VOL. 6

**AUGUST**, 1931

# HILGARDIA

# A Journal of Agricultural Science

PUBLISHED BY THE

California Agricultural Experiment Station

# CONTENTS

Distribution of Solid Matter in Thick and Thin Egg White W. F. HOLST AND H. J. ALMQUIST

Measurement of Deterioration in the Stored Hen's Egg W. F. HOLST AND H. J. ALMQUIST

Variability of Shell Porosity in the Hen's Egg H. J. ALMQUIST AND W. F. HOLST

UNIVERSITY OF CALIFORNIA PRINTING OFFICE BERKELEY, CALIFORNIA The titles of the Technical Papers of the California Agricultural Experiment Station, Nos. 1 to 20, which HILGARDIA replaces, and copies of which may be had on application to the Publication Secretary, Agricultural Experiment Station, Berkeley, are as follows:

- Effect of Sodium Chlorid and Calcium Chlorid upon the Growth and Composition of Young Orange Trees, by H. S. Reed and A. R. C. Haas. April, 1923.
- Citrus Blast and Black Pit, by H. S. Fawcett, W. T. Horne, and A. F. Camp. May, 1923.
- 6. A Study of Deciduous Fruit Tree Rootstocks with Special Reference to Their Identification, by Myer J. Heppner. June, 1923.
- 7. A Study of the Darkening of Apple Tissue, by E. L. Overholser and W. V. Cruess. June, 1923.
- Effect of Salts on the Intake of Inorganic Elements and on the Buffer System of the Plant, by D. E. Hoagland and J. O. Martin. July, 1923.
- Experiments on the Reclamation of Alkali Soils by Leaching with Water and Gypsum, by P. L. Hibbard. August, 1923.
- The Seasonal Variation of the Soil Moisture in a Walnut Grove in Relation to Hygroscopic Coefficient, by L. D. Batchelor and H. S. Reed. September, 1923.
- 11. Studies on the Effects of Sodium, Potassium, and Calcium on Young Orange Trees, by H. S. Beed and A. B. O. Haas. October, 1923.
- 12. The Effect of the Plant on the Reaction of the Culture Solution, by D. E. Hoagland. November, 1923.
- 14. The Respiration of Potato Tubers in Relation to the Occurrence of Blackheart, by J. P. Bennett and E. T. Bartholomew. January, 1924.
- The Moisture Equivalent as Influenced by the Amount of Soil Used in its Determination, by F. J. Veihmeyer, O. W. Israelsen and J. P. Conrad. September, 1924.
- Nutrient and Toxic Effects of Certain Ions on Citrus and Walnut Trees with Especial Reference to the Concentration and Ph of the Medium, by H. S. Reed and A. R. C. Haas. October, 1924.
- Factors Influencing the Rate of Germination of Seed of Asparagus Officinalis, by H. A. Borthwick. March, 1925.
- 19. The Relation of the Subcutaneous Administration of Living Bacterium abortum to the Immunity and Carrier Problem of Bovine Infectious Abortion, by George H. Hart and Jacob Traum. April, 1925.
- 20. A Study of the Conductive Tissues in Shoots of the Bartlett Pear and the Relationship of Food Movement to Dominance of the Apical Buds, by Frank E. Gardner. April, 1925.

# VARIABILITY OF SHELL POROSITY IN THE HEN'S EGG<sup>1</sup>

H. J. ALMQUIST<sup>2</sup> AND W. F. HOLST<sup>3</sup>

# INTRODUCTION

In connection with the formation of the egg in the domestic hen, Surface (1912) state that the uterus, which is the shell-forming part of the oviduct, possesses at least two kinds of glands, the function of which is to furnish shell-forming material. From one kind calcerous matter is secreted, from the other mucus. The result is a shell which, according to Lillie (1919), consists of three layers, the mammillary layer, the intermediate spongy layer, and the surface cuticle. This heterogeneous envelope is permeable to gases. Lillie explains this characteristic on the basis of a supposed 'network' of pores in the spongy layer, connecting the conical inner ends of the mammillae with pores of the cuticle. This conception of shell porosity no doubt originated with Landois (1865).

The application of the term 'spongy' to the intermediate layer of the egg shell was due to an entirely faulty and misleading experimental procedure. Egg shells were treated with dilute mineral acids. As a matter of course small bubbles of gas, carbon dioxide, appeared scattered all over the exposed shell surface. These bubbles, however, were wrongly interpreted to indicate cavities in the shell and were further assumed to be interconnected by a network of fine channels. Thus, unfortunately, the name 'spongy layer' was introduced into ornithological terminology. Clevisch (1913), in the course of his much more thorough studies of the subject, found the intermediate layer to represent calcium carbonate crystals, densely knitted together by what appeared to be albuminous material. While observing no evidence of a porous network in the intermediate layer he did find

<sup>&</sup>lt;sup>1</sup> Contribution No. 12 from the Division of Poultry Husbandry, Agricultural Experiment Station, University of California.

<sup>&</sup>lt;sup>2</sup> H. J. Almquist, Research Assistant in Poultry Husbandry.

<sup>&</sup>lt;sup>3</sup> W. F. Holst, Assistant Professor of Poultry Husbandry, and Associate Poultry Husbandman in the Experiment Station.

it to be penetrated by small, definitely tube-like passageways. These passageways or pores were closed at the outer ends by the cuticle which appeared as a nonporous, structureless deposit or seal of a thin and delicate character. It could easily be assumed that a number of outside influences might partly or wholly remove this deposit, thereby exposing the pores.

Investigations by Rizzo (1899) showed that the number of channels in the shells of hen's eggs is very large, varying in the neighborhood of seven thousand per egg. His experimental method was such as to detect all pores, whether originally closed or not. Rizzo used a dye solution, with which he filled the empty egg shell. He then sealed and warmed it. The duration of these experiments, together with the elevated temperature and the pressure developed by expansion of the filling fluid on warming would be expected to cause penetration by the dye at all possible points. Thus the results of Rizzo would represent a uniform upper limit of porosity which might be termed the total or 'potential porosity.' It is apparent from his work, however, that at a somewhat smaller number of points the dye solution used penetrated without assistance to the exterior of the shell, indicating that some of the channels were partially or totally unobstructed at the time of examination. The actual porosity was, therefore, somewhat less than the total or potential porosity found.

On the reasonable supposition that the calcareous portion of the shell is traversed by a large number of these small channels, many of which may be obstructed by organic matter such as the cuticle, it appears that porosity may be a variable, *even in a given individual egg*, depending upon the natural characteristics and treatment of the egg. Thus, if it be recognized that porosity may increase, the limiting value approached may be expressed as the 'potential porosity,' that is, the condition found by Rizzo.

Storage of the egg in air may be expected to lead to drying and shrinking of the external seal, the cuticle, in this way partially opening the already existing and numerous channels. From this physical conception of porosity it will readily be seen that porosity variation of at least three types may result; namely, variation with time, variation with treatment, and variation which may be expected to exist between individual eggs.

In discussing egg-shell porosity, therefore, it is essential to give a definition of the term 'porosity,' although of necessity it will be an arbitrary definition. The term, as used in this publication, is meant to characterize the condition of the egg shell with regard to the number and distribution of those small channels or pores which, at the time of inspection, offer free passageway to water vapor, air, and carbon dioxide.

The present study, dealing with porosity conditions of hens' eggs investigated under what is believed to be a somewhat original viewpoint, should be of considerable fundamental importance. It is hoped that the information obtained may aid the study of egg quality. This is because porosity—or lack of porosity—must be assumed to be one of the important factors bearing on commercial egg quality and particularly on the preservation of eggs. Of course, other factors, in the egg itself or connected with the condition under which the egg is being stored, must also be assumed to be of significance.

## METHODS OF MEASURING SHELL POROSITY

Several methods of measuring egg-shell porosity described in the existing literature were tried out but were later discarded. It is undesirable to apply to the shell any method involving an increase or decrease of pressure, because there is great likelihood of altering the existing porosity by dislodging the pore-filling material. For this reason the method which consists of placing the shell under water in a closed vessel and counting the bubble streams as pressure is reduced is open to objection. It is also unsuitable because of the difficulties in counting the bubble streams and the lack of a permanent record in the shell itself after the test has been made. The use of water or of aqueous solutions is in itself questionable since the effect of water in swelling the mucus in the pores, and later perhaps dissolving it, cannot be determined. Attempts were made to measure the rate of diffusion of gases through the empty shell. The results were inconsistent, largely owing, it was found, to the variation in the degree of dryness of the shell, a variation which can not easily be controlled.

Rizzo's use of a dye solution had great advantages, although his method of application was not satisfactory for the purposes of this work. The method described by Weston and Halnan (1927) of first painting the outside of the shell with a starch solution and later the inside of the shell with a solution of iodine represented an improvement over Rizzo's procedure. The method finally developed and adopted for routine observations in this laboratory, however, consisted of immersing the egg for 2 minutes in a solution of methylene blue in 95 per cent alcohol (3 grams per litre). After immersion the egg is allowed to dry, which requires but a short time. The shell

is then carefully split into halves and the contents are poured out. If the inside of the shell is wiped dry as soon as the egg contents have been removed, a clear and permanent picture of the porosity of the shell remains. An additional immersion of 3 minutes does not bring out more pores, but the spots where penetration has already taken place become enlarged because of diffusion of the dye in the shell membrane.



Fig. 1. Shell-porosity standards: the insides of shells adopted as standards of comparison. The numbers 1 to 9 represent increasing shell porosity as shown by the increasing number of small spots on the interior of the shells. Each small spot indicates an open pore. In each porosity class are included a large and a small shell, both halves of each shell being shown. Air-space ends are placed on the left.

In order to classify the shells examined for porosity in this manner a set of standards, covering the range of porosity encountered in this work, was selected and given arbitrary numbers. Later determinations of porosity were compared with these standards and assigned the corresponding numbers. The standards are reproduced in figure 1. The main advantages of the method are speed, permanence of record, and availability of the egg contents for further inspection. As justification for this method of estimating porosity the following considerations may be advanced:

1. The protein materials which comprise the nonmineral substance of eggs are not soluble in alcohol of this concentration (95 per cent). The mechanism by which the dye penetrates the shell is therefore not one of dissolving the protein-like, pore-filling substance.

2. The alcohol might exert a dehydrating and coagulating action on the cuticle. This would produce an effect similar to ordinary drying and shrinking in reducing the effectiveness of this pore-sealing material. However, it does not seem that this type of action could be effective in the short period required for the test; furthermore, if such action were effective, then, in view of the fact that the potential porosity of all normal egg shells is a fairly uniform and high value, all eggs tested would be expected to show a much greater and more uniform response to the treatment than is actually found to be the case. The variations of porosity and the differences in distribution, as presented later in this article, would be either much less prominent or nonexistent.

3. Observations which have been made by partially submerging emptied half shells in the dye solution have shown that penetration begins at once and that the spots produced merely enlarge during the remainder of the test period.

4. Finally, the relation which the measured porosity has shown to the rate at which eggs lose weight seems to justify confidence in the idea that this method of estimating shell porosity is based upon a fundamental property of the shell, namely, the effectiveness of the shell channels as free pores.

The loss of weight in stored eggs is undoubtedly controlled by the shell porosity. To check the method just described the relation between weight loss and estimated porosity was examined in a number of eggs.

In table 1 are summarized data secured under the direction of the authors.

Eggs were stored 5 to 6 days at 86° F and at constant humidity. Loss of weight was determined by accurate weighings and expressed as percentage of fresh weight lost per egg per day. The porosity numbers were assigned as described previously in this article. The results of the two determinations were collected for some time in an independent manner, then brought together, calculated to a common basis, and compared.

	Porosity numbers*									
	1	2	3	4	5	6	7	8	9	
Total number of eggs of each porosity rating	1	3	16	20	22	18	16	15	11	
Average per cent of weight lost per egg per day	0 212	0.442	0 502	0.532	0 573	0.603	0.691	0.917	1.047	

TABLE 1	
THE RELATION OF SHELL-POROSITY NUMBER TO THE MEAS	SUREI
LOSS OF WEIGHT IN HEN'S EGGS	

\* See figure 1 for sl ell-porosity standards.

The table clearly illustrates a parallel trend in assigned porosity numbers and measured loss of weight. If the loss of weight is plotted against porosity number (fig. 2), it will be seen that a practically linear relation exists over the range 2 to 7. The loss of weight increases sharply with the porosity numbers 7, 8, and 9, indicating that these notations represent greater increments in porosity than do the preceding ones. Perhaps this range should be subdivided into a somewhat greater number of comparative standards to achieve greater consistency with the lower porosity numbers.



Fig. 2. The agreement between the assigned shell porosity numbers and the measured rate of losing weight in 122 hens' eggs at 86° F.

It cannot be claimed for this method of evaluating porosity that an absolute measure is achieved. Although tests may be conducted in a uniform manner the final ratings depend upon personal judgment. Human errors may be reduced by working with larger numbers of eggs and in a random manner so that preconceived ideas may not prevail in classifying a particular egg or the egg from a particular hen. As far as possible this was done. It is a matter of practical impossibility to make an actual count of pores in a large number of egg shells. The method of classification just described sacrifices accuracy for speed in the hope that, on the average and with large numbers of eggs, results may still be significant.

The procedure used gives an approximate quantitative measure of variability in shell porosity. It has also shown certain changes in shell porosity with time and temperature not previously reported.

### RESULTS

Eggs from a group of more than 50 hens were examined. Included in this group were eggs showing wide differences with respect to shell characteristics, such as thickness and smoothness. In all more than 500 shells were examined for porosity. At the same time data were taken regarding shrinkage and keeping qualities. The latter work is still in progress.

Fresh Eggs.—The examination of fresh eggs demonstrated a striking variability in porosity in eggs from different birds. Eggs from the same bird were usually found to be fairly uniform in this respect.

Statements in the literature create the impression that shell porosity is greater or that pores are larger at the air space (Rizzo, 1899; Dunn, 1923; and Swenson and Mottern, 1930), but observations made in this laboratory on eggs one day or less in age indicated that this condition is far from being general. There is no apparent fundamental reason for the existence of greater porosity at air spaces in fresh eggs, but this may be expected in older eggs, for, at the air space, the shell is not in contact with watery material but is bounded on both sides by gases. This probably leads to a faster drying of the shell and of the pore-filling material and a corresponding faster opening up of the pores in this part of the egg.

Figure 3, A and B, represents shells in which very little porosity was found at the air space. The position of the air space is indicated by the circle. Both halves of the shell are shown, the air space ends being placed on the left in these and in all other cases.

Figure 3C illustrates some of the few cases in which porosity was found to be distinctly greater at the air-space end. It will be noted that the great degree of porosity is not necessarily confined to the shell portion forming the air space.



Fig. 3. Abnormal distributions of porosity in shells of fresh eggs. Both halves of each shell are shown. The air spaces occupied the regions indicated by the circles.

The standards (fig. 1) illustrate the type of porosity most commonly found in fresh shells. About 80 per cent of the shells examined showed a uniformly distributed porosity of this nature. The variability of this porosity in fresh eggs is indicated by the standards. All degrees of porosity represented by these figures were found in fresh shells, the condition most frequently met, however, being represented by porosity numbers 4 and 5.

Stored Eggs.—In order to note possible changes of shell porosity during storage, eggs were stored at different temperatures—at room temperature (about  $68^{\circ}$  F), at  $86^{\circ}$  F, and at  $102^{\circ}$  F. At the last two

temperatures the humidity was maintained at about 75 per cent. Examination of the egg shells after storage revealed certain significant facts.



Fig. 4. Increases in porosity found at room conditions over a twenty-five day period. Egg age increases in five-day increments from top to bottom of figure. Air-space halves are placed on the left. All shells in a are from one hen, all shells in b from another.

While heretofore porosity has been considered a rather fixed characteristic of the shell, it was apparent that porosity is not necessarily constant, that it may increase with the age of the egg, and more rapidly with higher storage temperature. A few exceptions to these findings resulted when eggs from the same hen started at a rather high initial prorosity and maintained this at about the same level over a period of 25 days, when stored at room conditions.

The increases in porosity as observed may partly explain the abnormally poor keeping quality of eggs which have been removed from storage. In particular eggs stored under conditions of low humidity would probably show an increase in porosity which would greatly favor their deterioration upon removal from storage.

In figure 4 are shown two series of shells, each series from an individual bird, which illustrate porosity increases encountered in eggs stored at room conditions over a period of 25 days. The shells are 5 days apart in storage age.



Fig. 5. Distribution of porosity in fresh eggs and eggs stored for six days at 86° F and at 102° F.

It is possible that the apparent increases in shell porosity with age may be due to differences in initial porosity. The changes noted, however, were found to be general, and for the most part the porosity in the older shells is far greater than the average initial porosity of eggs from the same hen. A more uniform distribution of the pores in the shell is invariably associated with an increase in degree of porosity.

In order to express the effects of storage on porosity at the various temperatures quantitatively, the results of an investigation have been shown graphically in figure 5. In this figure, one curve represents the distribution of porosity in 72 fresh shells, one day or less in age, which came from a group of 12 hens used in this work. Six eggs were used from each bird. A second curve shows the distribution of porosity found in an equal total number of shells and with the same individual number from the same birds, after the eggs had been stored for 6 days at 86° F. The third curve shows results from inspection of random eggs, largely from the same hens, after 6 or 7 days at 102° F. The first and second curves are entirely comparable. The shift of the most probable porosity toward a higher value after 6 days' storage is significant of a tendency toward an increase in porosity. It is also noteworthy that the porosity of eggs at incubation temperature (102° F) tends to reach a still higher value in a similar period of time.

## SUMMARY

A new method for the study of egg-shell porosity has been suggested.

The shell porosity in fresh eggs, i. e., the initial porosity, with but few exceptions, has been found to be low.

Egg shells are subject to changes in porosity when the eggs are stored. Shell porosity may increase with duration of storage, more rapidly at higher temperatures, and approach a maximum which is nearly uniform for all eggs with regard to degree and distribution.

Egg-shell porosity appears to be nearly uniform for the eggs of a particular hen, but shows differences for different individuals.

Porosity in fresh egg shells is rather uniformly distributed. It is not generally greater in the air-space region of the egg.

## LITERATURE CITED

#### CLEVISCH, A.

1913. Beiträge zur Struktur und Physiologie der Vogeleischalen. Aus dem zoologischen Institut der Universität Bonn. 48 p. Hannover, Germany.

#### DUNN, L. C.

1923. The variation of eggs in the rate at which they lose weight. Poultry Science 2 (6):199-204.

#### LANDOIS, A.

1865. Die Eierschalen der Vogel in histologischer Beziehung. Zeit. f. Wissen. Zool. 15:1-31.

#### LILLIE, F. R.

1919. The development of the chick. 2d ed. 463 p. Henry Holt and Co., New York.

### Rizzo, A.

1899. Sul numero e sulla distributione dei pori nel guscio dell'ovo di gallina. Ricerchie del Anat. Lab. Roma. 7:171-199.

SURFACE, F. M.

- 1912. Histology of the oviduct of the hen. Maine Agr. Exp. Sta. Bul. 206: 395-430.
- SWENSON, T. L., and H. H. MOTTERN.

1930. The oil absorption of shell eggs. Science 72(1856):98.

WESTON, W. A., R. D., and E. T. HALNAN.

1927. "Black spot" of eggs. Poultry Science 6(16):251-258.