ON THE DIALYSABILITY OF THE GROWTH-ACTIVATING PRINCIPLE CONTAINED IN EXTRACTS OF EMBRYONIC TISSUES.

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Since it was first observed by Carrel and Burrows (1) in 1911 that extracts made from embryonic tissues had a great stimulating action upon the growth of cells under artificial cultivation, very little study has been devoted to the investigation of the nature of the substance responsible for this activity. Burrows has found evidence to show that this substance is formed by cells in proportion to the oxygen absorbed by them, and has termed it archusia (S) (2). In his recent book, Fischer (3), in addition to summarizing what is known regarding its physicochemical properties, describes a series of experiments with it which he undertook but from which he obtained very indefinite results. In fact it still remained obscure whether the so called activating substance was of a crystalloidal or colloidal nature. Consequently, with this in view it was decided to examine an active embryonic tissue extract by a process of dialysis in order to determine whether the active molecules concerned are capable of diffusing through a collodion membrane impermeable to proteins. Certain preliminary experiments on these lines which suggested that this might be so were carried out previously (4).

Method.

The method for the estimation of growth is essentially that described by Wright (4), though it was varied in some details.

The technique used was the ordinary hanging drop culture in a hollow ground slide. The cells used in the experiments were emigrant cells from the hearts of

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embryo chicks of between 8 and 11 days of incubation. Ebeling has found that these cells are second only to a permanently cultivated strain of fibroblasts in the uniformity of their rate of multiplication.

The medium used was obtained by mixing fowl plasma with an equal volume of a buffered saline solution[1] which was slightly modified from the one devised by Pannett and Compton (5).

For each series of experiments some twenty to thirty cultures of heart muscle were set up in this medium and were so cultivated for about 48 hours. At the end of this time they were examined microscopically, and those showing good halos of cells emigrating from the implanted fragment were selected and set aside for recultivation, the remainder being rejected.

In this way a number of cultures were obtained which possessed roughly the same number of emigrant cells and therefore were potentially equal from the point of view of subsequent growth. These selected slides were then recultured by the addition to each culture of a small droplet of the medium under examination. This droplet, which was roughly equal in volume to that of the plasma originally used to make the culture, was carefully spread by the aid of an iridectomy knife in a thin uniform layer over its surface. These slides were then reincubated for a further 18 to 20 hours, at the end of which time they were rapidly washed in saline and fixed in Bouin's solution. After staining in hematoxylin and mounting in balsam the preparations were examined under an oil immersion lens and the number of mitoses in each culture counted. In this way the extent of growth in each type of medium was determined.

The growth-stimulating media were obtained in the following way. Under sterile precautions chick embryos were removed from their shells, placed in Petri dishes, and there finely minced with scissors. The resulting pulp was transferred to a centrifuge tube and rotated rapidly for several minutes. The supernatant fluid was then removed with a pipette and diluted in the desired proportion with the saline solution previously described.

Dialysis was carried out through collodion membranes which were made with attention to the points emphasized by Gates (6). The collodion solution was

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[1] The preparation of the saline used was as follows:

Solution A.	NaCl	8.00	per cent.
	KCl	0.42	" "
	$CaCl_2$	0.20	" "
Solution B.	$Na_2HPO_412H_2O$	0.43	" "
	$NaH_2PO_4H_2O$	0.043	" "

8 cc. of Solution A were added to 88 cc. of freshly distilled water in an Ehrlenmeyer flask. 4 cc. of Solution B were measured into a second flask. After sterilization of both in the autoclave, Solution B was added to A with a sterile pipette. This constitutes the solution used. Its pH varied from 7.5 to 7.7.

obtained by dissolving 8 gm. of collodion in a mixture of 50 cc. each of ether and absolute alcohol. The actual membrane was prepared in the interior of a 100 cc. beaker, and after being allowed to drain for about 1 minute the beaker was filled with 95 per cent alcohol. After about 10 minutes this was replaced by water. The membrane, which then came free, was removed from the beaker and allowed to stand in distilled water.

For the process of dialysation one of these membranes was mounted on a glass ring about 4 cm. in diameter and 2½ cm. deep, being held in place by a rubber band. Actual dialysis took place in a small glass crystallizing dish about 4½ cm. in diameter, the ring and membrane being supported above the floor of the dish by a bent glass rod. The apparatus, set up in this form in a suitable protective container and containing a small quantity of distilled water, was then sterilized by autoclaving.

The extract used in the experiments was placed in the outer crystallizing dish so that should small tissue fragments be contained in it they should not fall upon the membrane and so mechanically impair diffusion. Dialysis was in every case carried out against the saline solution already described.

In these experiments three media had their growth-stimulating activity assessed: the *saline;* the *extract,* from the under side of the membrane, after dialysis had taken place; and the saline solution from the upper side of the membrane which consequently contains whatever substances have passed through it. This latter solution I shall refer to subsequently as the *diffusate.*

The biuret reaction was used to test for the presence or absence of proteins in the diffusate.

EXPERIMENTAL RESULTS.

Experiment 1.—The embryonic tissue extract was made from five 8 day old embryos and after preparation was diluted with saline to 40 per cent. 3 cc. of diluted extract were dialysed against 3 cc. of the saline for 72 hours. The biuret reaction was *negative.* The cells used were from a 9 day embryo.

No. of Mitotic Figures in Cultures.

Saline.	Extract.	Diffusate.
12	31	28
12	34	15
11	16	42
1	41	18
4	21	67
0	67	24
Average.....7±2	35±5	32±5

Experiment 2.—The embryonic tissue extract was made from five 10 day old embryos and after preparation was diluted with saline to 40 per cent. 6 cc. of diluted extract were dialysed against 6 cc. of the saline for 72 hours. The biuret reaction was *negative*. The cells used were from a 9 day embryo.

No. of Mitotic Figures in Cultures.

Saline.	Extract.	Diffusate.
Average.....2	24	26

Experiment 3.—The embryonic tissue extract was made from three 10 day old embryos and after preparation was diluted with saline to 50 per cent. 4 cc. of the diluted extract were dialysed against 4 cc. of the saline for 72 hours. The biuret reaction was *negative*. The cells used were from an 11 day embryo.

No. of Mitotic Figures in Cultures.

Saline.	Diffusate.
Average.....2±1	62±9

In this experiment a comparison was only made between saline and diffusate.

CONCLUSIONS.

These experiments appear to indicate that whatever substance in embryonic tissue extracts excites mitosis, and therefore would appear to be the growth-stimulating substance, is capable of passing through a collodion membrane which is impermeable to proteins, at all events in such concentration as would give rise to any biuret reaction. The substance is therefore diffusible.

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