allied chemical constitution are heated together in sealed tubes with an excess of either phosphorus pentachloride or pentoxide, a series of colloidal substances are formed which, when freed from the contaminating phosphoric acid, and dissolved in concentrated ammonia, give opalescent solutions that, on evaporating down *in vacuo*, yield substances closely resembling in physical, chemical, and physiological properties certain proteids.

These colloidal substances, although they differ from one another in minor details, are usually distinguished by the following characteristics :—

1. They are soluble in warm water, forming opalescent lævorotatory solutions.

2. The resulting solutions yield the principal colour reactions hitherto deemed diagnostic of proteids.

3. In the absence of salts, solutions of these colloids do not coagulate on heating. In the presence of a trace of a neutral salt they coagulate on heating at temperatures very similar to proteid solutions.

4. Fractional heat-coagulation shows the colloidal solutions are a mixture of different substances.

5. The different constituents of the colloidal solution exhibit different physiological action.

6. In the presence of an excess of neutral salts, or of salts of the heavy metals, the colloidal solutions behave in a manner similar to proteid solutions.

7. When introduced into the circulation of pigmented rabbits, dogs, and cats, certain of these substances (viz., the colloids designated A, B, C, α and β) produce intravascular coagulation of the blood in a manner similar to a nucleo-proteid. They also hasten the coagulability of the blood withdrawn from the carotid, and will, when slowly injected intravenously in minute quantities into dogs, produce a retardation of the coagulability of the intravascular blood, *e.g.*, a "negative phase."

8. Apparently these colloidal substances are, owing to both their physical and chemical properties and their physiological behaviour, the nearest synthesised bodies at present known to proteids.

"An Experimental Examination into the Growth of the Blastoderm of the Chick." By Richard Assheton, M.A. Communicated by Adam Sedgwick, F.R.S. Received November 12,—Read December 10, 1896.

In making an experimental study of the growth of the blastoderm of the chick, I had two chief objects in view :

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(1) To test by actual experiment Duval's* theory of the formation of the primitive streak.

(2) To try and determine experimentally whether the whole or only part of the actual embryo is developed by the activity of the primitive streak. And further, if only a part, to determine its limits.

With regard to the first question it may be remarked that Duval's account is generally accepted, although perhaps greater stress is laid upon it by foreign and American writers than by embryologists in this country.

According to Duval's account, there is in the freshly laid and unincubated egg a groove which separates the blastoderm from the yolk. The groove, he says, is broader and more conspicuous at the posterior margin than at any other point. This he compares to the anus of Rusconi or blastopore of the segmenting frog's egg.

During the first few hours of incubation the edge of the blastoderm is said to advance over the yolk at every point except at this most posterior margin bounding the groove, which he regards as equivalent to the frog's blastopore. At this spot there is no advance. The portions of the edge of the blastoderm adjoining this part swing round to meet each other in the middle line, and eventually fuse and form what Duval calls the "plaque axiale."

This structure is in reality the primitive streak, and, according to Duval, it becomes visible as such during about the tenth to fifteenth hours of incubation by reason of the subsequent hollowing out of the subjacent yolk by the extension backwards of the sub-germinal cavity.

Such a mode of growth would be very extraordinary and interesting if true, and would be very acceptable to those who believe that the growth in length of the vertebrate embryo is caused by a concrescence of two at first separated germinal rims.

Naturally this account of the formation of the primitive streak as given by Duval is frequently quoted by the many adherents to the concrescence theory.

During the last few years experimental methods have been introduced much more freely into investigations of animal development. Foremost amongst the workers upon these lines is Dr. Wilhelm Roux, who experimented by destroying certain cells of the segmenting eggs of frogs, and noting the result after some days of development. He has been followed in similar work by Morgan and Ume Tsuda and others.

The eggs of frogs have been the object of experiment of a different

* "De la Formation du Blastoderme dans l'Œuf d'Oiseau," 'Annales des Sciences Naturelles, Zoologie,' vol. 18.

kind, such as that of Professor Oscar Hertwig, who studied the various monstrosities obtained by mechanical compressions, by supermaturation of the ovum, and addition of various salts to water in which the eggs were developing. Similar work has been done upon sea-urchin's eggs by several biologists (Pouchet and Chabry, Herbst, &c.).

There are other most valuable records of the results obtained by separating the several spheres of the early stages of segmentation of eggs of Ctenophores, Echinoderms and Amphioxus by Chun, Driesch, Wilson, and others.

Kastschencko, by injuring portions of the germ ring of Elasmobranch embryos, has produced very valuable evidence in connexion with the concrescence theory, and Morgan has by similar methods examined the development of Teleosteans.

As far as I know, an experimental study of the development of the avian blastoderm has not hitherto been made.

The method adopted, which is very simple, was as follows. The egg was first of all opened at one side, and a bristle inserted into the yolk at some distance away from the blastoderm, to mark its anterior and posterior axis.

The yolk, with its surrounding albumen, was then turned out into a glass vessel having a rather greater capacity than that of an ordinary egg shell.

The yolk was arranged so that the blastoderm floated uppermost, and a wire or celluloid ring was placed over it to prevent the yolk from floating to the surface.

A fine sable hair was then inserted in the blastoderm, and its position measured by a micrometer eye-piece and recorded in tenths of a millimetre. The vessel was filled up with albumen and covered with a glass lid, and placed in the incubator at a temperature of 104° F.

Under these conditions, although development was slower than under normal conditions, many embryos reached, after about forty-eight hours, an age equivalent to a normal thirty to thirty-six hours' chick with nine or ten pairs of mesoblastic somites.

To come now to the results of the experiments, it is clear that if Duval's theory is correct a hair inserted in the area opaca at the point a (fig. A (i)) ought to appear, in a specimen in which the primitive streak is formed, somewhere in front of the primitive streak. It, however, does not; it appears in the area opaca behind the primitive streak at a, fig. A (ii).

So again if the primitive streak is formed by the concrescence of the posterior margin, the sables inserted at the posterior edge at XX should either appear in the primitive streak or else prevent its formation.

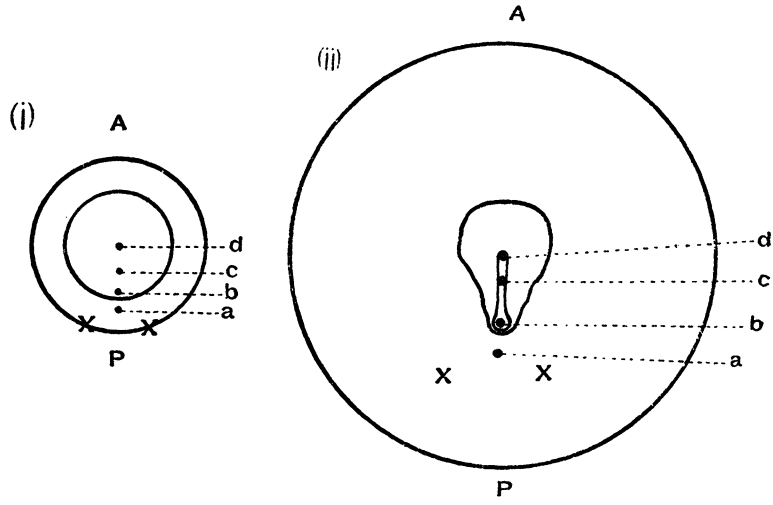

FIG. A.—(i) Diagram of the unincubated Blastoderm of a Bird. (ii) Diagram of the Blastoderm after the complete Formation of the Primitive Streak.

On the contrary, they are found far behind the primitive streak in the area opaca.

These facts seem to show that the primitive streak is not formed from the posterior edge of the blastoderm as Duval maintains.

As a rule, in the unincubated blastoderm the area opaca and area pellucida are very fairly well defined.

If, when this is the case, a sable hair is inserted just within the area pellucida at the point b, or if, when there is no such distinction, the sable is inserted about one quarter the distance from P to A, the sable hair is found, after the development of the primitive streak, piercing the posterior end of the primitive streak—whereas, according to Duval's account, it ought to be somewhere in front of the primitive streak.

If a hair is inserted in the median line rather further towards the centre of the blastoderm, it is found near the middle of the primitive streak, or, if placed about half way between the inner edge of the posterior part of the area opaca and the centre of the blastoderm (as at c), it is found in the anterior part of the primitive streak; and, when the sable is inserted at the centre of the blastoderm, it appears at the front end, or just in front of the primitive streak (fig. A, d).

The foregoing proves, I think, conclusively, that the primitive streak is developed from that portion of the unincubated blastoderm which lies between the centre of the blastoderm and the posterior

margin of the area pellucida. The area opaca takes no part at all in the formation thereof.

I may add that from a careful examination of surface views of living and preserved specimens, and from sections, I find it just as difficult to corroborate Duval's account of the formation of the primitive streak as I do from the experimental study I have just described.

I come now to the second part of the inquiry; namely, what part of the actual embryo does the primitive streak give rise to?

A sable hair inserted at the centre of the blastoderm appears at the anterior end of the primitive streak.

If such a specimen is allowed to develop for some hours longer, until the medullary plate and medullary groove are clearly formed, these structures are found to be in front of the sable hair; that is to say, the sable hair is still at the front end of the primitive streak (fig. B). If a specimen, in which the sable hair has been inserted at the same spot—that is to say, at the centre of the unincubated blastoderm—is left until several pairs of mesoblastic somites have appeared, the hair is found at the level of the most anterior pair of somites (fig. B (iii)).

From these specimens it seems clear that all those parts in front of the first pair of mesoblastic somites (that is to say, the heart, the brain, and medulla oblongata, the olfactory, optic and auditory

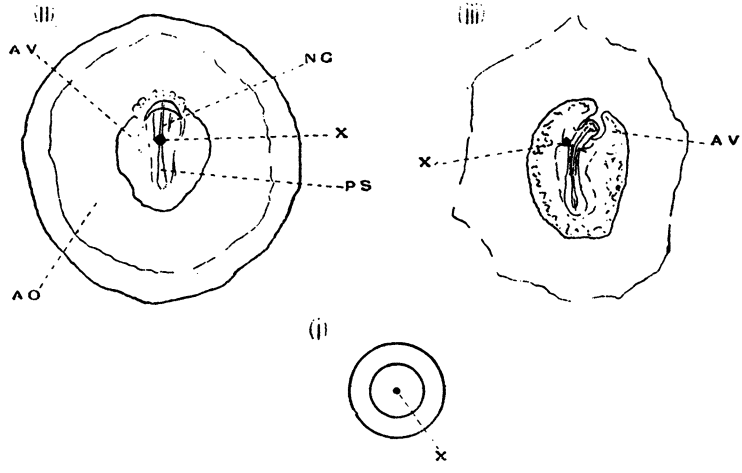

FIG. B.—(i) Diagram of unincubated Blastoderm.
(ii) Blastoderm after 24 hours' incubation.
(iii) Blastoderm after 40 hours' incubation.

AO, area opaca; AV, area vasculosa; NG, medullary groove; PS, primitive streak; X, point of insertion of sable hair.

organs and fore gut) are developed from that portion of the unincubated blastoderm which lies anterior to the centre of the blastoderm, and that all the rest of the embryo is formed by the activity of the primitive streak area.

I have found it very difficult to determine, exactly, the anterior limits of the embryo in the unincubated blastoderm. This, no doubt, is due to the fact that, for the production of the anterior end of the embryo, very complicated foldings of the blastoderm are called into play, and the insertion of a bristle or the infliction of any injury to the delicate parts of the blastoderm involved in the process, almost entirely prevents anything like a normal course of development.

However, such little success as I have had gives the following results:—A hair inserted at the most anterior border of the area pellucida is found far in front of the primitive streak.

A hair inserted only slightly in front of the centre of the blastoderm appears (in a specimen in which the medullary folds are just becoming visible) in the medullary plate in front of the primitive streak. In older specimens, after the head-fold has been formed, the embryos are extremely abnormal when the sable has been inserted in the region under discussion.

Indeed, very few will develop as far as the formation of the head-fold.

The only facts I can derive from the insertion of sable hairs in this area are:—

(1) That it interferes very seriously with the course of development.

(2) That the bristle appears inside the two anterior horns of the area vasculosa.

(3) That if placed some little way anterior to the centre it is found apparently in front of the embryo, but it interferes so greatly with the head-fold that it is difficult to say whether it has, or has not, perforated the anterior part of the embryo.

I have shown that a hair inserted between the centre of the blastoderm and the hinder margin of the area pellucida is found after about twenty hours of incubation in the primitive streak. When a specimen in which the sable has been similarly placed is allowed to develop until several mesoblastic somites have been formed, it is found to be posterior to the first formed mesoblastic somites.

For instance, in the specimen with me, the blastoderm measured 4·3 mm. in diameter. The sable was inserted 1·3 mm. from the posterior edge of the blastoderm. After forty-one hours of incubation seven pairs of mesoblastic somites had been formed, and the sable hair was a short distance posterior to the 7th pair of somites.

From such specimens as these we are, I think, bound to conclude

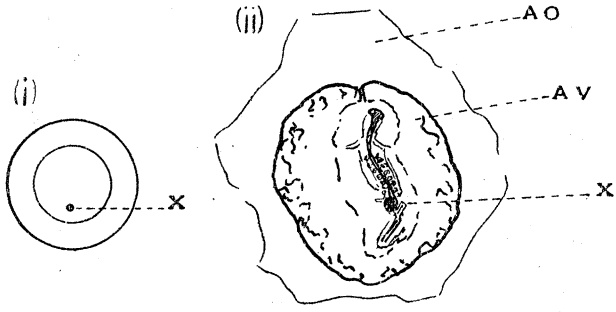

FIG. C.—(i) Diagram of unincubated Blastoderm.
(ii) Blastoderm of Chick with seven pairs of Mesoblastic Somites.

that the primitive streak is converted directly into a part of the embryo, that is to say, the part of the embryo posterior to, and including the first pair of mesoblastic somites.

With regard to the area vasculosa, my experiments seem to indicate that the part of the blastoderm which becomes area vasculosa is that part which lies on the inner edge of the posterior part of the area opaca of the unincubated blastoderm. It is along this edge where, according to Koller,* a white crescent is always visible.

Koller, further, asserts that this white crescent is grooved. From this crescent and groove Koller derives the primitive streak and primitive groove by the conversion of the transverse crescent and groove into a longitudinal streak and groove.

I think that all recent authors are agreed that it is not grooved, and most admit that it has nothing to do with the primitive streak.

It is, however, quite true that a crescentic whiter area is sometimes visible here, but in, I think, the majority of cases there is nothing of the kind to be seen.

When it is present a sagittal section of the hinder part of the blastoderm seems to reveal its nature. In such a section a mass of inner-layer cells, which would, perhaps, be more properly described as a band of yolk containing numerous nuclei, although quite sharply marked off from the underlying yolk-mass, can be detected. This area corresponds in position to that part of the blastoderm from which, according to experiments made with bristles, the area vasculosa is derived (figs. D, E).

A sable hair inserted in the yolk beyond the limits of the blasto-

* "Beiträge zur Kenntniss des Hühnerkeims im Beginne der Bebrütung," 'Sitz. Akad. der Wissensch.,' Wien, vol. 80, 1879. "Untersuch. über d. Blätterbildung im Hühnerkeim," 'Arch. f. mikr.-Anat.,' vol. 20, 1881.

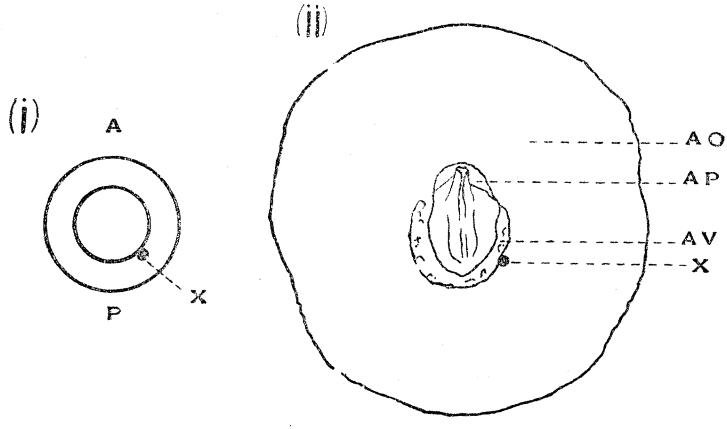

FIG. D.—(i) Diagram of unincubated Blastoderm.
(ii) Blastoderm after 24 hours' incubation.

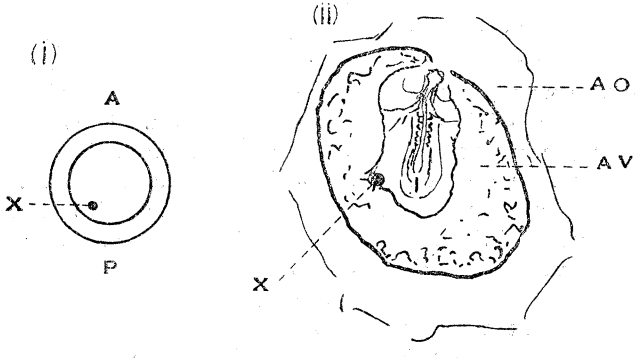

FIG. E.—(i) Diagram of unincubated Blastoderm.
(ii) Blastoderm with five Pairs of Mesoblastic Somites.

derm, if placed close to the edge of the blastoderm generally hinders the development of that side. But if placed at some distance from the blastoderm, it is eventually passed by the advancing edge of the blastoderm, and is found within the area opaca, though usually a streak is left between it and the edge of the blastoderm.