another dominant site of $\varkappa$-chain accumulation except for nuclear pores (Figure 2).

Ribosomes in direct relation with nuclear membrane were often stained with the same enzyme label. Sparse and poorly developed Golgi complex was always unresponsive either to peroxidase-conjugated anti-α or anti-$\varkappa$ antibody.

SCHAPIRO et al.[9] reported that polysomes are the active unit of gamma globulin synthesis in 2 transplantable murine plasma cell tumors, and heavy and light polypeptide chain molecules are formed separately on polysomes of different size, i.e., on 270s and 190s polysomes, respectively. WILLIAMSON and ASCONAS[10] obtained similar observations in their ^{14}C-amino acid incorporation experiment using ascitic murine plasma cell tumors.

We could not determine so far whether observed differential localization of heavy and light polypeptide chain, in our culture line revealed by electron microscopy, is the morphological counterpart of biochemical results by those authors, or whether this phenomenon is an inherent property of culture cells.

Zusammenfassung. In langfristiger Kultur wurde mit peroxidasekonjugiertem Antikörper (monospezifisch gegen H- und L-Kette) die submikroskopische Lokalisation der Immunoglobulin H- und L-Kette in den menschlichen Lymphoblastoidzellen an das endoplasmatische Retikulum gebundene beziehungsweise freie Polysoma gefunden.

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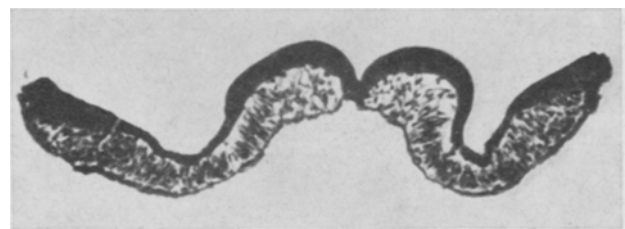
Separation of Germ Layers in Presomite Rat Embryos

The method of excision of presumptive ectodermal, endodermal and mesodermal areas from early amphibian embryos and the subsequent cultivation of isolated pieces under various experimental conditions has contributed a great deal to the understanding of some fundamental phenomena occurring in early development[1,2]. In avian and mammalian embryos any attempt to isolate particular prospective organ-forming areas must include the separation of germ layers from one another. By reason of the intricate mechanism of the formation of germ layers, some relevant experiments on avian[3-5] and mammalian[6,7] embryonic shields cannot be considered as true separation of definite germ layers. It was the purpose of the present investigation to apply the common method of enzymatic 'splitting off' of epithelia to the separation of germ layers in presomite rat embryos.

Pregnant females of randomly bred albino rats were killed by ether 9 days after mating and the embryos were isolated in sterile Tyrode's solution. The majority of them belonged to stages 14 and 15 in the normal series of NICHOLAS[8]. The mesodermal wings approached each other underneath the neural groove, opposite to the primitive streak. The extraembryonic part was cut off and the cup-shaped egg cylinder was transformed into a flat bi-lobated shield by a longitudinal cut (Figure). Prepared in this manner the shields were submitted to the action of enzymes. A solution containing 0.5% trypsin (cryst., lyophyl., Worthington) and 2.5% pancreatin (Difco) in the calcium- and magnesium-free Tyrode's saline was used. The embryonic shields were kept in it for 30 min at 4 °C (in the refrigerator[9]). The action of enzymes was blocked by a mixture of Tyrode's saline and horse serum which was substituted by the pure saline after 15–20 min. After this treatment the ectoderm detached spontaneously from the underlying mesoderm except for the region of the primitive streak where a complete separation had to be achieved by tungsten needles. The very thin embryonic endoderm did not detach spontaneously, but it could be easily rolled up from the mesoderm. In this way the embryonic shield was divided into 4 parts: the ectodermal and endodermal layer, and 2 mesodermal wings. The histological control showed that a complete separation of germ layers had taken place.

In order to test the viability and developmental capacities of embryonic cells after the treatment with enzymes, single germ layers or 2 of them in different combinations were implanted by a braking pipette under the kidney capsule of adult male rats. After 15 days the hosts were killed, the grafts were removed and submitted to the routine histological procedure. In all, 96 grafts were made



Cross-section of a stretched 9-day-old rat embryonic shield. × 100.

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and examined histologically. For the time being only a preliminary survey of results will be given.

The majority of grafts (90%) developed into masses of normal embryonic tissues ranging in shape and size from small discs to big tumorous masses reminiscent of the grafts of the entire rat egg cylinders. The only exception was the isolated endoderm which developed in only 2 out of the total 12 grafts.

The *ectoderm* differentiated regularly into typical ectodermal derivatives (neural tissue, epidermis). The mesodermal tissues (adipose tissue, cartilage, bone, muscle) were also present, but their incidence was not as high as in the grafts of the ectoderm + mesoderm combination. Derivatives of the endoderm were never found in ectodermal grafts.

The isolated *endoderm* did not differentiate at all. In the 2 successful endodermal grafts mentioned above, some mesodermal derivatives (cartilage, muscle) were also found. It was obvious that in these cases the endodermal layer was 'contaminated' with some adherent mesodermal cells. In the grafts of the endoderm and mesoderm, endodermal derivatives (intestine, respiratory epithelium, glands) differentiated regularly.

In grafts of the isolated *mesoderm*, the brown adipose tissue was the most constant differentiation. In some grafts, however, the derivatives of the ectoderm and the endoderm were also present. If grafted in combination with the ectoderm or the endoderm, the mesoderm differentiated into all characteristic derivatives which can be found in grafts of the entire egg cylinder.

The data reported show that the common method of 'splitting off' of epithelial layers from the underlying mesenchyme by the enzymatic digestion of their basement membranes can be applied with success to the separation of germ layers in early rat embryos. This procedure does not affect either the viability of embryonic cells or their ability to differentiate into normal tissues in homotypic grafts.

The main obstacle to a complete isolation of the *entire* germ layers was the existence of restricted areas of intensive migration of cells (primitive streak, Hensen's node) in which the germ layers being still in dynamic continuity with one another have not yet attained their individuality. In these areas a 'contamination' of the particular germ layer with adherent cells of the neighbouring one could not be excluded. Under these circumstances, the histodifferentiation in grafts of single germ layers cannot be considered as an adequate result of their auto-differentiative capacities.

In the present experiment, the principal aim of grafting the isolated germ layers was to test their viability and general ability for further development. In the future work special care will be taken to isolate and graft only the areas in which the formation of definite germ layers has already occurred.

Résumé. Les feuillets du cylindre-œuf du rat ont été disjoints par une solution composée de deux enzymes: 0,5% de trypsine et 2,5% de pancréatine. La séparation a été achevée par une aiguille de tungstène. Les feuillets ainsi détachés retiennent leur pouvoir de croissance et de différenciation comme homogreffes, sous la capsule rénale.

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The Role of the Diffusible Factor Released by the Egg Jelly in Fertilization of the Toad Egg

It has previously been reported that the gelatinous envelopes around the oocytes of the toad *Bufo arenarum* contain a diffusible substance, readily extractable by distilled water or balanced salt solution, which seems to be essential for fertilization[1]. Since this diffusible factor is quickly released from the jelly, it has been assumed that this principle would activate free spermatozoa before they penetrate the jelly[2]. On the other hand, some evidence indicates a small molecular size and a basic character[3]. Taking into account these properties, it was desirable to investigate if the role played by the diffusible factor in fertilization could be the result of an alkalizing effect on the insemination medium. Some experimental results supporting this assumption are reported in the present paper.

Material and methods. Bufo arenarum oocytes were obtained from the ovisacs of females treated with suspensions of homologous hypophysis preserved according to PISANÓ[4]. Sperm suspensions (about 10^6 cells/ml) were prepared by dilacerating the testes in 10% amphibian Ringer solution without bicarbonate.

Results were estimated using the fertilization rate method, which consists, essentially, in interrupting fertilization at different intervals of time by means of lauryl sulfate[5]. After insemination, egg cords were immersed for 5 sec in 0.1% lauryl solution followed by a quick wash in tap water. Control experiments showed that this treatment has no harmful effects on egg development. Further details will be found in the description of each experiment.

Results and discussion. As a first step, it was necessary to ascertain whether, under normal conditions, the diffusible factor had some influence on the pH of the inseminating medium. In fact, since various products, including appreciable amounts of proteins, are simultaneously released from the jelly after immersion in water, the presence of the diffusible factor is not enough to predict any pH effect on the surrounding medium. This was investigated by extracting the diffusible factor following the stepwise procedure as is routinely performed in this laboratory, and measuring the pH values of washing solutions by means of a Zeromatic pH meter. About 2000 oocytes were extracted by 3 consecutive washes with 200 ml of Ringer solution of 15 min each, with occasional gentle shaking. Under these conditions, fertilizability of washed oocytes decreases progressively being completely lost at the end of the last extraction

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