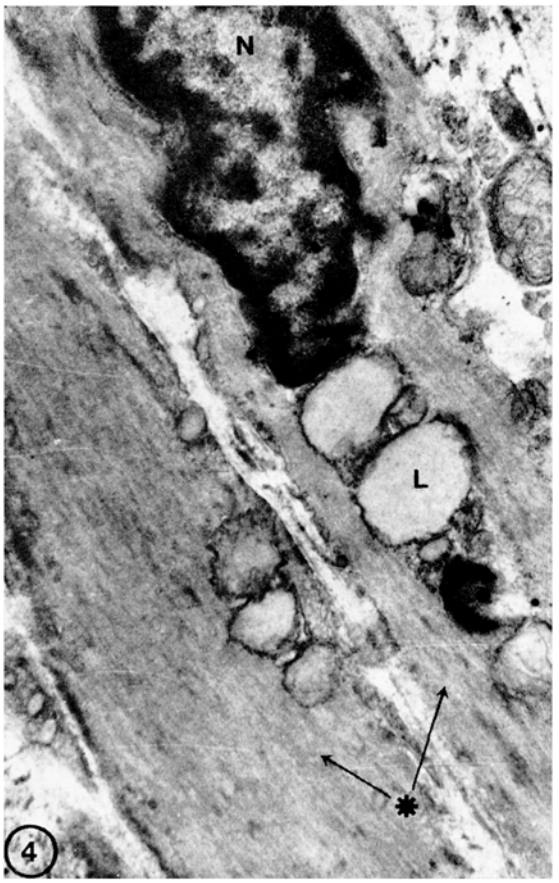

Fig. 4. Rabbit ovary. High magnification of 2 modified smooth muscle cells in the theca interna of an antral follicle. The cytoplasm contains a big nucleus (N), numerous myofibrils (*→) with scattered clear lipid droplets (L). ×22,700.

Owing to the distribution and significance of the smooth muscular tissue in the ovary, their direct role in the follicular dehiscence and atusia still remains debatable.

Riassunto. Mediante l'impiego del microscopio elettronico viene dimostrata la reale esistenza di piccoli gruppi di cellule muscolari lisce, non in rapporto con vasi, in differenti zone dell'ovaio (stroma, corpo luteo, teca, ghiandola interstiziale) del gatto, del coniglio e del topo. Le cellule muscolari subiscono caratteristiche modificazioni in relazione al ciclo ovarico.

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Differentiation of Endodermal Tissues in Homografts of Primitive Ectoderm from Two-Layered Rat Embryonic Shields

Recently Nicolet[1] showed that in chick embryonic shields the definitive endoderm (presumptive gut epithelium) arises by invagination of cells from the epiblast in the anterior part of the primitive streak. The possibility that an analogous mechanism exists during the germ layer formation in mammalian embryos was indicated by Grobstein[2] in 1952. He mechanically removed the outer cell layer (primitive endoderm) of mouse embryonic shields on the 7th day of pregnancy (variable cylinder length and developmental stage) and grafted the precultured clusters of primitive endoderm-deprived shields into the anterior chamber of the eye of adult mice for 30 days. The epithelium of the gut differentiated within these grafts at a high incidence. The author concluded that 'the inner cell layer, or primitive ectoderm, of the mouse embryonic shield cannot be regarded as a germ layer with capacities sharply limited to ectodermal differentiation'.

In a recent communication we showed that particular germ layers of presomite rat embryos can be separated from one another by treatment with proteolytic enzymes. This procedure does not affect the viability of embryonic cells and their ability to differentiate into normal tissues after transplantation[3].

In the experiment we are reporting here the albino rats of the inbred Fischer strain were used. The embryonic shields were all at the pre-primitive streak stage, and the outer cell layer was removed following pre-treatment with enzymes. The clustering and pre-culturing of embryonic shields were avoided and the grafts were transferred underneath the kidney capsule of adult rats.

Pregnant females were killed by ether 8 days after mating and the embryos were isolated in sterile Tyrode's saline. Only the egg cylinders belonging to the stage 12 of Nicholas[4] (pre-primitive streak) were selected for the experiment. They consisted only of an inner (primitive ectoderm) and an outer cell layer (primitive endoderm). The ectoplacental cone and the Reichert's membrane were removed and the embryonic shields with their extraembryonic parts were treated with enzymes[3]. The action of enzymes was blocked by a mixture of saline and a

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Differentiation in renal homografts of separate primitive germ layers isolated from pre-primitive streak rat embryonic shields

Graft	No. of cases	Skin	Neural tissue	Primitive gut				Cartilage	Bone	Muscle		
				Respiratory tube	Oesophagus	Intestine	Total			Smooth	Skeletal	Heart
Primitive ectoderm	15	–	12	13	8	11	14	11	3	13	2	12
Primitive endoderm	11	–	–	–	–	–	–	–	–	–	–	–

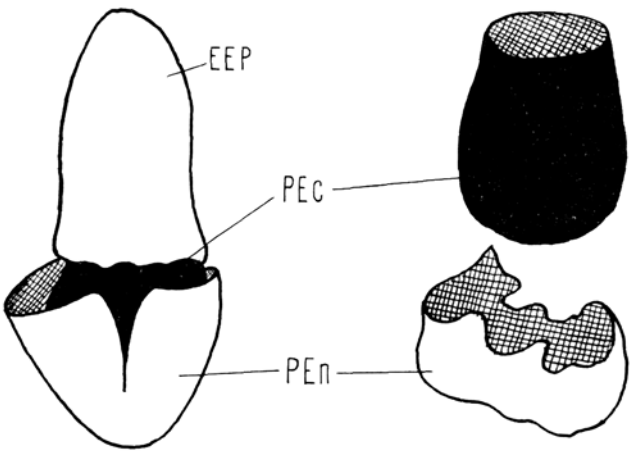

Fig. 1. Diagram illustrating the separation of primitive germ layers. PEc, primitive ectoderm; PEn, primitive endoderm; EEP, extra-embryonic part.

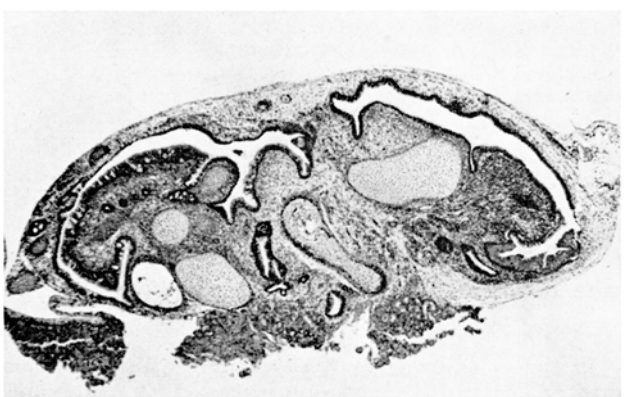

Fig. 2. Predominant differentiation of the respiratory tube (ciliated columnar epithelium, glands, smooth muscle, cartilage) within a graft of the primitive ectoderm. ×14,5.

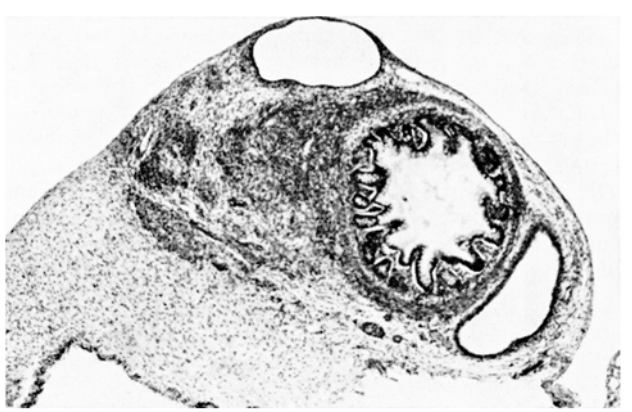

Fig. 3. Differentiation of intestine within a graft of the primitive ectoderm. ×23.

few drops of calf serum which was substituted by a pure saline after 5 min. The outer cell layer was then easily removed with tungsten needles and the remaining inner cell layer was separated from the extraembryonic part by a transverse cut (Figure 1). Both the cup-shaped inner and the shrunken outer cell layer were grafted separately underneath the kidney capsule of young male rats belonging to the same strain. After 15 days the hosts were killed, the grafts were removed and subjected to the routine histological procedure. 26 grafts in all (15 + 11) were made. The results are shown in the Table.

The primitive endoderm did not differentiate at all. All grafts were completely resorbed. On the contrary, in the grafts of the primitive ectoderm, characteristic tissues originating from all 3 germ layers were present. The most conspicuous feature was the regular appearance of the derivatives of the primitive gut. They differentiated as organotypic associations of endodermal (epithelia) and mesodermal (cartilage, muscle) tissues (Figures 2 and 3). Among non-endodermal tissues the complete absence of the skin and the low incidence of bone and skeletal muscle were obvious.

In spite of some differences in the design of experiment, the results of the present investigation confirm the main conclusions from GROBSTEIN's[2] experiment. They speak in favour of the presumption that the 2 cell layers of the pre-primitive streak rat embryo cannot be regarded as definitive ectoderm and endoderm to which the third layer (mesoderm) has only to be added to complete the final pattern of the embryonic shield. With regard to its histogenetic capacities the inner cell layer is still omnipotent at this stage. The definitive individualization of classical germ layers will probably be attained by segregation of cells of the inner layer during the next developmental steps. Further investigation is necessary to elucidate whether or not the morphogenetic mechanism during the formation of definitive germ layers in the rat egg cylinder is analogous to that occurring in the chick blastoderm.

Zusammenfassung. Die innere und die äussere Zellschicht 8 Tage alter Rattenkeimscheiben wurden enzymatisch voneinander getrennt und unter die Nierenkapsel erwachsener Ratten verpflanzt. Die isolierte innere Zellschicht (primitives Ektoderm) differenzierte sich nicht nur in ektodermale und mesodermale, sondern auch in typisch endodermale Gewebe (Darmepithel). Es wird daraus gefolgert, dass in diesem Entwicklungsstadium die innere bzw. äussere Zellschicht noch nicht als das definitive Ekto- bzw. Endoderm angesehen werden können.

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