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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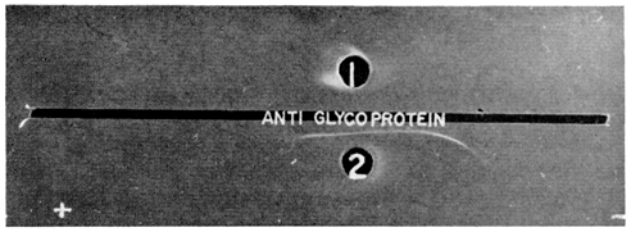

Fig. 1. Immunoelectrophoretic analysis of total soluble extract of the tumour induced by 2-AAF showing β-electrophoretic mobility of the glycoprotein. (1) Normal rat serum. (2) Total soluble extract of the tumour. Antiserum – antiglycoprotein serum prepared in goat containing monospecific antibody.

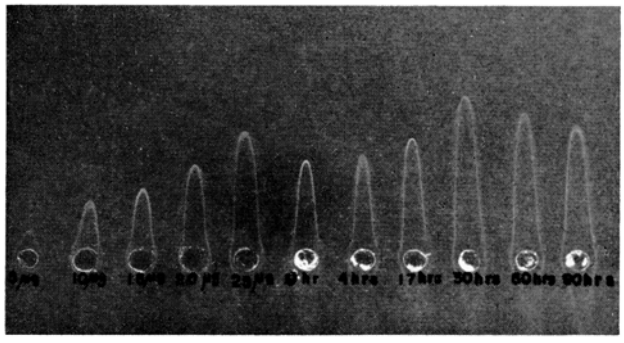

Fig. 2. Biosynthesis of the glycoprotein in vitro during period of incubation by spleen cultures as evaluated by electrophoresis in agarose containing monospecific antibody. Note the linear increase in the height of the peak in the first 5 wells according to the increasing quantity of purified glycoprotein which serve as standard. Experimental samples contain 500 μg of total protein from the culture medium taken at different periods of incubation.

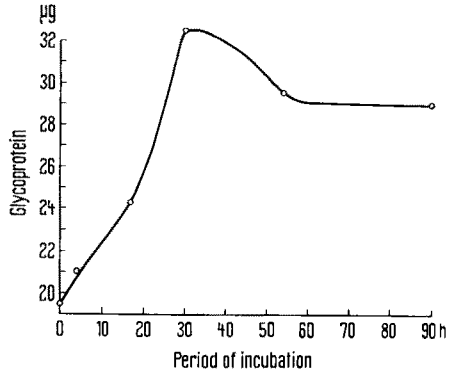

Fig. 3. Graphical representation of Figure 2 showing maximum concentration of glycoprotein after 30 h of incubation and reduction thereafter.

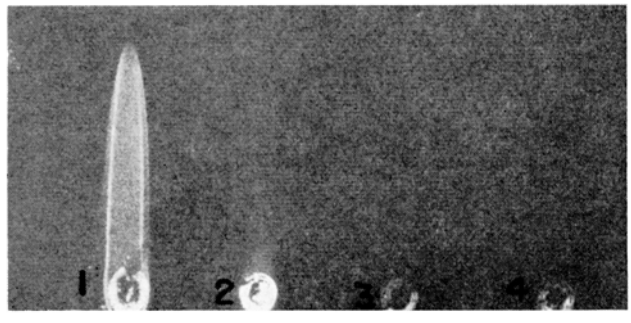

Fig. 4. Autoradiographic image of the agarose plate demonstrating (1) incorporation of labelled amino acid in the line of precipitation in control cultures, (2) absence of radioactive line in cultures with puromycin concentration of $6 \times 10^{-3} M$ and (3, 4) dead tissue.

and embryonic liver. Cultures of kidney, muscle and adult liver failed to show biosynthesis in vitro. Concentration of the glycoprotein as expressed per 500 μg of total protein from the medium was found to be maximum in the sample taken at the incubation period of 30 h (Figures 2 and 3).

Puromycin when used in a concentration of $3 \times 10^{-3} M$ caused 70% inhibition of the biosynthesis of the glycoprotein, but when used in a concentration of $6 \times 10^{-3} M$ caused 100% inhibition (Figure 4).

Discussion. Immunological significance of the glycoprotein under investigation has been discussed before[1].

Application of a specific analytical method gives us an insight into the study of biosynthesis of the protein in vitro. The results of the experiment on autoradiography with respective control of cultures of dead tissue and with puromycin provide conclusive evidence that this protein was synthesized by bone marrow, spleen, lung and embryonic liver (Figure 4).

This method of quantitative estimation of the glycoprotein using electrophoresis on agarose containing monospecific antibody seems very precise[7]. Gradual decline in the biosynthesis of the protein observed after 30 h closely relates to the time with the decline of nutrients from the medium.

Agarose plate when exposed to sensitive X-ray film can give quantitative and qualitative results at the same time. Combination of these methods can be beneficially applied to study the synthesis of various proteins in vitro by different tissues and also to study the effect of certain antimetabolites. Such studies are in progress at present in our laboratory[8].

Résumé. La biosynthèse d'une glycoprotéine, isolée à partir d'une tumeur induite au 2-amino-acetylfluorène, a été étudiée in vitro, par la culture de la moëlle osseuse du rat, du poumon et du foie embryonnaire. Cette étude a été faite à l'aide d'acides aminés radioactifs dans le milieu et, subséquemment, par radio-immunoélectrophorèse. Le dosage quantitatif de la biosynthèse in vitro, par les tissus, a été fait par la méthode électrophorétique d'agarose contenant un anticorps monospécifique préparé chez la chèvre, contre la glycoprotéine pure. La spécificité de la méthode a été éprouvée en utilisant, comme contrôle, l'incubation des tissus morts et en ajoutant de la puromycine dans le milieu.

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