immune response took place also when antigen and polyanion were given by different routes.

JOHNSON et al.[5] have reported that polyriboadenylic-polyribouridylic acid enhances immune response only wehn it is given close to the time of antigen injection. According to Figure 3, under our experimental conditions,

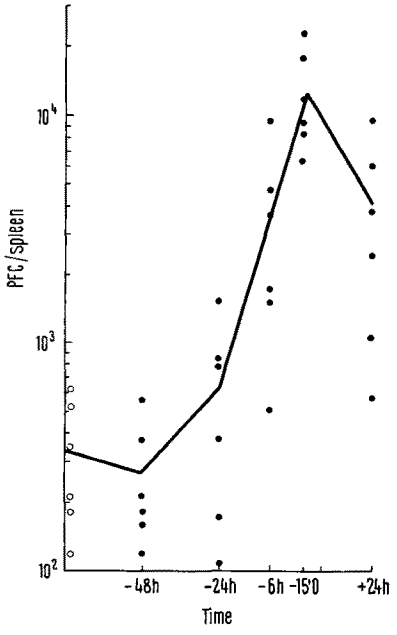

Fig. 3. The effect of timing on the adjuvant action of polymannuronic-guluronic acid (pMG). Groups of mice (6 mice per group) were injected with 4 mg pMG per mouse at various times prior or after antigen inoculation. Controls (○) and pMG-treated (●) mice were inoculated with 2×10^6 sheep red blood cells per mouse. Plaque-forming cells (PFC) were assayed 4 days after antigen inoculation.

using pMG instead of polyriboadenylic-polyribouridylic acid as adjuvant and SRBC instead of bovine-γ-globulin as antigen, similar but not identical results were observed. pMG enhances immune response, when given 6 h or 15 min prior to the antigen inoculation. However, pMG given 1 day after immunization causes significant enhancement of PFC-response. pMG injected 1 or 2 days before SRBC inoculation has no significant effect on PFC-response. It is of interest to note that pMG did not cause increase in spleen weight and erythropoesis in the spleen as observed with pyran copolymere, a carboxylate polymere[6] and polyacrylic acid[2], both known to stimulate immune response in mice towards SRBC. BRAUN et al.[1] discussed possible modes of action of polynucleotides on antibody formation. We recently[2] discussed possible modes of action of polyanions on immune response at cellular and at subcellular level, but experimental data available at present do not yet permit interpretation of the effects observed.

Zusammenfassung. Bei Mäusen, die mit suboptimalen Antigendosen geimpft wurden, erhöhte Polymannuron-Guluronsäure (Alginsäure) die Anzahl der 19S-antikörperbildenden Zellen in der Milz bis auf das 44-fache.

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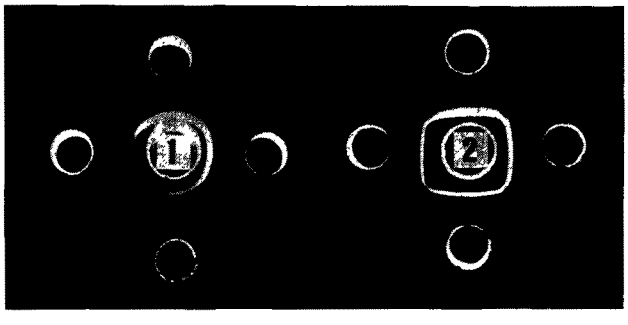
[5] A. G. JOHNSON, J. SCHMIDTKE, K. MERRITT and I. HAN, in *Nucleic Acids in Immunology* (Eds. O. J. PLESCIA and W. BRAUN; Springer-Verlag Inc., New York 1968), p. 379.

[6] W. BRAUN, W. REGELSON, Y. YAJIMA and M. ISHIZUKA, Proc. Soc. exp. Biol. Med. *133*, 171, (1970).

Einfluss der Ionenstärke auf das Immunpräzipitationsvermögen von Amphibien-Antikörpern

Antigen-Antikörper-Komplexe lassen sich methodisch einfach und dennoch spezifisch als Präzipitate durch das Verfahren der Immundiffusion nachweisen[1]. Von serologischen Arbeiten mit antikörperhaltigen Warmblüterseren ist jedoch bekannt, dass der Ablauf von Antigen-Antikörper-Reaktionen in vitro von der Ionenstärke des Reaktionsmilieus beeinflusst werden kann[2-4]. Das zunehmende allgemeine Interesse für die Immunitätsentwicklung bei Kaltblütern erfordert daher die Kenntnis einer eventuellen Abhängigkeit des Immundiffusionsergebnisses von der Ionenstärke[5] des verwendeten Puffermilieus, wenn statt Warmblüter-Antikörper Amphibien-Antikörper verwendet werden.

Der Vergleich von Kaninchen-Antikörpern und Erdkröten-Antikörpern in ihrem Immunpräzipitationsverhalten mit Rinderalbumin als Antigen in Ouchterlony-Platten (0,85% Ion-Agar in Sörensen-Phosphatpuffer mit Ionenstärke I = 0,150; 0,100; 0,050; 0,025; 0,005 oder in Aqua bidestillata, d.i. I = 0,000) ergab unterschiedliche Abhängigkeiten von der jeweils vorliegenden Ionenstärke. Während nämlich das Auftreten von Rinderalbumin-Kaninchen-Antikörperpräzipitaten ohne wesentliche Unterschiede über den gesamten I-Bereich von 0,000–0,150 verteilt war, traten optimale Präzipitate mit den Kröten-Antikörpern nur in zwei verschiedenen Berei-



Präzipitationslinien nach einer Reaktion von Kröten-Antikörpern (1) bzw. Kaninchen-Antikörpern (2) mit Rinderalbumin verschiedener Konzentrationen.

[1] Ö. OUCHTERLONY, Progr. Allergy *5*, 1 (1958).

[2] W. ATCHLEY, N. BHAGAVAN und S. MASOUREDIS, J. Immun. *93*, 701 (1964).

[3] R. SAISON und D. INGRAM, Nature, Lond. *197*, 296 (1963).

[4] H. RAPP und T. BORSOS, J. Immun. *91*, 826 (1963).

[5] P. R. LEWIS, Biochem. J. *52*, 330 (1952).

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

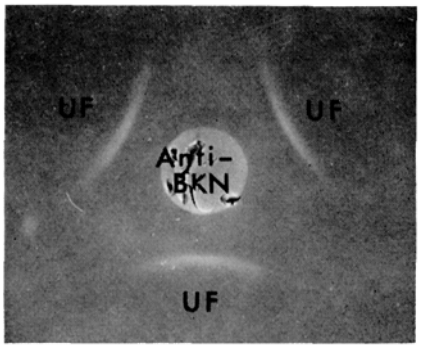

Fig. 1. Immunodiffusion pattern: anti-BKN X UF (see text).

[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			