culture[8] and diminished insulin content of cultivated mice islets[4]. It was found that the insulin release from fresh and cultured isolated islets of normal mice in short-time incubation was equal if the glucose concentration in the medium was low, and was diminished by high glucose concentration during the whole time of cultivation[4].

The present results suggest that the organ culture technique was successfully varied by use fo glass fibre vlies on metal grid. The β-cells of isolated cultivated rat islets retain their characteristic responses to glucose for a period up to 10 days and longer[9]. The β-cells were not damaged by being maintained at a high glucose concentration. The conditions are suitable for cultivation of adult rat islets and for investigation of insulin secretion for a long time.

IRI (μU/ml/24 h) released into the medium (5 mM glucose) by rat islets

Days in culture	50 Islets	20 Islets
1	1390 ± 232 (13)	1226 ± 321 (13)
2	1396 ± 199 (23)	887 ± 88 (16)
3	1213 ± 186 (26)	1285 ± 206 (14)
4	783 ± 87 (24)	1042 ± 202 (15)
5	820 ± 80 (23)	1193 ± 165 (16)
6	458 ± 69 (15)	1010 ± 171 (13)
7	723 ± 167 (12)	494 ± 120 (16)

50 or 20 islets at the beginning of the culture period.

Zusammenfassung. Inseln adulter Wistarratten wurden auf Glasfaservlies auf Metallgitter unter den Bedingungen der Organkultur gezüchtet und der Einfluss der Inselzahl unter basalen und stimulierten Bedingungen auf die Insulinsekretion in 24 h-Perioden während der Kultivierung untersucht.

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[8] I. HILWIG and S. VRBANEC, Z. Zellforsch. *103*, 410 (1970).
[9] B. ZIEGLER, R. BUTTER, H. J. HAHN und G. A. SPECK, VIII. Jahrestagung der Biochemischen Gesellschaft der DDR, Oberhof (1972).

Effects of 2-BR-α-Ergokryptine (CB 154) on Sex-Linked Rejection of Pituitary Isografts in Female C57BL Mice[1]

In 1955 EICHWALD et al.[2] first demonstrated a sex-linked rejection of skin grafts in C57BL mice. This phenomenon was interpreted by 2 possibilities: androgen dependency[3] and Y-linked antigenicity[3,4]. Thereafter, a sex-linked rejection of various tissues has been reported (skin[5,6]; thymus[7]; lymph node[8]; mammary gland[9–11]; lung, salivary gland, blood, spleen and liver[12]; hypophysis[13,14]).

Prolactin may be selectively and excessively secreted from pituitary grafts at sites remote from the hypothalamus[15,16]. Pituitary isografts enhance mammary carcinogenesis in all strains of mice, except C57BL mice[17]. In our laboratory, it was observed that pituitary isografts from male donors were not always rejected by female C57BL hosts when their mammary glands were not stimulated by those grafts[18], which suggested some relationships between sex-linked pituitary graft rejection and prolactin secretion from surviving grafts. CB154 has been reported to inhibit prolactin secretion both from pituitary glands in situ and from ectopically placed pituitary grafts[19,20]. Therefore, it was of interest to investigate the effects of CB154 on the sex-linked rejection of pituitary isografts in female C57BL mice.

Materials and methods. The animals used were 6 to 11 months old normal C57BL mice raised in our laboratory. Vaginal smears were taken daily from all females until the termination of experiments. 39 out of 60 proposed female host mice, which have shown regularly-cycling vaginal smear patterns after 41 days of smearing (Period I), were selected. These animals received single pituitary isografts from untreated adult male siblings into the dorsal triangular portions of mammary fat pads on both sides of their fourth mammary gland fat pads under Nembutal anesthesia. They were then divided into 2 groups: 1. Control group – 19 mice received no further treatments, and 2. Experimental group – 20 mice received daily s.c. injections of the CB 154 solution (0.2 mg of CB 154 suspended in 0.1 ml of 0.9% saline solution per mouse per day) for 45 days (Period II).

In the experimental group, the transplantation sites were surgically exposed for macroscopic investigation of pituitary isografts on the day following the last CB 154 injection. One of two transplantation sites in each host was left undisturbed when actively surviving pituitary graft tissues were evident. The dorsal half of the fourth mammary gland fat pad on the opposite side was surgically removed and fixed for histological investigation.

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[2] E. J. EICHWALD and C. R. SILMSER, Transplantn Bull. *2*, 148 (1955).
[3] T. S. HAUSCHKA, Transplantn Bull. *2*, 154 (1955).
[4] G. D. SNELL, Transplantn Bull. *3*, 29 (1956).
[5] R. T. PREHN and J. M. MAIN, J. natn. Cancer Inst. *17*, 35 (1956).
[6] L. SACHS and E. HELLER, J. natn. Cancer Inst. *20*, 555 (1958).
[7] B. B. HIRSCH, Transplantn Bull. *4*, 58 (1957).
[8] M. FELDMAN, Transplantn Bull. *5*, 15 (1958).
[9] K. HOSHINO, J. natn. Cancer Inst. *30*, 585 (1963).
[10] R. L. MORETTI and P. B. BLAIR, Transplantation *4*, 596 (1966).
[11] A. G. ERWIN, Proc. Can. Fedn biol. Soc. *10*, 97 (1967).
[12] E. J. EICHWALD, C. R. SILMSER and N. WHELLER, Ann. N.Y. Acad. Sci. *64*, 737 (1957).
[13] K. HOSHIMO and J. E. MOORE, Int. J. Cancer *3*, 374 (1968).
[14] K. HOSHINO, Proc. Can. Fedn biol. Soc. *14*, 142 (1971).
[15] J. W. EVERETT, Endocrinology *54*, 685 (1954).
[16] D. G. MONTEMURRO and W. U. GARDNER, Endocrinology *73*, 174 (1963).
[17] O. MÜHLBOCK, Adv. Cancer Res. *4*, 371 (1956).
[18] K. HOSHINO, unpublished data.
[19] R. YANIA and H. NAGASAWA, Experientia *26*, 649 (1970).
[20] N. NAGASAWA and R. YANAI, Endocr. jap. *17*, 233 (1970).

able I. Vaginal smear data obtained from mice carrying pituitary isografts and treated with and without CB 154 compound

A) Frequency of occurring vaginal estrous stage

Group	No. of mice	Period I (41 days) Prior to pituitary isografting	Period II (45 days) 1st 45 days after pituitary grafting	Period III (45 days) 2nd 45 days after pituitary grafting
Control	19	15.9 ± 1.36%	11.2 ± 1.92%	17.6 ± 3.45%
Experimental	20	17.0 ± 1.35%	30.0 ± 5.61%	22.0 ± 2.66%
Difference between C and E		non significant	$P < 0.001$	non significant

B) Frequency of occurring proestrous-II, estrous, and metestrous-I stages

Group	No. of mice	Period I	Period II	Period III
Control	19	35.8 ± 3.07%	20.5 ± 3.12%	32.4 ± 5.61%
Experimental	20	39.0 ± 2.26%	52.2 ± 2.0%	37.5 ± 4.12%
Difference between C and E		non significant	$P < 0.001$	non significant

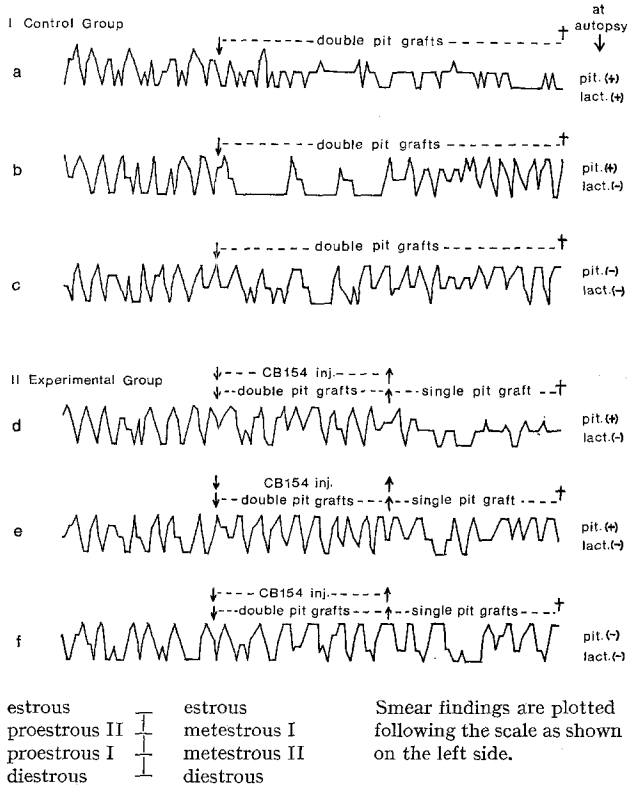

Fig. 1. Vaginal smear patterns selected from each of the six different groups are shown to demonstrate typical changes occurring in C57BL female mice.
A downward arrow indicates the time of either pituitary isografting or the first injection of CB154.
An upward arrow indicates the time of either the surgical removal of a pituitary isograft or the last injection of CB154.
A cross sign indicates the time of sacrifice. Under the heading 'at autopsy', (+) indicates either the recovery of pituitary isografts or the macroscopic presence of milk in mammary glands.

This procedure resulted in all host mice carrying actively surviving single pituitary grafts. From the day of operation, CB 154 treatment were discontinued for 45 days (Period III) at the end of which period they were autopsied. All control and experimental mice were sacrified on the same day and skinned for observation of the mammary glands; the tissues at the pituitary transplantation sites were removed and fixed in chrome alum solution for aldehyde-thionin-PAS-Orange G-hematoxylin staining to recover anterior pituitary cells which survived on transplantation. All mice were maintained in a uniformly controlled animal laboratory with an illumination of 12 h light and 12 h dark cycle, and provided with Purina Mouse Chow and water ad libitum.

Results. Vaginal smear cycles were classified as follows: diestrous, proestrous-I, proestrous-II, estrous, metestrous-I, and metestrous-II. Smear data are summarized in Table I. No significant differences were observed between the control and experimental groups during Period I and Period III, and also between Period I and Period III in both groups. During Period II, while the experimental mice were treated with CB 154, vaginal estrus occurred significantly more frequently, which clearly indicated the suppression of prolactin secretion from both the host's in situ pituitary glands and the ectopic grafts. The recovery rates of pituitary isografts, which had been confirmed by histochemical investigation of serial sections of the tissues obtained from the transplantation sites, and their influences on vaginal smear patterns are shown in Table II.

The survival rate of pituitary grafts at the end of Period II was 72.5% (29/40 grafts in 20 mice). During the next 45 days following cessation of CB 154 treatment (Period III) 60% of the persisting pituitary grafts were rejected. In mice rejecting grafts, vaginal smear patterns changed to pseudopregnancy-type in 2 to 3 weeks after the cessation of CB 154 and continued for 10 to 20 days, then returned to normally cycling patterns (Figure 1f). Most of the hosts carrying surviving pituitary grafts showed continuous pseudopregnancy-type smear patterns

Table II. The fate of pituitary grafts and their influences upon vaginal smear patterns

Group	Pituitary grafts recovered at autopsy	Pituitary grafts recovered and smears showing pseudopregnancy type patterns	Pituitary grafts recovered but smear patterns returned to regular cycles after pseudopregnancy type
Control	12/38 grafts (31.6%) in 9/19 mice (47.3%)	5/38 grafts (13.2%) in 4/19 mice (21.0%)	7/38 grafts (18.4%) in 5/19 mice (26.3%)
Experimental[a]	8/20 mice (40.0%)	7/20 mice (35.0%)	1/20 mice (5.0%)

[a] Each host carried single pituitary grafts.

(Figure 1d). One of 8 experimental mice which carried surviving grafts did not maintain pseudopregancy-type patterns and returned to normal cycles (Figure 1e), which was indicative of the lack of prolactin secretion. Therefore, only 7 of 20 grafts (35%) were actively secreting prolactin even after the cessation of CB 154 treatment. With the same criteria, only 21% of control mice carried pituitary grafts which ceased to secret enough prolactin but survived on transplantation (Figure 1b).

Discussion. The present experiments demonstrated that sex-linked rejection of pituitary glands isografted from adult male donors to adult female C57BL mice is due to pituitary tissues of male mice as well as to some unknown mechanisms involved in the secretion of prolactin from pituitary grafts derived from male C57BL mice. The difference in the constituents of prolactin secreted by C57BL mice between the 2 sexes is unlikely to account for this phenomenon. Possibly, some unknown substances are secreted along with prolactin. These substances may possess male specific antigenicity. An alternate explanation is the possibility that male grafts require an adequate stimulation by androgens. This possibility was originally postulated for a sex-linked skin graft rejection[3,12], but is not experimentally supported[21,22]. Furthermore, this hypothesis could not explain the positive effect of CB 154 in reducing rejection rates of pituitary isografts as observed in the present experiments. Further studies are warranted for the elucidation of the mechanisms of this sex-linked pituitary graft rejection phenomenon.

Zusammenfassung. Eine geschlechtsbedingte Unverträglichkeit von Hypophysen-iso-transplantat unter ausgewachsenen weiblichen C57BL Mäusen wurde durch simultane Behandlungen mit CB 154 bedeutend reduziert und nach Unterbrechung der Behandlung erhöht.

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[21] E. J. EICHWALD, C. R. SILMSER and I. WEISSMAN, J. natn. Cancer Inst. *20*, 563 (1958).
[22] S. E. BERNSTEIN, A. A. SILVERS and W. K. SILVERS, J. natn. Cancer Inst. *20*, 577 (1958).

Continuous Registration of the Pancreatic Blood Flow After Intravenous Application of Glucose

For the evaluation of the actual pancreatic insulin output under in-vivo-conditions one must know the pancreatic blood flow[1,2]. After rapid elevation of glycemia, the pancreatic circulation increases too[3-5]. But up to now all in-vivo-procedures for characterization of the dynamics of insulin secretion have been incomplete because they neglect both the real hemodynamics and the amount of insulin reaching the organ with the arterial blood stream. Therefore in these experiments the pancreatic blood stream was measured continuously during hyperglycemia and compared to the alternations of blood glucose and insulin concentrations.

Material and methods. Alsatian dogs of both sexes (25.6 ± 1.1 kg body wt., age 24 ± 3 months) in general anesthesia (N_2O/O_2 after introduction with hexobarbital, relaxation and endotracheal intubation, no premedication) have been used. They got indwelling plastic catheters both into the A. coeliaca via (A. lienalis) and into the V. portae near the liver (via V. lienalis). Flow probes of an electromagnetic blood flowmeter were arranged both on the A. pancreaticoduodenalis superior (= A. gastropancreatica) near the parenchyma of the pancreas and on the A. femoralis. Both probes were connected with a double-channelled electromagnetic flowmeter recording continuously (Nycotron 372 S, Norway). After the operative preparation (90 min) and the stabilization period (30 to 60 min), the animals were injected with glucose (0.5 g/kg = 1.25 ml/kg of a 40% solution) within 25 sec. In addition to the flow measurements, arterial blood pressure (Biometer BM 101, GDR), arterial blood glucose[6] and portal venous immunoreactive insulin (IRI)[7] have been analyzed. For statistical evaluation the actual amount of blood stream was taken from the original registration curves for that experimental time when chemical analyses were performed. At the end of the experiments, by the injection of China-ink into the A. gastropancreatica that

[1] R. METZ, Diabetes *9*, 89 (1960).
[2] H. S. SELTZER, J. clin. Invest. *41*, 289 (1962).
[3] I. MANDELBAUM and C. R. MORGAN, Ann. Surg. *170*, 753 (1969).
[4] P. LEFEBVRE et A. LUYCKX, J. Ann. Diab. Hôtel Dieu *10*, 139 (1969).
[5] A. M. RAPPAPORT, T. KAWAMURA, J. K. DAVIDSON. B. J. LIN, S. OHIRA, M. ZEIGLER, J. A. COODLING, J. HENDERSON and R. E. HAIST, Am. J. Physiol. *221*, 343 (1971).
[6] P. KÖHLER, Z. ges. inn. Med. *17*, 674 (1962).
[7] U. FISCHER, H. HOMMEL, M. ZIEGLER and R. MICHAEL, Diabetologia *8*, 104 (1972).