

When the chicks hatch, they are hardened off in a part of the passage where the air is cooler than in the hatching rooms. Then they are distributed to the women who rear them. Expert operators are said to hatch 90% of fertile eggs.

Strong (64) thinks that artificial incubation in China probably antedated that in Egypt, and the Chinese now incubate eggs of hens and ducks by practically the same methods used by their ancestors.

D. G. Brill (10) reports that in some sections of China the incubation houses have three walls of sun-dried bricks and mud, or of reeds and mud, the fourth being a wall of the dwelling. The only door opens into a living room. The roof is of loosely laid tile with matting inside.

The ovens are round enclosures of bricks and mud, having an opening in the side for convenience in building the fire. In the top is fitted a conical earthen tray, several inches of ashes being placed in the part nearest the fire. A large basket, holding 1,300 eggs, fits into the tray. The eggs are piled into the basket and covered with a coiled arrangement of ropes made of old cotton, twisted and bound with rice straw. This cover is somewhat convex, standing up several inches from the top of the eggs. A small fire of charcoal or rice straw within the enclosure supplies the heat.

The eggs are taken from the baskets and cooled two to four times a day, according to the temperature, and are tested the fourth day by looking through them at a light. The good eggs are returned to the baskets for seven days longer—eleven days in all. After that time

they are put on one or the other of two platforms which are built into the upper part of the incubating house. The floors of these platforms are of reeds laid half an inch apart and covered with matting. The eggs lie on the matting, and old quilts or cloth are spread over them, more covering being added if they become too cool. The operator tells the temperature by touching an occasional egg to his eyeball. The only artificial heat supplied at this stage of incubation comes from the ovens below, incubation being carried forward principally by conservation of the heat given off by the developing embryos. The eggs on the platform are turned and shifted two or three times a day. When the chicks hatch they are kept in baskets under a cooler temperature until they are ready for distribution to the women who rear them.

The Chinese are said to hatch, on an average, 70% of total eggs, and more than 90% of fertile eggs. Less than 60% of total eggs hatched is considered a poor result.

Thorne (68), quoting consular reports, says that in some sections of China eggs are placed in roasted unhusked rice which has been cooled to a lukewarm temperature. A three-inch layer of rice is put into the bottom of a wooden tub; then a layer of 100 eggs; then a two-inch layer of rice, and more eggs, until the tub contains six layers of rice and five of eggs. The whole is covered with a cotton mattress. The eggs are removed, and the rice heated, every twenty-four hours. Each time the eggs are returned to the tub, the bottom layer is placed on top, each successive layer being one space lower in the tub than before, and the eggs which have been in the center of the tub, being put at the outer edge.

In Europe, during 1494, King Alphonse II. of Naples (64) established an egg oven, and the following year, the Duke of Florence also

built an oven, bringing an expert from Florence to operate it. That these attempts were not successful was thought to be due to different climatic conditions, the hatching ovens being specially adapted to the Egyptian climate. Late in the Fifteenth Century (4), Charles VIII. of France attempted artificial incubation by the Egyptian method, as did also Francis I. of France in 1540. These experiments evidently were no more successful than those in Italy, as the work was carried no further.

M. de Reaumur, Royal Academy of Science (Paris), Fellow of the Royal Society (Paris), etc., published in January, 1749, two volumes of discourses on the "Art of Hatching and Bringing up Domestick Fowls by Means of Artificial Heat." These discourses were translated and abstracted the following year by Mr. Trembly, F. R. S. (London) and published. (53).

M. de Reaumur saw the advantage possessed by the Egyptians in a cheap and plentiful supply of poultry and eggs, and realized that this was due to artificial incubation. He also saw that a method of incubation which might succeed admirably in the equable climate of Egypt, might not prosper in France; so he set about to find some method better adapted to his conditions.

He knew that "several ancient authors" had "related that eggs might be hatched by keeping them in dung, but none of these authors have told us that they ever practiced it themselves." When he attempted this method of incubation, he apparently came to doubt that the ancients ever hatched out many chicks in this way. He found that the heat often became sufficient to cook the eggs; but even if the heat were moderated the embryos always died at early stages of development.

He tried for almost a year, never being able to hatch a single chick, and concluded from his observations that the excessive moisture was fatal to the embryos. Then he imbedded one end of a tight cask in manure, in a location where there was free circulation of air to carry away the moisture and odors, and where the cask was protected from rain. In the cask he put shallow baskets of eggs. The cover of the cask was closed, or opened partially or entirely, as temperature necessitated. To his great joy, some chicks appeared in due time.

By many further trials, he became convinced that the odors, as well as the moisture, were detrimental, and changed the shape of his "stove" to a tight chest having the open end in a room entirely removed from that containing the manure. This arrangement was apparently a success.

Later, the idea occurred to M. de Reaumur to utilize the surplus heat of bakers' ovens in the same way as the heat of fermenting manure. He built "stoves" in the space above bakers' ovens, conserving and moderating the heat so that incubating proceeded satisfactorily, even if the oven were heated for baking only every second day. He used the thermometer he had invented for taking the temperature in his "stoves". M. de Reaumur claimed to be able to hatch a larger percentage of eggs with his stoves than with hens, especially in "small batches". He admitted that there must be some loss with larger lots of eggs, but states that "Sister Mary of the Community of the Infant Jesus, who had applied herself with great diligence and exactness to the care of her stoves, had once from three hundred eggs, two hundred and ninety-six chickens; so that she lost only four, and even those not till the time when they were actually pecking of their shells."

In 1777, Dr. Bonnemain constructed his incubator, a complicated affair which supplied eggs and chickens to the Paris market, as well as to the Court, as late as 1814. (4) This was heated by the circulation of hot water through pipes, and had a regulator of metallic composition which expanded at a certain temperature sufficiently to close the furnace door. (64).

After de Reaumur's work, many ideas on artificial incubation seem to have been tried out, both in England and France. Most of the incubators were heated by burning oil or gas, and consumed so much fuel that the air about the eggs was seriously vitiated. Moreover, the machines were delicate and costly, and so difficult to operate that none but an expert could run them.

Olivier de Serres speaks of one incubator, (4) a portable metal oven heated by lamps, in which the eggs were covered by feathers. It was not a success.

In 1821-24, J. H. Barlow (3) published articles telling of a steam hatching apparatus which he had invented and for which he claimed great merit.

Mr. Bucknell, (9) also of London, constructed his Eccaleobion ("I call forth life") and published a book about it in 1839. This machine is said to have had a most efficient regulator (64) but was so complicated that it was not very useful except in a small way.

Mr. Cantelo, (23) in 1846 invented a Hydro-incubator, the warm medium of which was heated water flowing over glass. This apparatus supplied the heat <u>above</u> the eggs, and had a piece of coarse canvas below the egg tray, which allowed circulation of air from beneath. The Hydro-incubator was tested in London (1849) against other incubators, and was so successful that it soon replaced all other types

of machines for hatching. It was not too complicated and expensive for common use, and at once popularized artificial incubation.

M. Rouillier (23) exhibited in Paris (1877) a Hydro-incubator which dispensed with lamps. It consisted of a large tank of three horizontal compartments above an egg drawer. The compartments of the tank were connected with each other by small holes in their common walls, so that fluid put into the upper compartment would gradually filter into the others. The entire tank was filled with hot water, and the eggs put into the egg drawer when the temperature had reached the required degree. Every 24 hours (oftener, if necessary) the water was drawn from the top compartment, and boiling water put in. This filtered downward, keeping the lowest tank (that nearest the eggs) at the proper temperature for incubation. Tank and egg drawer were well protected from outside temperature variations.

The same year, Thomas Christy of London published a pamphlet or book telling of his hydro-incubator. It had a circulating boiler and tank similar in many respects to the tank machines in present use.

In the United States (4), two patents were granted in 1847 for methods of incubation. One of these was granted to L. G. Hoff, of Albany, New York. His idea was to place rubber bags of hot water over the eggs.

An incubator invented by Jacob and Henry Graves was exhibited in Boston, Massachusetts, in 1868. This was heated on all four sides and the top, and had an automatic heat regulator. The regulator consisted of a glass syphon two feet long which was filled with alcohol as far as it extended inside of the machine, the part on the outside being filled with mercury. In the mercury was a wired cork. The

expansion of the heated alcohol was expected to raise both mercury and cork, the cork being so attached as to cut off the heat and open the ventilator simultaneously. Since the alcohol was below the egg chamber, the eggs became overheated before the regulator would work.

Between 1870 and 1894, 135 patents on incubators were granted by the U. S. Patent Office. One of these machines was heated by hot water pipes imbedded in soapstone. It had no regulator, but was provided with a ventilator over each egg drawer. The incubator was lamp-heated, and evaporating pans of water supplied moisture to the air entering the egg chamber.

Another incubator (Rankin's) was regulated by the expansion of water. A brass float was raised as the water heated, and, at a certain expansion, tipped a horizontal bar controlling apparatus designed to diminish the lamp flame.

## Types of Modern Incubators.

The more modern incubators are of two general types:

(1) Those heated by hot water.
(2) Those heated by hot air.

Hot water machines may have one large partitioned tank, or the system may consist of a boiler and a series of pipes to carry and return the water.

Hot air machines may be heated by "radiation", the heat and circulation in the egg chamber being controlled by a heated metal plate above the egg chamber, or they may be "diffusive" heaters, the heat coming from hot pipes and penetrating to the egg chamber through layers of felt or other diffusing media. Combinations of the different heating methods are often employed by manufacturers.

Incubators are also classified as:

(1) Dry machines--constructed to be operated without extra moisture supplied to the air of the egg chamber.

(2) Moist machines--designed to be operated with extra moisture supply.

Nearly all incubators have some sort of automatic temperature regulator. Capsule regulators operate by expansion of a liquid inside a sealed metal container. Bar and disc regulators usually depend on the different expansion of metals. Regulators may control the temperature by adjusting the size of the lamp flame, by directly governing the supply of heated air to the egg chamber, or by opening ventilators.

## Problems of Incubation.

Certain problems must be solved in order successfully to incubate eggs in the modern incubator. Successful incubation produces well-developed, vigorous chicks from a good percentage of fertile eggs set, if the eggs are fresh, and are from strong healthy stock kept under favorable conditions.

The problems of incubation include the influence of:

(1) Temperature
(2) Turning the eggs
(3) Position of eggs
(4) Airing and cooling of eggs
(5) Ventilation and purity of air supply
(6) Humidity and moisture supply.

Temperature-

One primal necessity for incubation of eggs is heat. Embryonic development proceeds to the many-celled stage before the egg is laid. This development must take place at the temperature of the hen's

body. After laying, the egg becomes dormant, no further development occurring until heat is supplied.

The temperature of incubation was long thought to be the same as of the human body—about 98° Fahrenheit. De Reaumur (53) used a reading on his thermometer corresponding to 96°-97° F. He also gave directions for a practical heat gauge as follows: "Take only a lump of butter the size of a walnut melted with half as much tallow, and put them together in a small transparent vial. The heat - - - will render the mixture in the bottle liquid as oil, if too great, or the lump will remain fixed in one spot if too small, but it will flow like a thick syrup on inclining the bottle if the stove is of a right temper. All of which one may be assured of by trying the heat of the bottle in one bosom." He cites the story related by Pliny of the Empress Livia, who "for an augury" hatched an egg in her bosom, and tells of a lady of his acquaintance who hatched the eggs of a goldfinch in like manner.

Cantelo (23), however, asserted that the blood heat of birds approached 106° F. and kept the temperature of his Hydro-incubator as nearly as possible to that standard.

The temperature for the incubation of hen's eggs is now generally accepted as 103° F. with the bulb of the thermometer touching a fertile egg, or just below the level of the top of the eggs.

Eycleshymer (28) found the temperature in the hen's nest, and the temperature of the eggs gradually to increase as incubation progressed. His results are the following:

Temperature averages

| | First week | Second week | Third week |
|---|---|---|---|
| Nest | 101.5° F. | 103.0° F. | 104°-106°F. |
| Egg | 100.0° | 101.0° | 102°-103° |

(In center of egg contents.)

Actual experimental data as to the extremes of temperature which eggs may endure without fatal results is lacking, but it has long been noted that slight variations in temperature do not seem to influence the hatch, and that larger variations either way from the standard are not necessarily fatal, unless continued for a considerable time. Too high temperature early in the incubation period may, however, destroy or weaken many of the embryos, while too low temperature is likely to delay hatching, even if it does not injure the developing chicks. Most authorities recommend that the temperature be slightly low for the first few days, increasing gradually until running evenly at 103° F. The third week, a continued temperature of 104° F. is probably not harmful. At this stage, the heat of the developing embryos helps to warm the egg chamber, necessitating a lower lamp flame.

Many have observed that the hen which steals her nest usually brings off a better brood than one which has incubated under supervision. Jackson and Cochel (43) thought this might be due to the fact that the hen warms the eggs in the nest for a while nearly every day, and conducted experiments to test the idea. Some eggs were warmed one-half hour; others one hour; and still others two hours daily. They were incubated with eggs kept in the usual manner. The eggs warmed one hour a day during the "holding" period hatched somewhat better than those warmed a longer or shorter time, or than those not warmed.

Turning the eggs-

By observation of hens sitting on marked eggs, (53, 28, 31) it has been found that the hen turns the eggs and moves them about in the nest at least five times during the day; and at every observation made during the night, the eggs have been partially turned and shifted in the nest. The hen may, of course, do this for her own comfort and

convenience, but whatever inducement leads to the action, she usually exposes all of the eggs at some time during the twenty-four hours to all conditions prevailing in the nest. De Reaumur moved his baskets of eggs about in the "stoves", partially turning them each day, but does not seem to have rotated the eggs in the baskets.

Since the germinal disc, or "germ spot" always floats upward, no matter how often the egg is turned, it is evident that the contents of the egg are moved about in the shell when the egg is turned, and no opportunity is given for the germinal disc, or the organs of the chick, as they develop, to adhere to the membranes of the egg.

Dareste (28) considered the principal cause of the death of embryos during the first week of incubation to be the adhesion of the allantois to the yolk, and consequent rupture of the vitelline membrane. Eycleshymer also experimented in this direction and found that a large part of the embryos of unturned eggs either stuck to the shell membranes or had the allantois grown fast to the yolk. From his observations, he concludes that more frequent turnings give better results, even as many as five times a day.

The general practice is to turn the eggs twice a day after the second day of incubation; the trays being reversed and eggs shifted on the tray at each turning. Since the allantois begins to develop at the 48-hour stage of the embryo, the idea of turning the eggs at about that time may have good foundation; though the hen does not wait that long before turning and shifting her clutch. In artificial incubation, the eggs are not turned after the close of the 18th day, the incubator being closed from that time until the hatching is well on toward completion. The incubators are probably kept closed at this time to prevent lowering of humidity in the egg chamber.

The eggs should be turned without sudden jarring, lest the shock might rupture some membrane of the egg or embryo. The hands should be free from lamp oil, and thoroughly cleansed, before turning the eggs.

Position of eggs-

M. de Reaumur (53) states that "The natural position of the eggs is to lie along, but they will hatch, nevertheless, in whatever position they shall have been placed." Eycleshymer's (28) experiments convinced him that there is little difference in hatching between oblique and horizontal position of eggs in the incubator, unless the supply of oxygen is inadequate. In that case, better results were obtained from the oblique position, perhaps because the head of the embryo was brough into closer contact with the air cell of the egg.

In the hen's nest, the plane of the egg is determined largely by the depth of the nest. In a shallow nest, some are inclined at a considerable angle.

Airing and Cooling the eggs-

The hen leaves the nest primarily to seek food, and exercise , and the time of her absence is usually regulated by the convenience and abundance of food. Incidentally, the eggs are aired and cooled, usually once a day after the first day or two of incubation.

Observations of the behavior of the hen in this respect were made by de Reaumur, (53) and he reflects that the degree of cooling to which the eggs are exposed by the hen gives reason not to be too solicitious about small temperature variations in artificial incubation. He does not appear to regard cooling as a necessary factor.

Some maintain that the eggs are sufficiently aired in turning twice a day, especially for the first week of incubation. Most authorities advocate cooling once a day, the length of the cooling period depending on the temperature and the stage of incubation. The cooling time should be gradually increased as the hatch progresses, but discontinued after the 18th day. It is, perhaps, generally conceded, though not proved, that the greatest benefit in airing and cooling may be the extra amount of fresh air which the eggs receive in this operation.

Ventilation and purity of air supply-

De Reäumur (53) closed the shells of eggs with varnish, and found they would not hatch; he tried to incubate eggs imbedded in fermenting manure, and covered in water, but got no chicks in either case. This failure to hatch he attributed to excess of moisture within the egg rather than to lack of air, though he observed that the egg absorbed air through the shell, and exhaled (or perspired) other substances than water, which he considered poisonous to the embryo. However, he provided circulation of air in his ovens, so supplying oxygen as well as dissipating bad odors and excessive moisture.

Dareste (28) closed all apertures in an incubator and all the embryos died.

Gerlach (28) found that a diminishing air supply during incubation dwarfed the embryos. He then scraped the shells of some eggs very thin in places, and incubated these eggs with normal ones. For the first two days all developed alike, but from that time the embroys of the scraped eggs grew twice as fast as the normal eggs. A removal of part of the shell without rupturing the membranes resulted in the same astonishing growth.

The embryo absorbs air through the shell until near pipping time, when the lungs begin to function. Tegetmeier (58) noticed a creaking sound within the egg before the shell was broken, and, by carefully perforating the shell, was able to see that the beak of the chick had pushed through the inner membrane of the air sac, and that the chick was apparently breathing the air contained therein. De Reäumur (53) had previously made the same observations, and had also noted that the voice of the chick could often be heard before the shell was at all perforated, indicating a functioning of the lungs at that stage. When the shell is finally broken, the chick breathes the outside air.

Oxygen is essential for the chemical processes in the growing embryo. Some of the final products of metabolism are Carbon-dioxide ($CO_2$) and water. Oxygen is inhaled in the air, and $CO_2$ and part of the water are sent out through the shell. Some of the water may be used to maintain the necessary moisture content of the developing tissues.

Carbon-Dioxide in incubation-

Professor James Dryden (24) found that $CO_2$ in the air of the egg chamber was not in as great proportion as in the hen's nest. He concluded from this result, and from the fact that better hatches were obtained from the nest than from the incubator, that the presence of $CO_2$ could not be responsible for chicks dead in the shell, as had been suggested, and contemplated the possibility that the action of this gas might even be beneficial in hatching.

In pursuance of this idea, tests were made in the Chemical Laboratory of the Utah Experiment Station, by Widtsoe and Stewart, on the action of $CO_2$ on egg shells. The egg contents were blown from the

shells, and the shells placed in bottles containing $CO_2$. Shells which were filled with water, the opening being sealed with wax, and dry shells which were put into bottles containing both $CO_2$ and water, were so decomposed as to be easily broken and crumbled. Dry shells in a dry atmosphere of $CO_2$ were apparently not affected.

From this, it was thought that the action of $CO_2$ in presence of moisture might produce an easier exclusion of the chick.

The next step was a determination of moisture in the incubator and the nest, with the result that the condition of humidity called medium moisture was found to approach the humidity of the hen's nest. This gave better results in artificial hatching than higher or lower humidity.

Acting on the theory that an extra supply of $CO_2$ might be a beneficial factor in artificial incubation, experiments were conducted at Ontario Agricultural College, attempting to find some inexpensive and practical method of supplying this gas in incubators. Moisture pans were filled with buttermilk which had been inoculated with bacteria so as to furnish comparatively large amounts of $CO_2$; artificial $CO_2$ was run into the egg chamber; lamp fumes were conducted into the egg chamber; water into which $CO_2$ had been run, water into which lamp fumes had been conducted, and clear water, were used in moisture pans. Incubators having the buttermilk in the moisture pans gave slightly better results in hatching than those having clear water; but these were more successful than the other machines. Lamp fumes appeared to be distinctly detrimental to the hatch. Analyses for lime and phosphoric acid were made on fresh eggs, and the lime content was determined of chicks at different stages of incubation, and under different incubation meth-

ods. No consistent increase in the lime content of the chick was found, which could be attributed to the presence or absence of applied $CO_2$ or moisture. (35)

Professor Lamson (47) of the Connecticut (Storrs) Experiment Station arranged an incubator in such a way that he could establish a known rate of air circulation from outside the room into the egg chamber. $CO_2$ was, in most cases, controlled by ventilation. By observation of the incubating hen, he found that $CO_2$ increased progressively in the nest, the highest proportion being present the 17th and 18th days of incubation. The increase of $CO_2$ in the incubator was similar to that in the nest, and the amount of $CO_2$ in the egg chamber could be controlled by ventilation to approximately the proportion found in the nest. A nest containing boiled eggs did not show this regular increase of $CO_2$. Bohr and Hasselbach (47) found the increase of $CO_2$ to be in direct proportion to the increase of weight in the embryo, and this was confirmed in Professor Lamson's work.

Pfluger, Wolberg, Strassburg, and others (47) agree that in cell respiration the interchange of gases is altogether by diffusion. Bohr maintains that gaseous interchange in the lungs is essentially a secretory process. The chick has no lung respiration until pipping time. If the interchange of gases between the egg and the surrounding atmosphere is by diffusion, then an increase in concentration of $CO_2$ in the egg chamber would tend to decrease the transpiration of $CO_2$ from the egg. It appears probable from experiments (47) that a parge proportion of $CO_2$ in the air does have a marked inhibitory action on embryonic respiration, the embryos dying near the end of the incubation period, perhaps from asphyxiation.

About 60 parts of $CO_2$ in 10,000 of air were found in the hen's nest, and this proportion did not prove injurious in the incubator. Only slight effect was observed until the $CO_2$ reached 150 parts in 10,000; above that concentration there was a rapid decrease in the percent hatch. When $CO_2$ was added to the air in the incubator at pipping time and continued during hatching the decrease in the number of chicks hatched was in direct proportion to the amount of $CO_2$ introduced.

Professor Lamson concludes that the presence of carbon dioxide is not necessary to successful incubation, but that, under ordinary conditions of incubation, not enough of the gas is likely to be present seriously to injure the hatch. Increased ventilation decreases the amount of $CO_2$ in the egg chamber, and vice versa. Applied carbon dioxide was not found beneficial at any stage of the hatch.

Eycleshymer (28) says that all incubators seem deficient in ventilation, and establishes his position by obtaining better hatches from an incubator having accessory ventilation than from a similar machine with ordinary ventilation. However, sufficient ventilation to remove all $CO_2$ from the egg chamber would undoubtedly cause too great evaporation of the eggs.

Humidity and Moisture supply-

Early in De Reaumur's work (53), he became convinced that too much moisture was detrimental to incubation, and protected his eggs from the moisture of the manure. Still there was excess of moisture, so he concluded that the eggs must perspire during incubation. He reduced the humidity by ventilation and circulation of air.

Dareste (28) found that excessive moisture favored parasitic growths in the albumen of the egg. On the other hand, the ancient

Egyptians placed shallow pans of water in their hatching chambers to supply humidity (64).

Martin Doyle (23) says (1857): "As to the necessity of moisture, the most modern London experimenter - - - has stated that a fowl does not sweat, but, like the dog, throws off a quantity of moisture from the mouth, from which a small drop of moisture may be seen to fall". Yet M. Parmentier considered this moisture necessary to sustain the condition of damp animal heat natural to the sitting hen. This involves a question for deeper physiologists than we are."

It is now conceded that a certain degree of humidity in the incubator is necessary to best results in hatching (25, 26, 35). The humidity can not, of course, introduce moisture into the egg, once it has evaporated, but regulates evaporation. Humidity of the air should stop considerably short of the saturation point for most of the incubation period; though some believe that enough moisture should be present during hatching to produce a dew on the glass of the incubator door, and would put steaming hot water in the moisture tray just before closing the machine for the hatching. (22).

Humidity in the egg chamber may come from the following sources:

(1) Humidity of air introduced from outside the machine.
(2) Evaporation of eggs.
(3) Extra moisture supply.
(4) Insensible perspiration of hen's body (natural incubation)

Humidity may be controlled by:

(1) Humidity of air.
(2) Circulation of air.
(3) Thickness of egg shells.

It is said by some experts that the moisture of the egg itself

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"Cantelo

provides sufficient humidity for incubation, needing only to be conserved and regulated by operation of ventilators. Others argue for plenty of ventilation, regulating the evaporation of eggs by providing more moisture to the air in the egg chamber.

Some sort of hygrometer is commonly used to measure the degree of humidity. De Reaumur's hygrometer consisted of a cold egg placed in his "stove" when the temperature was right for incubation. If in fifteen minutes, the egg shell was moist, he considered the "stove" too damp. (53).

The wet-and-dry bulb thermometer (frame hygrometer) is now frequently employed in incubators. It consists of two thermometers, mounted side by side, which read exactly alike under dry conditions. One bulb is covered by wicking, one end of which is immersed in water. as the water evaporates from the wicking, this thermometer registers lower because heat has been used in evaporization of the water. The lower the humidity of the air, the more rapid the evaporation and the lower the reading on the wet-bulb thermometer. Thus the difference between the reading of the two thermometers is inversely proportional to the humidity of the air. A moisture gauge is sometimes employed. This consists of a wet-bulb thermometer which is so [illegible] graded and marked that the column indicates the relative humidity.

Experiments at Ontario Agricultural College (35) and Connecticut (Storrs) Experiment Station (47) indicate that the frame hygrometer does not give an accurate index of relative humidity in the egg chamber unless fanned to cause a better circulation of air. However, records made by these hygrometers operating under similar conditions would be comparable, and might serve as an index by which to gauge moisture conditions during incubation. In experiments at Oregon Agricultural Col-

lege, a wet-bulb reading of about 87.6° against a dry-bulb reading of 103° F. gave better results in hatching for that climate than higher or lower reading. Comparison of results of experiments conducted in different locations (24, 25, 26) seem to indicate that, in a moist climate, a "medium" moisture supply may give about the same relative humidity in the egg chamber as "maximum" amount of added moisture in a less humid locality. Humidity is said to be greater in the hen's nest than in the moist incubator as ordinarily run, though it is possible to produce a greater degree of humidity than in the nest (25). Humidity in the egg chamber is greater when moisture is supplied than in a machine of the same type without applied moisture.

## Part III.

## The Present Experiment.

It has been thought that the advantages claimed for incubation by hens and moist incubators as compared to incubation by dry incubators might be due to the greater use of lime from the egg shell by the developing chick. Previous work[‡] at the Chemical Laboratory of the Oregon Agricultural Experiment Station, in collaboration with the Poultry Department, failed to show the presence of greater proportions of lime in chicks from the moist incubator and the nest than in those from the dry incubator. The work did show, however, that the degree of humidity in the air surrounding the eggs might be an important factor in embryonic development; agreeing in this respect with previous practical and experimental results in incubation secured by the Poultry Department. Following this line of investigation, and considering the claims that hens hatch more and better chicks than incubators and that moist incubators

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[‡]Unpublished data.

hatch more successfully than dry incubators, this experiment was undertaken. The object of the experiment was to ascertain whether there would be physical and chemical differences in the development of the chicks at pipping time to parallel results obtained in hatching. This experiment also was carried on by the Chemistry Department of the Oregon Agricultural Experiment Station in collaboration with the Poultry Department.

The Poultry Department furnished the records of incubation for this study as well as the eggs and chicks for chemical analysis. The incubators were operated by C. C. Lamb, of the Poultry Department. After the eggs pipped, they were immediately etherized, and taken at once to the Chemical Laboratory, where the shells were removed and the preparation for chemical work begun at once.

A partial report of the experiment is given in this paper.

## Conditions of Experiment.

The eggs used were from one flock of fifty cross-bred pullets, fed on a ration including grain, moist mash, hopper-fed beef scrap, grit, charcoal, etc., with plenty of water and green food. The fowls had the freedom of a yard 100 x 200 feet in area.

Incubation extended over the period from February 20, 1914, to July 6, 1914. The experiment was divided into three series, each series consisting of two or three hatches, and each hatch employing a dry incubator, a moist incubator, and a number of hens. The eggs were so selected that four eggs from each laying hen represented were taken. One was put into the dry incubator, one in the moist incubator, one in the hen's nest, and the fourth was prepared at once for fresh egg analysis. The eggs were comparable as to age when set, none being old enough to

have been injured by keeping. The incubators used were all of one type, constructed to operate with applied moisture. In this report, the terms dry incubator and moist incubator are used to indicate that, of companion machines, one was operated with, and the other without, applied moisture. Since all of these incubators were designed for moist hatching, the term dry hatching does not in this instance, refer to incubation in machines constructed to operate without applied moisture.

The first series included eggs set February 20, February 24, and March 3; 360 incubated eggs, equally divided among the moist incubator, the dry incubator, and the nests. Series #2 embraced incubation trials of April 18, April 28, and May 8, using 370 eggs. Series #3 had only two hatches, those of May 26 and June 15, using 246 eggs.

## Temperature and Humidity.

Frame hygrometers were used to indicate humidity in the incubators and incubator room, but humidity was not determined in the nest. Temperature in the incubator was registered by a separate thermometer. No water was put into the moisture trays of the dry incubators, but enough was added to those of the moist incubators to keep the reading of the wet-bulb thermometer at near 88° when the dry-bulb thermometer registered 103° Fahrenheit. Below are given the average temperatures, and the relative humidity of the dry and moist incubators and the incubator room.

Table I.

Average Temperatures and Relative Humidity of the Incubator room of the Moist and Dry Incubators for each Series.

| | Temperature | | | Relative Humidity | | |
|---|---|---|---|---|---|---|
| | 7 A. M. | 12 M. | 5:30 P. M. | 7 A. M. | 12 M. | 5:30 P.M. |
| | Degrees Fahrenheit | | | Percentages | | |
| Series #1 | | | | | | |
| Room | 55.22 | 57.59 | 60.29 | 82% | 77% | 78% |
| Moist Incubators | 102.99 | 102.45 | 103.23 | 55 | 57 | 55 |
| Dry Incubators | 102.86 | 102.63 | 103.39 | 46 | 45 | 46 |
| Series #2 | | | | | | |
| Room | 57.03 | 66.01 | 66.65 | 77 | 60 | 67 |
| Moist Incubators | 102.74 | 102.92 | 102.83 | 56 | 56 | 53 |
| Dry Incubators | 102.77 | 102.79 | 102.97 | 43 | 43 | 42 |
| Series #3 | | | | | | |
| Room | 60.46 | 67.71 | 69.63 | 78 | 67 | 72 |
| Moist Incubators | 102.62 | 102.48 | 103.20 | 56 | 58 | 58 |
| Dry Incubators | 102.42 | 102.63 | 103.04 | 44 | 47 | 45 |

Wet and dry bulb readings from which the relative humidity was estimated were unfanned.

The temperature of the room increased as the season advanced, but incubator temperatures remained quite even. Humidity of the incubator room recreased as the temperature increased, but the variation of humidity in the machines was not great, the humidity of the moist incubator being greater than of the dry incubator, and less than the humidity said to prevail in the hen's nest, under favorable conditions, 60% (84) to 70% (35). Humidity in the dry incubator was always less than in the moist incubator.

Weight and Loss of Weight in Eggs.

Each egg weighed immediately before sitting and again on the eighteenth day of incubation. The following table gives the weight in grams for each hatch and the percentage loss in weight for each sitting.

Table II.

Weight and Loss of Weight in Eggs. Weights in Grams.

| | Hens | | | Moist Incubators | | | Dry Incubators | | |
|---|---|---|---|---|---|---|---|---|---|
| | Fresh | 18th Da | %Loss | Fresh | 18th Da | %Loss | Fresh | 18th Da | %Loss |
| Series #1 | | | | | | | | | |
| 2/20/14 | 52.74 | 46.56 | | 52.45 | 46.69 | | 53.01 | 45.30 | |
| 2/24/14 | 52.30 | 46,03 | | 53.15 | 47.30 | | 52.82 | 44.53 | |
| 3/ 3/14 | 52.96 | 47.42 | | 53.31 | 48.25 | | 52.83 | 45.03 | |
| Average | 52.67 | 46.67 | 11.39% | 52.97 | 47.41 | 10.50% | 52.89 | 44.95 | 15.01% |
| Series #2 | | | | | | | | | |
| 4/18/14 | 50.76 | 43.81 | | 50.12 | 43.95 | | 51.39 | 42.74 | |
| 4/28/14 | 49.72 | 43.56 | | 49.48 | 43.78 | | 50.19 | 41.17 | |
| 5/ 8/14 | 49.89 | 42.72 | | 48.97 | 42.51 | | 49.24 | 39.93 | |
| Average | 50.12 | 43.36 | 13.49% | 49.52 | 43.41 | 12.34% | 50.27 | 41.28 | 17.88% |
| Series #3 | | | | | | | | | |
| 5/26/14 | 49.72 | 43.38 | | 49.56 | 43.35 | | 49.62 | 40.68 | |
| 6/15/14 | 50.53 | 44.44 | | 50.44 | 44.21 | | 49.79 | 42.01 | |
| | 50.12 | 43.91 | 12.39% | 50.00 | 43.78 | 12.44% | 49.70 | 41.34 | 16.82% |

The eggs used in each series were quite uniform in weight. The loss of weight during incubation in the moist incubator and the nest did not differ greatly, the balance swinging sometimes one way and sometimes the other in individual hatches. The eggs in the dry incubator had, in every series, four or five percent greater loss in weight than those in the moist incubator or the nest.

Results of Incubation.

When each egg pipped it was removed from the incubator or the

nest to be prepared for chemical examination. The tabulation given below shows results of incubation.

Table III.

Summary—Results of Incubation.

| | Hens | | Dry Incubators | | Moist Incubators | |
|---|---|---|---|---|---|---|
| | Number | Percent | Number | Percent | Number | Percent |
| **By Series** | | | | | | |
| **Series #1** | | | | | | |
| Eggs set | 120 | | 120 | | 120 | |
| Tested out | 3 | 2.50 | 6 | 5.00 | 10 | 8.33 |
| Dead in shell | 3 | 2.50 | 13 | 10.83 | 11 | 9.16 |
| Broken | 1 | 0.83 | 0 | — | 0 | — |
| Pipped | 113 | | 101 | | 99 | |
| % pipped (to total eggs) | | 94.16 | | 84.16 | | 82.50 |
| % " " fertile" | | 96.58 | | 88.59 | | 90.00 |
| **Series #2** | | | | | | |
| Eggs set | 124 | | 123 | | 123 | |
| Tested out | 12 | 9.67 | 15 | 12.19 | 7 | 5.69 |
| Dead in shell | 14 | 11.28 | 31 | 25.20 | 4 | 3.25 |
| Broken | 6 | 4.84 | 0 | — | 1 | 0.81 |
| Pipped | 92[u] | | 77[uu] | | 111 | |
| % pipped (to total eggs) | | 74.19 | | 62.60 | | 90.24 |
| % " " fertile " | | 82.14 | | 71.30 | | 95.60 |
| **Series #3** | | | | | | |
| Eggs set | 82 | | 82 | | 82 | |
| Tested out | 8 | 9.75 | 13 | 15.85 | 2 | 2.44 |
| Dead in shell | 3 | 3.66 | 12 | 14.63 | 1 | 1.22 |
| Broken | 4 | 4.89 | 2 | 2.44 | 1 | 1.22 |
| Pipped | 67 | | 55 | | 78 | |
| % pipped (to total eggs) | | 74.19 | | 62.60 | | 90.24 |
| % pipped (to fertile" | | 82.14 | | 71.30 | | 95.60 |
| **Summary—All tests** | | | | | | |
| Eggs set | 326 | | 325 | | 325 | |
| Tested out | 23 | 7.05 | 34 | 10.46 | 19 | 5.84 |
| Dead in shell | 20 | 6.13 | 56 | 17.23 | 16 | 4.92 |
| Broken | 11 | 3.37 | 2 | 0.61 | 2 | 0.61 |
| Pipped | 272 | | 233 | | 288 | |
| % pipped (to total eggs) | | 83.43 | | 71.69 | | 88.69 |
| % pipped " fertile " | | 89.76 | | 80.08 | | 94.12 |

[u]One hen did not set well.

[uu]Part of eggs left out over night the 18th day.

Fertile eggs = eggs not tested out
Broken eggs not counted as tested out.

It will be noted that the results given in this table are for the pipping stage, and so can not be regarded as expressing what might have occurred had the chicks been allowed to come through the exclusion period.

There was some variation in the comparative results obtained in incubation for the different series, but usually the dry incubators did not give as good results as the moist incubators or the hens. In the final summary of all tests, the moist incubators ranked first, with the hens a close second, and the dry incubators considerably in the rear.

One hatch of the second series was not very successful on account of the unfaithfulness of one of the hens, and the accidental chilling of the eggs in one of the incubators. This being the case, a report of incubation is given below which discards the unsuccessful hatch.

Table IV.

A Report of Incubation, Discarding the Hatch of April 18.

| | Hens | | Dry Incubators | | Moist Incubators | |
|---|---|---|---|---|---|---|
| | Number | Percent | Number | Percent | Number | Percent |
| Series #2 | | | | | | |
| Eggs set | 82 | | 82 | | 82 | |
| Tested out | 5 | 6.09% | 10 | 12.19% | 2 | 2.44% |
| Dead in shell | 5 | 6.09 | 6 | 7.31 | 3 | 3.66 |
| Broken | 4 | 4.88 | 0 | — | 1 | 1.22 |
| Pipped | 68 | | 66 | | 76 | |
| % pipped (to total eggs) | | 82.92 | | 80.49 | | 92.68 |
| % pipped " fertile " (Taking out broken eggs) | | 88.31 | | 91.66 | | 95.00 |

| | Hens | | Dry Incubators | | Moist Incubators | |
|---|---|---|---|---|---|---|
| | Number | Percent | Number | Percent | Number | Percent |
| Summary—All tests | | | | | | |
| Eggs set | 284 | | 284 | | 284 | |
| Tested out | 16 | 5.63% | 29 | 10.21% | 14 | 4.93% |
| Dead in shell | 11 | 3.87 | 31 | 10.91 | 15 | 5.28 |
| Broken | 9 | 3.17 | 2 | 0.70 | 2 | 0.70 |
| Pipped | 248 | | 222 | | 253 | |
| % pipped (to total eggs) | | 87.32 | | 78.17 | | 89.08 |
| % pipped " fertile " | | 92.54 | | 87.06 | | 93.70 |

In this case also, the final summary of all series shows that the moist incubators had the largest percentage of eggs pipped and the dry incubator the smallest. In justice to the hen it must be said that her slight disadvantage in this experiment was undoubtedly due to the larger number of eggs broken during incubation.

## Chemical Work.

### (1) Preparation of Material—

The fresh eggs were separated, whites and yolks weighed, then dried and weights again taken. The whites were dried in warm fanned air and weighed, but the yolks were checked as to dryness by heating in a water oven at a temperature not above 60° C. before weighing. The incubated eggs were removed from the nest at pipping and the chicks exposed to an atmosphere of chloroform. The shells and their attached membranes were removed at once, and the chicks placed in weighed and marked watch glasses for drying. They were kept in warm fanned air until apperently dry, then placed in the water oven for a definite time at a temperature not above 70° C. Moist and dry weights were taken and the amount and percent of moisture calculated.

In previous work, Mr. R. H. Robinson of the Chemical Laboratory of the Oregon Agricultural Experiment Station had discovered that the unabsorbed yolk of the dried chick could be easily and completely separated from the tissue, thus giving a better opportunity to determine differences in embryonic development at a definite stage of incubation. In the present experiment, it was thought best to take advantage of this possibility, so the unabsorbed yolks were separated from the tissue, the dry weight of the yolk determined, and the weight of tissue calculated by difference.

(2) Preparation of Samples for Chemical Work—

It will be remembered that in selecting eggs for each hatch, four eggs from each laying hen represented were taken, three being incubated and the fourth kept for chemical analysis. In making up the samples for each series, the records were examined to see which of the laying hens represented in the hatches of a series had all three of their incubated eggs, or two of the three, recorded as having pipped. Where such a record was found, the two, or the three chicks, as the case might be, were set aside for the composite samples of the incubated eggs; the fourth, the fresh egg, was used in the fresh egg samples.

For illustration, we will suppose that Hen #670 had an egg in the dry incubator, one in the moist incubator, and one in the nest for each hatch of Series #1. Below is represented a possible result:

Illustration:

Series #1

Eggs of Hen #670

| | Dry Incubator | Moist Incubator | Hens | Fresh Eggs |
|---|---|---|---|---|
| Hatch #1 | A | B | C | D |
| Hatch #2 | A' | B' | C' | D' |
| Hatch #3 | A" | B" | C'' | D" |

Let A, A', and A", etc., represent the eggs.

Eggs marked (x) did not hatch.

Eggs A, B, C, D, and A', C', D' were used in the proper samples, but all in Hatch #3 were discarded. Table V. shows the number of eggs set, the number which pipped, and how many of the incubated eggs were used in the chemical work.

Table V.

Number of Eggs used in Incubation and in Chemical Analyses of Incubated Eggs.

| | Eggs Set | Eggs Pipped | Eggs Used |
|---|---|---|---|
| Series #1 | | | |
| Hens | 120 | 113 | 44 |
| Dry Incubators | 120 | 101 | 41 |
| Moist Incubators | 120 | 99 | 43 |
| | 360 | 313 | 128 |
| Series #2 | | | |
| Hens | 124 | 92 | 34 |
| Dry Incubators | 123 | 77 | 44 |
| Moist Incubators | 123 | 111 | 42 |
| | 370 | 280 | 120 |
| Series #3 | | | |
| Hens | 82 | 67 | 34 |
| Dry Incubators | 82 | 55 | 33 |
| Moist Incubators | 82 | 78 | 34 |
| | 246 | 200 | 101 |
| Total | 976 | 793 | 349 |

Samples for Dry Incubators, Moist Incubators, sitting Hens, Fresh Egg-Yolks, and Fresh Egg-Whites were prepared for each series.

In preparing the composite samples, the dry material was run repeatedly through a food chopper, and then, if necessary, reduced to the requisite fineness by pulverizing in an agate mortar. When sufficiently finely divided, the entire sample was thoroughly mixed and

put into a closed glass jar for a storage semple. A portion of the storage sample was further dried in a desiccator over sulphuric acid and under reduced pressure, then transferred to a covered glass jar and sealed with paraffin wax. These working samples supplied the material for chemical analysis, being renewed as necessary by drying more of the storage samples.

(3) Chemical Methods—

Total determinations of nitrogen, ash, lime (CaO) and phosphoric acid ($P_2O_5$) were made. Total nitrogen was determined by the Kjeldahl method on samples of one-half gram or one gram dry material.

Ash was determined on one-gram samples by burning at dull red heat over the Bunsen flame until complete charring had occurred, then reducing to whiteness in the muffle furnace. In ash determination of the fresh egg samples, it was found necessary to use three-gram samples, and to determine the ash by leaching. The material was burned to charring over the Bunsen flame, the soluble salts leached off, and the residue burned free of carbon in the muffle furnace. The filtrate and the carbon-free portion were then combined, and the moisture evaporated over the steam bath, with a final careful heating to dull red heat over the Bunsen flame.

Lime was run on the ash samples by McCrudden's Method (Jour. Biol. Chem. Vol. 10, p 187), the calcium being precipitated as calcium oxalate, dissolved in sulphuric acid, and titrated against standard potassium permanganate.

The samples for phosphoric acid were oxidized by means of magnesium nitrate over flame, the phosphorus determination being by the usual molybdate precipitation and final weighing as magnesium pyro-

phosphate. (Reference—Sherman, Organic Analysis; Lincoln and Walton, Quantitative Chemical Analysis). In oxidation of the fresh egg-whites with magnesium nitrate, difficulty was experienced in maintaining a sufficiently low temperature, after the oxidation had been initiated, to prevent loss of material. After repeated trials, it was found possible to moderate and control the heat by placing the platinum dish, containing the sample which had previously been moistened with magnesium nitrate solution, on a piece of heavy asbestos board about six inches square. The Bunsen flame was carefully applied to one corner of the board, allowing the flame to play along the lower surface of the board, or entirely removing it at intervals, as was necessary to maintain the proper degree of heat.

## Results Obtained.

(1) Fresh egg analysis—

Table VI. gives the weights of fresh eggs and parts of eggs, and the percentages of moisture, yolk, and white for each series.

Table VI.

Weights of Fresh Eggs, and of parts of Eggs, and percentages of Moisture, Yolk and White

| | Albumen | | | Yolk | | | Total Egg Content | | |
|---|---|---|---|---|---|---|---|---|---|
| | Moist wt. | Dry wt. | Wt. water | Moist wt. | Dry wt. | Wt. water | Moist wt. | Dry wt. | Wt. water |
| | gms | gms | gms | gms | gms | gms | gms | gms | gms |
| Series #1 | 27.383 | 3.614 | 23.769 | 17.851 | 8.996 | 8.855 | 45.234 | 12.610 | 32.765 |
| Series #2 | (24)26.731 | 3.387 | 23.344 | (25)17.092 | 8.624 | 8.468 | 43.823 | 12.011 | 31.812 |
| Series #3 | 26.560 | 3.445 | 23.115 | 16.852 | 8.767 | 8.085 | 43.412 | 12.212 | 31.200 |

Percentages

| | % total moist wt. | % to total dry wt. | % water to moist wt of albumen | % to total moist wt. | % to total dry wt | % water to moist wt of yolks | % to moist wt. |
|---|---|---|---|---|---|---|---|
| Series #1 | 60.536 | 28.659 | 86.802 | 39.464 | 71.341 | 49.605 | 72.434 |
| Series #2 | 60.997 | 28.199 | 87.329 | 39.003 | 71.801 | 49.544 | 72.592 |
| Series #3 | 61.181 | 28.209 | 87.029 | 38.819 | 71.791 | 47.976 | 71.869 |

The fresh eggs were fairly uniform in weight, though those of the first series were somewhat heaviest and those of the third series slightly lightest. The percentage of moist yolk to total egg contents decreased in the same order, the converse being, of course, true of the albumen. The percentage of moisture in the yolk decreased in the same order, but not in the same degree.

Let us compare these results with seasonal variations in hatching.

Table VII.

Seasonal Results of Incubation[1]

| | Series #1 | Series #2 | Series #3 |
|---|---|---|---|
| Eggs set | 360 | 246 | 246 |
| Eggs pipped | 313 | 210 | 200 |
| % pipped to total eggs | 86.95% | 85.36% | 81.30% |
| % " to eggs not tested out | 91.79% | 91.70% | 89.69% |

Here we find as the season advanced a slight decrease in the percentage of eggs pipped, both as to total eggs and to eggs not tested out; but differences were so small as to be of little apparent significance. The moist eggs had approximately 60 to 61 percent of their contents in albumen and 39 to 40 percent in yolks. The dry eggs, on the other hand, had 28 to 29 percent of albumen and 71 to 72 percent of yolk. The percent moisture of the albumen was nearly twice as high as that of the yolk. The total egg contents contained about 71.5 to 72.5 percent water.

The results obtained in analysis of fresh eggs are shown in Table VIII., expressed as grams and as percents, and calculated to the weight of the eggs.

[1] Hatch of April 18th discarded.

Table VIII.

Analyses of Fresh Eggs—Results Expressed in Grams and Percent.

| | Egg (total contents) | | | | Albumen | | | | | | Yolks | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Moist wt. | Dry wt. | Moisture | Dry wt. | Total N | Crude protein (nx6.25) | Ash | (CaO) Lime | $P_2O_5$ | Dry wt. | Total N | Crude protein (nx6.25) | Ash | Lime | $P_2O_5$ |
| | gms | gms | gms | gms | gms | gms | gms | gms | gms | gms | gms | gms | gms | gms | gms |
| Series #1 | 45.234 | 12.586 | 32.648 | 3.614 | 0.4643 | 2.912 | 0.2119 | 0.0031 | 0.0136 | 8.972 | 0.4493 | 2.8081 | 0.2706 | 0.0350 | .2508 |
| Series #2 | 43.823 | 12.011 | 31.812 | 3.387 | 0.4366 | 2.729 | 0.2027 | 0.0035 | 0.0129 | 8.624 | 0.4404 | 2.7525 | 0.2613 | 0.0379 | .2121 |
| Series #3 | 43.412 | 12.212 | 31.200 | 3.445 | 0.4431 | 2.769 | 0.2085 | 0.0028 | 0.0114 | 8.767 | 0.4523 | 2.8269 | 0.2779 | 0.0377 | .2492 |

Percentages (to dry material)

| | to moist wt. | % | % | % | % | % | % | % | % | % | % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Series #1 | 72.176 | 12.848 | 80.578 | 5.863 | 0.0860 | 0.376 | 5.0078 | 31.298 | 3.016 | 0.390 | 2.796 |
| Series #2 | 72.592 | 12.889 | 80.572 | 5.984 | 0.1033 | 0.381 | 5.1067 | 31.917 | 3.030 | 0.440 | 2.459 |
| Series #3 | 71.869 | 12.862 | 80.377 | 6.052 | 0.0822 | 0.331 | 5.1591 | 32.245 | 3.110 | 0.430 | 2.843 |

In this case, also, differences were so small as to be practically negligible. In fact, consideration of the above tabulations would tend to establish the opinion that the hen lays an egg of practically constant composition, the chief variation being in the moisture content and the size of the egg. The same conclusion might be drawn from a study of results obtained by Williard and Shaw (73).

(2) Analysis of Incubated Eggs—

There was considerable variation in the proportions of unabsorbed yolk and tissue in the chicks of the different series, and also in the chicks resulting from the different methods of incubation. Table IX. shows these differences.

Table IX.

Percentage of Unabsorbed Yolk and Tissue in Dried Chicks.

| | Percent of Unabsorbed Yolk | Percent of Tissue |
|---|---|---|
| Dry Incubators | 43.49% | 56.51% |
| Moist Incubators | 41.43 | 58.57 |
| Hens | 42.04 | 57.96 |
| Series #1 | 43.01 | 56.99 |
| Series #2 | 41.39 | 58.61 |
| Series #3 | 43.48 | 56.52 |

The chicks of Series #2 apparently had advanced further in development at pipping time than those of the other series. It will be remembered that the fresh eggs of this series had slightly a lower percentage of moisture in the yolks, a somewhat higher moisture content of the albumen and of the total egg, and a rather larger percent of yolk in the dry egg. However, results of incubation did not show any special advantage for this series.

Comparing the different methods of incubation, we find that the chicks from the moist incubators had a lower percent of unabsorbed yolk and a higher percent of tissue than those from the dry incubators or the nests. A reference to Table X. will show the chemical composition of the albumen and tissue, calculated to the weight of the chicks and expressed in grams and percents; the results being shown for the different series and for the different methods of incubation.

Table X.

Summary—Chemical Composition of Pipped Eggs—Expressed in Grams and in Percent.

| | Chick | | Unabsorbed Yolk | | | | | | | Tissue | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Moist wt. | Dry wt | Moisture | Dry wt | Total N | Crude protein nx6.25 | Ash | Lime (CaO) | $P_2O_5$ | Dry wt | Total N | Crude Protein (nx6.25) | Ash | Lime (CaO) | $P_2O_5$ |
| | gms | gms | gms | gms | gms | gms | gms | gms | gms | gms | gms | gms | gms | gms | gms |
| Dry Incub | 34.192 | 10.247 | 23.943 | 4.5560 | 0.3409 | 2.1304 | 0.1856 | .0590 | .0806 | 5.691 | 0.5270 | 3.2939 | 0.4409 | 0.1338 | 0.1597 |
| Moist " | 36.270 | 10.330 | 25.940 | 4.2799 | 0.3114 | 1.9462 | 0.1759 | .0573 | .0750 | 6.050 | 0.5763 | 3.6019 | 0.4577 | 0.1366 | 0.1717 |
| Hens | 35.986 | 10.365 | 25.621 | 4.3575 | 0.3154 | 1.9714 | 0.1737 | .0580 | .0757 | 6.008 | 0.5694 | 3.5589 | 0.4592 | 0.1346 | 0.1710 |
| Series #1 | 37.286 | 10.804 | 26.482 | 4.6463 | 0.3457 | 2.1604 | 0.1816 | .0577 | .0817 | 6.158 | 0.5880 | 3.6750 | 0.4795 | 0.1424 | 0.1706 |
| Series #2 | 34.551 | 10.042 | 24.509 | 4.1562 | 0.3059 | 1.9120 | 0.1733 | .0566 | .0743 | 5.886 | 0.5533 | 3.4579 | 0.4379 | 0.1328 | 0.1675 |
| Series #3 | 34.610 | 10.096 | 24.514 | 4.3909 | 0.3161 | 1.9756 | 0.1804 | .0600 | .0753 | 5.705 | 0.5315 | 3.3219 | 0.4404 | 0.1316 | 0.1644 |

Percentages (to dry weight)

| | % moisture to moist wt | % | % | % | % | % | % | % | % | % | % |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Dry Incub | 70.031 | 7.483 | 46.760 | 4.074 | 1.294 | 1.769 | 9.260 | 57.879 | 7.747 | 2.351 | 2.806 |
| Moist " | 71.519 | 7.276 | 45.473 | 4.110 | 1.340 | 1.753 | 9.525 | 59.521 | 7.565 | 2.258 | 2.838 |
| Hens | 71.197 | 7.238 | 45.250 | 3.987 | 1.331 | 1.737 | 9.477 | 59.236 | 7.643 | 2.270 | 2.846 |
| Series #1 | 71.240 | 7.440 | 46.497 | 3.909 | 1.242 | 1.758 | 9.548 | 59.678 | 7.786 | 2.312 | 2.770 |
| Series #2 | 70.936 | 7.360 | 46.003 | 4.169 | 1.362 | 1.788 | 9.455 | 58.748 | 7.440 | 2.256 | 2.846 |
| Series #3 | 70.829 | 7.199 | 44.991 | 4.109 | 1.366 | 1.713 | 9.316 | 58.229 | 7.719 | 2.308 | 2.882 |

(a) Seasonal variation.

The undried chicks of the first series were about 2½ grams heavier than those of the other series. The original eggs of this series were approximately 2½ grams heavier than those of the second and third series. Consequently, there must have been, in this case, more food material in the original egg for the use of the chick than was present in the eggs of the other series, as the eggs of all the series were practically uniform in percentage composition. Table X. indicates that the chicks of this series contained a higher percent of total nitrogen to total dry weight and more, in actual weight, of total nitrogen and phosphoric acid. An examination of Table IX. reveals, howevr, that though the chicks were heavier, and did actually contain more total nitrogen and phosphoric acid, they were not so far advanced in development at the pipping stage as those of the second series, if we consider yolk absorption an index of development.

(b) Variations which may be due to methods of incubation.

Before drying, the chicks from the moist incubators averaged a trifle over 2 grams heavier than those from the dry incubators, and slightly heavier than those from the nests, though the original eggs were of about the same weight. The chicks contained nearly 2 grams more moisture than those from the dry incubators and slightly more than those from the nests. Differences between the chicks from the moist incubators and the nests were always small; but a comparison of the chicks from the dry incubation with those from the moist incubators and the nests shows a marked disadvantage for dry incubation. Indeed, the chicks from the dry incubators could usually be selected from among the others, on removal from the shell, because of the larger amount of unabsorbed yolk exposed from the abdomen.

The average dry weight of unabsorbed dry yolk in chicks from the dry incubator was greater than in the other chicks of the experiment, notwithstanding the smaller total dry weight of the former. Variations continued consistently throughout the list of total analyses. The dry incubator chick had more total nitrogen, ash, lime, and phosphoric acid in the unabsorbed yolk, and less of these materials in the tissue, than were found in the other chicks. The percentages of the different materials to total dry weight followed in the same order, showing a less complete utilization of the food materials of the egg, to the stage of pipping, under conditions of dry incubation than in moist incubation or hen hatching. Tables III. and IV. show differences in pipping to correspond with variations noted above, the moist incubator and the hen being about equally successful, and the dry incubator considerably less efficient.

A comparison of differences between embryonic development in the moist incubator and the nest is a more difficult matter. In this experiment, results show a slight advantage for the moist incubator to the pipping stage of embryonic development, but the variations are so slight that they may well be considered as within experimental error.

## Summary and Conclusions.

Do we, then, find physical and chemical differences in the chick to parallel results obtained in incubation by different methods? The following facts are justified by the data, conditions of the experiment being apparently equally favorable to the different methods of incubation.

(1) The eggs in the dry incubator gave a considerably lower percentage of pipping than those in the moist incubator or the nest.

(2) Chicks incubated in the dry incubators were smaller than those incubated in the moist incubator and the nest, and contained a lower percentage of moisture.

(3) Chicks from the dry incubator had a larger amount (and percent) of unabsorbed yolk, and less tissue, than those from the moist incubator or the nest.

(4) Unabsorbed yolks of chicks from the dry incubator contained a higher percent of total nitrogen, ash, lime, and phosphoric acid than those of chicks incubated by other methods.

(5) Tissue of chicks incubated under dry conditions contained a lower percent of total nitrogen, ash, lime, and phosphoric acid than those of chicks incubated under conditions of greater humidity.

(6) Chicks incubated in the moist incubator and those incubated in the nest showed very slight physical and chemical differences. From the above facts it would appear:

(a) That the consistent differences found in actual amounts of the various materials present, and in the percentage composition of the unabsorbed yolk and tissue of chicks which came to the pipping stage under different conditions of humidity in the air immediately surrounding them, would indicate a less complete development at the stage of pipping of chicks incubated under dry conditions than of others which had developed in the more humid atmosphere of the moist incubator and the nest.

(b) That where the percent of incubated eggs which came to the pipping stage was greatest, the apparent development had reached the most advanced stage, as indicated by the highest utilization of the egg materials in the tissue of the chick.

(c) That in this experiment, as regards the number of chicks

brought to the pipping stage and the chemical composition of the chicks there was practically no difference between the moist incubator and the sitting hen.

Further light may be thrown on the subject by Bert Pilkington, Assistant Chemist of the Oregon Agricultural Experiment Station, in his work on separation of fat, protein analysis, and amino acid separation, using portions of the same working samples as were employed in the above total determinations.

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