

corn starch and baker's yeast are readily agglutinated by A and that boiled starch is precipitated by A. Glycogen is very completely precipitated at neutrality. As little as 0.01 mg of glycogen in 5 cc of solution eventually forms a perceptible haziness after adding a few mg of concanavalin A. At first it appeared likely that the agglutination of erythrocytes might be due to a reaction between concanavalin A and the glycogen present. Cow erythrocytes are not agglutinated by A, but after adding traces of glycogen, A agglutinates them readily. However, agglutinatable red cells, such as those of the horse, dog and cat, appear to contain too little glycogen to permit this hypothesis to be accepted. Furthermore, the agglutination of such cells is not prevented by previous incubation with salivary or pancreatic amylase, as would be expected if glycogen were the substance reacting.

We have found that the erythrocytes of the horse, dog and cat, after laking, give a fairly heavy precipitate with concanavalin A, whilst laked cow, goat, sheep and human erythrocytes, which are not agglutinatable, or which are agglutinated with difficulty by A, give no such precipitate. Evidently the erythrocytes which agglutinate do so because of the production of this precipitate. We have not yet succeeded in isolating the substance which forms the precipitate. It is thermolabile and appears to be a protein, but is not hemoglobin.

Joos³ has claimed that the agglutination of bacteria by immune serum is dependent upon the production of a chemical compound, and we believe that the agglutination of erythrocytes is caused by the formation of an insoluble compound composed of the unknown substance, or substances, and concanavalin A. The unknown substance can not be said to exist in the dissolved state; nevertheless we consider it to be an hydrophyllie colloid. Upon addition of concanavalin A, chemical combination occurs and a hydrophobic compound is formed. This hydrophobic compound offers no impediment to agglutination if salt is present. The red cells of the cow do not contain a substance capable of combining with concanavalin A, but are assumed to contain a hydrophyllie colloid which prevents spontaneous agglutination. When glycogen is added to cow cells, it is adsorbed upon their surfaces and subsequent addition of A forms a hydrophobic compound. If amylase is added to cow cells thus agglutinated, the glycogen is rapidly digested and the cells can be partly resuspended by shaking.

The most difficult point requiring explanation is why hydrophyllie substances present in the surfaces of erythrocytes prevent spontaneous agglutination. We assume that such hydrophyllie compounds attract films

of water which act as envelopes and thereby prevent neighboring cells from making contact.

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CHANGES IN THE POSITION OF CHICK EMBRYOS AFTER THE EIGHTEENTH DAY OF INCUBATION¹

It has been demonstrated repeatedly that many chick embryos dying on the eighteenth day of incubation or later, and examined generally on the twenty-second day, are not in the normal position for hatching. New evidence indicates that there is a decided change in the position of the chick embryo after the eighteenth day of incubation and that certain of the previously designated malpositions found in chick embryos are but a natural occurrence in normal development.

Extensive studies have been made by various investigators on the frequency of these so-called embryonic malpositions in the egg of the domestic fowl. In general embryos dying on or after the eighteenth day of incubation have been grouped together, according to their respective positions. Such a classification assumes that no profound change would take place in the position of the embryo after the eighteenth day of incubation. That such is not a correct assumption will be pointed out in this study.

The eggs examined in the present investigation were from a group of inbred single comb White Leghorns with an inbreeding coefficient ranging from 25 to 78 per cent., non-inbred White Leghorns and from a White Leghorn and Light Brahma cross. A total of 1,011 live embryos were available for examination. The eggs were divided into two lots and incubated at two different periods. The seven positions studied were classified as follows: I, normal hatching position; II, head between thighs; III, head in small end of egg; IV, head turned to left; V, normal but beak away from air cell; VI, feet over head, and VII, normal but head above the wing.

In the first phase of this study 51 live embryos were examined on the nineteenth day of incubation and only 7.8 per cent. of these were in the normal hatching position. More were in position VII, which is normal, except that the head is above the wing instead of under it. Fifty per cent. of the embryos were in either position II, III or IV. On the twentieth day of incubation 50.1 per cent. were in the normal hatching position, while 32.9 per cent. were in position VII. Only 16.1

³ A. Joos, *Zeit. für Hygiene*, 36: 422, 1901.

¹ Journal Paper No. J-192 of the Iowa Agricultural Experiment Station, Ames, Iowa. Project No. 54.

per cent. were in either position II, III or IV. A χ^2 test for significance shows that the difference in the results, including all positions between the nineteenth and the twentieth day of incubation, is highly significant. It is evident that there is a decided shifting of embryonic position after the nineteenth day of incubation.

An examination of all embryos dying after the eighteenth day of incubation and examined on the twenty-second day of incubation shows that most of the so-called embryonic malpositions occur from the eighteenth to the twentieth day of incubation, with the exception of position VII. None of the dead embryos in this group were in the normal hatching position until the twentieth day of incubation. Also few of the embryos were in malpositions on the twenty-first day of incubation.

The data of the second phase of this study substantiate the examinations made in the first phase. There is a very decided change in embryonic position from the eighteenth to the twentieth day of incubation. An examination of 117 live embryos on the eighteenth day of incubation failed to show any individuals which could be classified in the normal hatching position. An examination of 100 live embryos on the nineteenth day showed 25 embryos in the normal hatching position. Thus there is a very decided increase of embryos found in the normal hatching position on the nineteenth day of incubation and a corresponding decrease in the number of embryos found in the so-called malpositions. On the twentieth day there is a marked change in embryonic position from that found on the eighteenth and nineteenth days, for 76 live embryos out of 103 examined were in the normal hatching position.

The results obtained from these embryonic examinations present a new problem on the concept of embryonic malpositions. Previous available evidence would indicate that embryonic malpositions are the immediate cause of death of the embryo, particularly after the eighteenth day of incubation. It is a well-known fact that embryonic mortality is generally the highest during the eighteenth and nineteenth day of incubation. Seemingly, then, malpositions might be one of the reasons for this high eighteenth and nineteenth day embryonic mortality. If, however, malpositions cause the death of the embryo very few of the eggs used in this study would have hatched. The position most prevalent on the eighteenth and nineteenth day of incubation is that of the head between the thighs, which has been designated as position II in this study. It is obvious that if the embryo persists in this position up to the twenty-first day the egg can not hatch.

The evidence presented is conclusive that embryonic changes take place within the egg after the eighteenth day of incubation and that certain of the so-called malpositions are but a natural occurrence in the normal development of the chick embryo. If embryonic death occurs during the approximate eighteenth day period the embryo will probably not be in the normal hatching position. It is very probable that certain of the previously designated embryonic malpositions are not necessarily lethal, but that death is caused by some other agency and the embryo is in its normal position for its particular stage of development.

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MATERNAL BEHAVIOR IN MALE RATS¹

MATERNAL behavior has recently been described² in *virgin* rats and attributed to the lactogenic hormone of the anterior pituitary. This paper proposes to show that maternal behavior can be induced experimentally in *male* rats and that this can occur in the absence of mammary development.

Chronic administration of bovine anterior pituitary implants (Table I), or complete thyroidectomy without treatment (Table II), induced adult male rats to

TABLE I
EFFECT OF CHRONIC ADMINISTRATION (60 DAYS) OF
BOVINE ANTERIOR PITUITARY IMPLANTS ON
ADULT MALE RATS (5 MOS. OLD)

No. rats	No. implants	Total mg tissue implanted	Weight in mg				Maternal behavior
			Hyp.	2 Adr.	2 Thyr.	2 Testes	
10	14	7,100	14	103	36	2,825	Yes
10*	9	36	43	3,171	No

* Controls.

behave differently from their normal brothers and virgin sisters, as evidenced by the following actions: (1) making a nest for young rats put in their cages, and lying over them for hours³; (2) nest-building and nesting even in absence of young; (3) settling down

¹ From a dissertation submitted in partial fulfilment for the degree of doctor of philosophy in anatomy, University of California.

² B. P. Wiesner and N. M. Sheard, "Maternal Behaviour in the Rat," London, 1933. O. Riddle, E. L. Lahr and R. W. Bates, *Proc. Soc. Exp. Biol. Med.*, 32: 730, 1935.

³ The young suck the skin and hairs of the abdomen, and are permitted to hang on the hairs when the foster parent arises.