It has recently been shown that thymus-dependent cells play an essential role in the response to oxazolone[15]. We have previously indicated[14] that DMBA given at birth acts on the thymus, and consequently mainly on the long-lived thymus-dependent lymphoid cell population carrying immunological memory[16,17], that in rodents have a potential life span of some months[18]. Therefore, if some thymus cells have escaped from the effect of the carcinogen, we can assume that at 20 to 30 days of age, at least part of the thymus-dependent cell population is present and DMBA fails to exert an inhibitory effect on cellmediated immunity.

Between 90 to 100 days, i.e. up to an age corresponding approximately to one-third of the life span of a mouse, the long-lived thymus-dependent lymphocytes could be almost completely eliminated by the effect of DMBA given at birth, and by the involution of the cells derived from those which escaped the effect of the carcinogen. Their depletion explains the failure of the adult mice given DMBA at birth to show a normal delayed hypersensitivity response.

We have previously shown that DMBA injected at birth in mice greatly reduces the primary immune response prior to the appearence of the tumours[13]. The present results indicate that such treatment also reduces cell-mediated immunity. The reduction of both humoral and cell-mediated immune responses is thus compatible with the suggestion that there is a relationship between immunodepression and the high frequency of tumours that DMBA treatment induces[13,14,19].

Riassunto. Una iniezione neonatale di 100 μg di DMBA in topi BALB/c e Charles-River deprime la risposta immunitaria, a tipo ipersensibilità ritardata, in animali di 90–100 giorni di età, mentre non pare agire come fattore deprimente in topi valutati 20–30 giorni dopo la nascita ed il trattamento oncogeno.

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[1] J. K. BALL, N. R. SINCLAIR and J. A. MCCASTER, Science *152*, 650 (1966).
[2] J. STJERNSWÄRD, J. natn. Cancer Inst. *36*, 1189 (1966).
[3] J. STJERNSWÄRD, J. natn. Cancer Inst. *37*, 505 (1966).
[4] J. STJERNSWÄRD, Cancer Res. *26*, 1951 (1966).
[5] J. STJERNSWÄRD, J. natn. Cancer Inst. *38*, 515 (1967).
[6] J. STJERNSWÄRD, J. natn. Cancer Inst. *40*, 13 (1968).
[7] N. E. CREMER, D. O. TAYLOR and S. J. HAGENS, J. Immun. *96*, 495 (1966).
[8] M. H. SALAMAN and N. WEDDERBURN, Immunology *10*, 445 (1966).
[9] B. V. SIEGEL and J. I. MORTON, Proc. Soc. exp. Biol. Med. *123*, 467 (1966).
[10] B. V. SIEGEL, Immunology *10*, 559 (1966).
[11] W. S. CEGLOWSKI and H. FRIEDMAN, J. natn. Cancer Inst. *40*, 983 (1968).
[12] G. CHAN, M. W. RANCOURT, W. S. CEGLOWSKI and H. FRIEDMAN, Science *159*, 437 (1968).
[13] C. BARONI, G. BERTOLI and N. FABRIS, Tumori *54*, 117 (1968).
[14] H. RAPPAPORT and C. BARONI, Cancer Res. *22*, 1067 (1962).
[15] A. J. S. DAVIES, L. CARTER, E. LENEHARS and V. WALLIS, Immunology *17*, 111 (1969).
[16] J. F. A. P. MILLER and D. OSOBA, Physiol. Rev. *47*, 437 (1967).
[17] J. F. A. P. MILLER and G. F. MITCHELL, Transplant. Rev. *1*, 3 (1969).
[18] S. H. ROBINSON, G. BRECHER, S. I. LOURIE and J. E. HALEY, Blood *26*, 281 (1965).
[19] C. BARONI and F. CEFIS, Tumori *49*, 373 (1963).

Further Studies on Bovine Red Cells Having a Different Glycoprotein Coat

In a previous study[1] antigenic properties of bovine neuraminic acid (NA) containing erythrocyte mucoid preparations have been reported, using reagents for the blood group antigens in all bovine blood group-systems, whereas immunization procedures with the same glycoproteins were less successful[2]. A next step was the investigation of some heterophilic receptors of the NA-free mucoid[3]. Finally, a correlation was found between the thickness of the outer NA-containing glycoprotein layer of the red cells (rbc) and different forms of their agglutinability with special regard to 'incomplete' antibodies[4].

NA-containing receptors – like MN in human rbc – have not been detected, except myxovirus-receptors[1]. A comprehensive review of different receptors (virus, biological, serological and pharmacological), where NA is involved, has been given elsewhere[5]. Recently a contribution has been made[6] involving NA containing blood group receptors in the bovine isoantigen system. It was observed that NA is involved in the specificity of the F-antigen in bovine rbc. The assumption is based on the following experimental data: a) The F-antigen is inactivated by neuraminidase and b) in F/F homocygote rbc-stroma the NA-content is larger than in F/V rbc and in the latter larger than in V/V rbc.

In spite of these convincing data, however, the following should be taken into consideration: 1. Obviously the F-antigen having NA is not part of the outer mucoid layer and accordingly deeper in the membrane because otherwise a) F should be partly removed by proteolytic enzymes and b) the corresponding antibody should be an agglutinating one because of the superficial 'outside' localization of the antigen c) the mucoid should be a better inhibitor (it inhibits only weakly).

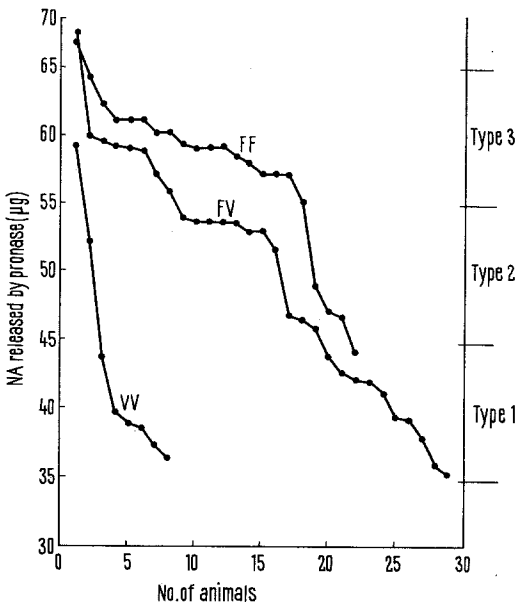
2. Accordingly the F-antigen could belong to the NA-containing glycolipid fraction of the stroma. This would imply a) removal by glycolipid extraction (methanol/chloroform) b) crossreaction of the anti-F with other

[1] G. UHLENBRUCK and D. O. SCHMID, Z. ImmunForsch. *123*, 466 (1962).
[2] H. HANSEN and G. UHLENBRUCK, Z. ImmunForsch. *131*, 453 (1966).
[3] G. UHLENBRUCK and M. KRÜPE, Z. ImmunForsch. *125*, 285 (1963).
[4] G. UHLENBRUCK, G. V. F. SEAMAN and R. R. A. COOMBS, Vox sang. *12*, 420 (1967).
[5] G. UHLENBRUCK and W. GIELEN, Fortschr. Neurol., *38*, 202 (1970).
[6] C. L. HATHEWAY, D. F. WESELI, T. M. LUDWICK and H. C. HINES, Vox sang. *17*, 204 (1969).

rbc, for instance cat, having the same NA containing glycolipid pattern[7,8].

3. It is known that the NA of bovine rbc consists of 2 forms, N-acetyl-NA and N-glycolyl-NA[1]; therefore a) do different amounts of these 2 NA occur in the F/F, F/V and V/V rbc, in other words: can it be that F/F rbc have only N-acetyl-NA, whereas for instance V/V have only the N-glycolyl compound? b) Do different forms of heterophile cryptantigens[3] appear in F/F and V/V cells after neuraminidase treatment? c) Is there any crossreaction between anti-F and other antibodies or heterophilic agglutinins against NA-containing receptors[5]?

The aim of this investigation was to see whether there is any relationship between the NA containing F-antigen or the F/V-system and the different quantity of the rbc glycoprotein coat, as has been stated earlier[4]. The Figure shows that the pronase-releasable amount of glycoprotein NA demonstrates no direct correlation to the F/F or V/V genetic background, although it is remarkable that F/F cells usually have a high amount of NA (Type 3), whereas in F/V and V/V rbc more examples of the thin glycoprotein layer cells do occur (Type 1). The method of this quantitative NA estimation has been described in full detail earlier[7,9]. Similar results have been obtained by using a combined method with neuraminidase and α-chymotrypsin[7,9] and measuring approximately the total NA.



Different amounts of pronase released glycoprotein NA from F/F, F/V and V/V bovine rbc. The µg NA values correspond to 0.5 ml of packed rbc, Type 1, 2 and 3 show the range of values for the 3 agglutination types according to Coombs[4], corresponding to thin, medium and thick glycoprotein coat.

From these experiments, it may be concluded that the F/V blood group system in bovine rbc obviously does not exclusively govern the expression of the outer NA glycoprotein coat of the cells, but does apparently, as previously stated[6], also influence the stroma (glycolipid?) associated NA, the more as we found some loss of NA glycoprotein during stroma preparation. Therefore the protease-released NA cannot be regarded as a suitable tool for investigating this blood group system, because no direct correlation to the F/V system could be found.

Zusammenfassung. Untersuchungen über den pronaselabilen Anteil der äusseren neuraminsäurehaltigen Glykoproteinschicht verschiedener Rindererythrozyten ergeben bei den einzelnen Tieren zwar signifikante Unterschiede, die jedoch in keiner direkten Beziehung zu den ebenfalls neuraminsäurehaltigen F-Antigenen dieser Zellen stehen.

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2 February 1970.

[7] G. Uhlenbruck, A. Rothe and G. I. Pardoe, Z. ImmunForsch. *136*, 79 (1968).
[8] G. Wintzer and G. Uhlenbruck, Z. ImmunForsch. *133*, 60 (1967).
[9] G. Uhlenbruck and H. J. Sehrbundt, Bibl. haemat. *32*, 337 (1969).
[10] Acknowledgment. The help of Mrs. M. Heggen is gratefully acknowledged. The work was supported by Deutsche Forschungsgemeinschaft.

Correlation between Electrophoretic Mobility and Heavy Chain Sub-classes of Residual IgG from Patients with Severe Hypogammaglobulinaemia

Previous studies[1] of normal IgG by isoelectric focusing established that fractions of pH near 7.0 were rich in IgG2 and that IgG1 and IgG3 – rich fractions were obtained at pH's higher than 8.0. This result is consistent with the reported fast mobility of IgG2 monoclonal proteins in relation to proteins of other sub-classes[2,3].

In a recent study (results to be published) we have demonstrated abnormalities in the distribution of IgG heavy chain sub-classes in sera from patients with severe hypogammaglobulinaemia.

Although most residual IgGs from hypogammagloublinaemic sera show an electrophoretic mobility similar to that of normal IgG, in some instances proteins showing abnormal electrophoretic mobility have been described[4–6]. In the present study we tried to correlate the electrophoretic mobility of residual IgG with the heavy chain sub-class distribution.

Material and methods. 11 sera containing 80–120 mg/100 ml of IgG were chosen out of a series of 16, previously

[1] A. Howard and G. Virella, Proc. XVIIth Coll. Prot. Biol. Fluids, Brugge 1969 (Pergamon, London 1970), p. 369.
[2] J. Gergely, G. A. Medgyesi and D. R. Stanworth, Immunochemistry *4*, 369 (1967).
[3] R. Jefferis, P. D. Weston, D. R. Stanworth and J. R. Clamp, Nature *219*, 646 (1968).
[4] R. Hong and R. A. Good, Science *156*, 1102 (1967).
[5] M. Seligman, G. Meshaka and F. Danon, Rev. fr. Etud. clin. biol. *12*, 604 (1967).
[6] H. Golebiowska and D. S. Rowe, Clin. exp. Immun. *2*, 275 (1967).