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Neural Induction by Inorganic Matters, with Special Reference to the Mechanism of Induction Through the Introduction of a Foreign Object into the Blastocœle

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With Plates XIX, XX and 6 Figures in the Text

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Introduction

The formation of neural plate in the development of Urodeles is at all times closely associated with an invagination of the archenteron, the dorsal wall of which comes into contact with the ectoderm. It can also be induced at abnormal positions of the embryo through the transplantation of the dorsal lip of the blastopore (SPEMANN and H. MANGOLD, 1924). The formation of neural plate cannot therefore be explained as a spontaneous activity of the ectoderm; the explanation is rather to be sought either in developmental capacities innate to the ectoderm which is aroused through contact with the dorsal wall of the archenteron, or in the capacity of the not yet specialized ectoderm for the formation of a neural plate. In the former case, since the material itself contains a store of several different potencies, by determination special for an induction is meant, as a matter of course, an operation which realizes one of them and suppresses the rest, whereas in the latter case the material may have no tendencies for development of its own, but is capable of accomplishing anything, and is forced, by determination which here acts as active instruction, to leave the state of indefinite ectoderm and acquire a definite organization. No matter which one of these interpretations may be adopted, i. e., selection or instruction, some active agent must have played a rôle. So it has been concluded from most of the experiments up to the present that neural induction is the result of some chemical influence exerted by the dorsal wall of the archenteron, the active agent being considered to be a substance (cf. SPEMANN, 1936: Die Mittel der Induktion, pp. 142-158). It was also shown by the result of experiments

that the potency for neural induction is not species-specific (GEINITZ 1925), that the induction is by no means a phenomenon of a character tissue-specific to the dorsal wall of the archenteron (SPEMANN and GEINITZ, 1927; O. MANGOLD and SPEMANN, 1927). Further, if by the term induction is meant merely the development of a neural plate or other structure comparable to it, this can be brought about not only with various organs and tissues of the same species, but also with those of other vertebrates (UMANSKI, 1932; WOERDEMAN, 1933; HATT, 1934; HOLTFRETER, 1934), and even with a piece of tissue taken from an invertebrate (HOLTFRETER, 1934). It is also known that the induction is in no way associated with the physiological function of the tissue which is employed as the inducing agent (MARX, 1931), since the effect may be the same whether the latter is alive or not (HOLTFRETER, 1933; WEHMEIER, 1934). Especially noteworthy is the fact that portions of an embryo which, when alive, are incapable of neural induction, or other tissues of similar character, come for the first time to be possessed of such potency, when they are boiled or treated with some sort of chemicals (HOLTFRETER, 1934). Further additions of new facts are hardly necessary to lead us to suppose that what serves here as the active agent for induction is a substance which is universally distributed in the animal kingdom, a chemical substance which is resistant to both chemicals and heat. Thus, through the enthusiasm of NEEDHAM, WADDINGTON and their collaborators (1934, '35) in searching for the effective constitutent, a sterol-like body was obtained from ether or petrol-ether extract, and they (1935) were further rewarded with success in inducing a neural plate in a *Triton*-embryo with the use of two synthetized phenanthrene compounds, 1 : 9-dimethylphenanthrene, 9 : 10-dihydroxy-9 : 10-di-*n*-butyl-9 : 10-dihydro-1 : 2 : 5 : 6-dibenzanthracene, and in less marked degree also by 1 : 2 : 5 : 6-dibenzanthracene. Before this BARTH (1934) had already shown the possibility of inducing the formation of a secondary neural plate in an early embryo of *Amblystoma* by his so-considered cephalin extracted from mammalian brain. On the other hand, FISHER and WEHMEIER (1935) found that the formation of a neural plate could often be induced by thymus-nucleic acid prepared from thyroid or pancreas, to the presence of which, they are convinced, the active agent of induction owes its property of resisting high temperatures as well as treatment for long hours with ether, acetone, alcohol etc. without losing its potency. The effective principle that remained was called "nucleoproteid fraction" as distinguished from the ether extract previously mentioned. Thus it seems at present quite unreasonable to continue in the effort to find the one and only effective principle for induction.

However, NEEDHAM *et al.* (1935) are of the opinion that thymus-nucleic acid owes its potency for neural induction not to any property of its own, but to some impurity contained in the preparation, and further that the induction with cephalin by BARTH can similarly be attributed to the impurity of the preparation. Thus he and his associates still contend that the induction must be due to a particular substance. On the other hand, FISHER

and his associates (1935) showed that neural induction is further possible with the use of many kinds of higher fatty acids; denied the specificity of the substances concerned, ascribing induction to the influence of acid radical which is common to all; and finally arrived at the conclusion that the process of induction is simply the effect of acid stimuli delivered to the ectoderm.

Though, in the above investigation, the fatty acids which are used are postulated to be of higher order as compared with their acid radical and to be in liquid state, scarcely anything more is stated as to the nature of the stimuli. Here arises first the question, what kind of relation exists between what is called by these authors acid stimuli and hydrogen ion concentration of the substances? Regarding this point, FISHER *et al.* express the opinion that fatty acids with acid radicals of lower order and synthetized acids with side chains are injurious to embryos; that those acids which are water-soluble and those substances which show acid reaction are without effect in induction experiments. But that this is not necessarily the case is clearly indicated in our experiments described on the following pages.

This investigation was started to make further inquiry into the problem above presented: the relation between the induction and the hydrogen ion concentration of the substance introduced. We were soon convinced that preference should be given to tests using very simple inorganic substances rather than those using complex organic ones. Accordingly, this line of inquiry was given to one of my students as a subject of study, and progress to a certain extent was made in his experiments (TANAKA, 1935, '36). But, since many questions were unanswered by his results, the problem was again attacked with the assistance of Mr. Y. TOKUOKA, my private assistant, the experiments already made were repeated, and new ones were added to complete the research. The results have been briefly reported in the journal "Growth" (vol. 2, pp. 49-54. 1938), but as there remain many things to be added, a paper on the subject is here offered giving some details of each of the experiments. Here at the publication of this paper, my sincere thanks are due to Mr. TOKUOKA, who has devotedly assisted me in the whole course of the work.

Method

As method of study, substances to be tested were introduced into the blastocœle of blastulas or early gastrulas of the red-bellied newt, *Triturus pyrrhogaster* (BOIE), which is the most common in this district. If the substance was in fluid state, fuller's earth or *kôya-dôfu* (about the properties of this material refer to p. 313) was used as the carrier. After the operation each embryo was kept separate on silk cloth in a small glass vessel. Until some recognizable change appeared on the surface of the embryo, observations were taken every day, records and, if necessary, sketches being made. When a definite time had elapsed, the surviving embryos were fixed in SMITH's fluid and histological changes of the ectoderm and the interior of the embryo were studied in sections stained simply with gentian-violet.

Outline of the results

Many of the embryos which were operated on, died during early development. Only about 20% of them remained alive until they reached the stage at which the primary neural plate was fully formed and in some specimens, almost came to the point of being converted into the canal. It is especially noteworthy that these surviving embryos belonged for the most part to those which had thrown out the introduced substance, while of those which retained it completely not all embryos indicated any change on the ectoderm, but only some tenths manifested recognizable changes of varying degrees. The variability of these changes was of a wide range, namely from a mere local overpigmentation of the surface on the one hand to a partial formation of a neural plate on the other. These changes on the ectoderm might take place in embryos of early developmental stages in a comparatively wide sphere, in one case on the flank and in another on the ventral surface of the embryo; whereas they were localized in embryos of later stages almost without exception on the thoracic region just behind the heart. As to whether this was due to the fact that those parts which had undergone changes migrated to that region of the embryo or whether those embryos only which had received changes on that region of the body were able to survive, we are not here in a position to speak.

Operations were made in each of the four successive periods of development between the beginning of gastrulation, where invagination of the archenteron was about to occur, and the stage where the dorsal lip of the invagination was bent downwards to become hoof-shaped. When the operation was made in any of the first three of these periods, that is until the ends of the dorsal lip began to be bent downwards, the result was almost uniform, the induction test often turning out positive; but when it was done during the last period, where both ends of the dorsal lip had already curved to become semicircular or hoof-shaped, positive result of the induction test could no longer be obtained except in a very few cases.

It may not be superfluous to add here, that as was observed in similar experiments by the previous authors, at least three different stages of the histological changes of the ectoderm can be distinguished: first, a mere local thickening of the ectoderm attended with abnormal proliferation of cells (cf. fig. 8, pl. XIX); second, the comparatively regular arrangement with elongation of cells in such a thickening, or the so-called differentiation of nervous tissue (cf. fig. 14, pl. XX); and finally, formation of a typical neural plate (figs. 9 & 11, pl. XIX). A more advanced stage of fusion of neural folds of each side with the result of a tubular formation was rarely attained, a few examples only being obtained through the entire course of our experiments (figs. 12 & 13, pl. XX). The neural plate, it may be added, which was thus obtained through the induction, was as a rule very large, covering in some cases the whole surface of the flank of an embryo (cf. figs. 7 & 10, pl. XIX).

Induction and the hydrogen ion concentration of the substance to be introduced

(This series of experiments was conducted by Mr. S. TANAKA, and here only his results are cited almost as they are verbally told.)

Solutions possessing pH values 6.4-6.0 and 8.0 respectively were made up by mixing approximate proportions of aquatic Na_2HPO_4 and KH_2PO_4 according to the instructions for preparing SÖRENSEN's phosphate buffer solutions. They were kneaded separately into a paste of moderate consistency with fuller's earth of unknown nature. These were then used as the agent for induction and introduced into the blastocœle of embryos. Fuller's earth was used in this way as the carrier for no particular reason other than convenience; at least in the beginning of our investigation there was no thought of its having different significance from that of kaolin which BARTH used as carrier. It was at that time far from our imagination that neural induction might be possible through inorganic matters.

It was found as a result of experiment that, out of the surviving embryos in the group of pH 6.4-6.0 eight were possessed of a differentation resembling nervous tissue in the modified portion of the ectoderm, and again three out of these were provided with a structure which might be regarded as a neural plate. In the second group of pH 8.0, the number of the surviving individuals was approximately the same as in the preceding, namely 28. A differentation of nervous tissue could be recognized in the modified region of the ectoderm in five of these individuals, in two of which, again, a very marked formation of neural plate could be found. Fig. 1 in the previous publication (Growth, vol. 2) represented one such specimen.

New, if we compare the results of these two sets of experiments, and if the assumption is made that the carrier by itself is strictly neutral and has no relation whatever to induction, it naturally follows that neural induction is equally possible whatever the hydrogen ion concentration of the inductive substance may be in the acid or alkaline side of the reaction. As important point which must here be taken into consideration is the hydrogen ion concentration in the blastocœle of embryos, which is estimated by BUYTENDIJK and WOERDEMAN (1927) in blastulas of *Triton taeniatus* to have pH values 8.4-8.6 or still higher. It must be questioned, therefore, whether the hydrogen ion concentration of the introduced material could remain unaltered under the influence of that of the blastocœle if the ion concentration of the latter is so low as this. It may be conjectured at least from my own experience in injecting hydrochloric acid of known concentration into the blastocœle of sea-urchin blastulas that the blastocœlic fluid has a remarkable buffer action. (Reference may also be made to HIRABAYASHI's experimental results (1937) on *Toxopneustes pileolus*.) Therefore, it is quite open to question whether the above introduction into the blastocœle of phosphate mixtures with different hydrogen ion concentrations could have any effect on the transformation of the ectoderm into nervous tissue. However, it is an established fact that the formation of neural plate was in fact

induced, at least in some cases, through the introduction of such preparations as we are here considering. But, if the induction has, for the reason we have just stated, no connection whatever with the acidity or alkalinity, in other words the hydrogen ion concentration, of the phosphate mixtures employed, it must be due to the properties of the salts themselves or, though this was quite outside our expectations at that time, to the carrier.

Hence, to inquire first whether the fuller's earth which served as the carrier possesses by itself the capacity of neural induction, it was kneaded into a paste with an ordinary RINGER's solution and introduced as in the previous experiments into the blastocœle of early gastrulas. The result was as follows: Out of the 35 specimens which survived, a modification on the ectoderm was observed in six embryos, in some examples of which again a differentiation of nervous tissue from the ectoderm was apparent and moreover in a case represented in fig. 7 on the plate a well-developed neural canal was found in the middle of the differentiated tissue (neural plate) covering a wide area. Thus the formation of neural plate does not arise, as we anticipated at the beginning of this series of induction tests, under the influence of this or that concentration of hydrogen ions made up by mixing two kinds of phosphates, nor under the influence of the salts themselves, but the secret lies beyond all questions in the fuller's earth which was here employed as the carrier. A new question may be raised through the result of this experiment, whether in reality the induction above described of a secondary neural plate by the use of cephalin was due, as BARTH contends, to the particular effect of that substance which was extracted from the brain. Most regrettable is the fact that he did not make the control experiment of introducing kaolin alone.

Introduction of fuller's earth of varying properties

This mineral substance, when desiccated, generously adsorbs various kinds of gases as well as moisture. The degree of adsorption varies, however, according to locality and, even if it is from the same source, to the depth at which it originates. This adsorptive capacity of the earth is also to a certain extent related to the reaction it presents for litmus solution. This is shown in the following test. Samples of fuller's earth were taken at levels of different depths, namely at intervals of one foot, in the same district (Ôsawa, Niigata Prefecture) which supplied us exclusively with the material for our study. These, dried separately and powdered, were kept overnight in a desiccator. Two grams were taken of each sample, shaken with the addition of 100 c.c. of distilled water, left to stand again overnight and then five drops of litmus or phenolphthalein solution added. Titrations were made either by N/100 NaOH or by N/100 HCl according as the reaction indicated was acid or alkaline. The results were plotted in fig. 1, which shows that the quantity of moisture adsorbed in a definite time (6 hours) is inversely proportional to the degree of acidity or alkalinity. Hence fuller's earth is most rapid in adsorbing moisture, when it is neutral in reaction.

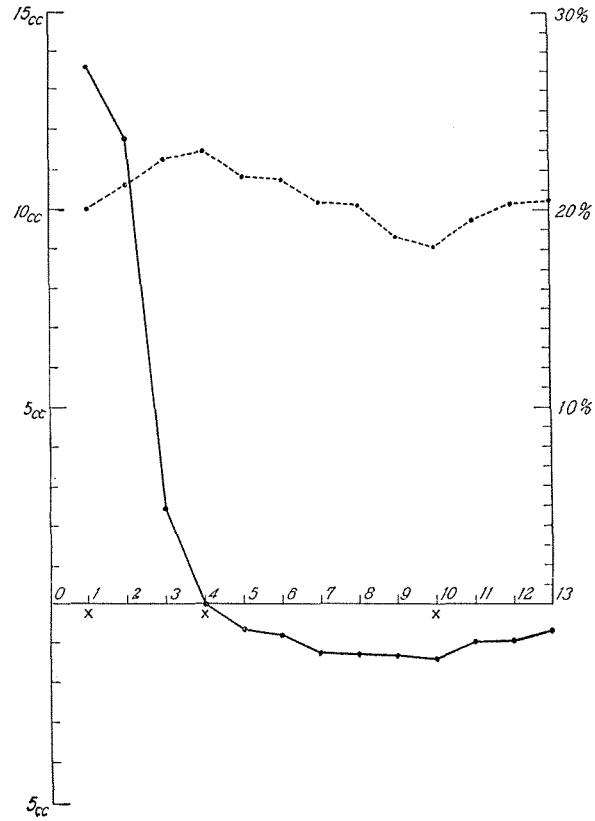

Fig. 1. Graphic representation of the relation established between the quantity of moisture adsorbed by Ôsawa fuller's earth and the degree of acidity or alkalinity of the earth at the end of 6 hours. Measured by H. Isobe (1928). *cc* in ordinate indicates quantity required of N/100 NaOH or N/100 HCl for titration, *%* adsorbed moisture in percent. Letters on co-ordinate represent depth from which the samples of the earth originate, those marked by × being used in the experiments.

After a survey of some of the properties of fuller's earth had thus been made, those portions of it were chosen, which were of the greatest acidity, of the greatest alkalinity and of neutral reaction, that is those taken at levels respectively 1, 10 and 4 feet deep. Each of these was first reduced to powder and having been kneaded into a paste in the same manner as previously described, with such a quantity of distilled water as to acquire a moderate consistency, was introduced into the blastocœle of young embryos.

Out of 114 embryos which had received introduction of fuller's earth from the depth of one foot 24 remained alive until the time of fixation. Six of them which exhibited most remarkable changes on the ectoderm were cut into sections and examined under the microscope. At the spot where the introduced material was in contact with the ectoderm a local thickening

with marked proliferation of cells was perceptible. In some specimens it was clearly differentiated into a sort of primordial nervous tissue with a comparatively regular arrangement of vertically elongated cells (see fig. 8, pl. XIX for example). Particularly in one of these cases (fig. 10, pl. XIX), at the end of a wide ectodermal thickening of this kind and in the proximity of the primary neural canal (nt_1) of the embryo, a supernumerary one (nt_2) that was fully formed could be detected. There are, however, many difficulties in considering that this mode of formation of a neural canal (and later on similar cases will also be encountered) is due exclusively to the effect of the material introduced. For structural abnormalities were evidently seen to have arisen in that region of the archenteron of embryos (*ar*) into which the foreign object was introduced.

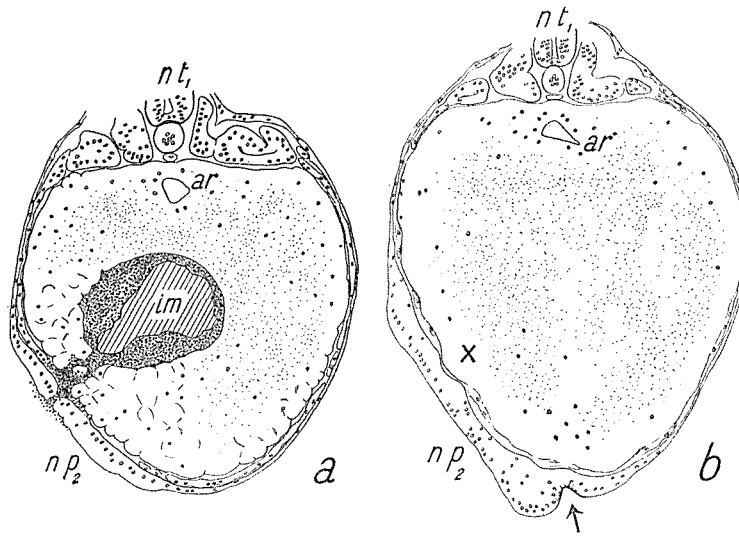

Fig. 2. Neural plate induced in *Triturus*-embryo through the introduction of neutral fuller's earth derived from the depth of 4 feet, *a* shwing the section of an embryo at the level where the implanted earth (*im*) is retained, *b* the same embryo at the level of the induced neural plate ($n\ p_2$) to the ventral side. × indicates the place where the implanted material is found nearest to it, the arrow the point where invagination takes place on the induced neural plate.

Into 209 embryos fuller's earth from the depth of 4 feet was introduced, and 49 with neural reaction survived until the time of fixation. Eight of these which underwent an especially remarkable change on the ectoderm were selected and cut into sections. Examination under the microscope revealed precisely the same results as in the previous cases of introducing acid fuller's earth derived from the depth of one foot; the region of the ectoderm which was in contact with the introduced material was considerably thickened and differentiated into something like a nervous tissue and, when

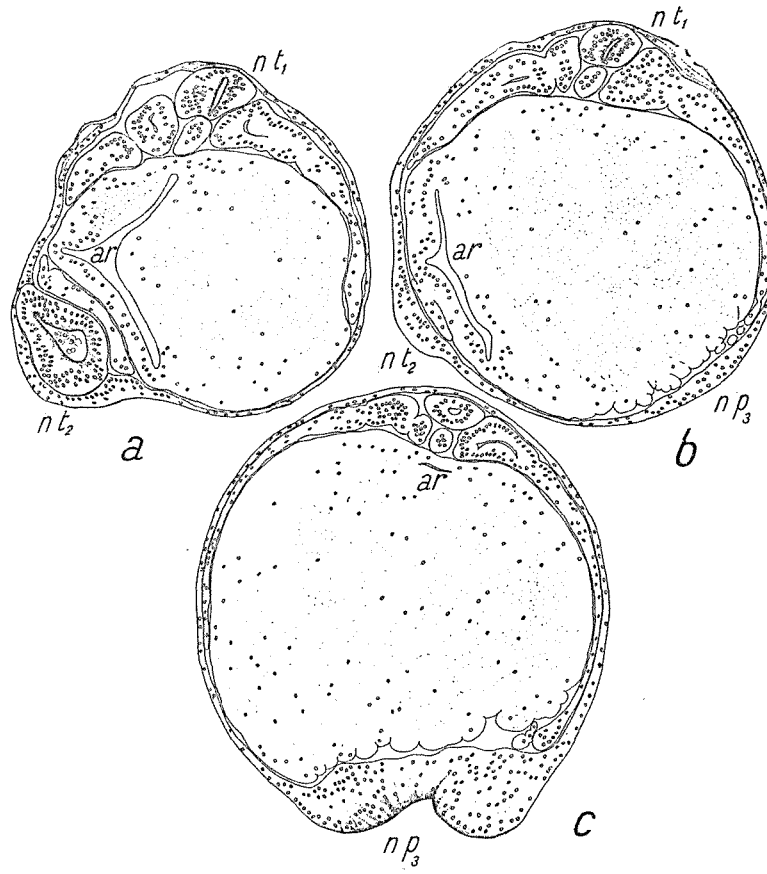

Fig. 3. Neural induction in *Triturus*-embryo through the introduction of alkaline fuller's earth derived from the depth of 10 feet. *a* showing the anterior neural induction (*n* t_2) possibly brought up by the deviated archenteron (*ar*) beneath it, *c* the posterior neural induction (*n* p_3) through the introduction of a foreign material, and *b* an intermediate zone between these two neural structures. (Fig. 3 *c* is represented by photograph in fig. 11. on the plate.)

in addition abnormalities occurred in the archenteron of the host embryo, a supernumerary fully formed neural canal made its appearance. Fig. 2 presented in the previous paper (Growth, vol. 2) was an example in which a very pronounced formation of neural plate was shown to have been induced at the same side where fuller's earth was introduced, though this substance was not represented in the figure. So the relation between the neural plate and the introduced material is illustrated here in fig. 2 in two figures drawn from two appropriate sections of the same series.

Introduction of fullre's earth with alkaline reaction derived from the depth of 10 feet, was carried out on 188 embryos, out of which 26 were

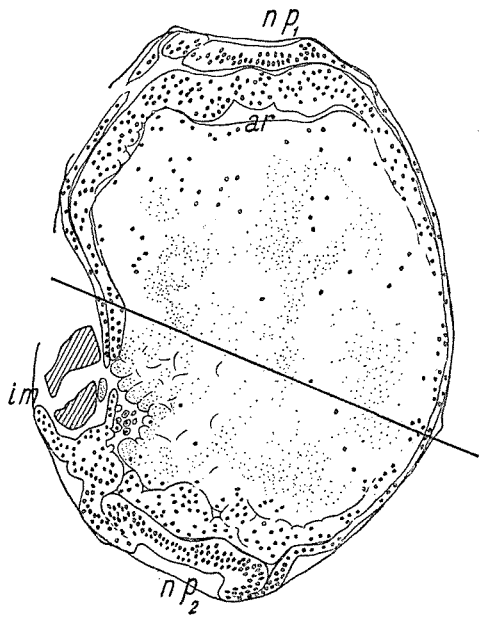

Fig. 4. Another specimen of the neural induction through the introduction of alkaline fuller's earth. The lower half of this specimen where the induced neural plate ($n\ p_2$) exists, is magnified by photograph in fig. 9 on the plate.

fixed. Five of the latter with especially marked changes on the ectoderm were investigated in sections. As a result it was found that in some of these cases the formation of a neural plate from the ectodermal thickening was the same as or more complete than in the foregoing series of experiments. In the specimens shown in figs. 3 and 4 lateral sides of the neural plate were so elevated that a central groove was formed, indicating evidently a stage in the formation of a neural canal. In fig. 4 (represented in fig. 3 in the previous occasion), however, the introduced material had been extruded out of the embryo and was enclosed in the ectodermal fold of the damaged portion. Although no great histological changes could be recognized on the ectoderm which was in immediate contact with the material, somewhat apart from that position, along the median plane and at the ventral side of the embryo, a neural plate of nearly typical character was found to be formed (see fig. 9 on the plate). The extrusion of the introduced material out of the embryo occurred probably at a comparatively later stage of development, the ectodermal region, with which the material had been in contact, having, it seems, already been influenced earlier in such a way as to undergo a change in thickness.

Thus the tests with fuller's earth, whether its reaction against litmus was acid, neutral or alkaline, all proved positive in inducing the formation of neural plate in early embryos of *Triturus pyrrhogaster*. It may therefore be concluded that fuller's earth is effective irrespective of its acidity or alkalinity or even neutrality; that the inductive power depends on some other property which is common to different varieties of the substance. However, it may be worth noting that the induction of neural plate by means of fuller's earth is, at least within the limits of our foregoing experiments, more complete by the alkaline than by the acid variety of the earth.

Now, what is here employed and known as fuller's earth is not a chemically simple substance, but represents a mixture of various kinds of minerals

yielded by weathering of rocks. According to my friend chemist Dr. H. ISOBE of the Institute of Physical and Chemical Research of Tokyo "though it resembles kalifeldspat (Si_3O_8AlK) and desmin ($Si_6O_{16}Al_2Ca_6H_2O$) in the proportion it contains of silica and aluminium both of which are considered as its essential components, those minerals are not possessed of a cubic form of crystals as fuller's earth is when the latter is heated to over 800°C. Coincidence may be found in this respect, namely in the shape of crystals, between fuller's earth and leucit (SiO_2O_2AlK), but chemical analysis reveals quite different results between the two". For the present let it suffice to say that the main constituent of the substance in question is generally considered to be a complex salt of silica containing aluminium. Hence, as the next step, tests were made for the purpose of inquiring to what extent the introduction of much simpler silica may be potent in inducing the formation of a neural plate.

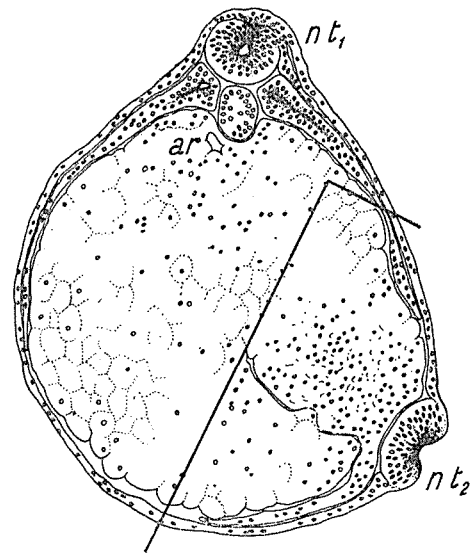

Fig. 5. Neural induction in *Triturus*-embryo through the introduction of Si_2O. The right half of the specimen which bears the induced plate almost closing to a tube ($n\,t_2$), is represented through photographic magnification in fig. 13 on the plate.

Introduction of silica

Commonly sold, powdered silica was immersed in a mixture of potassium bichromate and sulphuric acid for the purpose of purification, and then washed several days with running water, the vessel containing it being sometimes shaken, until the water in the vessel had no other reaction than original towards tests for hydrogen ion concentration and then the material was ready for use in our experiment. Operations were made on 157 embryos, out of which 69 survived until the time of fixation. Among the latter 36 presented some modification on the ectoderm. All these were cut into sections and examined under the microscope. A local thickening of the ectoderm accompanied with abnormal proliferation of cells was often found to occur at the place where the introduced material was in contact with the surface. Sometimes, histological differentiation of the tissue could evidently be identified with that of a neural plate. Furthermore, in the specimen shown in fig. 5 for example, the neural folds drew together considerably in the middle, so that they were on the point of becoming converted into a neural canal. The secondary neural plate, even in such a case, was attended towards the interior with a differentiation somewhat like the dorsal wall of the archenteron (see also fig. 13 on the plate).

Introduction of calcium carbonate

It was previously found by TANAKA (1936) that the neural induction was possible through the introduction of calcium carbonate. Induction tests essentially similar to his were repeated with the use of the same substance.

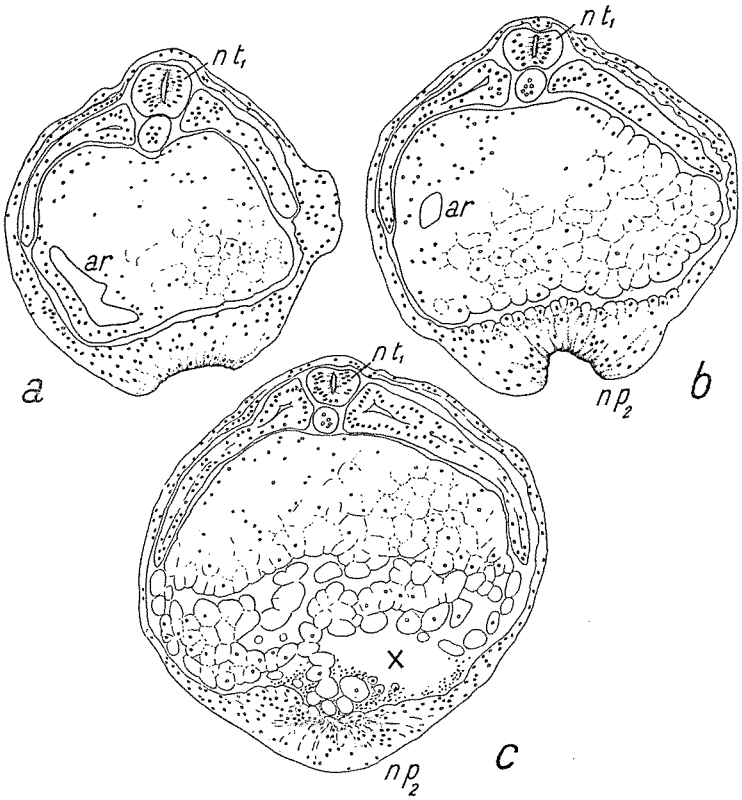

Fig. 6. Neural induction in *Triturus*-embryo through the introduction of $CaCO_3$, *a* showing the internal structure of such an embryo at the most anterior level of the induced neural plate (np_2), *b* the middle part where invagination for neural tube is most evident, *c* the posterior end of the plate, where the implanted material is found to be retained a little posterior to this level.

Here use was made of MERCK's preparation of $CaCO_3$, which was mixed with distilled water and kneaded to an appropriate consistency. 106 embryos were operated on and 30 of them fixed. 17 out of the latter with especially marked modifications on the ectoderm were cut into sections, investigation on which revealed as in the experiments above described, that the ectoderm became thickened where the introduced material was in contact with the surface, and further that the formation of a neural plate could evidently be observed in a few cases where the induction test gave especially favorable results. In the case shown in fig. 6 the neural plate had taken a step

further and become invaginated to form a groove in the middle and curved dorsally at both lateral margins, being apparently on the way towards the formation of a neural canal. TANAKA (l. c., fig. 1) reported a case in which the neural plate was completely closed and converted into a canal.

Induction by plant material

Thus it was shown that the formation of neural plate in Urodeles, the induction of which was believed hitherto only possible through the introduction of animal tissues or some kinds of organic substances, could also be induced through the introduction of inorganic or even mineral matters. Consequently, it was thought necessary, as our next step, to carry out renewed experiments on plant materials, which had been considered to be ineffective in this kind of inducing tests. A small piece of such materials as agar, boiled potato, bread, *kôya-dôfu* etc. was introduced into the blastocœle of early gastrulas. As a result, all the materials, with the exception of the last named, failed altogether to bring any positive effect, not even the change of overpigmentation being observed on the ectoderm in nearly all the cases. A similar test, it may here be noted, in which lampblack was used as the agent of induction, was attended also with negative results. Only in those cases in which a small piece of *kôya-dôfu* was introduced, modifications were recognizable on the ectoderm, which, when studied in sections, were found to be abnormal proliferation of the cells taking place at the point where the piece of *Kôya-dôfu* was in contact with the surface (figs. 15 & 16, pl. XX), and which, in some cases, could apparently be regarded as beginning of differentiation towards nervous tissue (fig. 15).

Kôya-dôfu is a very porous material made of *tôfu*, a junket-like stuff produced from bean proteins, being exposed to extreme cold, then steamed and dried. It is commonly sold in Japan as a well conservable food stuff. This material was first chosen, as already mentioned in the procedure of experiment, as a carrier of substances in liquid state. But it was later found that this material has for itself the capacity to provoke cell proliferation at that point of the ectoderm which is in contact with it. Hence, induction tests were conducted anew on a number of embryos. Of course, the material was, before use, thoroughly washed with water and boiled, furthermore, in distilled water. A piece of it was then introduced as usual into the blastocœle of early gastrulas. Operations were performed on 61 embryos, 23 of which survived several days. Marked modifications on the ectoderm were observed on 13 of these. Investigations in the sections of these specimens made it clear, as above stated, that proliferation of cells was provoked at the place where the ectoderm was in contact with the material. What is shown in fig. 16 on the plate is only one of such examples. In the case indicated in fig. 15, the ectodermal thickening is made up of cells which are grown longitudinally and in a regular arrangement, in other words, differentiated in such a way that it can be considered as nervous tissue. But so far as this specimen is concerned, it remains as a serious question whether or

not the differentiation to such an extent is really due solely to the action of the material introduced. For studies of the serial sections show us elsewhere that there is, to the interior of the ectodermal thickening, a cavity which is often continuous with the archenteron of the host embryo. So far as this is, in reality, an extension of a part of the archenteron, the formation of neural plate in this case should evidently not be attributed to the introduced material in contact with the ectoderm, but to the very influence of the dorsal wall of the archenteron, which comes to extend itself to that place of contact, or to a histological structure which is at least comparable to that.

General consideration of the results

Although, in this series of experiments, only three kinds of substances with differing properties are employed, namely fuller's earth, silica and calcium carbonate, the results obtained seem, at least, to show that the induction of neural plate in Urodelan eggs is possible through the introduction of an inorganic substance into the blastocœle. From the fact that the induction tests prove positive, though slightly different in degree, whether the fuller's earth is acid or alkaline, it is evident at least that the stimulus for neural induction has nothing to do with the hydrogen ion concentration of the material concerned. From the additional fact that the induction test proves likewise positive when fuller's earth is neutral, it follows, if the neural plate is regarded as coming into existence under the direct influence of the introduced material, that the inductive power cannot simply be due either to an acidity or alkalinity, but must be connected with the characteristics of the substances employed. It was also established in the succeeding series of experiments that induction of a neural plate, as well or even better formed than in the preceding, is possible through the introduction of SiO_2 and $CaCO_3$, both of which are practically insoluble in water, and which have nothing common in their chemical constitution. Hence, it must seriously be questioned whether neural induction in these cases was really due to the direct influence of the material introduced.

If the mineral substances named are introduced into the blastocœle, as when organic matters are employed, the formation of neural plate occurs, as a matter of course, always at or near the point of contact of the ectoderm with the introduced material. If this lies deep in the interior of an embryo, no modifications are recognized even at the point of ectoderm nearest to it. But there are two noteworthy exceptions: first, though the introduced material remains in an embryo at a considerable distance from the surface, if marked disintegration of endodermal (yolk) cells occurs around it, and a stream of their contents reaches just under the ectoderm as shown in fig. 2 a, thickening is provoked there and a kind of nervous differentiation resembling the neural plate is sometimes induced (fig. 2 b). Second the neural induction is brought about also when the introduced material does not give birth to the cellular disintegration inside of an embryo, but apparently its

presence is the reason that either a part of the archenteron is pushed away into an abnormal situation (fig. 3), or a histological structure comparable, at least, to the dorsal wall of the archenteron is called into differentiation as in the specimens of figs. 4 and 5. Especially marked in these instances is the closure of the neural plate into a canal (fig. 5 in the text and fig. 12 on the plate). Hence in the latter series of exceptional cases it is reasonable to conclude that the induction phenomena are due not to the direct influence of the material introduced but rather to the abnormalities—and especially those affecting the structure of the archenteron—provoked in an embryo by the introduction of a foreign object. From these considerations it is evident that the induction process here at work consists, in principle, of nothing more than the mechanism involved in the normal course of the formation of a neural plate through the contact of the dorsal wall of the archenteron. Regarding the case first mentioned, of the ectoderm coming into contact with the disintegration products of the endodermal cells, it must borne in mind that the endoderm which, when alive does not possess the power for induction, was shown by HOLTFRETER (1934) to be attended with positive results in induction tests after it had been killed. Hence, it may be allowable to presume that the endodermal cells also acquire similar power of neural induction when they disintegrate.

In those specimens, too, in which the introduced material lies in immediate contact with the ectoderm, more or less disintegration of the internal tissue including both ecto- and endodermal cells, is often found to have been produced at the point of contact. Since in the foregoing case, in which the introduced material remained inside of the embryo, the formation of a supernumerary neural plate was considered to be due to products of cellular disintegration, no contradictions will result, if we assume the induction here at work to be due also to the disintegration products of the tissue at the place of contact. From these points of view, what is, then, the part played in the induction phenomenon by the inorganic substances used here? May it be possible that the implant has no effect other than mechanical?

It was established by BALINSKY (1925-'31) that supernumerary limbs could be made to develop in *Triton*-embryos by a subcutaneous graft of ear-vesicle or nose-anlage (1933). The same induction was also shown to be possible by embedding a piece of celloidin (1927). It is almost impossible in this case to consider any specific effect of celloidin other than mechanical. Similarly, it is very difficult to conceive that SiO_2 or $CaCO_3$ can have any specific chemical effect on the neural induction in *Triturus*-embryos. That the introduction of mineral matters may effect neural induction, as our experiments have shown is possible, may rather be attributed to direct mechanical stimulus given to the ectoderm. Or it may be conceived as more probable that the very process of introduction of the test leads first to a destruction of the internal tissue, the disintegration products of which exert then an influence on the ectoderm at the time most favorable for the development of neural plate. If then, the formation of a neural plate is induced

through the agency, though indirect, of mechanical stimuli, what may be the cause of failure of those experiments that were conducted previously, in which starch, bread, yeast, agar, gelatine, lard, wax, coagulated egg albumin, coagulated blood of hen, powderd charcoal, etc. were introduced? The answer may be rather simple. So long as these substances themselves are not provided with a specific chemical capacity to deliver stimulus to cells, it may be supposed that their physical properties also do not possess power to cause disintegration of cells in the interior of an embryo. For even plant materials, of which the inductive power has always been denied in experiments, must be considered to be provided with it, if they are comparatively coarse like *kôya-dôfu* which may serve here as an example, to such an extent that an evocation on the part of the ectoderm to cellular proliferation can be recognized (figs. 15 & 16, pl. XX).

But we must not conclude from our foregoing simple results that the introduction conducted by HOLTFRETER and others, of pieces of various kinds of tissues derived from widely different kinds of animals, plays in much the same way as the above an indirect rôle in neural induction. It can hardly be doubted that some of them take, as these investigators state, a direct part in the formation of a neural plate from the ectoderm. But, at the same time, we cannot recognize as valid without further revision, the results as they interpreted them, to the effect that all the materials used might have in the same manner an inductive effect on the formation of a neural plate.

It was mentioned that embryonal tissues, which belong to the same species as the host, remained, when alive, without effect, but when killed by heat or with chemicals, they came to have effects upon neural induction. Whether this is due as SPEMANN (1934) supposes to the presence of an inhibitory substance in addition to the inductive one, or to the fact that the latter substance can first permeate out of the cell, when tissues are killed (HOLTFRETER, 1934), we have at present no opinion to offer. Nevertheless, it may be pointed out with regard to the latter point of view that the loss of inductive power on the part of tissues will probably be more advanced after an extraction lasting through long hours. But tissues, after being extracted, are hardly diminished in their power of induction. So at length if a somewhat bold proposal made here be allowed, it may be supposed that the introduction of the embryonal tissues of the same species of animal does probably the least harm to the host embryo, and is attended also with the least probability of inviting cellular disintegration inside of tissue. However, a piece of tissue once boiled or treated with chemicals is to be regarded as nothing but a sort of organic matter. Whether or not it can remain harmless to the host embryo as in the living condition, is an open question. Transplantations, which are made between individuals belonging to different species or with a wide difference in age, are often harmful not only to the graft but also to tissues of the host embryo. The harm which is experienced may not necessarily correspond in degree to the taxonomical relationship between the two, but the introduction, in general, of a piece of tissue per-

taining to another species of animal will probably invite great incompatibility between it and the host tissue, leading to death and disintegration of cells. In the case where an inducing substance is contained in the grafted tissue, the formation of a supernumerary neural plate is obviously to be attributed to the effect of the graft itself. In the case where the graft is not possessed of such potency, if an autolysis or necrosis on the part of the host tissue is provoked by its presence, the graft may also be said to be the cause, through indirect, of the induction of a secondary neural plate, as is the case with the introduction of inorganic substances. Therefore, it seems to be somewhat hasty to conclude that those various kinds of animal tissues and organic matters which, being introduced into the blastocœle of an embryo, are instrumental in inducing the formation of a neural plate, are all provided with the inductive power and are, hence, in possession of an inductive substance which is common to all. What is needed here is to inquire first of all in what manner the tissues play their part in the process of neural induction.

Here, attention should be called to the fact that in those specimens, in which neural induction is realized through the introduction of inorganic mineral matters, particulary in those in which a distinct neural plate is formed, a part of the archenteron of the host embryo is so extended under the mechanical effect of the introduced material that it reaches or approaches very near to the place where the graft is made, although this may not be a universal phenomenon. In the specimen represented in fig. 6, for example, formation of the secondary neural plate takes place between the ventral outgrowth of the archenteron in front and the position of the introduced material behind, but closely approximating to the latter. It will further be seen that, in the specimen represented in fig. 3 with the introduction of fuller's earth derived from the depth of 10 feet, a neural canal is induced in direct contact with a portion of the archenteron which is dislocated there (fig. 3 a), and behind it another neural plate (which is also represented in fig. 11 on the plate) makes its appearance independently of the other and far back at the position of the introduced object (fig. 3 c). Fig. 3 b shows an intermediate position between these two neural inductions. From these facts, it seems justifiable to conclude that the ectoderm activated through the introduction of a foreign object may in itself possess a faculty bordering upon neural differentiation, and can further be activated through an access of the archenteron to such an extent that it can achieve the most typical formation of a neural canal.

Resumé

1. Neural induction, which has been supposed to be exercised only through an introduction of animal tissues or particular kinds of organic substances, is shown to be realized also through an introduction of inorganic mineral matters.

2. The mineral substances which were used in the present experiments

as the inductive agents, were those of entirely differing properties; fuller's earth, silica and calcium carbonate. Practically, however, no differences in inductive power were observed among them.

3. It was shown, further, that the inductive power was independent of the hydrogen ion concentration indicated by these substances above mentioned.

4. Most of the plant materials used in induction tests for the formation of neural plate remained without effect, but those which were both coarse and hard enough to lead to destruction inside of an embryo and eventually to cellular disintegration, brought about cell proliferation of the ectoderm to a certain extent.

5. It may be concluded that the part played by such mineral or plant matters as are above mentioned in the tests for the neural induction is always effected, induction being observably realized only when the introduced material injures the internal tissue to destruction and eventually to extrusion of cellular contents. Especially, when a part of the archenteron draws up to the position where cellular destruction occurs cell proliferation on the part of the activated ectoderm proceeds to such an extent that the formation of a neural canal is the end result.

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Explanation of the Plates

Abbreviations: *ar*, archenteron; *im*, implant; *n*, cellular proliferation in connection with induction phenomena; *n* p_1, primary neural plate; *n* p_2, secondary or induced neural plate; *n* t_1, primary neural canal; *n* t_2, secondary or induced neural canal. × indicates the place where implanted material is to be found or the place nearest to it.

Fig. 7. A broad neural plate (*n* p_2) with a structure like neural canal (*n* t_2) in the middle and beneath it, is induced through the introduction of fuller's earth kneaded simply with RINGER's salt solution.

Fig. 8. Neural evocation of the ectoderm (*n*) through the introduction of acid fuller's earth (*im*).

Fig. 9. Lower half of the same specimen as shown in fig. 4 in the text in higher magnification.

Fig. 10. Neural induction by alkaline fuller's earth. Here also as in fig. 7 a broad neural plate (*n* p_2) is induced and a tubular formation (*n* t_2) occurs to the side of the primary neural canal (*n* t_1) of the host embryo. The archenteron (*ar*) is much deformed.

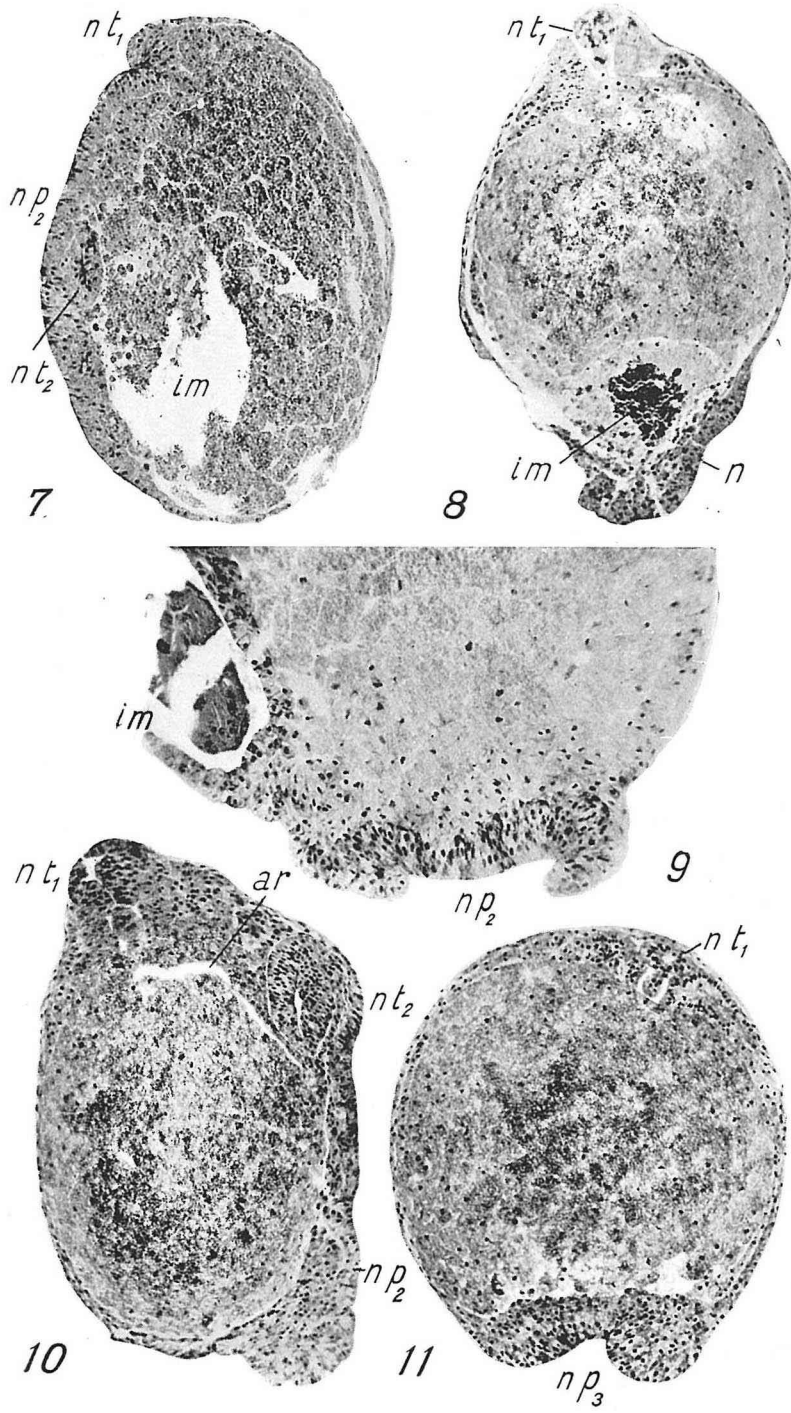
Fig. 11. Neural plate (*n* p_2) induced through the introduction of fuller's earth of the same quality as used in the specimen of fig. 10. The photographic representation of the same section as shown in fig. 3*c* in the text.

Fig. 12. Photographic representation of the right half of the same specimen as shown in fig. 5 in the text. The induced neural plate (*n* t_2) is much magnified.

Fig. 13. Neural induction by $CaCO_3$; the same as shown in fig. 6*b* in the text.

Fig. 14. Another specimen of the same induction by $CaCO_3$; here the induction (*n* p_2) is less perfect than in the preceding figure.

Figs. 15-16. Results of introduction of a plant material, *Kôya-dôfu* into the blastocœle of *Triturus*-gastrulas. *im*, implanted material. *n*, cellular proliferation of the evocated ectoderm.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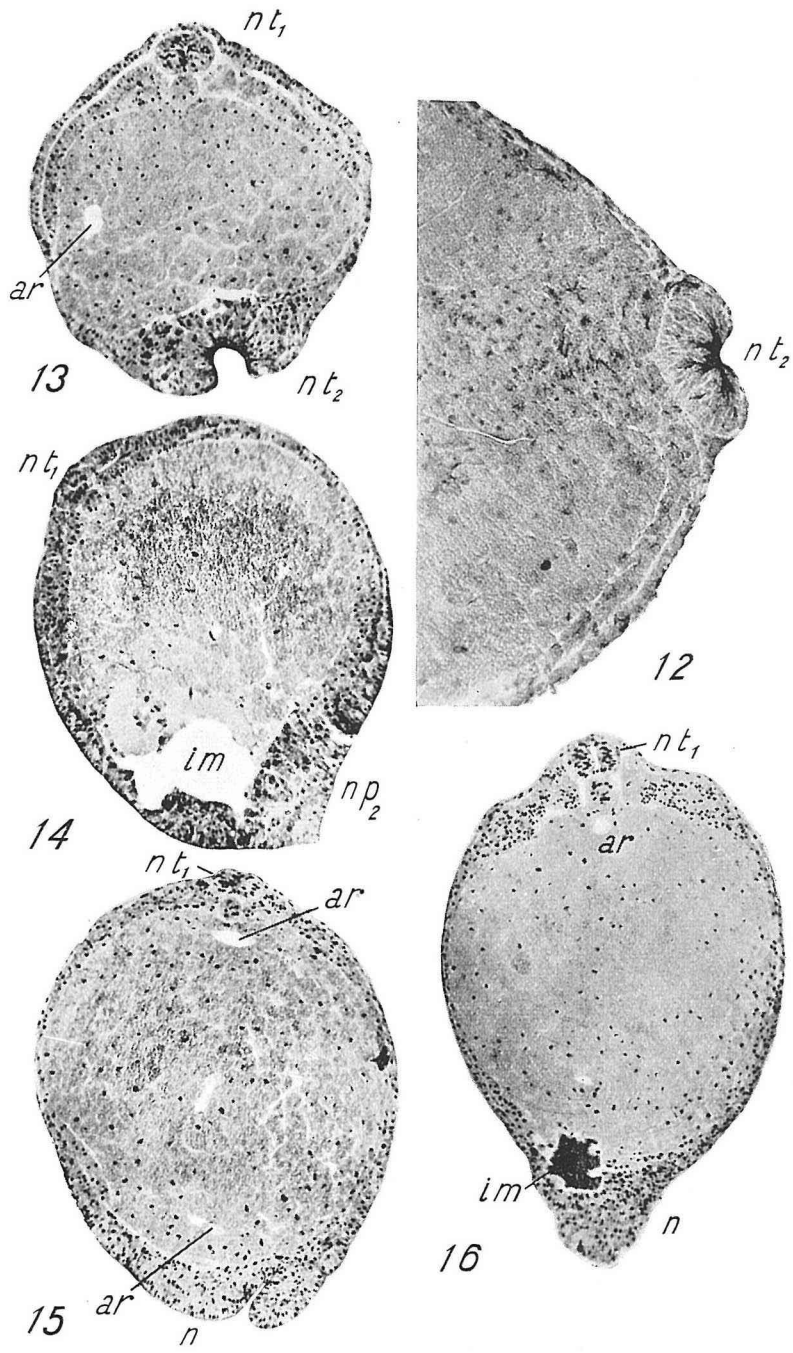
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