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The influence of the area opaca on the development of the young chick embryo

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INTRODUCTION

The area opaca of the chick blastoderm is generally regarded as being merely the primordium of the yolk sac. Thus it might be expected that during the early stages of development its role would be essentially to grow and to differentiate, rather than to exert any influence on the development of the area pellucida. Such a view would be supported by the fact that pieces of the area pellucida can differentiate in the absence of the area opaca if they are isolated on the chorio-allantoic membrane (Rawles, 1936) or *in vitro* (de Haan, 1964).

There are, however, reasons for enquiring whether the area opaca does exert some influence on the area pellucida. The first is that New (1959) has demonstrated that the blastoderm is normally under tension, and that this tension is produced by the peripheral cells of the area opaca which adhere to the inner surface of the vitelline membrane. One of the objects of this work has been to enquire what effect this tension has on the development of the embryo. The second reason is that it has been suggested that even before the extra-embryonic circulation has formed, yolky material may be passed from the area opaca cells to the area pellucida, perhaps by some process of phagocytosis (Schechtman, 1956). A second object of this investigation has been to test this hypothesis. Finally, it is known that explants of tissues growing *in vitro* may often develop better if the cell population is increased. A third object of this investigation is therefore to find out whether the area opaca exerts an effect on the area pellucida through increasing the cell population of the blastoderm.

MATERIALS AND METHODS

One hundred and sixty-six chick blastoderms were each incubated for about 24 h, by which time they had reached the full-length primitive streak stage, or

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the early head process stage, i.e. they corresponded to about stage 4 in the normal table of Hamburger & Hamilton (1951). Each blastoderm was explanted *in vitro* using the technique of New (1955). In this method the blastoderm lies with its ventral side uppermost and its dorsal side downwards against a piece of vitelline membrane. The latter is stretched out tautly and beneath it lies hen's egg albumen.

Control specimens were explanted *in toto*. The blastoderms were incubated for 24 h and then fixed in Bouin's fluid. They were dehydrated in ethanols, cleared in cedar-wood oil, embedded in paraffin wax and serially sectioned at 10 μ. The sections were stained with haematoxylin and eosin.

At the time of explantation, and before any operations were carried out, certain measurements were made on each blastoderm. These were: length of primitive streak, length of head process if present, length of area pellucida, width of the area opaca. Measurements made after incubation and before fixation were the length of embryo from the most anterior tip of the forebrain to the remnant of Hensen's node and the width of the area opaca. Subsequently, in a number of embryos selected at random from each group of experiments, the volume of neural tissue present after incubation was calculated as follows. Projection drawings were made at a magnification of $\times 200$ of the neural tissue in every sixth section. The areas of these drawings were measured using a Stanley planimeter. The total volume was then taken as:

$$\frac{(\text{sum of the areas}) \times (\text{distance between measured sections})\ \text{mm}^3}{200^2}.$$

The mitotic rate was calculated for the neural tissue in eighteen of these specimens. In each case, counts were made in every sixth section at a magnification of $\times 1000$. The mitotic rate was calculated as:

$$\frac{\text{Number of dividing cells}}{\text{number of non-dividing cells}} \times 100\ \%.$$

The pyknotic rate was also obtained using the same sections, and was calculated as:

$$\frac{\text{number of pyknotic cells}}{\text{number of non-dividing cells}} \times 100\ \%.$$

RESULTS

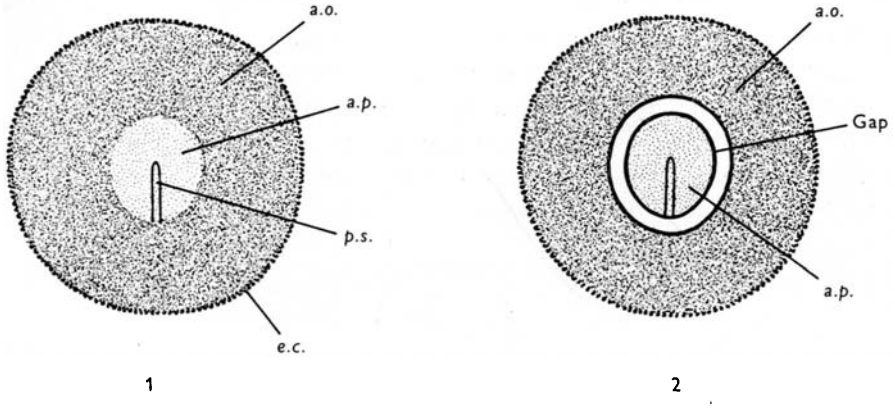
Group A: the normal embryo

Experiment I: control experiment

To obtain a standard for normal development thirty-two specimens were treated as controls (Text-fig. 1). After explantation, the edge cells at the periphery of each blastoderm were released from the vitelline membrane in order to place

the controls in a comparable situation to the experimental embryos, for these became detached from the vitelline membrane during operation (see below). New (1959) showed that if the edge of the area opaca was detached from the vitelline membrane, it subsequently re-attached after 2–3 h incubation, and this finding was confirmed with the control specimens.

Thirty of the control specimens developed well (see Plate 1, fig. A), each having a well-formed head, heart and 8–12 pairs of somites. They corresponded with stages 8–11 of Hamburger & Hamilton (1951). A circulation was present in six of them. Two of the control specimens were highly abnormal and were discarded.



Text-fig. 1. Experiment I. Unoperated control blastoderm explanted at the primitive streak stage. *a.o.*, Area opaca; *a.p.*, area pellucida; *e.c.*, edge cells; *p.s.*, primitive streak. Well-developed embryos usually formed from this experiment.

Text-fig. 2. Experiment II. The area pellucida cut free from the area opaca. Both regions shrank, leaving a gap between them. Thus the area pellucida is normally under tension.

Measurements made on the well-developed specimens showed that the embryos were about twice the length of the original primitive streak (or the primitive streak and head process). The blastoderm as a whole had increased in diameter by between 2 and 3 times and had usually covered the entire area of the piece of vitelline membrane on which it was explanted. Only the periphery of the area opaca was attached to the vitelline membrane.

After sectioning the volume of the neural tissue was obtained for five specimens (Text-fig. 10), and the mitotic rate and pyknotic rates were calculated for the same material (Text-figs. 11, 12).

Experiment II: tension experiments

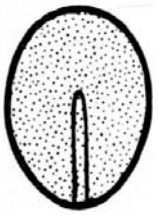
New (1959) showed that the blastoderm as a whole was under tension. To determine whether the area pellucida was under tension in unoperated normal blastoderms nine specimens were explanted as usual and incubated for 1–2 h, or until any wrinkles in the vitelline membrane had disappeared (see New, 1955). Measurements were then made of the maximum diameter of the area pellucida,

and of the maximum width of the area opaca. The area pellucida was now cut free from the area opaca and the same regions remeasured. Both area pellucida and area opaca were found to have shrunk (Text-fig. 2) and it was concluded that they had both been under tension. In a few specimens the area pellucida began to curl at the cut edge which made it difficult to measure; these specimens were discarded.

Group B: experiments in which the area opaca is reduced

Experiment III: total absence

In the total absence of the area opaca development was poor. The entire area opaca was extirpated and the area pellucida was cultured alone, ectodermal side against the vitelline membrane (Text-fig. 3). Care was taken to avoid including even small pieces of area opaca or excluding any of the area pellucida. Thirty-one specimens were treated in this way and after 24 h of incubation they had several features in common before fixation. In none could a well-formed



Text-fig. 3. Experiment III. The area pellucida cut away from the rest of the blastoderm and explanted on its own. Poor development took place.

embryonic axis comparable to the control embryo be seen, although in ten it was possible to discern some neural tissue. In twenty-four an apparently stunted disorganized mass had formed (Plate 1, fig. B), and in seven of these a number of cysts had developed; these specimens were all fixed. In the remaining specimens differentiation did not appear to have taken place at all, and these were discarded. In no case had the area pellucida expanded, and in some it had apparently contracted. For instance, the area pellucida in one specimen shrank from a a maximum length of 1·8 mm to 1·5 mm. The relationship to the vitelline mem-

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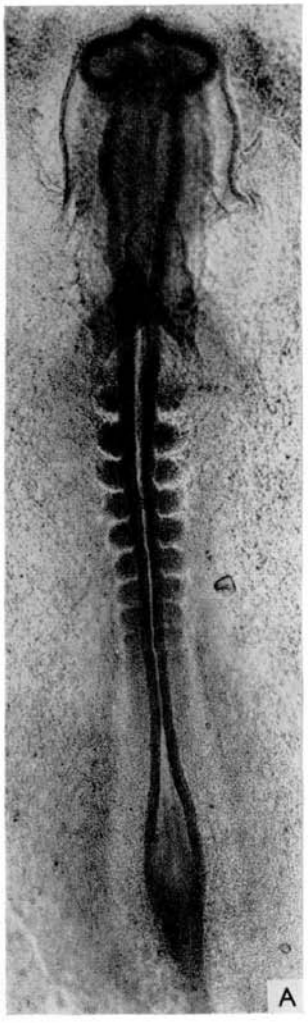
PLATE 1

Fig. A. Experiment I. Control embryo explanted at the primitive streak stage and incubated for 24 h. Whole mount × 30.

Fig. B. Experiment III. Area pellucida explanted alone at the primitive streak stage and incubated for 24 hr. Whole mount × 62.

Fig. C. Experiment VIII. Three area pellucidae explanted at the primitive streak stage and incubated for 24 h. Whole mount × 45.

Fig. D. Experiment VII. The rim of the area opaca was grafted on to the area pellucida and the latter was then incubated for 24 h. Whole mount × 30.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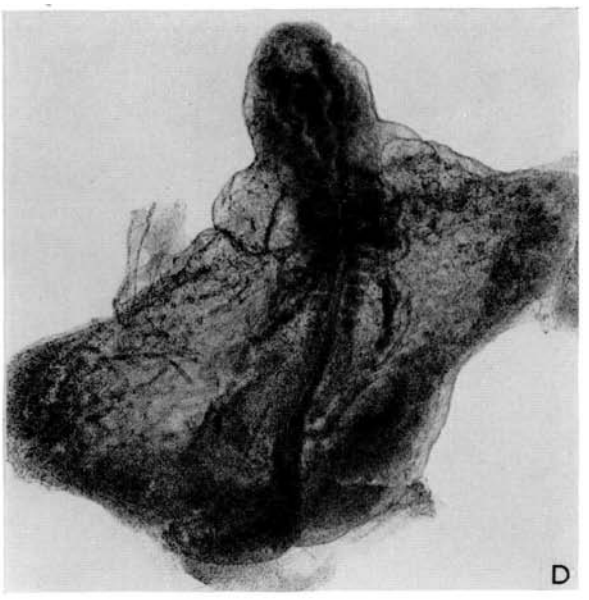
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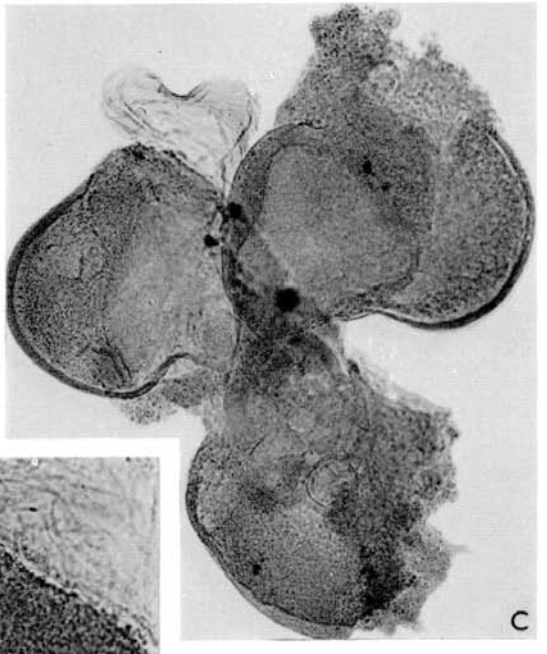
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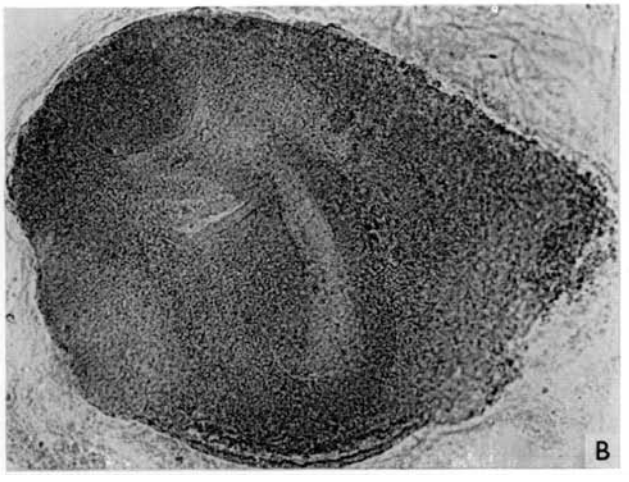
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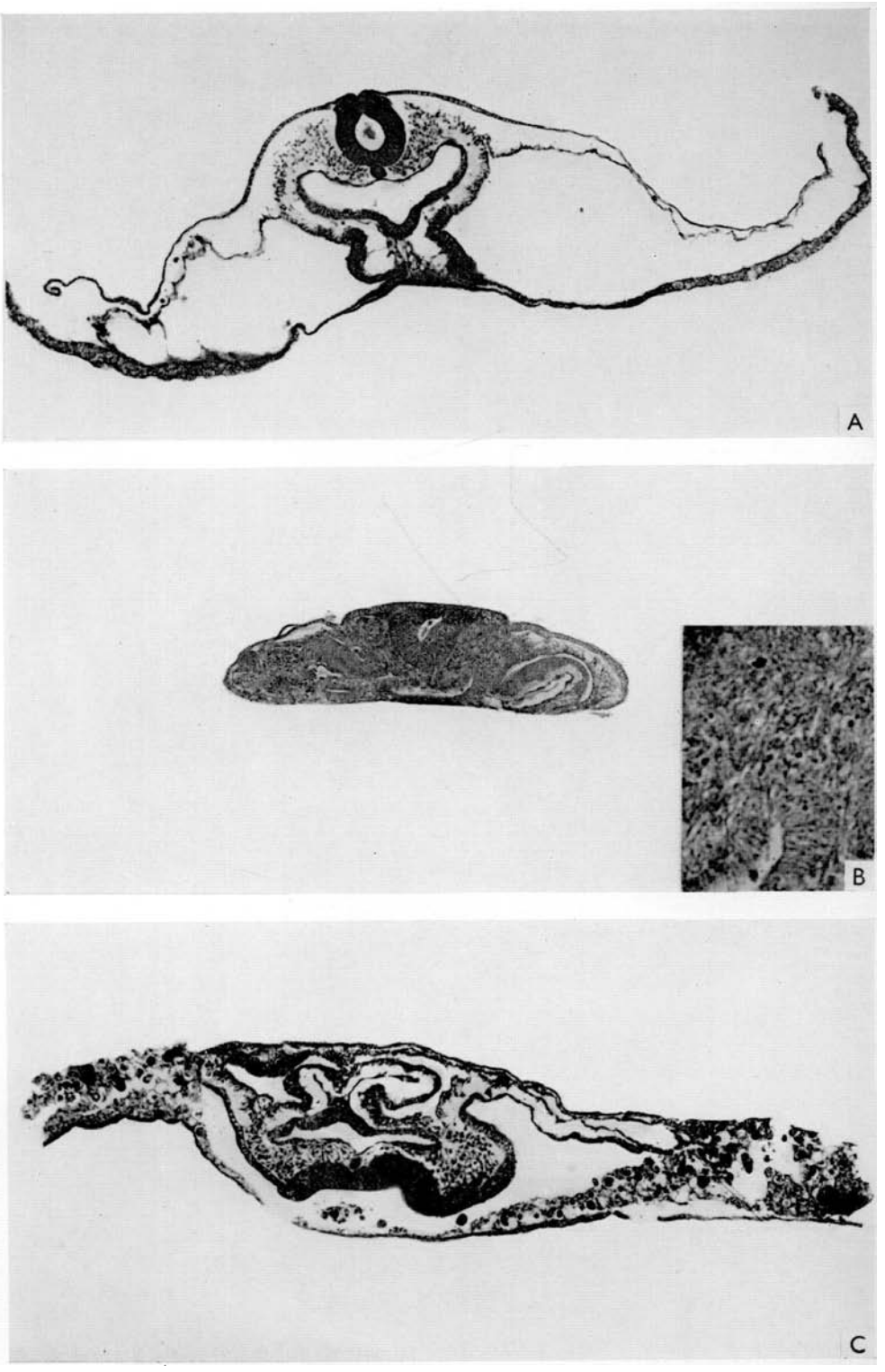


Three embryos sectioned at the level of the heart. All × 65.

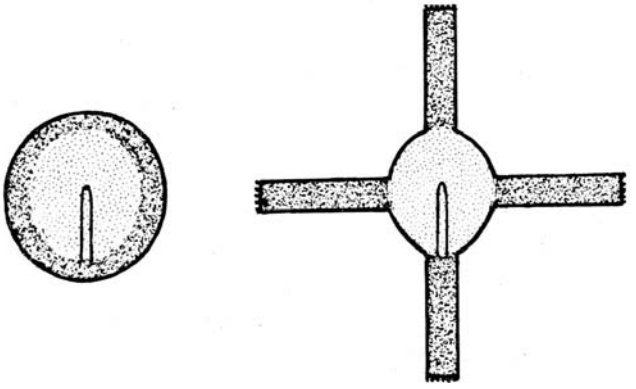
Fig. A. Experiment I (control).

Fig. B. Experiment III (area pellucida alone). Inset: enlargement of a part of this section to show dense packing of cells.

Fig. C. Experiment V (area pellucida on top of area opaca).

brane was of especial interest, however, for frequently the entire surface of the specimen was stuck to the membrane so firmly that it had to be dissected away from it.

When sections were examined, however, it was found that, with the exception of those that were badly distorted by cysts, an embryonic axis was present. In five it consisted of only a neural plate underlain by a notochord and densely packed unsegmented mesoderm. In twelve a more elaborate embryonic axis was present, though typically it lacked a head fold and the brain was distorted. Furthermore, the tissues tended to be packed abnormally close together (Plate 2, fig. B). Only seven specimens possessed several pairs of clearly segmented somites and these were all derived from blastoderms that had been at the head process rather than at the younger, primitive streak stage at explantation.



Text-fig. 4. Experiment IV. Between two-thirds and three-quarters of the area opaca was removed and the remainder explanted with the area pellucida. In group *a* the tissue was extirpated from the periphery of the blastoderm; in group *b* it was removed in segments. Good development usually took place.

The volume of neural tissue present was calculated for thirteen specimens (see Text-fig. 10). The volume of neural tissue obtained tended to be greater from head process specimens than from younger ones. Even when the head process specimens were included the mean volume of neural tissue was found to be less for this group of embryos than it was for the controls (see Comparison of Results, below). The mitotic rate of the neural tissue was estimated for six specimens (see Text-fig. 11). In two of them the mitotic rate was exceptionally low, but the mitotic rate of the remaining four was of the same order of magnitude as that of the controls. When the pyknotic rate for the neural tissue was estimated it was found to be very high compared with the controls (Text-fig. 12).

Experiment IV: partial absence

On reducing the quantity of area opaca present, it was found that the embryos were almost as well developed as the controls. The experiment was carried out

in two ways. In the first method, a ring of tissue corresponding to about two-thirds to three-quarters of the distal part of the area opaca was removed, and the area pellucida was cultured together with the remaining proximal part of the area opaca (Text-fig. 4*a*).

Six embryos were subjected to this operation and of these five developed well, having a well-formed head with brain, foregut and heart and possessing between 4 and 20 pairs of somites. The remaining specimen was small but appeared to be retarded rather than abnormal; it had only one pair of somites.

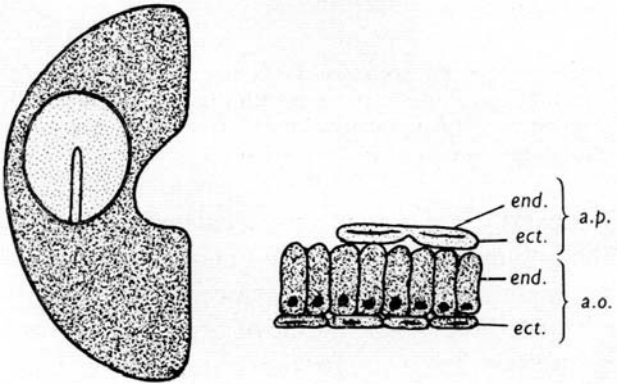
In the second method, two-thirds of the area opaca was again removed but in a segmental manner (see Text-fig. 4*b*). Only two embryos were included in this group. In both a well-developed embryo was formed corresponding to about stage 10. In section the two embryos were normal and the volume of neural tissue corresponded with that found for the first method.

In both methods some expansion of the blastoderm occurred, the maximum diameter being greater after incubation than immediately after explantation. The volume of neural tissue was however less than that in the controls (Text-fig. 10). Mitotic and pyknotic rates were not estimated.

Group C. Experiments to test whether the area opaca has a specific influence on the development of the embryo

Experiment V

To determine whether the area opaca could influence the area pellucida other than by tension, the area pellucida was cut away from the area opaca and was



Text-fig. 5. Experiment V. (*a*) The area pellucida cut away from the area opaca and then explanted on top of it. (*b*) shows the relationships of the tissues in section. *ect.*, ectoderm,; *end.*, endoderm. Good development usually took place.

then explanted on top of it with the ectodermal side of the area pellucida upon the endodermal side of the area opaca (see Text-fig. 5). It was found that although the embryos were smaller than the controls, the degree of differentiation was comparable.

Twenty-six specimens were treated in this way and in each case an embryonic axis developed that was recognizable before fixation. In fifteen specimens the edge of the area pellucida had fused with the area opaca (Plate 2, fig. C) and in the remainder it lay freely on top. All but two of the embryos possessed a neural tube, and most of these had between three and seven pairs of somites, lateral plate mesoderm and a short foregut. In six a simple heart had formed. None of the embryos possessed the densely packed mesoderm so characteristic of those in Exp. III. Cysts had distorted three specimens and two others had failed to develop at all, but, apart from these, differentiation appeared to be much better than in Exp. III.

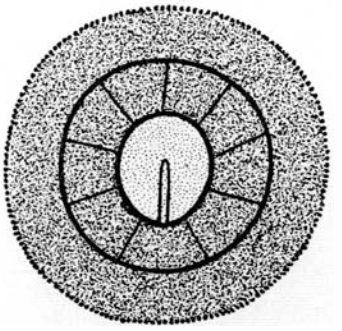
The volume of neural tissue (Text-fig. 10) and the mitotic rate (Text-fig. 11) were found to be comparable to that of the controls. The pyknotic rate (Text-fig. 12) was higher than in the controls.

Some expansion and/or change in shape of the area pellucida appeared to have taken place, though it was difficult to assess this in view of the fusion of the host and graft tissues.

In most specimens the area pellucida appeared to have exerted some influence on the area opaca (Plate 2, fig. C) for the yolky endoderm of the area opaca was considerably reduced.

Experiment VI

To determine whether the endoderm of the area opaca had a specific effect on the area pellucida a ring of area opaca endoderm corresponding to about the proximal third of the area opaca was extirpated (Text-fig. 6). The ectoderm was



Text-fig. 6. Experiment VI. A ring of endoderm (radiating lines in diagram) in the proximal part of the area opaca was extirpated. This endoderm regenerated however. Good development of the embryo occurred.

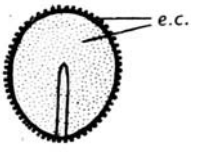
left intact but the peripheral cells of the blastoderm were released from the vitelline membrane. Six specimens were treated in this way. After 24 h incubation two were dead, but the remaining four had developed normally having reached stages 9 and 10 and having expanded to about twice their original diameter. These embryos thus corresponded well with the control specimens and

their volume of neural tissue was of the same order. It was found, however, that the endoderm of these specimens had regenerated during incubation so that it was decided that no conclusion could be drawn from this group and further specimens were not obtained.

The reverse experiment, of retaining the entire opaca endoderm whilst extirpating the proximal third of the ectoderm, was performed on six specimens, but since these all subsequently developed a mass of cysts, the experiment was abandoned.

Experiment VII

A strip of cells about 0·35 mm wide was dissected from the periphery of the area opaca. The remainder of the area opaca was then cut away from the blastoderm and the peripheral strip was grafted on to the area pellucida (Text-fig. 7). Care was taken to place the graft with its dorsal side against the vitelline membrane and to avoid including any endodermal cells of the area opaca.



Text-fig. 7. Experiment VII. The entire area opaca was extirpated, and then a strip of edge cells from its periphery were grafted on to the area pellucida. *e.c.*, Edge cells. Good development of the embryo frequently took place.

The purpose of this experiment was to test whether the embryo could develop well if the entire proximal part of the area opaca was extirpated. A second purpose was to test the effect of exerting tension on the area pellucida (see group E). Over 50 % of the embryos developed well.

Nine embryos were treated in this way. In all but one of them the graft became fused over most of its length to the area pellucida, and the periphery spread out so that the size of the blastoderm was greater after incubation than it was immediately after operation.

In five cases well differentiated embryos developed and had reached stage 9 or 10 after 24 h of incubation (Plate 1, fig. D) though they tended to be smaller than control embryos. In two specimens the embryo was retarded and abnormal, consisting of an ill-arranged mass of neural tissue and undifferentiated mesoderm. The remaining two specimens failed to develop.

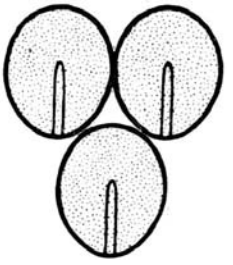
The volume of neural tissue, and mitotic and pyknotic rates were measured in the five specimens that developed well, and were found to be comparable to those of the controls (Text-figs. 10, 11, 12).

Group D: experiments to test the possibility that the cell population of the isolated area pellucida is too small to ensure good development

Experiment VIII

The purpose of this experiment was to increase the population of area pellucida cells without including any area opaca cells.

Three blastoderms were assembled in a single watch-glass and the area opaca was removed from each. The three area pellucidae were then arranged with their ectodermal surfaces against the vitelline membrane and their edges in contact (Text-fig. 7). Eleven such groups of explants were made, involving a total of thirty-three embryos.



Text-fig. 8. Experiment VIII. Area pellucidae, each devoid of area opaca, assembled in groups of three and cultured in contact with one another. Poor development took place.

After 24 h incubation the explants had generally become so firmly attached to the vitelline membrane that it was difficult to free them without damaging them. For this reason, no attempt was made to section all this group of embryos. No expansion appeared to have taken place. In all but two cases the three individuals had fused together, but it was possible to distinguish them from one another. No correlation was noticed in the degree of development of members of a group.

The development of these embryos was similar to that of the embryos in Exp. III. Like the latter, they often possessed closely packed mesodermal masses, and normal but simple neural tubes. In several embryos two or three pairs of somites were present. About half of the specimens failed to differentiate at all, and consisted largely of cysts.

Neural tissue volumes, mitotic and pyknotic rates were not calculated for this experiment in view of the close similarity of these embryos to those of Exp. III.

Group E: experiment to test the importance to the area pellucida of the tension exerted by the area opaca

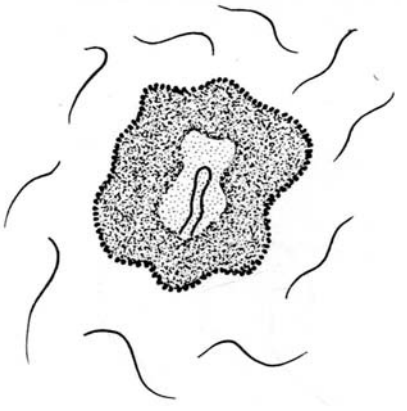
Experiment IX

The purpose of this experiment was to find out the effect of reducing tension on the area pellucida without removing any of the area opaca.

Each blastoderm was explanted without operation but the vitelline membrane

was arranged excessively slackly around the glass ring so that numerous wrinkles and folds were present (Text-fig. 9). Thirteen specimens were treated in this way.

After 24 h of incubation only one embryo had developed well; it possessed six pairs of somites and its area pellucida and area opaca had spread out as well



Text-fig. 9. Experiment IX. An entire unoperated blastoderm was explanted but the tension that normally exists within the tissues was eliminated by slackening the underlying vitelline membrane. Poor development took place.

Table 1. *General Summary of Results*

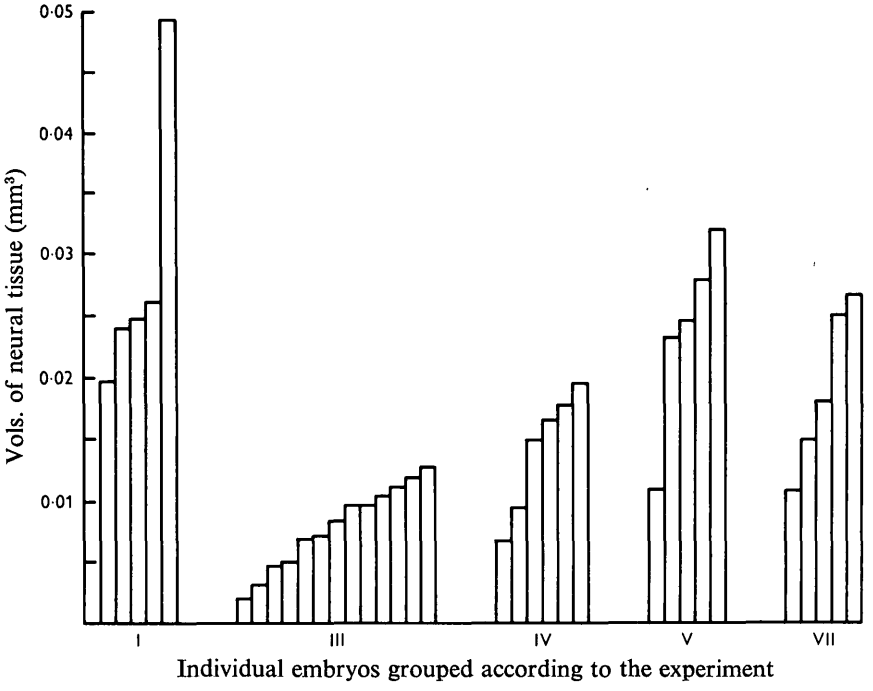
General development	Experiment	Differentiation: Good	Poor	None	Total	Tension
Good	I Unoperated control	30	2	—	32	Good
	V Area pellucida on top of area opaca	21	3	2	26	?
	VII Area opaca periphery grafted to area pellucida	5	2	2	9	Good
Good	IV Parts of area opaca extirpated	7	1	—	8	Probably moderately good
Poor	III Area pellucida alone	—	24	7	31	Poor
	VIII Three area pellucidae	—	About half the total	About half the total	11 × 3	
	IX Vitelline membrane slackened	1	3	9	13	

as those of the unoperated controls. The vitelline membrane of this embryo had perhaps been arranged less slackly than that of the other specimens for it had lost most of its wrinkles.

By contrast, none of the remaining 12 specimens had developed well. Four had formed large cysts, 5 had not developed any axial structures at all, and the

remaining 3 had differentiated to a state comparable with the embryos of Exp. III. In each of these 12 cases the vitelline membrane had remained slack and wrinkled and no expansion of the blastoderm had occurred.

Neural tissue volumes, mitotic rates and pyknotic rates were not calculated for these specimens in view of their close similarity to the embryos of Exp. III.



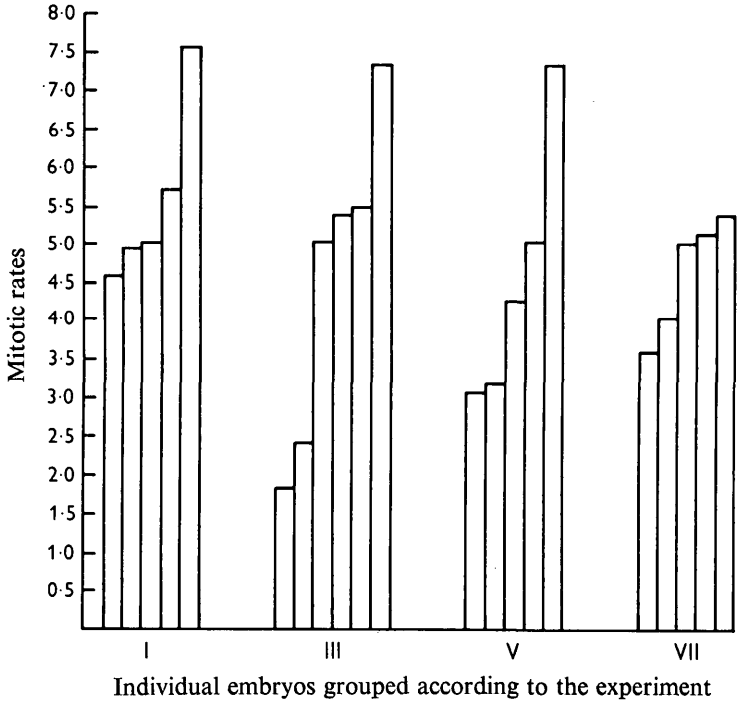
Text-fig. 10. Histogram to show the volumes of neural tissue in a number of embryos taken from different experimental groups. The volume of neural tissue in Exp. III was significantly lower than that in Exp. I ($t < 0{\cdot}001$), in Exp. IV ($t < 0{\cdot}01$), in Exp. V ($t < 0{\cdot}001$) and in Exp. VII ($t < 0{\cdot}001$). The volume of neural tissue in Exp. IV was also significantly less than that in Exp. I ($t < 0{\cdot}05$), but in no other experimental group was the volume of neural tissue found to be significantly small. In general, any embryo that had a large volume of neural tissue had undergone considerably more growth and differentiation of the brain than had an embryo with a small volume.

Comparison of results

The general results are summarized in Table 1. They fall into two categories, namely those where well-formed embryos generally developed even though they were sometimes rather small, and those where differentiation was so poor that it was difficult to recognize any axial structures except by sectioning the material. The extent of development did not appear to be directly dependent on the amount of area opaca present. In the discussion it will be suggested that the amount of area opaca present was important mainly in so far as it affected the tension exerted on the area pellucida. Where the tension appeared to be

good, differentiation and growth were good. Where tension was poor, differentiation and growth were poor.

As a basis for more precise comparisons the volume of neural tissue was calculated for a number of specimens (Text-fig. 10), and these respective volumes were compared by the *t* test.



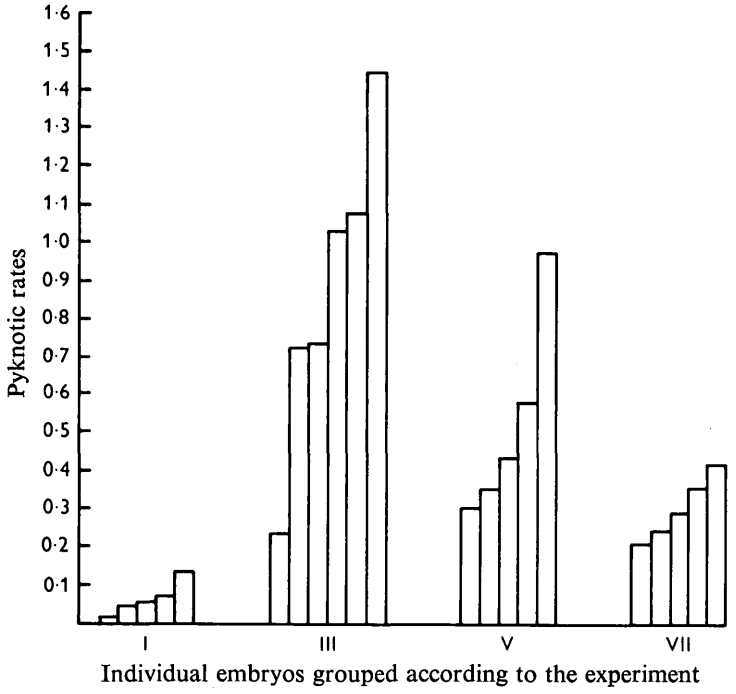
Text-fig. 11. Histogram to show the mitotic rates in the neural tubes of embryos taken from different experimental groups.

Despite striking differences in neural volume, the mitotic rate (see Text-fig. 11) in Exp. III did not differ significantly from that in Exps. I, V and VII. By contrast however, the pyknotic rate was found to be exceptionally high in Exp. III (Text-fig. 12).

A marked histological difference was apparent between those embryos where poor development occurred (Exps. II, III, VIII, IX) and those where good development took place. The cells of the poor embryos were abnormally closely packed together, intracellular spaces being reduced to a minimum (Plate 2, fig. B). There was, however, no evidence to suggest that the individual cells of these embryos were smaller in volume than those of the controls.

DISCUSSION

The main point to be considered in the discussion is why the area pellucida does not develop well under tissue culture conditions if the area opaca is not



Text-fig. 12. Histogram to show the pyknotic rates in the neural tubes taken from different experimental groups. The pyknotic rate in Exp. III was significantly higher than that in Exp. I ($t < 0{\cdot}01$), though not than that in Exp. V ($t < 0{\cdot}10$) or in Exp. VII ($t < 0{\cdot}10$).

present. The most important reason is probably that the tension normally provided by the area opaca is lacking.

(1) *Tension effects produced by the area opaca*

New (1959) demonstrated that a state of tension exists in the blastoderm. He showed this mainly by measuring the diameter of the blastoderm before and after releasing it from the vitelline membrane. After release, the blastoderm shrank. Similarly, in the present experiments we have shown that the area pellucida shrinks if it is cut away from the area opaca, and that the area opaca is also under tension.

New was able to show that this tension is produced by the edge cells of the blastoderm. These cells are normally attached to the inner surface of the vitelline membrane and as they move in a centrifugal direction they exert tension throughout the blastoderm. He suggested that the edge cells owe their properties to their morphological structure rather than to their position, and this was subsequently confirmed (Bellairs, 1963).

Under normal circumstances only the edge cells are firmly attached to the vitelline membrane. The more proximal cells are either unattached or possibly

very lightly attached (New, 1959). In particular, the area pellucida does not seem to be attached at all. It seems possible that the cells of the area pellucida are normally prevented from adhering because they are subjected to a centrifugal tension from the peripheral cells, and this leads them continually to shift their position against the vitelline membrane. Furthermore, it seems probable that it is only when they are prevented from adhering that normal growth can occur. Thus, if the area opaca is only partially removed some tension still remains; but if it is entirely removed there is no tension in the area pellucida and nothing to prevent it from adhering to the vitelline membrane. Our observations suggest that where the area pellucida becomes attached to the vitelline membrane in this way there is an interference with normal development.

It is now relevant to consider how tension exerts its effects in normal embryos. It might be expected that when tension is normal mitosis would be stimulated throughout the blastoderm, and that when tension is reduced the mitotic rate would fall. Indeed there is evidence in adult organs that local increases in tension may stimulate proliferation (summarized by Abercrombie, 1957). No direct support for this interpretation could be found however when the mitotic rates were obtained for the neural tissue of representative embryos (see Text-fig. 10).

By contrast, when the pyknotic rate was calculated for the same sections it was found to be significantly higher in the embryos of Exp. III than for those of control embryos (Exp. I) (Text-fig. 12). It seems possible therefore that lack of, or reduction of, tension in the area pellucida may result in conditions that lead to an increase in cell death. The mechanism of this effect, however, is open to speculation.

A second characteristic of many of the embryos that have suffered from reduced tension is the dense packing together of the cells, especially the mesenchyme cells. A similar observation was made by New (1959) on the edge cells of blastoderms that had failed to expand. It seems probable that reduction of tension inhibits the cells from adjusting their positions within a particular tissue and this in itself may interfere with generalized cell movements. It is clear that the major morphogenetic movements have taken place irrespective of the reduction in tension (e.g. regression of the node has usually occurred since the notochord is generally present) but other movements have not occurred normally (e.g. head-fold formation is often disturbed).

The importance of tension is further emphasized in those experiments in which cyst formation has occurred. Cysts were a common result of the pioneer explantation technique of Waddington (1932) in which the blastoderm was explanted on to a plasma clot, and on which no centrifugal expansion took place. Similarly, Butros (1963) who explanted pieces of area pellucida obtained many cyst-like embryos unless he grew the tissue on a substratum that encouraged spreading.

(2) *The effect of the area opaca on the nutrition of the area pellucida*

In addition to the tension effects discussed above, it is possible that the area opaca also influences the area pellucida by providing yolk materials for nutrition even before the stage of vascularization. The yolk of the area opaca is stored principally in its endodermal cells, but the results obtained from experiments in which these cells were extirpated were inconclusive (see Exp. VI) because of their extensive powers of regulation. More significant is the fact that the embryos in group VII developed better than those in group III even though in both groups the entire area opaca endoderm was absent. It is concluded therefore that the area opaca does not exert an important nutritive function on the area pellucida until a well-established circulation is present.

This conclusion supports the findings of Bellairs (1963), who deduced on the basis of an electron microscopical study that the area pellucida contains within its own tissues sufficient food stores to nourish it for about the first 48 h of incubation, and that not until the circulation is established does it receive food from the yolk sac.

By contrast, the area pellucida appears to have exerted an effect upon the area opaca in Exp. V, for there is a conspicuous thinning of the area opaca beneath the graft. This thinning is due largely to the removal of intracellular yolk and it seems likely that this is the result of the activity of enzymes produced by the area pellucida.

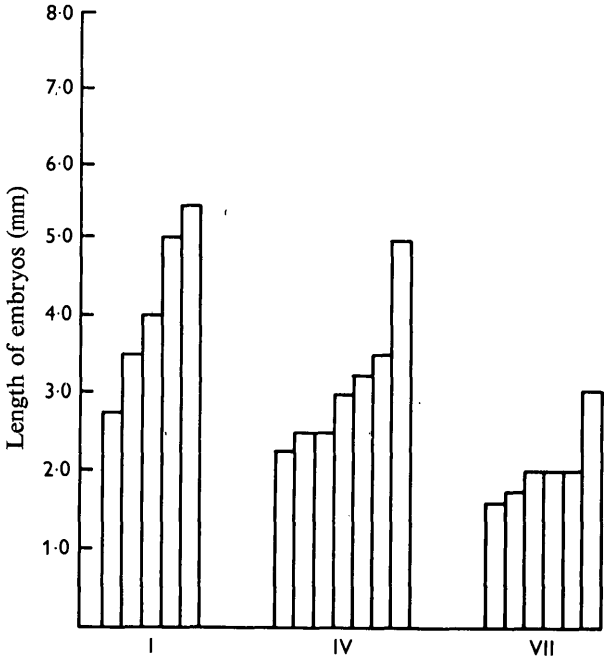
(3) *The area opaca and the problem of cell population*

There is now considerable evidence in the literature that for certain tissues to develop well the mass of cells present that have similar properties must be above a certain minimum (see e.g. Zwilling, 1960). The cells of the area opaca and of the area pellucida differ in their morphological structure as well as in their presumptive fates. Nevertheless, it seemed possible that the area pellucida cells when isolated from all other cells might constitute too small a population to differentiate well. The possibility was therefore considered that one of the functions of the area opaca was the non-specific one of increasing the cell population of the explant and perhaps in this way conditioning the culture. Against the hypothesis, however, is the fact that development was retarded in the embryos of both group VIII whose cell population was secondarily increased and of group IX whose cell population was not reduced at all. It is concluded that the size of cell population of the entire area pellucida is not of significance in the present experiments.

(4) *The area opaca and regression of the primitive streak*

When the regressing node has reached the posterior border of the area pellucida it continues to migrate for a short distance into the area opaca (Spratt, 1947). Thus, if that region of area opaca is removed, the node will be unable

to regress as far back in control embryos; this could lead to the laying down of an embryonic axis that is shorter than normal and less differentiated. However, the embryos of Exp. IV were less differentiated than the control embryos even though they were of comparable length (Text-fig. 13), not having been deprived of the relevant region of the area opaca. Thus, we conclude that inability to regress into the area opaca is not an important factor in determining the subsequent size of the embryo.



Text-fig. 13. Histogram to show the lengths of the embryos in three experimental groups.

SUMMARY

1. The effects exerted by the area opaca upon the area pellucida have been tested experimentally by interfering with the normal relationships between them and then growing the blastoderm in tissue culture by the technique of New.

2. If the entire area opaca was removed from a blastoderm at the full-length primitive streak stage, the embryo that formed was smaller than the controls and poorly differentiated.

3. New evidence supporting the hypothesis that the area opaca influences the development of the embryo by exerting a tension upon the area pellucida is presented.

4. Estimates of the mitotic rates provided no evidence to support the idea

that this centrifugal tension promoted a high rate of cell division. With reduction in tension, however, cell death increased.

5. No evidence could be found to suggest that the isolated area pellucida failed to grow and differentiate because its cell population was inadequate. When three area pellucidae were cultured in close contact with one another, growth and differentiation were no better than when one was cultured alone.

6. The hypothesis that the proximal part of the area opaca exerted a nutritive effect on the area pellucida was tested. This was supported by the fact that when the area pellucida was explanted on top of the area opaca, differentiation of the embryo was good. Furthermore, some digestion of the area opaca endoderm appeared to take place. On the other hand, such a relationship cannot be essential for development at this stage, since equally good development could occur when the proximal part of the area opaca had been removed.

RÉSUMÉ

L'influence de l'aire opaque sur le développement du jeune embryon de poulet

1. Les effets exercés par l'aire opaque sur l'aire pellucide ont été éprouvés expérimentalement en modifiant les rapports existants entre ces deux formations puis en cultivant le blastoderme selon la technique de New.

2. Si l'on enlève la totalité de l'aire opaque d'un blastoderme au stade où la ligne primitive atteint sa longueur maximum, l'embryon qui se forme est plus petit que les témoins et médiocrement différencié.

3. Des preuves nouvelles sont présentées en faveur de l'hypothèse selon laquelle l'aire opaque influence le développement de l'embryon en exerçant une tension sur l'aire pellucide.

4. L'évaluation des taux mitotiques n'est pas en faveur de l'idée que cette tension centrifuge détermine un taux élevé de divisions cellulaires. Toutefois la quantité de morts cellulaires augmente lorsque la tension diminue.

5. Aucun argument n'a pu être apporté suggérant que l'aire pellucide a une croissance réduite parce que la population cellulaire qui la compose est inadéquate. Lorsque trois aires pellucides sont cultivées en contact étroit les unes avec les autres, la croissance et la différenciation ne sont pas meilleures que lorsqu'une seule aire est cultivée isolément.

6. L'hypothèse selon laquelle la partie proximale de l'aire opaque exercerait un effet nutritif sur l'aire pellucide a été éprouvée. Ceci a été étayé par le fait que lorsque l'aire pellucide a été cultivée au dessus de l'aire opaque le développement de l'embryon a été bon. De plus une certaine digestion de l'endoderme de l'aire opaque est apparue. D'un autre côté une telle relation ne peut pas être essentielle pour le développement à ce stade, puisque d'aussi bons développements peuvent se produire lorsque la partie proximale de l'aire opaque a été enlevée.

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