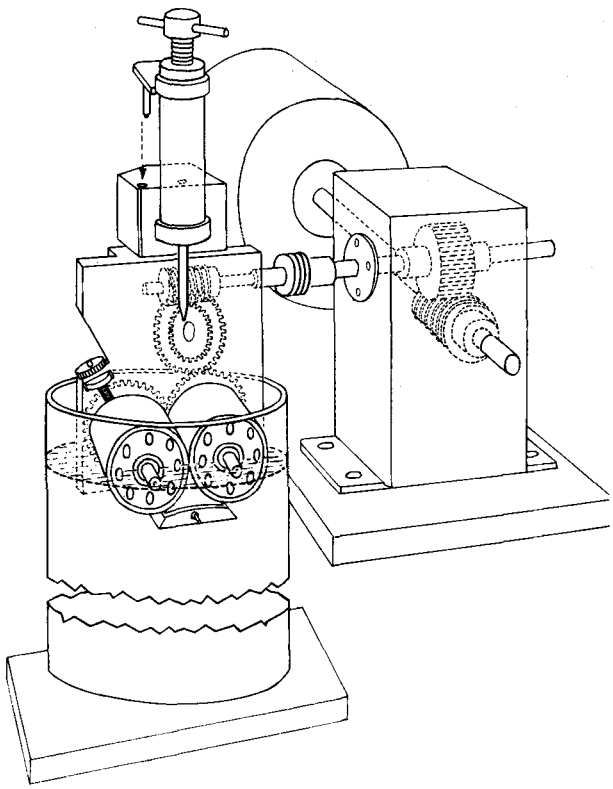
Metallblöcke presst. Das von uns entwickelte Gerät (Figur) gestattet es, diesen Vorgang kontinuierlich zu gestalten. Dabei wird das Gewebe zwischen zwei tiefgekühlte Metallwalzen (ca. – 190 °C) durchgedreht und fällt unmittelbar in das Kältemittel. Die Walzen tauchen zur Hälfte in flüssigen Stickstoff ein; Längsbohrungen sorgen für eine intensive Kühlung. Durch Anbringen von Blenden an den beiden Enden der Bohrungen (in der Figur nicht eingezeichnet) wird der Stickstoff bei der Rotation der Walzen mit nach oben genommen. Dadurch werden auch die jeweils aus dem Stickstoff herausragenden Teile der Walzen ständig gekühlt. Der Abstand der Walzen kann zwischen 0 und 1 mm variiert werden, ihr Durchmesser beträgt 4 cm, ihre Umlaufgeschwindigkeit 1 Umdrehung in 5 sec. Sie werden von einem Elektromotor (1,5 PS) über zwei Schneckengetriebe angetrieben. Das grob vorzerkleinerte Gewebe wird durch eine Metallspritze, deren Stempel durch eine Schraube bewegt wird, in den Spalt zwischen die Walzen eingebracht. Die Spritze mündet in ein kurzes Rohr von 5 mm innerem Durchmesser, dessen Ende flachgedrückt und dadurch dem Spalt zwischen den Walzen gut angepasst ist; es hat ca. 1 mm Abstand von dem Spalt. Durch eine geeignete Halterung lässt sich die Spritze leicht entfernen und wieder in die vorgesehene Lage bringen. Da das Material in dünnen Platten anfällt und sich somit durch eine grosse Oberfläche auszeichnet, wird auch die Gefriertrocknung begünstigt[3].



Gerät zum schnellen Einfrieren von Geweben. Die Frontplatte des Walzwerkes ist wegen der besseren Übersicht weggelassen. Nähere Erläuterungen im Text.

Summary. A new unit for rapid freezing of tissues and liquids in liquid nitrogen is described.

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A New Method of Multiple Grafting of Endocrine Glands into the IVth Ventricle of the Chick Embryo

The various methods designed for grafting tissues into the chick embryo have their inherent limitations. For instance, grafting onto the chorio-allantoic membrane on the 5th–6th day of incubation is technically possible only for *one* small implant, on the 8th–9th day for *multiple* grafts of a larger size, but in the latter case the time remaining until recovery is rather short.

Hamburger's method[1,2] of intracoelomic grafting onto the abdominal wall of the 60–100 h old embryo, has the disadvantage that the size and number of the implants are limited by the small capacity of the coelomic cavity at this age. A similar limitation holds for the intraocular grafting designed by May and Thillard[3]. After intracoelomic grafting as introduced by Dossel[4], the implants often fuse with various organs such as liver, mesonephros, mesenteries and intestines, which hampers recovery.

While studying the influence of embryonic pituitaries on the gonads of the chick embryo, the author[5] found that modifications brought about by older pituitaries could be effected by younger ones only when the number of implanted glands was increased. As, however, no satisfactory method was available for implantation of as many pieces as six, a new method was developed. For implantation the IVth ventricle is utilized of the 4–5 days old chick embryo, which constitutes a large cavity with an easily accessible and scarcely vascularized roof. The method appears to have sufficient applicability to be of interest to workers in other fields of research.

[1] V. Hamburger, J. exp. Zool. *77*, 379 (1938).
[2] V. Hamburger, J. exp. Zool. *80*, 347 (1939).
[3] R. M. May and M.-J. Thillard, C. r. Ass. Anat. *44*, 488 (1957).
[4] W. E. Dossel, Science *120*, 262 (1954).
[5] M. M. Groenendijk-Huijbers, Anat. Rec. *136*, 202 (1960).

The implantation proceeds as follows. After windowing the egg according to a method described previously[6], chorion and amnion covering the occiput of the embryo are torn with two pairs of scissors. In this way the roof of the IVth ventricle is exposed, exhibiting an almost avascular centre, encircled by branches of the posterior and anterior cerebellar arteries (Figure). The centre is easily pierced with a sharp glass needle, without bleeding. Then the edge of the opening is caught with a slightly bent forceps. In this way the head of the embryo is immobilized and the ventricular cavity is kept from collapsing. With another pair of forceps a number of implants are pushed into the lumen of the ventricle until it is all filled up. Care should be taken not to tear the opening in the roof, otherwise the implants may float out. In this manner the author[5] successfully implanted a maximum of 6–8-days old embryonic chick pituitaries. The mortality rate of the procedure is low and amounts to about 5%.

On autopsy at the end of the incubation period, the site of implantation is readily recognized by a defect in the skin of the occiput through which an encephalocele bulges. After careful opening of the tumour, the implants are found well vascularized and fused with one another and with the ventricular roof, protruding into the lumen of the ventricle.

The implants are fully functional, as is demonstrated in two sets of experiments: (1) Multiple embryonic chick pituitaries of 8–18 days old donor embryos, implanted into the IVth ventricle, produced similar ovarian modifications of the female hosts as after intracoelomic grafting: the right ovaries were enlarged and the ovarian medulla was cystically degenerated (GROENENDIJK-HUIJBERS[5]). (2) After implantation of a complete or half testis of 5–8 days old donor embryos, a complete or partial regression of the Müllerian ducts of the female hosts occurred, in a similar way as was previously described after chorio-allantoic and coelomic implantation of testicular tissue (GROENENDIJK-HUIJBERS[7]).

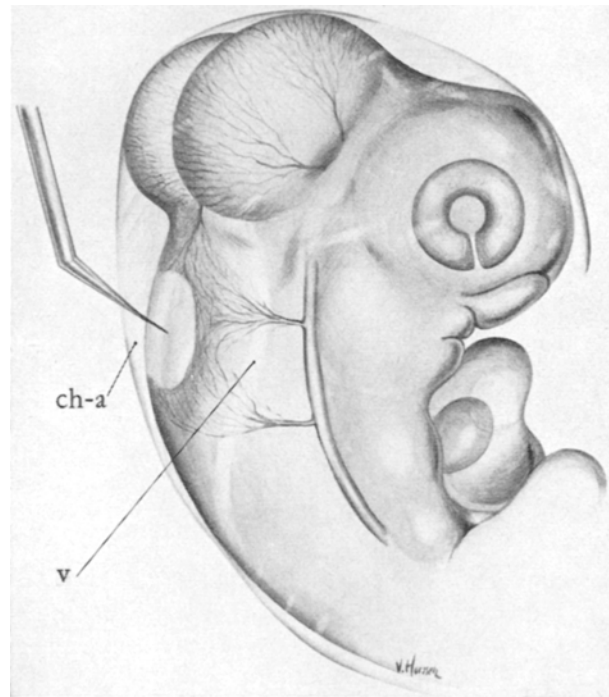
In order to evaluate the quantitative development of the intraventricular implants, volumetric estimates in paper weight (PW) were made of 6-day-old embryonic donor testes, which after implantation on the 4th day of incubation remained in the female hosts until autopsy on the 14th day of incubation. The mean PW values of the intraventricular grafts equalled the means of the intracoelomic ones (Table I).

[6] M. M. GROENENDIJK-HUIJBERS, Acta morph. neerl.-scand. *1*, 241 (1957).

[7] M. M. GROENENDIJK-HUIJBERS, Anat. Rec. *137*, 237 (1960).

Table I. Paper weight of 6-day-old embryonic testes, developed during a 10 day stay in female host embryos

Site of implantation	Size of testis on implantation	No. of cases	PW mean ± SD
Intraventricular	one complete testis	5	18.22 ± 5.73
	one half testis	5	17.50 ± 9.91
Intracoelomic	one complete testis	2	19.95 ± 9.97
	one half testis	2	15.40 ± 4.10



5-day-old chick embryo in paramedian position. Glass needle points to the avascular centre of the roof of the IVth ventricle. ch-a: chorion and amnion, which has to be opened in order to expose the roof. v: line of attachment of ventricular roof, indicating the large extent of the ventricular cavity.

Table II. Relation between volume of testicular implant (estimated in paper weight) at autopsy and degree of Müllerian duct regression in female chick embryos

Site of implantation	Age of testis on implantation, in days of incubation	Day of implantation	Regression of Müllerian ducts		
			Absent A	Incomplete B	Complete C
Intraventricular	5–8	4	PW < 6.4 (2)	11–13 (2)	> 15.3 (7)
Intracoelomic	5–8	4	PW < 6.1 (3)	13 (1)	> 12.5 (7)

Number in brackets indicates the number of cases. A, the amount of testicular tissue developed in the implant is *subliminal*; B, the amount is *liminal*; C, the amount is *minimal*.

In previous experiments[7], it was found that the degree of regression of the female Müllerian ducts could be related to the quantity of testicular tissue developed in the implants at autopsy. A subliminal amount of testicular tissue caused no regression, a liminal amount caused an incomplete regression, and a minimal amount was required to produce a complete regression.

A 6-day-old testis, implanted on the 4th day of incubation, usually caused a complete regression. In order to be able to assess the functional activity of the implanted testicular tissue, it was necessary to implant parts of 5–8 days old testes, which would cause varying degrees of regression. The preliminary results of these experiments are listed in Table II. As judged from the relation between the degree of regression of the host's Müllerian ducts and the quantity of testicular tissue developed in the implant expressed in PW, no difference in functional activity is found between intracoelomic and intraventricular implants. This observation provides sufficient evidence that the implants are functionally equivalent. Moreover, it is interesting to note that so far no influence is noticed of the different distance of the two sites of implantation from the target organ.

The experiments reported here illustrate that the intraventricular implantation is a valuable complement to other methods of implantation. Apart from its specific usefulness for developmental embryology, it may be of interest to workers in various fields of research, such as comparative endocrinology, neurology and cancer research[8].

Zusammenfassung. Methode zur Implantation mehrerer Gewebestückchen (Hypophyse und Testes) in den IV. Ventrikel 4–5 Bruttage alter Hühnerembryonen, deren funktionelle Vollwertigkeit demonstriert wird.

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The Wash-Out of Intraarterially Injected Krypton^{85} from the Intestine of the Cat

Techniques have recently been developed for quantitatively measuring blood flow and flow distribution in different tissues by recording the disappearance of radioactive inert gases injected intraarterially[1,2]. These methods are based on the theoretical considerations originally developed by Kety[3]. The tissue clearance technique seems a priori to be a suitable method for studying blood flow distribution within the intestinal wall since it contains anatomically well defined parts, such as the muscularis and the villi. In this preliminary report, experiments are described in which the above-mentioned technique has been applied to the intestine of the cat.

Methods. The experiments were performed on cats deprived of food for at least 24 h and anaesthetized with chloralose (50–70 mg/kg). Venous outflow from a section of the jejunum weighing 20–50 g was recorded by means of a drop recorder unit operating an ordinate writer. Arterial inflow pressure was monitored from the left femoral artery by a mercury manometer. Atropin was given (1 mg/kg) and the splanchnic nerves were cut bilaterally. Intravenous infusions of isopropylnoradrenalin to produce intestinal vasodilatation were made via a catheter in the left femoral vein.

0.4–0.8 ml (0.3–0.6 mC) of a saline solution containing the radioactive isotope Kr^{85} was given as a single injection, lasting 5–10 sec, through a small branch of the superior mesenteric artery. The γ-radiation was recorded by an external scintillation detector collimated in such a way that only radiation from the intestine was registered. The activity was recorded with a spectrometer and a linear ratemeter operating a Rikadenki recorder. In most experiments β-activity was also recorded by means of a Geiger-Müller tube (Philips No. 18509) placed either in the lumen of the intestine or against the outside of the gut in such a way that only activity from the intestinal wall was registered. The wash-out curve minus background was plotted semilogarithmically and the multiexponential curve was analysed by successively subtracting exponentials as originally proposed by Dobson and Warner[2,4].

Results. The Figure illustrates the data from a representative experiment. The upper panel shows a semilogarithmic plot of the Kr^{85} disappearance curve as monitored by the external gamma probe (heavy curved line). A continuous record of arterial blood pressure and venous outflow from the jejunum was simultaneously obtained (lower panel). Blood flow and blood pressure remained throughout constant except for a small, transient flow increase when injecting the isotope (time zero). The decay of radioactivity is multiexponential and is the sum of 4 monoexponential components indicated by the thin straight lines of the figure (components I–IV). Since the very slow component (IV) evidently represents blood flow of a tissue outside the intestinal wall, this component was constructed from the decay of the γ-radiation after the β-radiation, registered by a G-M tube in the lumen of the intestine, had returned to background level, indicating that no radioactivity remained in the intestinal wall (see discussion). The table in the upper right part of the Figure gives some pertinent data obtained from the wash-out curve. The Table summarizes 11 experimental runs.

Discussion. The experiments reported in this study indicate that the wash-out curve of intraarterially injected Kr^{85}, recorded from the intestine of the cat, can be

[1] J. A. Herd, M. Hollenberg, G. D. Thorburn, H. H. Kopald, and A. C. Barger, Am. J. Physiol. *203*, 122 (1962). – D. H. Ingvar and N. A. Lassen, Acta physiol. scand. *54*, 325 (1962).
[2] G. D. Thorburn, H. H. Kopald, J. A. Herd, M. Hollenberg, C. C. C. O'Morchoe, and A. C. Barger, Circ. Res. *13*, 290 (1963).
[3] S. S. Kety, Pharmacol. Rev. *3*, 1 (1951).
[4] E. L. Dobson and G. F. Warner, Am. J. Physiol. *189*, 269 (1957).