endroits les bords du feuillet épithélial et pénètrent, accompagnées de nombreuses cellules sanguines, dans la lumière vésiculaire. Elles s'infiltrent ensuite à l'intérieur de la masse cellulaire résultant du décollement de l'épithélium apical et provoquent sa lyse progressive. De même, les nodules épithéliaux entraînés précédemment dans le parenchyme, sont progressivement résorbés (Figures 2 et 3).

Après 6 jours de culture, l'épithélium ne forme plus qu'un mince feuillet monocellulaire et discontinu, il tapisse une vésicule interne large et vide.

Conclusions. Nos expériences d'associations, en culture in vitro, de foie et d'épithélium gastrique montrent qu'il y a incompatibilité entre les 2 tissus mis en présence. Cette intolérance se manifeste sous 2 aspects principaux: 1. Réaction propre de l'épithélium gastrique qui se caractérise: a) par l'hypertrophie des lacunes internes conduisant à la rupture de la zone apicale, puis à la formation de nodules au centre de la vésicule, enfin à la réunion de ces petits groupes en une masse épithéliale unique; b) par l'effacement partiel de la membrane basale. 2. Réaction du tissu hépatique: a) le foie encercle l'épithélium qui devient interne; b) il s'infiltre entre les cellules de l'épithélium et morcelle ce dernier en petits nodules qui sont ensuite entraînés à l'intérieur du parenchyme hépatique; c) le foie s'insinue aussi dans la lumière vésiculaire, où, avec l'aide des cellules sanguines, il provoque la lyse progressive mais totale de la masse cellulaire apicale détachée précédemment de l'épithélium gastrique.

Summary. In experiments of associations of gastric epithelium with liver, proceeding respectively from rabbit and quail embryos, the Feulgen and Rossenbeck's nuclear reaction permits us to distinguish the gastric nuclei from the hepatic nuclei and consequently, to observe the cellular movements in both associated tissues.

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The Margin of Overgrowth of the Embryonic Chick Blastoderm as a Study Model for Cell-To-Cell Contacts

Cell-to-cell contacts are important in the study of invasiveness of cancer cells. The margin of overgrowth[1] of the chick germ appeared to be a suitable site for the study of such relationships. The cells, forming the periphery of the germ, show a long cytoplasmic offshoot, comparable to the leading edge of a moving fibroblast in culture[2]. Moreover, during the first hours of chick germ development, they move over the vitelline membrane rather quickly. We therefore investigated how these edge cells would behave when in contact with various other cells.

Fresh eggs from commercial stock are incubated for 6 h. They then fulfill Vakaet's stage 2 to 3[3]. They are then explanted in vitro following New's technique[4]. It proved important in these experiments to use germs with a yolk-free margin of overgrowth. We therefore carefully removed the yolk from the peripheral parts of the germ.

The following grafts have been placed on the vitelline membrane near the margin of overgrowth; pieces of 9-day-old chick embryo mesonephros (12 cultures); pieces of 9-day-old chick embryo tibio-tarsal skin (12 cultures); pieces of millipore filter (8 cultures) and Hela cells from monolayer cultures (21 cultures).

Cultures are incubated at 37.5 °C for 24 h. Figure 1 presents the experimental design schematically. During the incubation, time-lapse cinematography has been performed, taking one frame every 30 sec. The following results are based mainly on these records. During incubation, the chick germ increased its overall diameter over $\pm$ 10 mm, through a general extension of the margin of overgrowth over the vitelline membrane. The mesonephros, the skin, and the millipore filter, do not inhibit the expansion of the germ (32 cultures). In all these cultures, the grafts are taken up by the peripheral parts of the germ.

Generally, the margin of overgrowth shows a normal evolution in these cases (23 out of 32 cultures). At most there is a little retardation in its development, which sometimes is still visible peripherally to the graft at the end of the culture (9 out of 32 cultures) (Figure 2).

Hela cells, on the contrary, do disturb the normal evolution of the margin of overgrowth. As soon as the edge cells make contact with the Hela cells, they sud-

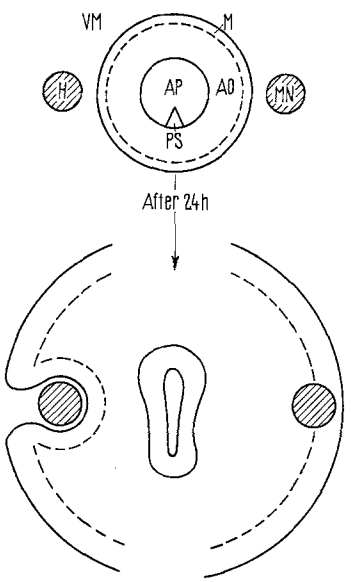

Fig. 1. Schematic representation of the evolution of the margin of overgrowth (M) in contact with Hela cells (H) and mesonephros (MN). PS, primitive streak; AP, area Pellucida; AO, area opaca VM, vitelline membrane.

[1] M. Blount, J. Morph. *20*, 1 (1909).
[2] R. Bellairs, A. Boyde and J. Heaysman, Wilhelm Roux' Arch. EntwMech. *163*, 113 (1969).
[3] L. Vakaet, J. Embr. exp. Morph. *10*, 38 (1962).
[4] D. New, J. Embr. exp. Morph. *3*, 326 (1955).

denly retract over a short distance. The parts of the margin of overgrowth lying close to the contact zone, develop normally. When however, during the course of expansion, they also touch the lateral rim of the bulk of Hela cells, they also exhibit an immediate retraction (Figure 2). At the end of the incubation, the Hela cells lie in a gap, partly surrounded by the edge cells (13 cultures out of 21). Sometimes the Hela cells are swept laterally by a normally developing margin of overgrowth (4 out of 21 cultures). If yolk is present between the edge cells and the Hela cells, thus impeding direct contact between them, the margin of overgrowth moves underneath the graft (1 out of 21 cultures). This observation confirms 7 preliminary results of cultures where the germs have not been freed of yolk before grafting. If fluid is present on the germ surface, the Hela cells lose contact with the vitelline membrane and float above the germ, without inhibition of the margin of overgrowth (3 out of 21 cultures). We never observed Hela cells to be incorporated by the germs.

We have been able to demonstrate differencies of behaviour, in the same germ, between Hela cells and mesonephros, or between Hela cells and skin, when grafted on separate sites near the margin of overgrowth.

It seems from these experiments that the movement of the edge cells is inhibited by Hela cells, but not by the normal cells used, proving that this inhibition cannot be explaned by mechanical factors only. Moreover, a direct contact between edge cells and Hela cells is necessary to cause this phenomenon, as shown by the yolk-contaminated cultures. This movement inhibition of the edge cells by the Hela cells does not necessarily point to

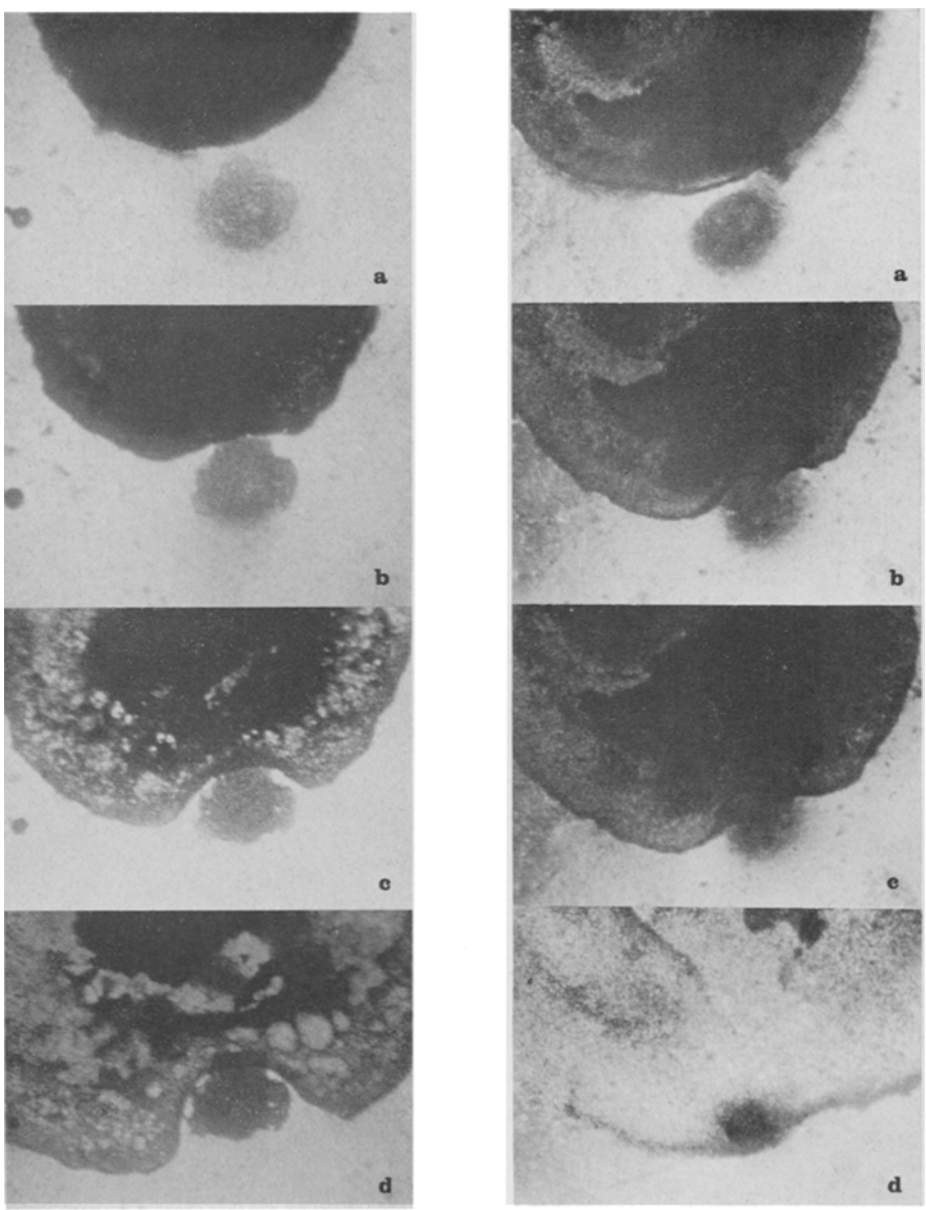

Fig. 2. Reproduction of time lapse cinematographic frames of cultures of Hela cells (left hand) and mesonephros (right hand) at the margin of overgrowth of young chick germs, before incubation (a), after 4 h (b), after 12 h (c) and after 20 h (d).

the contact inhibition phenomenon, as described in the well known studies of ABERCROMBIE and HEAYSMAN[5], although BELLAIRS and NEW[6] ascribe this property to the edge cells of the chick germ.

It would be interesting to find out how far the inhibition phenomena in this study model are specific for cancer cells. We are therefore testing, in further experiments, other cancer cells and their normal counterparts.

Zusammenfassung. Die Entwicklung vom «margin of overgrowth» junger Hühnerkeime ist verschieden nach Kontakt mit Helazellen und mit Mesonephros oder Hautgewebe; die ersteren hemmen die Entwicklung, die anderen werden vom Keim aufgenommen. Deshalb wird der «margin of overgrowth» als Modell für die Studie von Zellkontakten vorgeschlagen.

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[5] M. ABERCROMBIE and J. HEAYSMAN, Expl Cell Res. *5*, 111 (1953).
[6] R. BELLAIRS and D. NEW, Expl Cell Res. *26*, 275 (1962).

Heat-Labile Natural Anti-Digestive Antibodies in Animals

Evidence has recently been obtained of the presence, in animals[1,2] and in man[3], of digestive group systems. Natural digestive group antibodies have been shown to be heat-resistant. Although the signification of digestive group systems is still unknown, their theoretical interest may lead to further studies. Such studies should take into consideration the presence, in healthy animals from different species, of heat-labile anti-digestive antigens antibodies.

Material and methods. The immunofluorescent indirect method was applied to sera and alcohol fixed cryostat sections of the colon, the small intestine, and the stomach from various species. Experiments were conducted on 400 healthy rats from different strains (Wistar, Long Evans, Fischer inbred rats, Fischer inbred germ-free rats), on 100 healthy rabbits, and on 200 healthy mongrel dogs. Normal human sera from 200 blood donors, and sera and colons from 40 patients without digestive disease were also tested. All sera were tested before and after heating (30 min, 56 °C). Specific fluorescent anti-γ-globulins were prepared in the laboratory[4]. Control experiments were conducted with commercial fluorescent anti-rabbit and anti-human globulins (Microbiological Associates, Bethesda).

Results. In rats, specific fluorescent staining of the goblet cells from the colon, the small intestine, and the stomach was observed with all unheated fresh sera when tested on autologous tissue sections (Figure 1). The fluorescent staining, restricted to and present in all the goblet cells, was indistinguishable from that observed with a specific anti-rat colon rabbit immune serum[5]. Same positive reactions were observed with the same unheated sera when tested on isologous and heterologous rabbit, dog, and man digestive tract tissue sections. All these reactions were negative when the sera had been previously heated (Figure 2). These results have been obtained in all the animals from the different strains of rats under experiment, including germ-free rats. This heat-labile activity was not modified when unheated sera were stored at -20 °C (up to 6 months). It disappeared after absorption with iso- or heterospecific digestive mucosa dry powder. Absorption with any one of the species-specific dry powders removed the positive staining observed on the goblet cells of all the autologous, isologous, and heterologous digestive tissue sections.

Similar results were observed in rabbits. In this species, however, some differences may be seen in relation to the

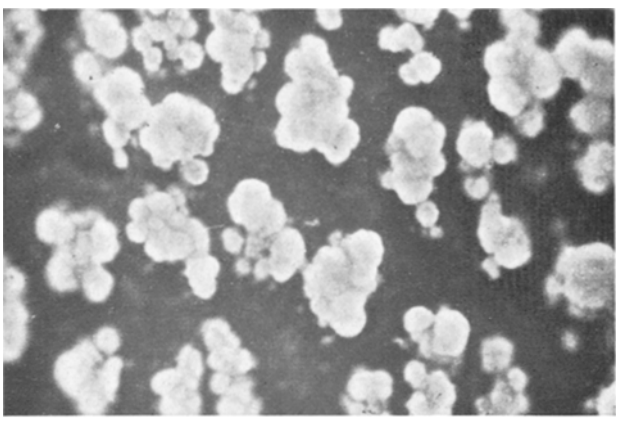

Fig. 1. Positive immunofluorescence staining observed on a rat colon with its own unheated serum.

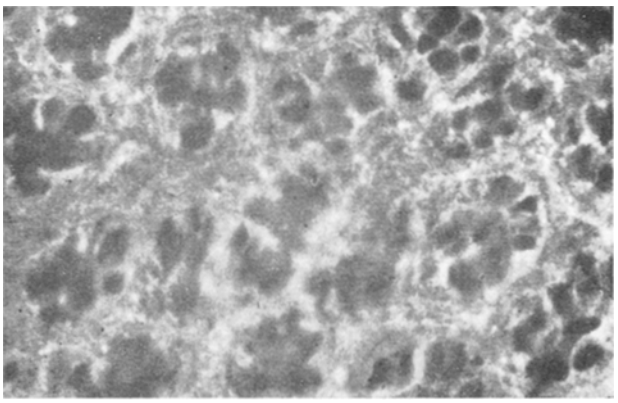

Fig. 2. Negative immunofluorescence staining observed on the same colon as in Figure 1 with the same serum heated for 30 min at 56 °C.

[1] A. ZWEIBAUM and V. STEUDLER, Nature *223*, 84 (1969).
[2] A. ZWEIBAUM and V. STEUDLER, Annls Inst. Pasteur *117*, 839 (1969).
[3] A. ZWEIBAUM and E. BOUHOU, Annls Inst. Pasteur *118*, 547 (1970).
[4] A. ZWEIBAUM, R. ORIOL PALOU and B. HALPERN, Annls Inst. Pasteur *115*, 789 (1968).
[5] E. J. HOLBOROW, G. L. ASHERSON and R. D. WIGLEY, Immunology *6*, 551 (1963).