

la même sur tous les champs d'un même animal. Le fait d'employer plusieurs doses permet de mieux graduer les résultats.

Les conditions d'irradiations ont été les suivantes: 50 kV – filtre 1 mm Al – C.D.A. 0,8 mm Al – 20 mA – D.F. 10 cm – champ 1 cm de diamètre – débit 852 R/min.

Résultats. Les résultats sont représentés dans la Figure. Chaque point est une moyenne de 50 observations. Les unités sont arbitraires; 100 est considéré comme épilation totale et 0 comme absence d'épilation. Sur chaque animal l'intensité de l'épilation a été estimée à plusieurs reprises en comparant les champs entre eux à partir de 8 jours après l'irradiation. Comme on peut le voir sur la Figure, la protection atteint un maximum 10 min après l'irradiation pour diminuer ensuite lentement. Après 60 min il persiste encore une protection appréciable. Les résultats correspondent plus à ceux de BACQ⁹, de GRAYEVSKY et al.⁴ étudiant la mortalité chez la souris et à ceux de BETZ et al.⁵ chez le rat, qu'à ceux de SMOLIAR¹ avec la cystéamine chez le rat. Les résultats ne sont naturellement pas tout à fait comparables, Smoliar ayant utilisé

la cystéamine comme protecteur et le taux de mortalité comme critère de protection¹⁰.

Zusammenfassung. An Cystamin-injizierten Ratten (i.p.) wurde das Ausmass der Schutzwirkung, in zeitlicher Abhängigkeit, bei lokaler Röntgenbestrahlung, auf das Haarwachstum untersucht. Eine maximale Schutzwirkung wurde nach 10 min gefunden. Sie nimmt von da an langsam ab und ist selbst 60 min nach der Cystamin-injektion noch nachweisbar.

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⁹ Z. BACQ, Bull. Acad. Méd. Belg. VI sér. 18, 426 (1953).

¹⁰ Ce travail a été réalisé partiellement grâce au Contrat Euratom no. 006-61-12-BIOB.

Antibody Production in Rabbits Following Immunization via the Lateral Ventricle of the Brain

In a previous paper¹ we have shown that repeated injections of heterologous erythrocytes into the lateral ventricle of the rabbit brain induce significant haemolysin production in the blood, whereas very weak or no antibody activity could be detected in the cerebrospinal fluid. It has been assumed that slow clearance of antigen from the cerebrospinal fluid to blood may act as a prolonged stimulus to antibody-forming cells. In the following study, small amounts of foreign protein were introduced into the lateral ventricle of the rabbit brain; antibody formation was estimated and compared with that produced by conventional routes of immunization.

Young adult chinchilla rabbits weighing 2.6 to 3 kg were used, and assigned to three groups. Group I consisted of 18 rabbits; each animal had a cannula permanently inserted into the lateral ventricle of the brain². The rabbits of this group were injected through the cannula with 0.1, 1.0 and 10 mg of human γ -globulin. Groups II and III were immunized intravenously and subcutaneously respectively; an immunization schedule identical to that of Group I was used. Twenty days after primary immunization, each rabbit received a single intraventricular, intravenous or subcutaneous booster injection of 1.0 or 10 mg of human γ -globulin. Blood samples were obtained 5, 8 and 11 days after both primary and secondary immunization. Anti-human γ -globulin activity of the sera was estimated by passive haemagglutination, using formalinized and tanned sheep erythrocytes as described elsewhere³. All antibody estimations were performed in duplicate. Control sera obtained from all rabbits prior to immunization were also tested for the presence of anti-human γ -globulin antibody. On the eleventh day after booster injection, cerebrospinal fluid was withdrawn from the cisterna magna with special precautions not to contaminate the cerebrospinal fluid with blood. Passive haemagglutination tests were also done on the cerebrospinal fluids. The brains in which cannulae were inserted were carefully inspected at autopsy in order to

ascertain whether infection occurred at the site of implantation.

The results of the passive haemagglutination studies are summarized in the Table. The primary immune response to intraventricular administration of the lower doses of human γ -globulin (0.1 and 1.0 mg) was more consistently detected than that noted in Groups II and III. Thus, eight of eleven rabbits of Group I produced antibody earlier and in significantly higher titer when compared with Group II (four of eleven) and Group III (one of thirteen). The primary responses were comparable in rabbits immunized intravenously and intraventricularly when a 10 mg dose of antigen was employed. Cerebrospinal fluid from rabbits of all experimental groups did not exhibit passive haemagglutination activity, either by the method described or by reaction of the fluid with human Rh(D) red blood cells coated with incomplete anti-D (indirect Coombs reaction).

It is well known that various routes of protein antigen administration may lead to quantitative differences in antibody formation. Although the low antibody titers were obtained in the present study, nevertheless the results show that the injection of antigen into the lateral ventricle of the rabbit brain represents an effective mode of stimulating the antibody-forming apparatus without using adjuvant materials, particularly when small doses of antigen are employed. These findings confirm those reported previously¹ on haemolysin production in cannulated animals. In addition, others have shown⁴ that smaller quantities of antigen may be required to induce

¹ B. D. JANKOVIČ, M. DRAŠKOVIČ, and K. ISAKOVIČ, Nature 191, 288 (1961).

² W. FELDBERG and S. L. SHERWOOD, J. Physiol. 120, 3P (1953).

³ B. D. JANKOVIČ, B. H. WAKSMAN, and B. G. ARNASON, J. exp. Med. 116, 159 (1962).

⁴ K. WESSELY, Münch. med. Wschr. 58, 1713 (1911). – R. THOMPSON and H. OLSON, J. Immunol. 65, 633 (1950). – A. C. BREEBAART and J. JAMES-WITTE, Am. J. Ophthal. 48, 37 (1959). – J. J. PARKS, H. M. LEIBOWITZ, and A. E. MAUMENEE, J. Immunol. 87, 199 (1961).

Primary and secondary antibody responses following intraventricular, intravenous and subcutaneous administration of antigen

Primary antibody response					Secondary antibody response				
Amount of antigen injected (mg)	No. of rabbits*	Mean peak antibody titer (log ₂)			Amount of antigen injected (mg)	No. of rabbits*	Mean peak antibody titer (log ₂)		
		5 days	8 days	11 days			5 days	8 days	11 days
<i>Intraventricular injection</i>									
0.1	3/5	0.6	1.4	1.0	1	4/5	1.6	1.0	0.8
1	5/6	0.4	1.5	2.0	1	5/6	3.2	2.5	1.8
10	6/7	0.6	1.8	2.2	10	7/7	4.3	4.0	3.4
<i>Intravenous injection</i>									
0.1	2/6	0	0	0.4	1	5/6	1.8	1.6	1.2
1	2/5	0	0	0.6	1	5/5	2.2	2.3	1.5
10	8/8	0.4	2.0	2.7	10	8/8	3.7	3.8	3.0
<i>Subcutaneous injection</i>									
0.1	0/6	0	0	0	1	0/6	0	0	0
1	1/7	0	0	0.3	1	2/7	0.3	0.4	0.3
10	5/8	0	0.4	1.5	10	5/8	2.3	1.8	1.0

* Numerator, number of rabbits with anti-human γ -globulin antibody; denominator, number of rabbits in group.

demonstrable antibody when injected intracorneally or intravitreally than when injected by other parenteral routes.

The mechanism by which foreign protein reaches immunologically competent cells from the cerebrospinal fluid is unknown. It was demonstrated in dogs that when radioiodinated human serum albumin is injected intrathecally, a cisternal fluid-plasma equilibrium is established in 16–20 h⁵. DUPONT et al.⁶ suggested that red blood cells and albumin are cleared from the cerebrospinal fluid by separate mechanisms. They postulated that red blood cells are phagocytized by the mesothelial cells which line the subarachnoid space. SIMMONDS⁷ demonstrated that the bilateral ligation of the cervical lymphatics does not affect the 'absorption rate' of the red blood cells from the brain cavity. Similar results were obtained on clearance of colloidal Au-198 particles from the cerebrospinal fluid⁸. However, these observations do not rule out the lymphatic pathways for the passage of soluble protein from the cerebrospinal fluid into the circulation.

The present study does not offer evidence on the immunological competence of choroid plexus cells. However, the possibility that choroid plexus may be a route by which protein particles cross the brain-blood barrier must be taken into account. It seems probable that choroid plexus may play a role by furnishing the site where the intraventricularly injected antigen meets the blood-borne immunologically competent cells. Indirect evidence in support of this hypothesis is the formation of 'round

space aggregates of cells', resembling lymphoid follicles, in the choroid plexus of animals developing experimental allergic encephalomyelitis^{8,9}.

Résumé. Des lapins ont été immunisés avec de petites quantités de γ -globuline humaine par voie intraveineuse, sous-cutanée et par le ventricule latéral du cerveau. L'expérience met en évidence que l'immunisation par voie intraventriculaire est la plus efficace pour la production des anticorps.

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⁵ R. A. FISHMAN, *Am. J. Physiol.* **175**, 96 (1953). – C. A. VAN WART, J.-R. DUPONT, and L. KRINITZ, *Proc. Soc. exp. Biol. Med.* **103**, 708 (1960).

⁶ J.-R. DUPONT, C. A. VAN WART, and L. KRINITZ, *J. Neuropath. exp. Neurol.* **20**, 450 (1961).

⁷ W. J. SIMMONDS, *Aust. J. exp. Biol. med. Sci.* **31**, 77 (1953).

⁸ B. D. JANKOVIČ and M. IŠVANESKI, *Int. Arch. Allergy* **23**, 188 (1963).

⁹ Supported by grants from the Yugoslav Foundation for Scientific Research.

Postsynaptic Inhibition in Motoneurons Evoked from the Lower Reticular Formation

Powerful descending inhibition can be evoked from the lower reticular formation¹. Stimulation of this region can give presynaptic inhibition through primary afferent depolarization and also inhibition of reflex paths at an interneuronal level², but there has been no clear demonstration of postsynaptic inhibition of motoneurons. Although hyperpolarizing responses can be evoked in moto-

neurons from the lower brain stem^{3,4}, it has been reported that these responses can neither be reversed on hyperpolarization of the membrane nor are they associated with the conductance changes typical of inhibitory postsynaptic potentials (IPSP)⁵. It will presently be demonstrated that large IPSPs can be evoked in motoneurons from the lower brain stem.

Intracellular recordings have been made from motoneurons in precollicular decerebrate cats either with intact spinal cord or with the spinal cord transected at