the blastocyst stage of development has an adverse effect on the further development of the rabbit embryos. In the first series, while all the controls showed visible normal implantations (Figure 2), none of the anti-BKN group had any comparable implanted embryos. Even where a few implantations were observed in the latter, the uterus was much smaller in size compared to the controls, and all the implantations were confined to one horn, A-8 and A-10.

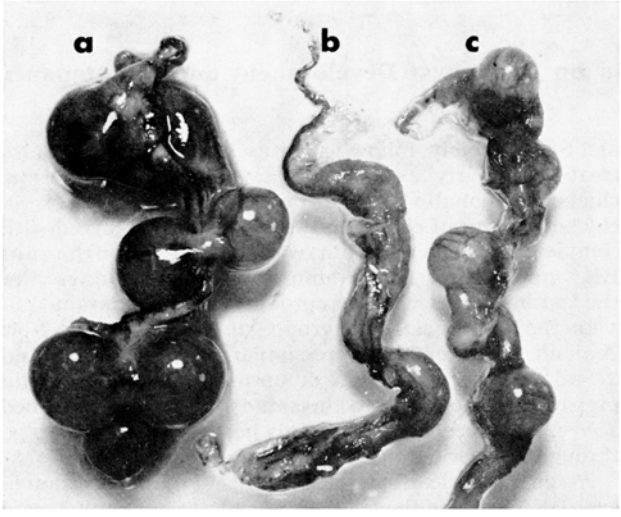

Fig. 2. Uteri taken from control and antiblastokinin-treated rabbits: a) control; b) and c) anti-BKN treated; a) and b) represent whole uteri whereas c) represents the horn that showed implantation sites.

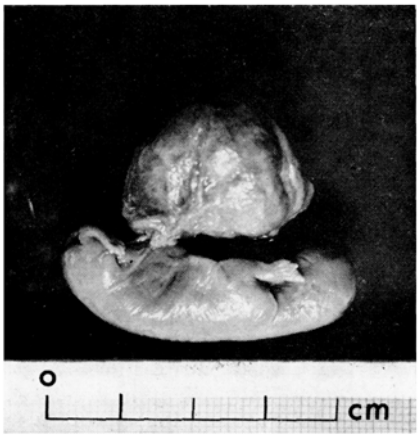

Fig. 3. A foetus expelled by antiblastokinin-treated rabbit (B-6, see text).

Similarly, in the second series, while all the controls had normal litters, none of the anti-BKN group had any comparable young. For instance, both the young delivered by rabbit B-6 were abnormally small and one of them (Figure 3) was still in the amnionic sac having been expelled as such 2 days later than the normal controls.

Discussion. It has been shown earlier[1] that blastokinin first appears in the pregnant rabbit uterus in detectable quantities on day 3 and peaks therein around day 5. Should blastokinin be biologically active in the formation and/or development of blastocysts in vivo, then, anti-BKN might be expected to exert its maximum inhibitory effect during this period embryonic development. The fact that administration of anti-BKN during this critical stage of embryonic development results in either abnormal development of the young or complete cessation of pregnancy strongly suggests that blastokinin plays a major role in vivo in the normal development of early rabbit embryos. The relative differences in the effectiveness of anti-BKN in the 2 series of experiments might be due, at least in part, to individual variations among the animals in the 2 strains.

Normally, blastocyst formation in the rabbit takes place around day 3 and implantation occurs around day 7 post coitum. The schedule of anti-BKN treatment employed was such as to provide the experimental animals with an injection of anti-BKN at each of the pre-blastocyst, early blastocyst and late preimplantation blastocyst stages. It is, therefore not possible to determine precisely whether blastokinin specifically facilitates the formation of blastocysts or aids in the development of early blastocysts prior to implantation. Perhaps it plays a role in implantation itself. Further experiments to clarify this point are currently in progress[4].

Zusammenfassung. Passive Immunisierung durch i.p. Verabreichung von Antiblastokinin an trächtige Kaninchen am 2., 4. und 6. Tag post coitum hat meistens eine Unterbrechung der Trächtigkeit im Implantationsstadium zur Folge oder führt in einigen wenigen Fällen zu missgebildeten Jungen. Aus den Ergebnissen kann man den Schluss ziehen, dass Blastokinin auch in vivo eine wichtige Rolle für die normale Entwicklung der Keime während der Frühphase der Trächtigkeit spielt.

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The Protective Effect of Estrogens Against Spontaneous Pancratic Islet and Renal Changes in Aging Male Rats

A considerable amount of work has been carried out on the influence of estrogens upon experimental diabetes and pancreatic islets[1-3]. A sex related spontaneous pancreatic islet change in aging male Sprague-Dawley rats has been described in our laboratories[4]. This appeared to be a good model to study the effect of estrogens on the pancreatic islets. Previously we reported a beneficial effect of conjugated equine estrogens (Premarin®) in this model in a 3-month test[5]. The present experiment was designed to further study this protective effect with different estrogens administered on a long term basis.

The other objective of the experiment was to study, beside the pancreatic islet protection, any possible protective effect of estrogens against the well known spontaneous renal alternations associated with aging[6].

Materials and methods. Two hundred thirty-seven 6-week-old male Sprague-Dawley rats (Charles River CD) were used in this study. The rats were divided into 10

groups and were treated daily for a period of 19 months as follows: 1. 3 groups with sodium equilin sulphate of 50, 500 and 1000 µg/kg in the drinking water; 2. 3 groups with ethynyl estradiol of 4, 40 and 200 µg/kg in the food; 3. 3 groups with sodium estrone sulphate of 50, 500 and 1000 µg/kg in the food. 1 group served as control. The animals received Purina rat meal.

The following parameters were studied in the experiment: weekly body weight and food intake, terminal blood glucose, organ weights of pituitary, adrenal, thyroid, liver and kidney and histology of pancreas and kidney. The incidence of the above-mentioned spontaneous pancreatic islet change was determined according to the principle outlined previously[7]. The histopathological status of the kidney was evaluated by the method of DURAND et al.[8].

Results. 1. The present study showed a high incidence of spontaneous pancreatic islet change (fibrosis and enlargement) in the control, low dose sodium equilin sulphate, low and middle dose ethynyl estradiol and low dose sodium estrone sulphate groups (Table I). However, in the middle dose sodium equilin sulphate and sodium estrone sulphate groups, the incidence rate significantly dropped and no pancreatic islet change was found in the high dose groups treated with any of the three estrogens. 2. In the aging animals there was evidence of senile glomerular and tubular changes. The estrogens in general in a dose related fashion also exerted a protective effect aginst these renal alterations (Table I). 3. The terminal blood glucose values were generally lower in the treated groups as compared to controls. 4. The body weight was significantly lower in a dose related fashion of all treated groups with the exception of rats treated with low dose sodium equilin sulphate and sodium estrone sulphate (Table II). A similar dose related significant reduction as compared to controls was also evident in the food intake in all treated groups. 5. As far as the organ weights are concerned there was generally a decrease of thyroid, liver and kidney weight and an increased weight of adrenals and pituitary with increasing dose of estrogens.

Table I. Effect of estrogens on the incidence of pancreatic islet changes and severity of progressive glomerulonephrosis in male rats

Group	Dose (µg/kg)	Pancreatic islet change No. of animals examined	Change (%)	Severity of progressive glomerulonephrosis (0–4)
Control		7/13	53	1.9
Sodium	50	8/16	50	1.5[b]
equilin	500	1/19	5[a]	0.8[b]
sulphate	1000	0/19	0[a]	0.6[b]
Ethynyl	4	6/19	31	2.2[b]
estradiol	40	7/16	43	1.6[b]
	200	0/20	0[a]	1.2[b]
Sodium	50	9/18	50	1.7[b]
estrone	500	2/16	8[a]	0.8[b]
sulphate	1000	0/19	0[a]	1.0[b]

[a] $p < 0.05$. [b] $p < 0.01$.

Table II. Body weights and food intake of male rats administered different estrogens for 19 months

Group	Dose (µg/kg)	Mean body weight (g) ± S.E. Initial	Terminal	Food intake (g) ± S.E. Average/rat/day
Control		169 ± 1.6	688 ± 23.2	24.8 ± 0.56
Sodium	50	170 ± 2.5	648 ± 18.0	22.2 ± 0.48[b]
equilin	500	184 ± 3.1	437 ± 16.1[b]	18.6 ± 0.39[b]
sulphate	1000	174 ± 1.9	367 ± 13.4[b]	16.5 ± 0.36[b]
Ethynyl	4	160 ± 1.9	582 ± 19.2[b]	21.4 ± 0.44[b]
estradiol	40	149 ± 1.5	483 ± 18.4[b]	18.6 ± 0.35[b]
	200	165 ± 1.2	381 ± 9.5[b]	15.7 ± 0.46[b]
Sodium	50	176 ± 2.4	671 ± 16.9	23.5 ± 0.15[a]
estrone	500	189 ± 2.5	540 ± 14.4[b]	20.6 ± 0.19[b]
sulphate	1000	176 ± 2.4	446 ± 9.0[b]	18.0 ± 0.14[b]

[a] $p < 0.05$. [b] $p < 0.01$.

Discussion. The present study shows that long term estrogen treatment beginning at an early age with sufficiently high doses confers a complete protection to aging male rats aginst the spontaneous pancreatic islet changes described by us previously[4]. Estrogens in a dose related fashion also proved to be protective against the senile alteration of the kidneys.

Some authors[1–3] claim a direct effect of estrogens on the pancreatic islets. In the light of the protective action of estrogens on both the pancreatic islets and renal integrity in our experiment one should consider the role of food intake in the mechanism of protection. A correlation seems to exist in our experiment between the reduction of both the food intake and the body weight gain on the one hand, and the lowering of the incidence of pancreatic islet fibrosis and decrease in severity of renal changes on the other with an increasing dosage of estrogen. This possibility is also supported by observations that under-nutrition[9] exerts a beneficial effect while over-feeding[10] has an aggrevating influence on senile kidney changes. BERG[11] entertains the theory of a possible auto-immune origin of the renal changes observed in aging nephrotic rats. In our previous report[5] we have also mentioned that the pancreatic islet change described by us may have an immunological component. Since estrogens suppress certain types of immunological response[12,13], this factor could not be ruled out as part of the mechanism of estrogen protection against pancreatic islet or renal changes associated with aging[14].

Zusammenfassung. Langzeitbehandlung mit Oestrogen bei von früher Jugend an daran gewöhnten männlichen Ratten ergibt einen vollständigen Schutz gegen Spontanfibrosis der Langerhansschen Inseln und ist ebenso wirksam gegen senile Nierenveränderungen.

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