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Further Experiments on the Transplantation of Neural Crest (Mesectoderm) in Amphibians.

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In previous experiments[1] the author has shown that when mesectoderm was excised in the cranial regions of early stages of embryos of *Amblystoma punctatum* deficiencies were produced in the branchial skeleton, Meckel's cartilage, palatoquadrate bar, anterior trabeculae and loose mesenchyme in the head. It was further found[2] that when groups of mesectoderm cells were transplanted to new positions on the side of the body they differentiated into cartilages in the presence of the strange mesentoderm (mesoderm) of the somites and in the absence of mesentoderm of the visceral arches.

A similar study has been extended to the Anurans. In *Rana palustris* the author reported[3] a migration of bands of mesectoderm over the mesentoderm of the visceral arches from the dorsal part of the cranial portion of the neural folds similar to the condition found in Amblystoma. The migration begins before the closure of the neural folds—an earlier stage than in Amblystoma. It was found in those studies[3] in *Rana* that when the mesectoderm was removed deficiencies were produced in the visceral skeleton on the operated side.

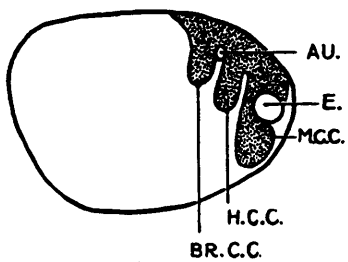

FIG. 1.

Camera-lucida drawing of *Rana palustris* embryo just before closure of neural folds showing extent of migration of mandibular (M. C. C.), hyoid (H. C. C.), and branchial (BR. C. C.) mesectoderm (stippled) at time of operation. AU., auditory placode. E., optic vesicle. X 15.

Further tests of the ability of the mesectoderm to form cartilages in the anurans have been made by a series of transplantation experiments upon early stages of *Rana palustris* in which one or more bands of these cells have been placed in new positions on the side of the body. The relative growth of the bands of mesectoderm at the time of operation is shown in Fig. 1. This is just before the closure of the neural folds and seems to be the most feasible time for removing the mesectoderm for transplantation. Very soon after this stage the mesectoderm over the branchial region splits into 4 bands, 1 for each branchial arch. Then the mesectoderm rapidly migrates further ventrally upon the surface of the visceral arches and soon takes a position upon their medial surfaces where it forms cartilage.

A large area of ectoderm over the branchial and mandibular regions was carefully excised so as to expose intact the bands of mesectoderm (neural crest) as they lie upon the side of the brain and upon the mesentoderm of the visceral arches. These bands are firm and compact and are easily lifted from the underlying mesentoderm of the arches as finger-like processes. One or two of these bands of mesectoderm were transplanted at one time and only that part of the band which had reached the mesentoderm of the visceral arches was grafted. Care was always taken not to remove with it the cells of the mesentoderm which are quite white in contrast to the grayish-brown mesectoderm cells. A wound previously prepared in the side of the body of another embryo at about the same stage of development received the transplant. In most cases this

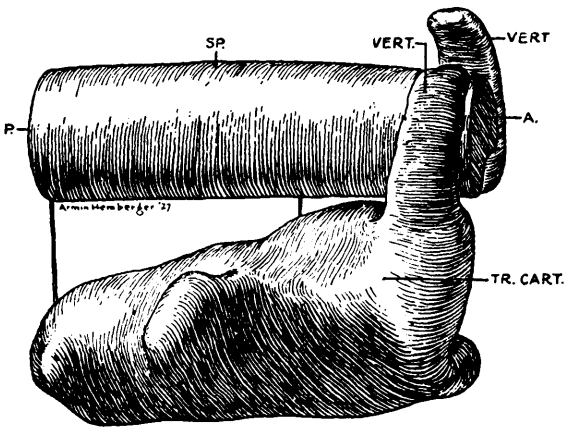

FIG. 2.

Showing from right side a wax reconstruction of a large cartilage (TR. CART.) developed from transplanted mandibular mesectoderm. It is flattended against side of notochord. The right vertebra (VERT.) is fused with its anterodorsal border. SP., spinal cord; A., anterior end; P., posterior end. X 100.

wound was prepared by excising most of the lateral and ventral portions of two or three somites and the overlying ectoderm. In a few cases a site much more ventral to the somites was selected. Into the wound was laid one or two bands of mesectoderm, oriented so that the long axes of the bands were parallel with the long axis of the body of the recipient of the graft. The wound was covered by a piece of ectoderm taken from the belly region of the donor of the transplant. This was allowed to heal in place in the usual manner.[1]

Most of the specimens were killed about 18 days after operation when cartilaginous tissue was well differentiated. Many specimens when sectioned revealed from 1 to 4 cartilages in the area of the transplant. These cartilages vary in size from pieces consisting of only a few cells to bars of cartilage over 500 microns in length (Fig. 2). They are in most cases elongated bars and usually their orientation corresponds to that of the bands of mesectoderm at the time of operation. The position of some of the cartilages and their occasional small size indicates that frequently the bands of mesectoderm cells were disoriented and sometimes broken up at the time of the operation when the glass weights were laid upon the transplant to hold it in position while healing took place.

Some of the cartilages of the transplant lie in loose connective tissue between the ventral portion of the somites and skin (Fig. 3). Others are partially imbedded in portions of the injured somites in the area of operation while many lie near or against the lateral surface of the notochord. The hyoid band of mesectoderm in its normal environment forms a large cartilaginous bar. In the cases

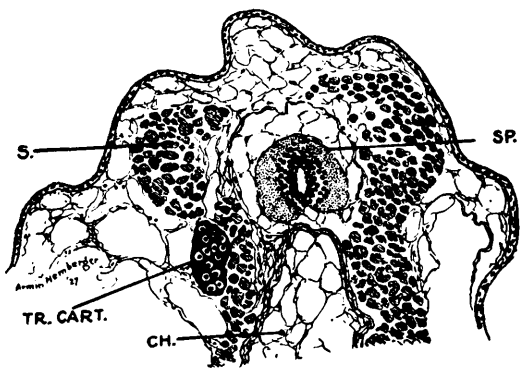

FIG. 3.

Showing a portion of a transverse section passing through one of four cartilages (TR. CART.) developed from grafted hyoid and branchial mesectoderm. It lies lateral to muscle of the injured somite (S.) deep under the skin. SP., spinal cord; CH., notochord. X 66.

to iodide and chloride in the form of their potassium salts, and made acid to a pH of 2.2 with equal amounts of 0.1 normal HI and HCl, equal molecular amounts of iodide and chloride were found in the gelatin solution after equilibration.

A series of 10 per cent gelatin solutions was made to contain

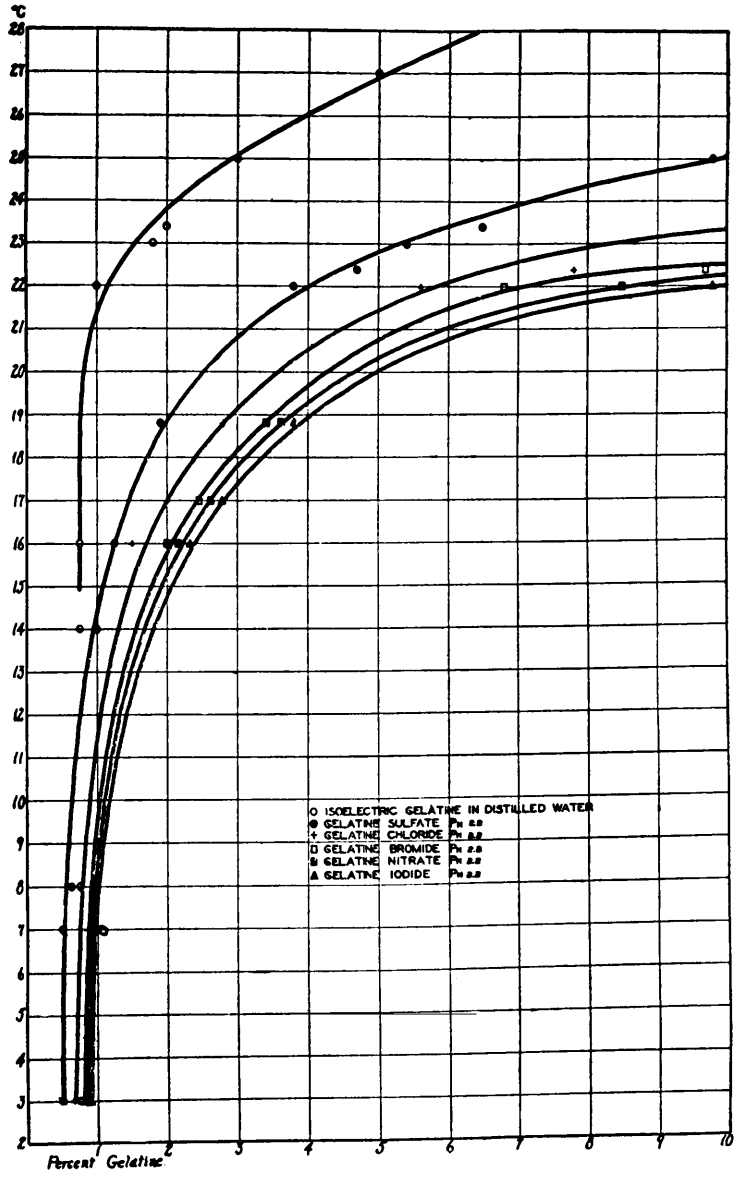

CHART 1.

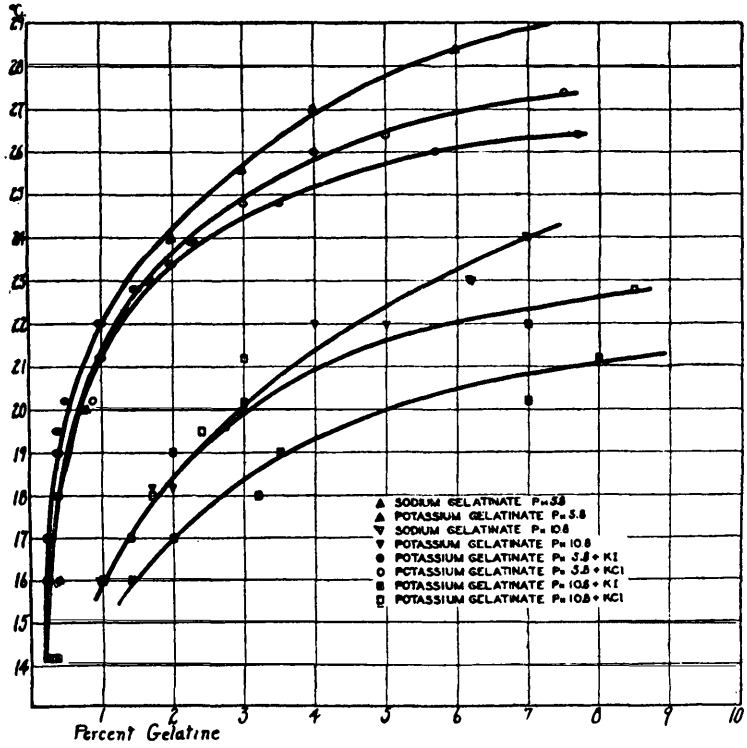

CHART 2.

sufficient HI, HCl, HBr, HNO_3, or H_2SO_4 to make the pH of each solution 2.2. For each of these gelatin solutions a series of 10 test tubes was assembled. Varying amounts of the gelatin solution were placed in the tubes, together with such varying amounts of the corresponding acid solution of pH 2.2 so that the final volume and pH were respectively 5.0 cc. and 2.2 for each tube. Thus five series of different gelatin-acid solutions all of the same pH, but with varying concentrations of gelatin were obtained. These tubes were placed in a thermostat controlled water bath for 24 hours at each temperature, starting at 37° C. In Chart I are shown curves based upon the greatest percentage of gelatin remaining in a fluid state at any given temperature, compared with ash free gelatin in distilled water.

Chart 2 shows similar curves obtained with KOH and NaOH solutions of pH 5.8 and 10.8 and with the additions of KI and KCl in amounts equivalent to the amounts of I^- and Cl^- ions in the series of tubes from which the data for Chart 1 are taken.

These experiments, though preliminary and not at present sub-

allotment of the oil mixture was increased to 21 drops, which represented 6 drops or 0.2 grams of butter fat. These animals also failed to grow and the mineral oil was again removed and growth was resumed. (See Curve III.)

The fourth group was given the same treatment, using the oil mixture which contained daily additions of .1333 grams of butter fat. These animals were allowed to remain on experiment for 56 days, while the mineral oil was alternately added and removed at stated intervals. The growth curves of the animals rose and fell with the omission and addition of mineral oil. (See Curve IV). To conserve space only representative curves are given.

It is quite evident from the results obtained that mineral oils possess the property of dissolving vitamin A from food materials, thereby depriving the body of this vitamin, even though vitamin A be present in excess of the body needs.

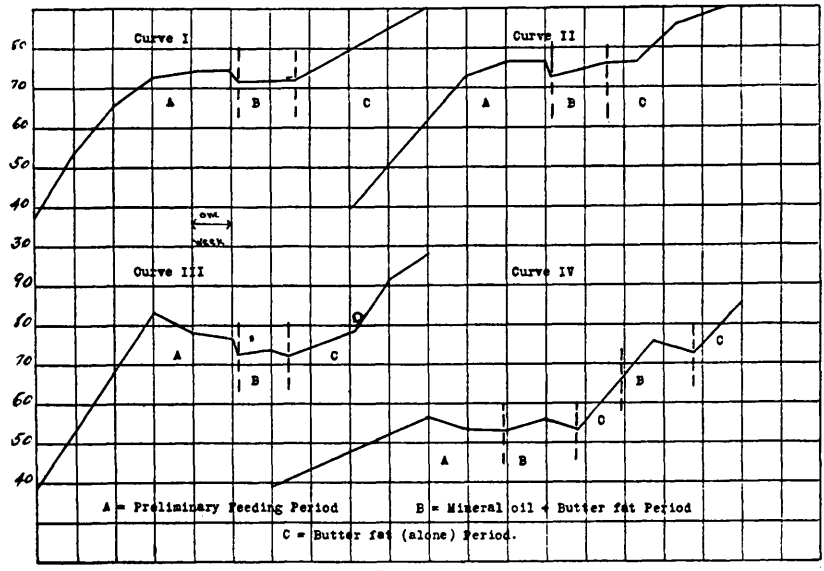

CHART 1.

The fifth group, consisting of 10 rats, was placed on experiment to study the calcifying potency of cod liver oil when fed in the presence of mineral oil. The same technique was followed as described in a previous paper[2] using the Steenbock yellow corn ration.

A mineral oil-cod liver oil mixture was prepared of 3.56 parts (by weight) of mineral oil to 1 part of cod liver oil. Previous experiments with this sample of cod liver oil (4 drops daily) had

yielded 41.5 per cent of (femur) bone ash after the animals had subsisted on the Steenbock ration for 21 days. In the study of the effect of mineral oil the animals received daily dosages of 15 drops of the mixture which contained 4 drops of cod liver oil weighing .1180 grams.

When the rats had subsisted for 21 days on the Steenbock ration, and the daily allotment of 15 drops of oil mixture, all animals were chloroformed and the femur bones were dried, extracted with alcohol and ether and ashed. The average percentage of ash obtained was 40.80, which approximates the results we obtain when cod liver oil is fed alone. Line tests were performed on all of the animals and the pictures indicated a degree of calcification identical with that obtained when cod liver oil is fed in the absence of mineral oil, thereby substantiating the results obtained by the ash method.

It is clear that mineral oil may act as a solvent for vitamin A, thereby depleting the ingested foods of their supply of this vitamin, but we have obtained no evidence to show that the calcifying potency of cod liver oil is appreciably affected by the presence of mineral oil. It is entirely possible that increased amounts of mineral oil might show a solvent effect on the antirachitic factor.

Work is now in progress to study the entire matter in greater detail, and with particular reference to the influence of mineral oil upon the digestion of fats and oils. We are also planning to study the effect of diets rich in vitamin deficient fats with the view of determining whether or not undigested glycerides may not have a solvent action on the fat-soluble vitamins in the food and body tissues.

Note: After this paper had been prepared and the work announced at the Rochester meeting of the Federated Societies for Experimental Biology (April 11-16, 1927), the work of Burrows, M. T., and Farr, W. K., appeared in this PROCEEDINGS (xxiv, 719, April, 1927), substantiating the findings described in this paper.

This is a preliminary report.

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[2] Dutcher, R. A., Creighton, M., and Rothrock, H., *J. Biol. Chem.*, 1925, lxvi, 401.