

Human complement C7 and C9 in fetal and newborn sera

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Adinolfi, M., and Beck, S. E. (1975). *Archives of Disease in Childhood*, 50, 562. **Human complement C7 and C9 in fetal and newborn sera.** Using specific immune sera, C7, C9, and C3 activator were detected in sera from human fetuses more than 16 weeks old and in newborn samples. Levels of C9 in cord sera ranged between 10 and 30% of those present in sera from adult subjects. The mean value of C3 activator was about half that in maternal blood. The mean level of C7 in newborns was nearly 70% of the amount in normal adults.

The availability of antibodies to specific components of human complement (C) and the analysis of *in vitro* cultures of fetal tissues have made it possible to study the onset and site of synthesis of these proteins during fetal life (Adinolfi, 1972; Rosen, 1974). The studies of the ontogeny of complement so far published have shown that C1, C3, C4, and C5 are produced by the human fetus at an early stage of gestation (Adinolfi and Gardner, 1967; Adinolfi, Gardner, and Wood, 1968; Gitlin and Biasucci, 1969; Colten, 1972, Köhler; 1973).

We report preliminary results of our investigations on the presence and estimation of C3 activator, C7, and C9 in sera from human fetuses and newborns.

Materials and methods

Twenty-four samples of cord blood were collected by syringe from the umbilical vein. Blood from normal adult individuals was taken by venepuncture. Serum was separated from clotted blood as soon as possible and the samples stored at -20° for no more than 2 months before being used. Blood was also collected by cardiac puncture from 5 fetuses whose ages, calculated from crown-rump length, ranged between 14 and 25 weeks. Most of the fetuses were obtained from therapeutic abortions.

Immune serum against C3 activator (factor B) was obtained from Behringwerke (Marburg, Germany) and it was raised against one of the breakdown products of factor B. The rabbit immune sera against C7 and C9 were made by injection of precipitin lines cut from immunoelectrophoretic plates using anti-C7 and anti-C9 (kindly donated by Dr. H. Y. Müller-Eberhard,

U.S.A.). Both antisera gave only one line of precipitation when tested by double diffusion in agar gel.

The presence of C7 and C9 in fetal and newborn sera was investigated using double diffusion in agar gel. Levels of C3 activator and C7 were measured by the single radial diffusion method, using a minor modification of the technique described by Köhler and Müller-Eberhard (1967). Levels of C7 were also estimated with the agarose plate technique (Lachman, Hobart and Aston, 1973) using guinea pig red cells and C56 euglobulin prepared from sera collected from normal women after childbirth. Concentrations of C9 were estimated by comparing the patterns of precipitations of serial dilutions of a normal adult serum with those of fetal and newborn samples.

In the present paper the amount of C3 activator is expressed in mg/100 ml, using a standard serum provided by Behringwerke (Marburg, Germany), and levels of C7 and C9 in fetal and newborn sera are expressed as a percentage of the amount detected in adult samples.

Results

C3 activator was detected in all 21 newborn samples tested and in sera from 5 fetuses of from 14 to 25 weeks of gestation. Individual levels of C3 activator in adult, cord, and fetal sera are shown in Fig. 1. The mean value of this protein in the newborn samples was 8.49 mg/100 ml. Levels of C3 activator were estimated in 2 fetal sera (3.7 and 1.5 mg/100 ml), the youngest fetus being 16 weeks old; in the other 3 sera the protein was detected but the levels could not be measured being less than 1 mg/100 ml.

Using double diffusion in agar gel, C7 was detected in all newborn sera tested. When levels of

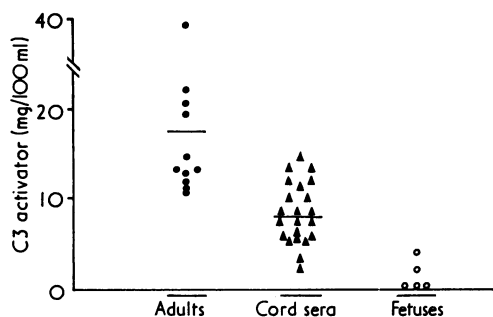


FIG. 1.—Levels of C3 activator (in mg/100 ml) in sera from normal adults, cord sera, and sera from fetuses between 14 and 25 weeks of age. In 3 fetuses C3 activator was detected by double diffusion but could not be estimated since the concentrations were lower than 1 mg/100 ml.

C7 in cord sera were estimated using the single radial diffusion technique, the areas of precipitation were occasionally ill defined in some plates. Nevertheless, levels of C7 ranged between 54 and 120% of the mean level of pooled serum from normal adults. C7 was detected in 3 fetal sera tested by double and single radial diffusion techniques. The youngest fetus in which C7 was detected was 16 weeks old.

C7 was also estimated by the agarose plate technique using C56 euglobulin; the mean level of C7 in 14 cord samples was 67.3% of the mean value detected in adult sera. In the 5 fetuses tested, aged from 14 to 25 weeks, C7 levels ranged between 20 and 56% of the normal mean concentration (Fig. 2).

C9 was detected in 11 newborn sera out of 24 tested by double diffusion in agar gel; in the remaining 13 sera, the concentration appeared to be less than 5% of the levels in normal adult samples. By comparing the lines of precipitation obtained using several dilutions of an adult serum, C9 levels

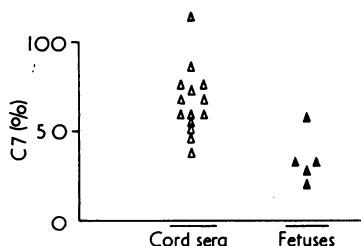


FIG. 2.—Levels of C7, expressed as percentage of the amount in sera from normal adults, in 14 cord sera and 5 fetuses. Estimation of C7 was carried out using the agarose plate technique.

in the 11 cord samples were found to range between 5 and 50% of those present in normal adults (Fig. 3). C9 was also detected in sera from 3 fetuses aged 18, 20, and 25 weeks, but was not observed in 2 sera from fetuses 14 and 16 weeks old.

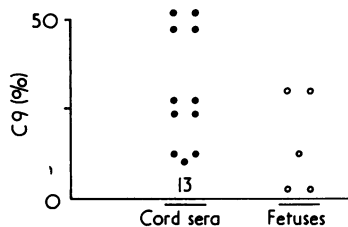


FIG. 3.—Levels of C9, expressed as percentage of the amount in sera from normal adults, in 24 cord sera and 5 fetuses. In 13 cord sera and 2 fetuses protein could not be detected using precipitation in agar gel.

Discussion

In recent years direct evidence that C1, C3, C4, and C5 are produced *in utero* has been obtained by analysis of the culture fluids of human fetal tissues incubated in media containing labelled amino acids. The presence in the culture fluids of newly synthesized specific components of complement was then established by the detection of haemolytically active proteins or by autoradiography of immunoelectrophoretic plates (Adinolfi, 1972; Rosen, 1974). Fetal synthesis of C3 and C6 has also been shown by detection of different genetic variants of these components of complement in pairs of maternal and cord sera (Propp and Alper, 1968; Alper, Hobart, and Lachmann, 1975).

In the present study, C3 activator, C7, and C9 were detected in newborn sera and in sera from fetuses over 16 weeks of age. The mean levels of C3 activator in newborn samples was about half the mean value detected in adult sera, agreeing with the ratios between cord and adult sera observed for C3, C4, and C5 (Fireman, Zuchowski, and Taylor, 1969; Adinolfi, 1972). However levels of C9 in cord sera seem to be lower than those present in normal adults.

Detection of C3 activator, C7 and C9 in sera from human fetuses and from newborn infants agrees with studies on the ontogeny of the late components of complement in other mammals. Geiger, Day and Good (1972a, b) have detected C3 activator, C6, C7, C8, and C9 in fetal piglets. Haemolytically active C3, C6, and C7 were observed in sera from pig fetuses at an early stage of embryonic life in low titres, but showed a striking

increase at about 110 days of gestation. By contrast, levels of C8 and C9 were found to increase regularly during embryonic life. Development of C3 activator appeared to precede the synthesis of C3. Early synthesis of components of complement has also been shown in fetal lambs, mice, and rabbits (Rice and Silverstein, 1964; Tachibana and Rosenburg, 1966; Adinolfi, 1972; Rosen, 1974).

Work is now in progress to determine the site of synthesis of C7 and C9 in human fetal tissues, using *in vitro* cultures. Preliminary data suggest that C7 is synthesized in the fetal liver.

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