chen auf, und zwar bei I = 0,000 und bei I = 0,100. Darüber hinaus imponierten die Immunpräzipitate aufgrund verschiedener Diffusionseigenschaften der Kaninchen- und Kröten-Antikörper sehr unterschiedlich (Figur).

Diese Ergebnisse deuten darauf hin, dass die Nachweisbarkeit der Amphibien-Antikörper-Antigen-Präzipitate mit Hilfe der Immundiffusion von der Ionenstärke des verwendeten Puffers abhängig ist.

Summary. Precipitating antibodies of amphibia are more dependent on the ionicity of the buffer system than the well known rabbit antibodies.

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Effect of Passive Administration of Antiblastokinin on Blastocyst Development and Maintenance of Pregnancy in Rabbits

The discovery of the presence of a specific glycoprotein[1,2], blastokinin, in rabbit uterine secretions in early pregnancy led to considerable speculation as to the possible overall role of this protein in the uterine environment. Although blastokinin has been demonstrated to facilitate the formation and development of blastocysts from late rabbit morulae in vitro[1], its role in this capacity in vivo could only be surmised. It is well known that immunological and immunochemical methods provide highly specific means of not only elucidating the scope and mechanism of action but also the means of controlling the activities of biologically active compounds. Hence, in the absence of any definitive information concerning other ways of regulating the synthesis and availability of blastokinin in the uterine environment, attempts were made to examine immunologically the biological activity of blastokinin in vivo.

The present communication reports the results of some preliminary experiments which strongly indicate that blastokinin plays an essential role in the uterine environment in the development of preimplantation blastocysts.

Materials and methods. In this study the technique of passive immunity was employed. The γ-globulin fraction, obtained by ammonium sulphate fractionation[3] of the serum of chickens (White Leghorn) immunized with purified rabbit blastokinin incorporated into complete Freund's adjuvant, was used as the source of antiblastokinin (anti-BKN). Based on the fact that only a single precipitin line was observed (Figure 1) when the preparation was allowed to react with crude uterine secretion (UF) from pregnant rabbits in Ouchterlony double immunodiffusion analysis, it was adjudged to contain antibodies only to blastokinin.

Two independent series of experiments were conducted. In the first series, 10 mature Dutch female rabbits were mated and divided into a control group and an anti-BKN group of 5 animals each. Each of the animals in the anti-BKN group was i.p. given 0.5, 1.0 and 1.0 ml of the anti-BKN preparation (12 mg protein/ml) incorporated into an equal volume of complete Freund's adjuvant on days 2, 4 and 6 respectively, following coitus. The controls were also treated similarly except that a preparation of normal chicken γ-globulin (11 mg protein/ml), obtained from chickens (White Leghorn) administered saline mixed with complete Freund's adjuvant, was substituted for the anti-BKN preparation. All the animals were killed 5 days after the last injection, and the reproductive tract examined.

In the second series, 2 groups of 3 each mature New Zealand white females were similarly given 1.0, 2.0 and 2.0 ml of either anti-BKN or normal chicken γ-globulin preparation according to the same schedule as described above. However, all the animals here were allowed to go through the period of normal pregnancy.

Results. It is clear from the Table that administration of anti-BKN prior to the formation of blastocysts and during

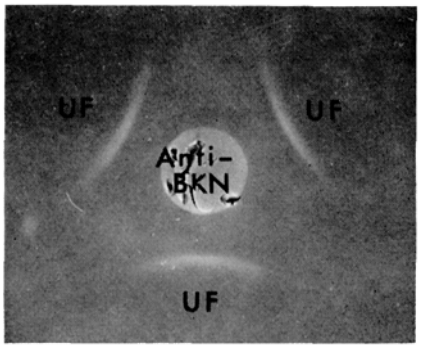

Fig. 1. Immunodiffusion pattern: anti-BKN X UF (see text).

[1] R. S. Krishnan and J. C. Daniel jr., Science *158*, 490 (1967).
[2] R. S. Krishnan and J. C. Daniel jr., Biochim. biophys. Acta *168*, 579 (1968).
[3] P. Stelos, in *Handbook of Experimental Immunology* (Ed. D. M. Weir, F. A. Davis Company, Philadelphia 1967), chapt. 1.

Effect of antiblastokinin on implantation and litter size in rabbits

No.	Treatment	Implantations	No.	Treatment	Litter size
Dutch			New Zealand		
A-1	control	10	B-1	control	7
A-2	control	8	B-2	control	8
A-3	control	9	B-3	control	5
A-4	control	8			
A-5	control	7	B-4	anti-BKN	0
			B-5	anti-BKN	0
A-6	anti-BKN	0	B-6	anti-BKN	2
A-7	anti-BKN	0			
A-8	anti-BKN	4			
A-9	anti-BKN	0			
A-10	anti-BKN	3			

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.

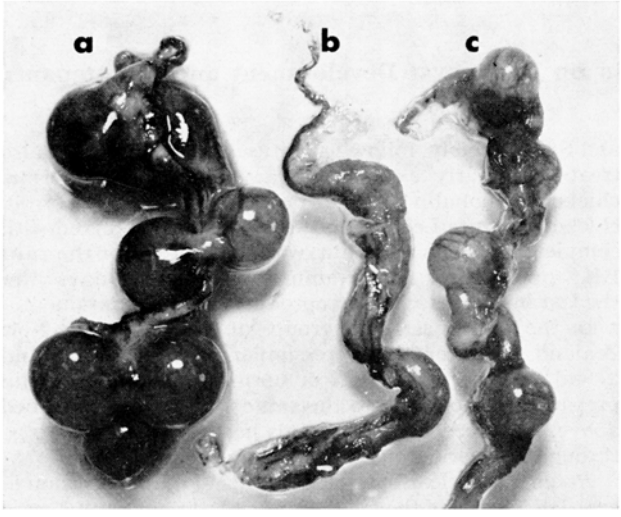

Fig. 2. Uteri taken from control and antiblastokinin-treated rabbits: a) control; b) and c) anti-BKN treated; a) and b) represent whole uteri whereas c) represents the horn that showed implantation sites.

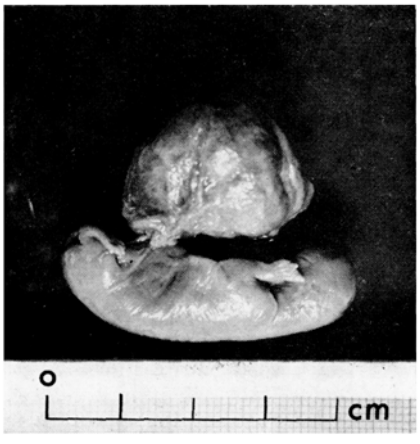

Fig. 3. A foetus expelled by antiblastokinin-treated rabbit (B-6, see text).

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