Control animals were anaesthetized with 1.5% halothane and sham injected with the appropriate solvents. 18 out of 21 rats treated with LH or LRH shed a full complement of ova (Table II); this finding indicates a neural site of action of the anaesthetic.

Recent evidence suggests that the mechanism of halothane anaesthesia involves a decrease in the amount of transmitter released from nerve endings[5]; similar findings have been reported for barbiturates[6]. It is thus conceivable that both drugs interfere with ovulation by reducing the synaptic activation of neurons involved, directly or indirectly, in the production of LRH; this mechanism of action may be a general property of anaesthetics. Therefore, the exploration of neural mechanism underlying spontaneous ovulation in the rat is unlikely to yield physiologically meaningful results if carried out under the influence of any general anaesthetic.

Zusammenfassung. Halothan-Anaesthesie während der sogenannten «kritischen Periode» der Proöstrusphase bewirkt eine zentrale Hemmung der Spontanovulation bei der Ratte. Wie Barbiturate und Urethan ist deshalb Halothan als Anaesthetikum zur Erforschung neuroendokriner Mechanismen nur in beschränktem Masse geeignet.

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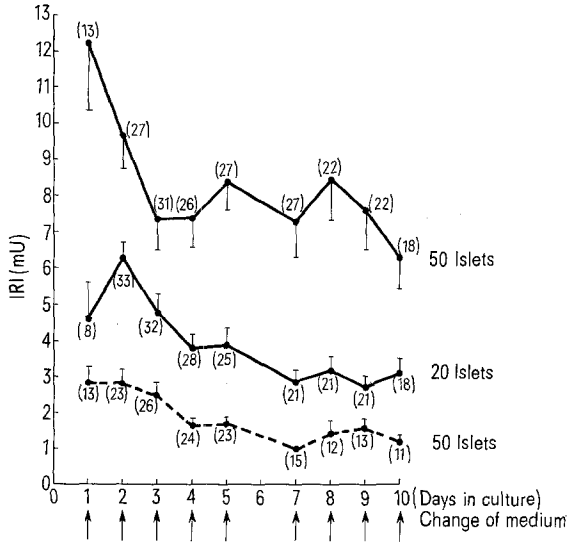
Insulin Release from Isolated Islets of Langerhans of the Rat in Organ Culture

The isolated islets of Langerhans of guinea-pig, mice and rabbits have been cultured in vitro[1–4]. There are only a few results on insulin secretion of cultured islets, especially on rat islets[5]. Our recent work attempts to study the possibilities of cultivation of adult rat islets, and to characterize the effect of different glucose concentrations in the nutritional medium on the insulin secretion within 24 h periods for a long duration of cultivation.

The islets of adult wistar rats (starved overnight), with a body weight of 160–180 g, were isolated by the modified method of Lacy and Kostianovsky[6]. The pancreas were aseptically excised, washed in Hank's salt solution and digested by shaking at 37 °C in Hank's solution containing 3.7 mg collagenase/ml for 20 min.

Enzyme treatment was interrupted by dilution with cold Hank's solution and the islets were subsequently sedimented at 4 °C and washed 5 times. The isolation of islets was carried out under sterile conditions by means of a stereomicroscope and the islets were collected in TC 199 medium (Difco Laboratories Detroit, Michigan).



Insulin (IRI) released into the medium by cultured rat islets [mU/culture bottle/24 h]. —, 16 m*M* glucose; ----, 5 m*M* glucose. Results as $\bar{x} \pm s\bar{x}$ with the number of observations in parentheses.

Groups of 20 or 50 islets were cultivated on glass fibre vlies ('Microlith', Schuller, Wertheim/Main) supported by metal grid in small culture bottles in 2 ml TC 199 with 10% inactivated calf serum, penicillin (100,000 U/l), streptomycin (145 mg/l) and hydrocortison (1 mg/l.) The glucose concentration was 5 m*M* or 16 m*M* during the whole cultivation period. The medium was changed every day.

The content of insulin in the medium was determined immunologically by back titration and alcohol precipitation[7], (standard: pork insulin Novo, 10–4000 μU/ml nutritional medium with 10% calf serum).

A high glucose concentration in the medium elicited a marked increase in the insulin release of cultivated islets (Figure) during the whole cultivation period ($P < 0.001$ for every day). With the high glucose concentration (16 m*M*) the medium insulin content of groups of 50 islets was significantly elevated in comparison with groups of 20 islets ($P < 0.01$).

The rate of insulin release calculated for one islet in groups with 50 or 20 islets was equal. There was no change, however, in the IRI values of groups of 20 or 50 islets after administration of a basal glucose concentration (Table), which means that the basal rate of insulin secretion calculated for one islet is diminished. The reason for this different behaviour is not clear. It may be that the negative feedback mechanism depends on the glucose concentration, on the time of incubation, and on the kind of medium or buffer.

With the duration of cultivation, the amount of insulin secretion is diminished. $P < 0.01$, first day, 50 islets; $P < 0.01$, second day, 20 islets. The present results agree well with the decreased secretion of monolayer

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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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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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