Summary. The effect of the alkylizing agent cyclophosphamide (100 mg/kg i.p.) on the cell cycle of the diploid (EAT dipl.) and hypertetraploid (ELT) Ehrlich ascites tumor growing in the peritoneal cavity of male NMRI-mice has been studied by autoradiography with H^3- and C^{14}-thymidine (double labelling technique and method of labelled mitoses). The results suggest that the proliferation kinetics of tumor cells after administration of cyclophosphamide is dependent essentially on the type of tumor strain tested.

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Immunoglobulins in Human Fetal Sera at Different Stages of Gestation

The synthesis of IgM has been demonstrated in the human fetus on the twelfth week of gestation, and that of IgG somewhat later[1,2]. Cells producing IgA have not been found prenatally, although at least one-third of neonates have this immunoglobulin in the serum[3,4]. IgG in the fetal circulation is derived almost totally from the mother, and its level rises evenly towards birth[5–8]. The occurrence of IgD at birth is doubtful[9]. Information about IgM and IgA in the fetal serum at different stages of development is scanty and merely qualitative[5]; only VAN FURTH et al.[1] have reported IgM levels from 0.010 to 0.146 mg/ml in 11 out of 15 fetuses with a crown-heel length of 22.5–39.5 cm.

We have studied IgM, IgA, IgG and IgD in a group of fetal umbilical cord sera from different stages of gestation, in a group of cord sera from putatively healthy full-term new-borns taken at delivery, and in corresponding maternal sera taken on the same day as the fetal and neonatal samples. The fetal sera were from legal abortions and premature births without any evidence of infection. They were collected carefully avoiding contamination with the maternal blood. The sera were stored at $-40\,^{\circ}C$ until examined. The level of IgM, IgA and IgG was determined by radial immunodiffusion in agar[10]. Commercial specific antisera were used: rabbit anti-IgM and anti-IgG from Behringwerke AG (lot Nos. 1374 C and 1334 B) and goat anti-IgA from Hyland Laboratories (lot No. GP 9-65). Afterwards we became aware that these antisera might give some cross reactions; all results less than 0.1 mg/ml have been confirmed by the use of antisera prepared by us. Our antiserum to IgM has already been described[11], anti-IgA and anti-IgG were prepared by immunizing rabbits with the respective immunoglobulins. IgA was isolated from human colostrum by a combined procedure[12,13]. IgG was purified by DEAE-cellulose chromatography of serum[14]. Anti-IgA was made monospecific by absorption with IgM and IgG, anti-IgG with IgM and IgA. The occurrence of IgD was examined by double diffusion in agar with rabbit anti-IgD from Behringwerke AG (lot No. 1310). Reference sera containing known amounts of IgM, IgA and IgG were supplied by Behringwerke AG and Hyland Laboratories. The variation coefficient for IgM quantitation was 15%, for IgA 18% and for IgG 8.3%, each calculated from 4–6 determinations of one fetal sample on different plates.

The results are shown in the Figures 1 and 2. The smallest fetus with determinable IgM was of a crown-heel length of 14.0 cm and weighed 75 g. Out of 26 fetuses below 25 cm, IgM was demonstrated in 13. It was always found in the fetuses greater than 25 cm (after the 20th week of gestation), and in the full-term new-borns. The neonatal level was 0.13 ± 0.14 mg/ml (mean $\pm$ S.D.) being one-eighth of the maternal concentration. A correlation between the fetal length and log IgM concentration could be observed ($r = 0.85$, $P < 10^{-6}$). IgA was demonstrated in the sera of 3 prematures; at the earliest in one of 41 cm and 1680 g. In the neonates it was found in 30 from 81 (37%). The neonatal mean value, including zeroes, was 0.02 ± 0.10 mg/ml. The maternal level was one hundred times greater. Concordant cord/maternal ratios and cord concentrations for IgM and IgA have been reported previously[4,15]. Neither the neonatal IgM nor IgA value was dependent on the maternal one. No correlation could be observed between the levels of IgM and IgA in the neonates.

Linear correlation was established between the fetal length and log IgG concentration ($r = 0.81$, $P < 10^{-5}$). IgG was found in all sera studied. The neonatal mean 12.0 ± 4.3 mg/ml was significantly higher than that of their own mothers, 8.6 ± 3.2 mg/ml ($P < 0.01$ by Student's t-test). These observations are consistent with previous results based on electrophoretic[5,6] and immunochemical[16,17] IgG determinations as well as with the transfer from mother to fetus of specific viral and bacterial antibodies of IgG class[18]. Agreement was also found with the conclusion[17,18] that maternal and neonatal antibody levels are not directly correlated.

[1] R. VAN FURTH, H. R. E. SCHUIT and W. HIJMANS, J. exp. Med. *122*, 1173 (1965).
[2] D. BUFFE and P. BURTIN, Annls Inst. Pasteur, Paris *112*, 468 (1967).
[3] O. VIVELL, T. SICK and G. LIPS, Klin. Wschr. *38*, 721 (1960).
[4] E. R. STIEHM and H. H. FUDENBERG, Pediatrics *37*, 715 (1966).
[5] G. DE MURALT, Helv. med. Acta *29*, suppl. 42 (1962).
[6] H. E. SCHULTZE and J. F. HEREMANS, *Molecular Biology of Human Proteins with Special Reference to Plasma Proteins* (Elsevier, Amsterdam 1966), vol. 1.
[7] J. R. HOBBS and J. A. DAVIS, Lancet *1*, 757 (1967).
[8] T. BERG, Acta paediat. scand. *57*, 369 (1968).
[9] D. S. ROWE, P. A. CRABBÉ and M. W. TURNER, Clin. exp. Immunol. *3*, 477 (1968).
[10] J. L. FAHEY and E. M. MCKELVEY, J. Immunol. *94*, 84 (1965).
[11] P. TOIVANEN and T. HIRVONEN, Scand. J. Haemat. *6* (1969), in press.
[12] H. AXELSSON, B. G. JOHANSSON and L. RYMO, Acta chem. scand. *20*, 2339 (1966).
[13] J. P. VAERMAN and J. F. HEREMANS, in *Protides of the Biological Fluids*, Proceedings of the 15th Colloquium, Bruges, 1967 (Ed. H. PEETERS; Elsevier, Amsterdam 1968), p. 615.
[14] J. L. FAHEY and E. W. TERRY, in *Handbook of Experimental Immunochemistry* (Ed. D. M. WEIR; Blackwell, Oxford 1967), p. 19.
[15] S. G. O. JOHANSSON and T. BERG, Acta paediat. scand. *56*, 572 (1967).
[16] P. F. KOHLER and R. S. FARR, Nature *210*, 1070 (1966).
[17] J. L. MICHAUX, J. F. HEREMANS and W. H. HITZIG, Trop. geogr. Med. *18*, 10 (1966).
[18] P. TOIVANEN, R. MÄNTYJÄRVI and T. HIRVONEN, Immunology *15*, 395 (1968).

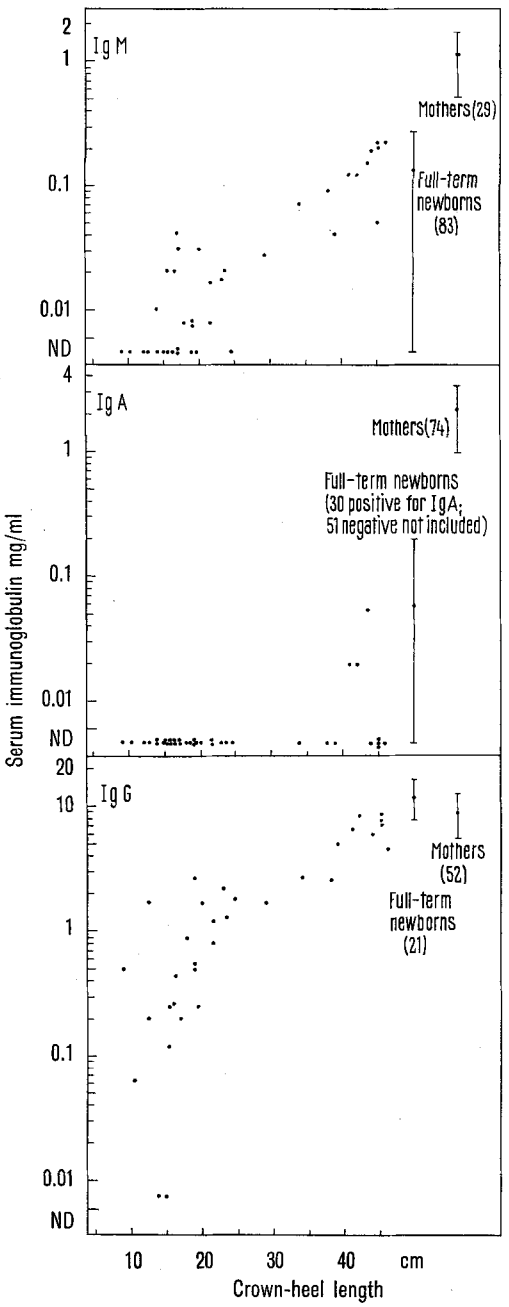

Fig. 1. Immunoglobulins in fetal, neonatal and maternal sera. IgM was determined from 38 fetuses, IgA from 37 and IgG from 33. The number of full-term new-borns and mothers included in the mean (± S.D.) is given in parentheses. Values below 0.01 mg/ml were not quantitable. ND, not detectable.

IgD was demonstrated by double diffusion in none of 11 fetal and 50 neonatal sera. In the maternal samples it was found in 46 out of 61 (75%), as has also been reported by others[9,15].

Although there is no doubt that the human fetus in utero is able to synthetize antibodies with varying specificities[11,19–22], the possibility of placental transfer of IgM and IgA cannot be excluded on the basis of our results. The fact that the level of both these immunoglobulins is known to increase evenly after birth, in contrast to the postnatal decline of IgG[4–8,15], makes the fetal origin

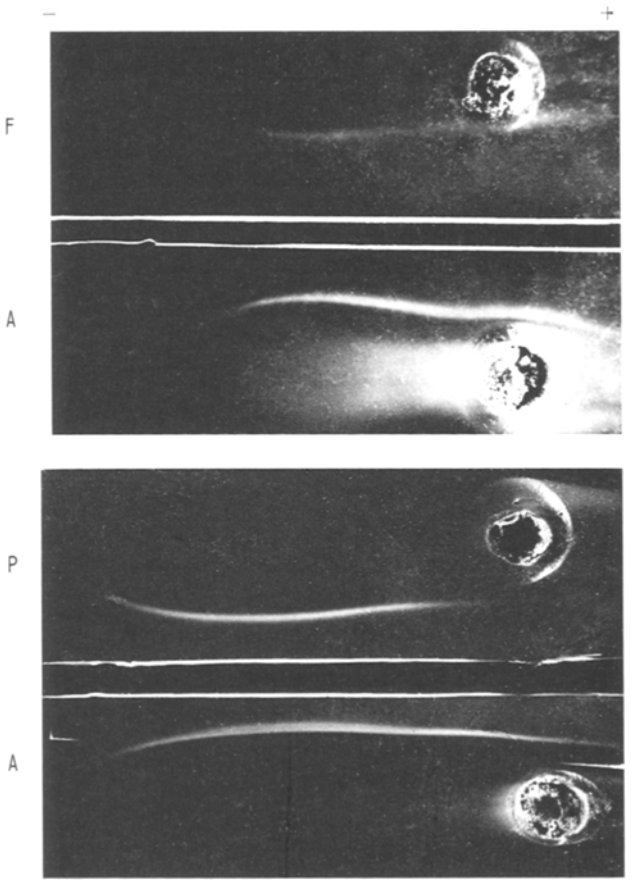

Fig. 2. Serum electrophoretic pattern of a fetus (F), a premature (P) and an adult (A) reacting with anti-IgM (upper) and anti-IgA (lower). The fetus was of a crown-heel length of 14.0 cm and the premature of 41 cm. Prior to the electrophoresis, these sera were concentrated about five-fold with the aid of lyophilization. The adult serum was unconcentrated.

obvious, however. Thus IgM production in the human fetus can be considered as a rule after the 20th week of pregnancy.

Zusammenfassung. Durch quantitative Immunodiffusion wurde der Gehalt an IgM, IgA und IgG in menschlichen Fötalseren untersucht. Der früheste Zeitpunkt, wo IgA entdeckt wurde, war bei einem prämaturen Embryo, der 41 cm lang war und 1680 g wog. In den Seren von am Termin geborenen Kindern konnte IgA in 37% nachgewiesen werden. IgG wurde in allen untersuchten Seren gefunden.

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[19] M. Adinolfi, Immunology *9*, 43 (1965).
[20] W. V. Epstein, S. W. Fong and M. Tan, Immunology *10*, 259 (1966).
[21] C. A. Alford, J. Schaefer, W. J. Blakenship, J. V. Straumfjord and G. Cassady, New Engl. J. Med. *277*, 437 (1967).
[22] J. A. Bellanti and A. L. Jackson, J. Pediat. *71*, 783 (1967).
[23] Supported by a grant from the Sigrid Jusélius Foundation.