affirmer que la sensibilité particulière de certaines molécules d'anticorps à l'agent réducteur correspond toujours à des anticorps 19 S.

Conclusion. Nos expériences montrent que dans les conditions utilisées la production d'anticorps des rats âgés est inférieure à celle des animaux jeunes et que cette différence provient d'une teneur plus faible en anticorps 7 S chez les animaux âgés. Elles permettent de constater toutefois que malgré cette diminution, les rats demeurent encore capables au cours de leur phase de senescence de produire des anticorps sériques à des taux relativement élevés.

Summary. Antibodies produced following injections of a proteinic antigen emulsified in Freund's adjuvant were studied by the passive hemagglutination test in some lots of WCF rats, varying in age. It is shown by treating sera with mercaptoethanol that older animals produce less 7 S antibodies than younger ones, and that, despite this difference, rats remain able to elaborate seric antibodies at relatively high rates during their senescence period.

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avec la collaboration technique de
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The Inhibition of Antibody Production in Mice Caused by the Injection of Specific Antiserum on the First Day of Life

Relatively few papers[1-3] concerning the problem of inhibition of antibody production caused by prior passive immunization have been published. The mechanism of this phenomenon has not been explained yet.

The aim of our research was the examination of the influence of the antiserum dose used in the passive immunizing of neonatal mice and of the manner of antiserum injection on the inhibition degree of immunogenesis.

Material and methods. In general, 196 1-day-old white mice were used for the investigation. 35 mice were control animals, the other mice were given intracardially, i.p. or s.c. the different doses of diphtheria anti-toxin serum (25, 50, 100 anti-toxin units – A.U.).

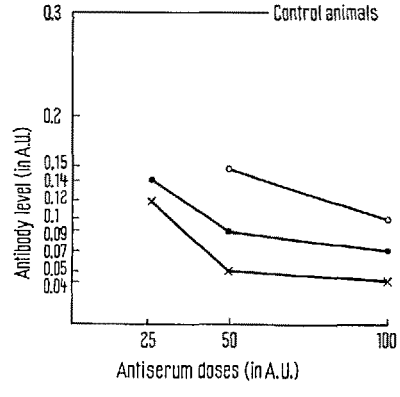
When the animals were 3 weeks old they were immunized with diphtheria toxoid; the mice passively immunized on the first day of life and the control mice were injected s.c. 3 times each week for 4 weeks with 2 Limes floculatio (Lf) toxoid (weekly toxoid dose was 6 Lf and general dose of diphtheria toxoid used for the immunization of mice amounted to 24 Lf).

Six days after the end of the immunizing cycle, the mice were bled by intracardial puncture and the diphtheria anti-toxins in the sera were determined by JENSEN's method on guinea-pigs. It should be pointed out here that we found diphtheria anti-toxins passively introduced into the neonatal mice to be absent in sera of the

Diphtheria anti-toxin level in the sera of mice passively immunized on the first day of life and in the control sera

Passive immunization of neonatal mice	Dose of diphtheria anti-toxin serum used in passive immunization of neonatal mice, in A.U.					
	25		50		100	
	No. of mice	Average anti-toxin titre after active immunizing, in A.U.	No. of mice	Average anti-toxin titre after active immunizing, in A.U.	No. of mice	Average anti-toxin titre after active immunizing, in A.U.
Intracardial	10	0.12	34	0.05	19	0.04
i.p.	–	–	19	0.15	15	0.1
s.c.	16	0.14	22	0.09	26	0.07

In the control animals which were not given diphtheria anti-toxins on the first day of life the antibody titre in serum induced by active immunization amounted to an average of 0.3 A.U./ml.



Comparison of diphtheria anti-toxin level in the sera of mice passively immunized on the first day of life and in the control animal sera. ×——× intracardial injection of specific antiserum; ●——● s.c. injection of specific antiserum; o——o i.p. injection of specific antiserum.

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3-week-old animals at the beginning of their active immunization.

From the results listed in the Table and graphed in the Figure the following conclusions can be drawn: (a) The inhibition degree of antibody production in 3- to 7-week-old mice which were given the specitic antibodies on the first day of life depends on antiserum dose. (b) Even massive doses of specific antiserum (100 A.U.) used in passive immunization of neonatal mice do not cause the complete 'paralysis' of the immunogenesis apparatus. (c) The best inhibition was achieved when intracardial passive immunization of the neonatal mice was carried out, the least inhibition was observed when the neonatal mice were given antiserum by i.p. injection. (d) The lack of diphtheria anti-toxins passively introduced into the neonatal mice when the animals began to be actively immunized gives evidence against the possibility of direct immunological reaction with antigen. In the passively immunized organisms, the mechanism of 'feedback' in the specific inhibition of immunogenesis would be indicated[2].

Résumé. L'injection de l'antitoxine diphthérique aux souris nouvelles-nées inhibe chez elles la production des anticorps lorsqu'elles sont, à l'état de maturité, immunisées à l'aide d'un toxoïde diphthérique. Nous avons examiné l'influence de la dose d'antisérum administrée aux animaux nouveaux-nées au cours d'immunisation passive ainsi que l'influence de la voie d'injection sur le degré d'inhibition de l'immunogenèse.

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In vitro Studies on the Biosynthesis of a Glycoprotein Associated with Normal and Tumoural Growth in the Rat

Isolation and characterization of pure β-glycoprotein from a primary tumour induced by 2-amino-acetylfluorene (2-AAF) in rats has been reported by us[1]. Studies on immunofluorescence have shown that this protein is present in cells of bone marrow, spleen, lung and liver which are connected to the reticulo-endothelial system.

With the aid of further investigations on the subject we have studied the capacity of the same tissues to biosynthesize this protein during incubation in vitro.

Materials and methods. The method of HOCHWALD and THORBECKE was used for tissue culture[2]. The medium of incubation consisted of Hank's balanced salt solution, amino acid mixture, vitamins and penicillin, supplemented with 0.5% ovalbumin[2,3]. Natural glycine in the medium was replaced by uniformly labelled glycine C^{14} (New England Nuclear Corp., 2–10 mc/mM) to give a final concentration of 2 μc/ml of the medium. The experiments were repeated by replacing glycine C^{14} with alanine C^{14} in the same concentration.

The tissue was dissected out aseptically from a rat weighing 60 g and minced. Tissue weighing 50 mg precisely was added to each tube containing 2 ml medium and incubated in the shaker at 37 °C for 90 h. Sterility of the cultures was tested and thereafter the medium with tissue fragments was frozen. It was thawed, centrifuged, and supernatant was dialysed against normal saline for 24 h. Samples were lyophilized and used for radioimmunoelectrophoresis[2,4,5] utilizing goat antibody prepared against pure glycoprotein.

To test whether the labelled amino acid is actually incorporated in the protein and not simply adsorbed on the surface during incubation[6] 2 controls were kept: multiple freezing and thawing of the tissues prior to incubation, and addition of puromycin in a concentration of $6 \times 10^{-3} M$ to a group of cultures to inhibit protein synthesis.

The method described by LAURELL was used for quantitative evaluation of biosynthesis[7]. Electrophoresis was carried out on agarose gel of uniform 1.5 mm thickness containing 1.5% antiserum prepared against pure β-glycoprotein in goat.

A standard curve was established in each experiment using precisely known quantities of pure β-glycoprotein in a progressively increasing manner from 5 to 25 μg. The height of the peak was directly proportional to the amount of antigen applied in each hole (Figure 2); 500 μg of the total protein from the culture medium in 10 μl volume were applied to each hole. The samples were taken out at different periods of incubation, i.e. 0, 4, 17, 30, 50 and 90 h, to study the rate of biosynthesis of glycoprotein in vitro.

The electrophoresis was carried out at 300 V and 35 mA for 7 h at 4 °C. The peaks were stained with Ponceau red, and the dried agarose plate was exposed to Kodak (industrial k k) X-ray film for 15 days for autoradiography.

Results. Immunoelectrophoretic analysis of total soluble extract of the tumour induced by 2-AAF with antibody prepared in goat against glycoprotein demonstrates that the glycoprotein is immunochemically pure as the antibody is monospecific (Figure 1). This Figure also illustrates the β-electrophoretic mobility of the protein.

The results obtained by electrophoresis carried out in agarose gel containing antibody showed that glycoprotein was synthesized by cultures of bone marrow, spleen, lung

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