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Effect of Protein Extracts of Neural Plate Plus Chordamesoderm on Presumptive Epidermis.

L. G. Barth and Samuel Graff.

From the Zoology Department and the Departments of Biochemistry and of Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia University.

The purpose of these investigations was to test crude protein extracts of the neural plate plus chordamesoderm for ability to induce neural tubes in the presumptive epidermis of three species of Amblystoma. The work was undertaken because the neural inductions obtained by protein residues after ether extraction, as described by Holtfreter,[1] were larger and better organized than those obtained with the ether soluble fractions as observed by Needham, Waddington, and Needham[2] or Barth.[3]

Extraction methods. One ml of neural plates plus chordamesoderm was extracted with 11 ml of 5% NaCl for 24 hours at 5°C. Stage 15 (Harrison stages) of *Amblystoma punctatum* was used for donors. The extract was then filtered through a Seitz filter and treated in 2 ways before using. Some was diluted with Holtfreter's solution which caused a precipitate to form. This precipitate with the solution was injected into the blastocoel of stage 10 (Harrison stages) by means of a micropipette. Another portion of the extract was dialized at 5°C and the resultant precipitate was injected.

The residue after extraction with NaCl was then further extracted with 0.05 N NaOH at 5°C and passed through a Seitz filter. Acetic acid was added to precipitate the proteins and this precipitate was injected using a micropipette or implanted by means of a forceps into the blastocoel.

The chloroform extract was made by shaking ground-up neural plate plus chordamesoderm with chloroform until a chloroform-protein gel was obtained. This gel was separated by centrifuging and the chloroform removed at 5°C. The residue was implanted into the blastocoel by means of forceps.

Buffer extracts were made with phosphate citric acid buffers at pH 7 by freezing and thawing tissues in centrifuge tubes. After centrifuging the clear layer of buffer solution was removed and injected.

Material. The presumptive epidermis of the gastrulæ stage 10 and 11 of *Amblystoma punctatum, A. opacum,* and *A. mexicanum* was used to test the inducing ability of the extracts. The proteins were usually precipitated from the extracts and the precipitate was then drawn up into a glass pipette controlled by means of a micromanipulator. The pipette was inserted through the vitelline membrane and into the blastocoel and a measured amount injected. The reactions of the presumptive epidermis were classified as (1) induction of a neural tube and (2) induction of aggregations of neural cells. Fig. 1, A, B, C, illustrates the variation in the neural tubes which were induced by extracts.

Results. Table I compares the effects of 5% NaCl extracts, 0.05 N NaOH extract, chloroform extract, and neutral buffer extract of the neural plate plus chordamesoderm. It is clear that only the NaOH extract induces neural tubes in appreciable numbers. NaCl extracts induced only one clear neural tube and 3 neural aggregations. The chloroform extract induced no neural tubes but consistently induced large masses of epidermal cells to aggregate around the implant forming a large growth on the side of the embryo. Buffer extract gave no consistent reaction except that it tended to produce cytolysis in a number of cases.

Comparison of extracts of anterior with posterior neural plate plus chordamesoderm. For this comparison ten embryos in early neural

[1] Holtfreter, J., *Arch. Entw.-mech.*, 1934, **132**, 226.

[2] Needham, J., Waddington, C. H., and Needham, D. M., *Proc. Roy. Soc. London B*, 1934, **114**, 393.

[3] Barth, L. G., *Biol. Bull.*, 1934, **67**, 244.

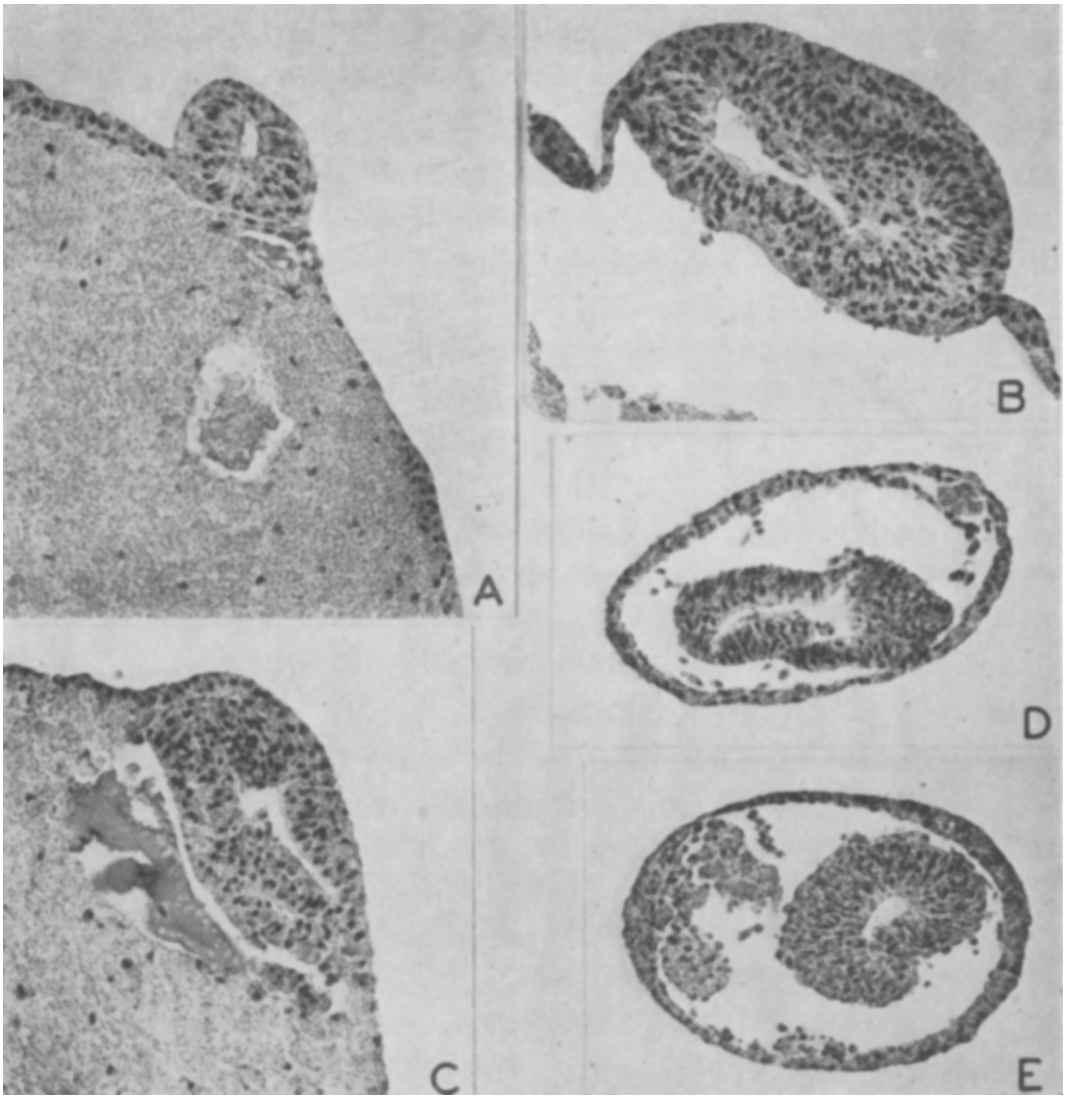

FIG. 1.
A, small neural tube induced in the ventral epidermis of the whole embryo by protein extract; B, C, large neural structures induced by protein extracts. D, E, neural structures induced in explants of the presumptive epidermis by frozen-dried preparations of the organizer.

TABLE I.
Comparison of Crude Protein Extracts.

Extract	No. cases	Neural tube	Neural aggregate
5% NaCl (diluted)	69	1	0
5% " (dialized)	35	0	3
0.05 N NaOH	69	26	9
Chloroform	48	0	1
Buffer pH 7	26	0	0

plate stage were prepared by removing the membranes and cutting out the neural plate together with the adhering roof of archenteron. These preparations were then divided into equal thirds by transverse cuts, allowing for the difference in width. The anterior and posterior thirds were used for extraction while the middle third was discarded.

The anterior and posterior thirds were then treated in exactly the same way. The water was pipetted off and 65 mm^3 of .05 N NaOH added and immediately frozen in a mixture of ether and solid CO_2. While still frozen the

tissue was ground up by means of a steel drill. The preparation was allowed to thaw and the process of freezing and grinding repeated. After thawing the preparation was centrifuged for 2 minutes at 10,000 R.P.M. This gave a layer of fat on the top and a layer of yolk granules and pigment on the bottom of the tube with a clear solution between. The clear fluid was pipetted off and the proteins precipitated with 65 mm^3 of .05 N acetic acid. If the drop of acetic acid is added gently without stirring, a firm precipitate is obtained which can be cut into fragments, picked up with a forceps and inserted into the blastocoel. After the precipitate formed, a few drops of Holtfreter's solution was added and the resultant pH was measured by the Beckman microglass electrode assembly. The pH was varied by addition of .05 N acid or alkali. The amount of precipitate which was implanted varied but averaged about 2 μg dry weight per embryo.

TABLE II.

Comparison of Extracts of the Anterior with Posterior Neural Plate Plus Chordamesoderm.

	pH	No. cases	Neural tubes	Neural aggregates
Anterior	6.5-7.0	30	7	4
Posterior	5.5-6.5	29	6	1
Anterior	7.5-8.0	40	17	1
Posterior	7.5-8.0	29	19	1

Results are given in Table II. Experiments in which the pH after precipitation with acetic acid was brought to 7.5 or 8.0 gave the best results. Observation of the living neurulæ showed small neural plates which rolled into tubes in the normal fashion. In some cases the injection mass broke up into 3 fragments and 3 small plates could be seen to fold into small tubes. The sections were studied carefully to see if there were any differences in the kind of neural tube induced by anterior as compared with posterior extracts and no differences could be detected. Both anterior and posterior extracts induced small neural tubes as in Fig. 1, A, and also large brain-like tubes as in 1, B. 1, C, shows clearly the protein precipitate adjacent to the induced neural tube. All of these tubes are completely independent of the normal primary neural tube which is not shown in the figure.

The effect of frozen dried preparations of the neural plate plus chordamesoderm on presumptive epidermis. In order to test the proteins in as nearly a native state as possible frozen dried preparations of the neural plate plus chordamesoderm were made by freezing neurulæ stage 15 (Harrison stages) in tubes immersed in ether-solid CO_2 mixtures followed by desiccation at low pressures. These dead dried neurulæ were very well preserved showing little or no shrinkage and the neural plate plus chordamesoderm was dissected out and applied to the inner (blastocoel) surface of explants of the normal presumptive epidermis of stage 10-11. The edges of the explant curl forming a cup-shaped preparation with the dried tissue on the inside.

Table III records the results of a comparison of the action of anterior and posterior dried preparations of the neural plate plus chordamesoderm and dried epidermis. Explants of the living presumptive epidermis having had no treatment with dried tissue were used as controls. Both anterior and posterior dried preparations induce neural tubes Fig. 1, D, E, and a careful study of the sections revealed no differences in the structure of the induced neural tubes. Dried epidermis also induced neural tubes which did not differ from the above. Controls with no treatment formed some neural tubes and this has been reported in detail by Barth.[4] Quantitatively the posterior neural plate plus chordamesoderm induced the highest percentage of neural tubes (31%), the epidermis was next with 26%, the anterior neural plate plus chordamesoderm gave 18% induction while the non-treated controls formed neural tubes in 13% of the cases.

TABLE III.

Reaction of Explants of the Presumptive Epidermis to Frozen Dried Tissues of the Neurula.

Tissue	No. cases	Neural tube	Neural aggregate
Anterior	37	7	2
Posterior	32	10	3
Epidermis	15	4	4
No tissue	38	5	1

[4] Barth, L. G., *J. Exp. Zool.*, 1941, **87**, 371.

Summary. Protein extracts of the neural plate plus underlying chordamesoderm induce the presumptive epidermis of the gastrula to form neural tubes. However no differences were obtained in the structure of the neural tubes which were induced by extracts of anterior as compared with posterior regions of the neural plate and underlying chordamesoderm. Preparations of the anterior and posterior regions of the neural plate and underlying chordamesoderm which were killed by freezing and subsequent drying induced neural tubes in the explanted presumptive epidermis of the gastrula. Quantitative differences in the frequency of induction were obtained with the posterior neural plate and underlying chordamesoderm inducing with the greatest frequency.

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Further Report on Malignancy Observed in Rats Injected with Crude Ether-Extracted Wheat Germ Oil.

LEONARD G. ROWNTREE AND WILLIAM M. ZIEGLER.

From the Philadelphia Institute for Medical Research, Philadelphia General Hospital.

In previous papers from this laboratory[1-3] the presence of a carcinogenic substance has been demonstrated in crude ether-extracted wheat germ oil. An interesting observation is that some of these tumors developed from feeding this oil, either by a forced feeding method or by mixing the oil with the diet. However, some were also produced by intraperitoneal injection of the oil. Since that time, several investigators[4-9] have attempted to produce neoplasms by the feeding of similar oils, but all have been unsuccessful. However, nothing has been further reported upon the production of malignant tumors by injection of a crude wheat germ oil.

In the past 3 years our attempts to produce tumors by feeding crude ether-extracted wheat germ oil have, as with other investigators, resulted in failure. On the other hand, by injection we have been able to produce a substantial number of tumors with some of the oils available, a fact which has been confirmed by the similar results of another investigator.[10]

Materials and Methods. Four strains of rats, Wistar, Buffalo, a hooded variety of our own breeding, and a strain of Wistar rat supplied by Eli Lilly and Company were used. Four different oils were used also. One was a percolated oil with the sediment removed, supplied by Eli Lilly and Company. This oil is identified by the letter L in the table. The 3 other oils were prepared by us by a Soxhlet type of extraction, one for 8 hours (8E), one for 12 hours (12E), and one for 24 hours (24 Ma). These were used as prepared, the sediment shaken each time before use, except that in one sample of the 24-hour oil the sediment was removed before use (24 MaC). Two series of control oils were also used. One was a purified expressed wheat germ oil supplied by Eli Lilly and Company. The other was sesame oil, divided into 3 parts, one part of which was used plain, another contained 2% of starch in suspension, and a third con-

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