These results demonstrate an instructive effect exerted by mammary mesenchyme on chick epidermis differentiation. Heterotypic interactions between the tissues result in a mammary-like morphogenesis which chick epidermal cells undergo. At first, a spherical epithelial bud develops, which becomes elongated before the sprouting of the secondary buds while its inner cells keratinize and synthesize glycogen. Since mesenchymal cells organize themselves into a connective sheath around the sprouts, inductive interactions occurring in the recombinants are similar to those taking place during normal mammary development. Mammary mesenchyme thus controls not only the branching pattern of the structures differentiated by chick epidermis, but also cell cytodifferentiation and metabolism. How mammary mesenchyme can influence chick epidermal cells has not yet been explained. We must conclude that chick cells possess some surface properties allowing them to interact with mammary mesenchyme, and the genetic information necessary for the building up of mammary-like structures. Experimental mammary-like morphogenesis takes place faster than normal mammary gland development in vivo (12 days instead of 16): the resting phase is therefore reduced with chick epithelium.

These results may be compared with those of KOLLAR[9]: 4- or 5-day chick embryonic mandibular epithelium interacts with the dental papilla of 15-day mouse embryo tooth germs, whose cells differentiate as odontoblasts while matrix material, characteristic of tooth development, forms a deposit. A genetic compatibility between chick and mammalian tissues is perhaps involved in these heterotypic interrelationships.

The competence of chick epidermal cells to react with mammary mesenchyme suggests that they can be influenced by an induce rable to promote a morphogenesis which does not normally occur. Experiments are underway to investigate if these mammary-like structures could be stimulated to secrete proteins under hormonal influence.

Résumé. Le mésenchyme mammaire d'embryon de lapin de 13 jours induit dans l'épiderme embryonnaire de poulet de 6 jours, la différenciation de structures ressemblant à des glandes mammaires fœtales, après 2 jours de culture in vitro et 10 jours de greffe sur la membrane chorio-allantoïdienne d'embryon de poulet. La répartition du glycogène, synthétisé par les cellules épithéliales de poulet sous l'influence du mésenchyme mammaire est identique à celle des glandes témoins, ce qui signifie que la ramification des bourgeons épithéliaux et le métabolisme des cellules épidermiques sont également sous le contrôle du mésenchyme mammaire.

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Phosphorylase in the Adult Rat Testis: The Use of Dextran in Histochemical Studies

Phosphorylase has been demonstrated histochemically in the adult rat testis using TAKEUCHI's technique[1-3]. In agreement with other authors[4] in a series of investigations using this technique, we were unable to confirm this finding[5]. More recently, however, modifications have been made to the original technique of TAKEUCHI[6-11], one of the most interesting of which is the use of dextran, first introduced by MEIJER[10,12]. The aim of the present investigation was to study the effect of dextran on the histochemical demonstration of phosphorylase in the adult rat testis.

Materials and methods. 30 adult rats (Wistar) were used in these experiments. Biopsy specimens of testicular tissue were immediately frozen at −70 °C on dry ice and absolute ethyl alcohol. 12 µm sections cut on the cryostat were incubated in a medium containing 0.2 *M* acetate buffer pH 5.6, glucose-1-phosphate, NaF and EDTA for the histochemical demonstration of active phosphorylase. The same incubation medium, with the addition of 20 mg AMP, was employed for the demonstration of inactive phosphorylase. Other sections were incubated in the same medium to which dextran (500,000 mol.wt.) had been added in the ratio 2 g/25 cm³ medium. The sections incubated at 37 °C for 1 h and washed in 40 % ethyl alcohol were fixed in absolute ethyl alcohol and stained by the Schiff-dimedone method[13]. Control sections were incubated in a medium without substrate.

Results. When dextran is used in the incubation medium, the reaction product of active phosphorylase is very faint and is visible as fine granules localized inside the tubules in close proximity to the tubular wall. The tubular wall itself and the interstitium also show a positive reaction. With dextran the reaction product of inactive phosphorylase is much stronger and appears as well-stained granules distributed throughout the tubule, being more evident close to the tubular wall and in the peripheral zone of the preparation (Figures 1 and 2). Also in this case, the reaction is strongly positive along the tubular wall and the interstitium. Sections incubated in the medium without dextran show no reaction.

Discussion. Phosphorylase appears to be histochemically detectable with the use of dextran and is visible in the form of fine granules inside the seminiferous tubule. In a previous series of investigations, in contrast to other authors[1-3], we were unable to detect phosphorylase histochemically in the adult rat testis. Our present research confirms this earlier finding. It is therefore feasible to hypothesize that dextran, a predominantly 1:6 linked glucose polymer, is able to serve as glucosyl-acceptor(primer) for the demonstration of phosphorylase activity[10,12]. This addition of dextran to the incubation medium thus appears to be important since it makes

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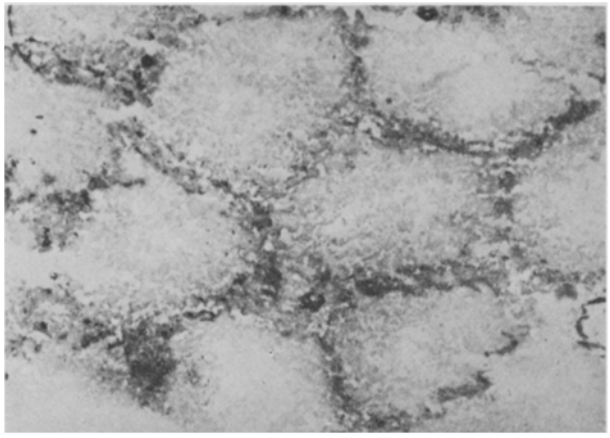

Fig. 1. Enzyme reaction of inactive phosphorylase appears as fine granules within the seminiferous tubule. Stronger positivity is visible in close proximity to the tubular wall and in the interstitium. ×140.

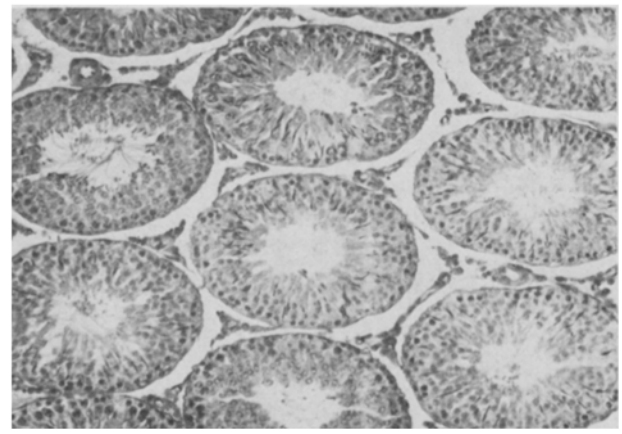

Fig. 2. Histological pattern of the same specimen. Hopa staining. ×140.

possible the histochemical demonstration of phosphorylase in those tissues, such as adult rat testis, which have a low content of glucosoyl-acceptor glycogen[14–17].

It is of interest to note, in contrast to normal human testis, that inactive phosphorylase is more abundant than the active form[13]. This difference may be due to the fact that the very low amount of glycogen and active phosphorylase is related to the cycle of rat spermatogenesis. In consequence of the short duration of the cycle[18], it is possible that metabolic processes are more rapid and so rapid synthesis of the glycogen triggers off immediate activation of the phosphorylase which in turn releases the energy needed in the metabolic processes of spermatogenesis.

Riassunto. Per la scarsa quantità di glicogeno presente nel testicolo di ratto adulto, non é possibile dimostrare istochimicamente la fosforilasi. Gli Autori affermano che solo con l'uso del destrano é possibile mettere in evidenza questo enzima. Viene inoltre ipotizzato una relazione fra glicogeno, fosforilasi e spermatogenesi nel testicolo di ratto adulto.

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Suppression of Cellular Immunity in vivo by Rifampicin

Several observations indicate an immunosuppressive action of rifampicin[1–4]. Also, a prophylactic activity against adjuvant arthritis in rats has been described[5]. In the light of recent reports on the possible consequences of chronic immunosuppression[6,7], such an influence may have practical implications. Therefore, I wish to communicate the effect of rifampicin on two manifestations of cellular immunity.

First, it was investigated whether the drug prolongs skin allograft survival in mice. Male C3H mice were grafted with BALB/c tail skin and treated daily from the day of transplantation with either 30 or 100 mg/kg of rifampicin (Rimactan®) orally. The control grafts (8 mice) displayed a survival time of 10.5 ± 0.9 (S.D.) days, while with 30 mg/kg/day, the grafts survived for 9.9 ± 1.0 days (10 mice) and with 100 mg/kg/day for 12.4 ± 1.9 days (6 mice; $p < 0.02$). However, the latter dose was tolerated badly and caused the death of 4 out of 10 mice within 1 week and had therefore to be reduced to 30 mg/kg/day.

Another manifestation of in vivo cellular immunity, tuberculin hypersensitivity, was also tested. Albino guinea-pigs were immunized with living lyophilized BCG vaccine[8] and challenged 4 weeks later with 750 IU tuberculin intracutaneously. The ensuing reaction was assessed by measuring the thickness of the skin fold over the reaction site[8]. In a group of 6 guinea-pigs treated with a single dose of rifampicin (30 mg/kg) applied orally 30 min before the antigenic challenge, the reaction intensity was reduced compared with the controls at 24 h by 69% ($p < 0.01$) and at 48 h by 87% ($p < 0.001$).

Thus, rifampicin slightly depresses transplantation immunity, but this effect is of doubtful practical importance, since it occurs only with dosages approaching the

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