The Effect of Estrogen and Castration on a Spontaneous Pancreatic Islet Change in Rats

A high incidence of spontaneous alterations of the pancreatic islets in mature and old Sprague-Dawley male rats, consisting of intra-insular, occasionally peri-insular fibrosis, and enlargement has been reported by us previously[1]. Functional studies have revealed a decrease in glucose tolerance in these rats as compared to animals of the same age[2]. The islet change has been considered to be the result of a failure of a compensatory mechanism to certain metabolic requirements. Since only male animals have shown this islet alteration it seemed to be important to investigate if there was a hormonal basis for this remarkable sex difference and particular emphasis was placed on the possible protective role of estrogens.

Method. 230 Sprague-Dawley, Charles River D, male rats of 20 weeks of age were investigated. Group 1 was treated with s.c. injections of 0.5 mg/kg conjugated equine estrogen, Premarin®, 3 times weekly throughout the experiment. Group 2 was castrated prior to the experiment and group 3 served as control. The body weights of the animals were recorded weekly. Groups of rats were sacrificed (Table I) at 30, 40 and 50 weeks of age. The pancreas was removed and processed for histopathological examination (Bouin's fixative, haematoxyline-eosine staining). Special granule stain for selected slides was also performed. The incidence of islet change was established according to the criteria described previously[2]. From animals sacrificed at 50 weeks of age, the pituitary gland, adrenals and thyroid were also removed, weighed and processed for histopathological examination.

Results. The results summarized in Table I show that the control animals at 30 weeks of age already had a 33% incidence of islet fibrosis and enlargement. This percentage rose to 51% by 40 weeks of age and remained constant until the end of the experiment.

The estrogen treated animals showed an incidence of 13% after 10 weeks of treatment. By 20 weeks of treatment the incidence was 17% and it remained at this level until the end of the experiment (30 weeks of treatment). All these values were significantly different from controls. (10, 20 and 30 weeks of treatment correspond to 30, 40 and 50 weeks of age, respectively.)

The incidence rate in the castrated animals was between the control and estrogen treated groups: 22, 43, 36% at the age of 30, 40 and 50 weeks, respectively. These figures were not significantly different from controls.

Compared to that of controls the body weight gain was significantly lower in the estrogen treated group throughout the experiment. It was also significantly lower in the castrated animals at 40 and 50 weeks of age (Table II).

There was no difference in thyroid weight between groups but the adrenal and pituitary weights were significantly higher in the estrogen treated and castrated animals (Table III).

Discussion. Our experiment has demonstrated that estrogen treatment, beginning at 20 weeks of age, markedly influences the development of the spontaneous pancreatic islet change occurring in our mature and old Sprague-Dawley male rats. A significantly lower incidence of islet change was noted in the estrogen treated group throughout the experiment. The effect of castration was much less apparent since only a slight reduction in the incidence was noticed in this group.

Our experiment also showed that estrogen treated and castrated rats gained less in body weight than controls. Furthermore, it was noted that the pituitary glands and adrenals were larger in estrogen treated and castrated animals than in controls.

[1] A. HAJDU and G. RONA, Diabetes *16*, 108 (1967).
[2] A. HAJDU, F. HERR and G. RONA, Diabetologia *4*, 44 (1968).

Table I. Incidence of pancreatic islet change in different groups of rats

Group	Age (weeks) 30	40	50
	Incidence/No. of rats (%)		
Control	10/30 (33)	15/29 (51)	12/24 (50)
Estrogen treated	4/30 (13)[a]	4/23 (17)[a]	4/23 (17)[a]
Castrated	6/28 (22)	9/21 (43)	8/22 (36)

[a] Significantly (X^2-test) different from controls.

Table III. Organ weights of different groups of rats[a]

Group	Organ weight (mg) ± S.E.		
	Pituitary	Adrenals	Thyroid
Controls	14.6 ± 0.37	50.1 ± 1.33	21.6 ± 0.57
Estrogen treated	16.2 ± 0.55[b]	58.4 ± 1.75[b]	20.8 ± 0.69
Castrated	20.4 ± 0.64[b]	55.6 ± 1.88[b]	22.4 ± 1.12

[a] Data refers to groups sacrificed at 50 weeks of age. [b] Significantly different from controls.

Table II. Body weights and weight gains in different groups of rats

Group	Body weight (g)			Δ Weight (g) ± S.E.		
	I[a]	II[b]	III[c]	I	II	III
Control	481; 524	486; 615	491; 634	43 ± 8.9	129 ± 10.2	143 ± 10.0
Estrogen treated	524; 526	546; 557	550; 577	2 ± 4.4[d]	11 ± 8.7[d]	27 ± 7.5[d]
Castrated	493; 553	498; 581	506; 611	60 ± 4.8	83 ± 7.1[d]	105 ± 11.9[d]

[a–c] Refers to mean body weights at start (20 weeks) and termination, [a] 30 weeks, [b] 40 weeks, [c] 50 weeks of experiment. [d] Significantly different from controls.

Our results are in agreement with those of HOUSSAY[3], FOGLIA, SCHUSTER and RODRIGUEZ[4], LEWIS, FOGLIA and RODRIGUEZ[5], and RODRIGUEZ[6]. These authors demonstrated that the incidence of diabetes in the rat, produced by subtotal pancreatectomy is much higher in males than in females. They also reported that estrogens conferred marked protective effect in their diabetic animals, while castration of male rats only slightly reduced the incidence.

It has been theorized that the protective effect of estrogens could be either the result of altered food intake, a direct effect on the islets, an action mediated through the pituitary gland and adrenals or an indirect effect on the peripheral carbohydrate metabolism. HOUSSAY and co-workers critically examined the role of these factors[3,6] and by forced and paired feeding ruled out a principal role of altered food intake. It was also shown by adrenalectomy that this gland does not play an essential role. Furthermore, no strict correlation was found between protective action and pituitary enlargement. Although the role of the peripheral carbohydrate metabolism has not been clarified fully, HOUSSAY and RODRIGUEZ concluded that the protection is chiefly due to direct effect on the islets. Whether in our experiment the lower incidence of islet change in the course of estrogen treatment is due to a correction of certain abnormal metabolic conditions in mature and old male rats or a direct effect on the pancreatic islets or both remains to be seen. Although in our previous work no correlation was found between inflammatory (immunological?) changes (insulitis, peri-insulitis) and islet fibrosis, a suppressive action of estrogen on these processes cannot be ruled out[7,8].

Zusammenfassung. Die bei alternden männlichen Sprague-Dawley-Ratten auftretende spontane Fibrose und Vergrösserung der Langerhansschen Inseln kann durch eine im Alter von 20 Wochen eingeleitete Östrogenbehandlung verhindert werden. Diese Schutzwirkung der Östrogene kann Ausdruck eines direkten Einflusses auf die Inseln oder eines indirekten Einflusses durch den peripheren Kohlehydratstoffwechsel sein. Eine Kastration vermochte diese Veränderungen an den Inseln nur wenig zu verhindern.

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Fractionnement des protéines bactériennes par électrophorèse en gel d'acrylamide-agarose

Nous nous sommes proposés de fractionner par électrophorèse en gel d'acrylamide-agarose les protéines endocellulaires libérées par désintégration sonique de divers bacilles Gram négatifs.

Matériel et méthode. 15 souches bactériennes appartenant aux espèces suivantes: *Escherichia coli, Proteus vulgaris, Serratia, Pseudomonas aeruginosa, Alcaligenes denitrificans,* sont cultivées à la température de 37 °C dans un milieu à base de peptone pancréatique de caséine et d'extrait de viande (Difco).

Les bactéries sont recueillies soit après 18 h de culture, soit au cours d'une phase définie de la croissance[1]: phase exponentielle, phase maximum stationnaire.

L'extraction des protéines bactériennes s'effectue en tampon *Tris*-glycine pH 8,7 à l'aide d'un insonateur Sonifier S 125. L'électrophorèse en gel d'acrylamide-agarose est réalisée selon la méthode d'URIEL[2] avec les modalités suivantes: tampon discontinu *Tris*-glycine pH 8,7; différence de potentiel 6,7 V/cm; traces de noir amide dans le réservoir pour contrôler la longueur de migration; durée d'électrophorèse: 30 min à 4 h 30; fixation et coloration des protéines par le noir amide en solution acétique.

Résultats. Comme le montre la Figure 1, les séparations électrophorétiques diffèrent selon les concentrations en acrylamide utilisées, chacune présentant des caractéristiques qui lui sont propres. Les séparations les meilleures et les plus reproductibles ont été généralement obtenues avec les concentrations en acrylamide de 5 et 7%.

La qualité des séparations et le dénombrement des fractions dépendent de la concentration finale en protéines et de la longueur de migration – d'où la nécessité d'étudier une souche bactérienne en faisant varier ces différents paramètres. Dans les conditions habituellement utilisées la concentration finale en protéines a été de 2 mg/ml et la longueur de migration du noir amide de 10–12 cm.

A pH 8,7 la plupart des protéines bactériennes se dirigent vers l'anode, mais certaines d'entr'elles migrent vers la cathode. Une trentaine de fractions peuvent être ainsi distinguées. La mobilité relative de chaque fraction peut s'exprimer par rapport à la distance parcourue par le noir amide. La mobilité, le nombre et la répartition des fractions diffèrent selon l'espèce bactérienne étudiée. La Figure 2 montre à titre d'exemple, le fractionnement obtenu avec divers bacilles Gram négatifs. On remarque notamment que l'électrophorégramme d'*Alcaligenes denitrificans* présente 5 fractions migrant vers le pôle négatif.

Des variations s'observent également selon la phase de croissance considérée. Les Figures 3 et 4 permettent de comparer les distributions électrophorétiques obtenues avec la même souche de *Proteus vulgaris* en phase exponentielle et en début de phase stationnaire. La Figure 3 montre les différences observées dans les conditions habituelles d'électrophorèse. La Figure 4 correspond à une durée d'électrophorèse de 40 min; on révèle ainsi l'exis-

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