introduced postnatally significantly increased the length of the estrus-metestrus phase. That irregularities in gonadotropic hormone release were involved in the effects of postnatal heat stress in the former report[13] were supported by the additional findings that postnatal heat stress delayed vaginal opening, increased the length of time to conception and depressed the onset of pregnancy. Measurements of gonadotropin secretion were not made in the present study, nor were they reported for the prepubertally heat-stressed mice[13]. However, Benson and Morris[14] exposed adult rats to 4-h periods of heat stress daily during days 7–11 of pregnancy and reported that the adrenals were hyperactive, as indicated by elevated weights and elevated serum corticosterone; moreover, pituitary and serum follicle-stimulating hormone, which normally begins to fall by day 12 of pregnancy, remained high.

Recently Ward and Weisz[15] reported that prenatal stress significantly alters circulating levels of gonadal and adrenal steroids during prenatal and early postnatal periods. Furthermore, prenatal stress significantly increases steady state concentrations of dopamine in the arcuate nucleus of female offspring as adults by 153%[4]. Concentrations of catecholamines in the arcuate nucleus are known to influence gonadotropin secretion from the anterior pituitary gland[6]. It is possible therefore that prenatal stress disturbs the hormonal milieu of the female fetus during a critical hypothalamic differentiation stage, resulting in a disruption of estrous cycling in adulthood.

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PRO EXPERIMENTIS

An improved method of transplantation on chicken chorioallantoic membrane (CAM)

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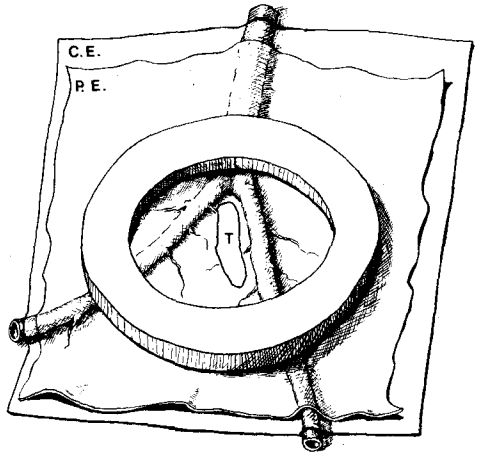
Summary. An improved method for grafting on the chicken CAM is described and compared with other CAM grafting methods.

The first use of the chorioallantoic membrane (CAM) of the chick embryo for transplantation studies probably dates back to the experimental work of Murphy and Rous[2]. Since then, improvements of the CAM grafting technique have been introduced by various investigators[3–5]. Embryo containing eggs, incubated for 8–10 days, are in the best condition for receiving grafts to the chorioallantois[4,6]. By transplantation on CAM, usually much larger and more complex structures can be grown than is possible by isolating organs in vitro[6]. However, the development of the transplants on CAM does not always occur as regularly as with the latter method[7]. Our experiments were undertaken with the aim to improve the CAM transplantation technique, classically described by Harris[8].

Material and methods. The CAMs of fertile eggs from White Leghorn chickens, incubated for 8–10 days at 38.5 °C, were used in this study. On these CAMs, whole embryonic chicken or quail ovaries, testes, adrenals or parts of these organs were transplanted for 8–10 days. A 1st group of transplants were grafted according to Harris[8], a 2nd group with O'Hare's modified technique[7], and a 3rd group with our method.

Results and discussion. The method of Harris[8], when applied as such, often gives very irregular results. Indeed, in a variable number of cases, the host embryo dies during the days following the transplantation. The reason for this death is not always clear. Some sets of chicken eggs seem to be very sensitive, other not at all. Death of the host embryo can usually be avoided by: 1. puncture of the egg shell over the air space, near its highest point (the major blood vessel branches of the chorioallantois, after their determination by candling, being oriented to a topmost position); 2. the creation of the artificial air space above the chorioallantois[5], whilst the blunt pole of the egg is slightly elevated. The oblique position of the egg on its holder may not be altered before the puncture hole over the air space is sealed with transparent tape. By this procedure, the contents of the egg are prevented from bulging excessively into the air space. Another drawback of the classical CAM grafting technique is, that graft 'takes' are erratic, with many grafts undetectable at the end of the transplantation period[7]. O'Hare seems to have overcome this difficulty by placing the grafts between a small piece of cellulose ester (Millipore) filter and the surface of the CAM. This method ensures a more direct contact of the transplant with the chorionic ectodermal layer of the CAM. This facilitates the penetration of the graft by the host's blood vessels. Indeed, after the transplantation, as the CAM ages, its capillary network migrates through the chorionic ectodermal layer of cells[9]. In principle the method of O'Hare is excellent; however it has 2 drawbacks: 1. after the application of a piece of cellulose ester filter material on top of the transplant, the latter is no longer visible and its further evolution can no longer be followed at the surface of the CAM; 2. the

Millipore filter often adhers tightly to the transplant and may become embedded in the CAM. Deformation of the transplant cannot always be avoided. The inclusion of the piece of filter material into the graft may interfere with histological sectioning. The disadvantages of O'Hare's modification can be avoided by the use of a thin film of polyethylene instead of cellulose ester filter material. Square films (side approximately: 10–15 mm, weight: 1,5–2 mg) of polyethylene (trade-mark: Glad, Union Carbide) are pre-sterilized by immersion in 70% ethanol for several h. After drying in a sterile Petri dish, they are ready for use. The transplant or association of transplants is placed in the freshly prepared artificial air space at the surface of a vascularized area of the CAM, and then covered by such a sterilized film of polyethylene (figure). To assure a better contact of the transplant with the underlying CAM, a small ring cut from a silicone rubber tube is placed on the polyethylene film with the graft in a central position. The ring (weight: 30–40 mg) has an external diameter of approximately 8 mm and a lumen width of 4–5 mm. With this accommodation of the CAM grafting, the number of successful transplantations is much higher than with the classical method. For this there are several reasons. Since the polyethylene film is waterproof, the exsiccation of the graft and graft site is avoided. The weight of the rubber ring on the polyethylene sheet usually prevents slipping of the graft on the CAM and assures optimal contact of the transplant with the chorionic ectoderm. The evolution of the transplant can easily be followed, since the polyethylene film is perfectly transparent. During the transplantation period, the polyethylene film always remains stretched at the surface of the CAM and never becomes enclosed in it. So the localization of the graft remains easily detectable, even after a prolonged sejourn on the CAM, and nearly always the polyethylene film can easily be separated from the underlying graft and CAM.



The polyethylene-ring technique of CAM transplantation: view of the graft site after excision. T, transplant; P.E., polyethylene film; C.E., chorionic ectoderm.

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A quick and modified Winkler-method for measuring O_2-concumption of aquatic animals[1]

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Summary. A modified procedure for measuring O_2-consumption, based on Winkler-method, is described. Instead of KI and HCl (or H_2SO_4) triphenylmethane-dye leukoberbelinblue I and citric acid are used.

Besides the Winkler-method, the so-called O_2-electrode is applied for the estimation of oxygen consumption of aquatic animals[2]. Although the chemical method is simple in practice, some interferences occur if the sample is taken from lakes or rivers as well as marine habitats. Generally in Winkler-method, the measuring of optical density is preferred to titration with $Na_2S_2O_3$. However, the vapours of strong acids like HCl or H_2SO_4 destroy the sensitive parts of the photometer, even when the cuvettes are carefully covered. The advantage of the polarographic method is undoubtedly a continuous estimation of O_2-concentration as a function of time, but suitable equipment like amplifier and recorder are required for the purpose.

In view of these complications, we present here a quick and modified Winkler-method using the dye leukoberbelinblue I[3] and citric acid instead of expensive KI and strong acids like HCl or H_2SO_4. In our chemical method, the compound MnO_2 $(OH)_2$ oxidizes the triphenylmethane-dye at pH 3.0 to a deep blue berbelinblue I which has a maximum absoprtion at the wavelength of 620 nm. The optical density of the blue solution of this dye is a measure of O_2-concentration in water.

Material and methods. The O_2-consumption of the fish *Idus idus* L. (Cyprinidae) was measured by the set-up (figure 1) as described[4–6]. A respiratory chamber (made out of transparent plastic material) with a diameter of 7 cm and measuring 20 cm in length was used.

In the present experiments, 3 fishes of similar size (3–4 g b.wt) were introduced into the respiratory chamber covered with a black foil.

O_2-consumption of the fish *Idus idus* L. as a function of adaptation-temperature (experimental temperature 25 °C)

Adaptation-temperature [°C]	O_2-consumption [ml $O_2 \cdot g^{-1} \cdot h^{-1}$]
10	0.32
20	0.22