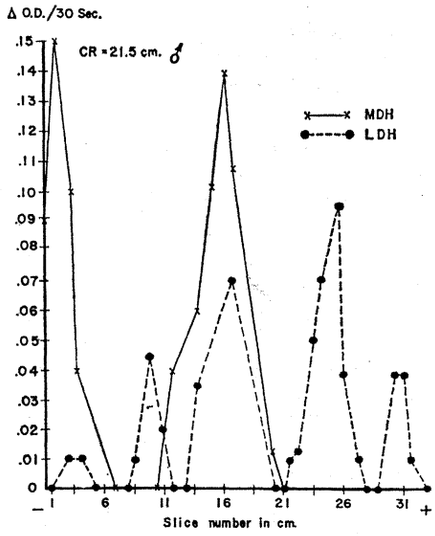

Fig. 1. Electrophoretic separation of lactic and malic dehydrogenases in the nonparticulate fraction of heart homogenate from a human fetal specimen measuring 21.5 cm from crown to rump.

peaks of MDH were always present. The electrophoretic pattern of an enzyme preparation from the heart of a 21.5 cm crown-rump specimen (Fig. 1) demonstrates the five lactic dehydrogenases and two malic dehydrogenases typically found. The protein preparation was inserted in a slit in the starch block at 11 centimeters. Thus, under these conditions of electrophoresis, three LDH peaks migrated toward the anode and two toward the cathode. One MDH peak migrated toward the anode, the other toward the cathode.

Each peak of malic and lactic dehydrogenase was tested for its rate of reaction with the thionicotinamide analog of DPN (TNDPN) and the 3-acetylpyridine analog of DPN (APDPN) as well as DPN itself. From the ratios of these activities we concluded that the two MDH peaks were the same in all tissues. The anodic peak has a TNDPN/DPN ratio of 0.54, whereas the cathodic peak has a TNDPN/DPN ratio of 0.22. The APDPN/DPN ratios were 0.44 for the cathodic MDH and 0.34 for the anodic MDH. Kaplan and Ciotti (*9*) reported APDPN/DPN ratios ranging from 1.2 to 4.7 for the MDH present in mitochondria of rabbit tissues. The APDPN/DPN ratio of MDH activity in the unfractionated soluble fraction ranged from 0.52 to 1.1 in the various tissues tested. The highest ratios for both mitochondrial and soluble fractions were found in the heart. These different ratios could be due to varying amounts of two or more enzymes, each with characteristic APDPN/DPN ratios. The five LDH peaks appear to be the same in all tissues and at all ages, on the basis of their rates of migration relative to the rates of the two MDH peaks and their activities with the DPN analogs. There was, however, no such clear-cut difference in activity with the analogs as that observed with the MDH peaks and TNDPN. Although the same five peaks of LDH and two peaks of MDH appeared in all the tissues studied, there were variations in the relative amounts of enzymatic activity in the different fractions (Table 1).

The same two peaks of MDH activity were found in embryonic chick heart, liver, brain, and skeletal muscle and in adult rat liver, heart, and kidney. The two peaks in these tissues were similar to the two peaks found in human fetal tissues both in their electrophoretic mobility and in their rates of reaction with pyridine nucleotide analogs. There appears to be a greater similarity between the LDH enzymes from different organs within the same animal than there is between those from the comparable organs in human and chick.

In the human fetal tissues the several molecular forms of lactic and malic dehydrogenase were qualitatively the same but there were quantitative differences in the relative amount of enzyme activity associated with the different peaks in different tissues, and in the same tissue at successive stages of fetal development.

B. WIGGERT
C. A. VILLEE

Department of Biological Chemistry, Harvard Medical School, and *Research Laboratories, Boston Lying-in Hospital, Boston, Massachusetts*

References and Notes

1. E. S. Vesell and A. G. Bearn, *Ann. N.Y. Acad. Sci.* **75**, 286 (1958); B. Hess, *ibid.*, p. 292; B. R. Hill, *ibid.*, p. 304.
2. P. G. W. Plagemann, K. F. Gregory, F. Wroblewski, *J. Biol. Chem.* **235**, 2282 (1960); B. Hess and S. I. Walter, *Ann. N.Y. Acad. Sci.* **94**, 890 (1961); F. Wroblewski and K. F. Gregory, *ibid.*, p. 912; J. S. Nisselbaum, M. Schlamowitz, O. Bodansky, *ibid.*, p. 970.
3. E. Vesell, *Ann. N.Y. Acad. Sci.* **94**, 877 (1961).
4. C. L. Markert and F. Møller, *Proc. Natl. Acad. Sci. U.S.* **45**, 753 (1959); H. Fine, N. O. Kaplan, S. White, *Federation Proc.* **21**, 409 (1962).
5. L. B. Flexner, J. B. Flexner, R. B. Roberts, G. De La Haba, *Develop. Biol.* **2**, 313 (1960).
6. We thank Prof. J. Lind, Karolinska Sjukhuset, Stockholm, and Prof. C. Gemzell, University of Uppsala, for specimens, and Prof. C. Schmitterlow, Kungl. Veterinärhögskolan, Stockholm, for laboratory facilities. One of us (B.W.) is a predoctoral fellow of the U.S. Public Health Service.
7. H. Bloemendal, "Starch electrophoresis I. Starch block electrophoresis," in *Chromatographic Reviews*, M. Lederer, Ed. (Elsevier, Amsterdam, 1960), vol. 2.
8. J. B. Nielands, in *Methods in Enzymology*, S. P. Colowick and N. O. Kaplan, Eds. (Academic, New York, 1955), vol. 1, p. 449; R. G. Wolf and J. B. Nielands, *J. Biol. Chem.* **221**, 61 (1956).
9. N. O. Kaplan and M. M. Ciotti, *Ann. N.Y. Acad. Sci.* **94**, 701 (1961).

20 September 1962

A Protein Present in Fetal but Not in Maternal Rat Serum

Abstract. Immunoelectrophoretic comparison of maternal and fetal rat serum proteins with antiserum to adult rat serum proteins showed a unique protein in the fetal serum. The fetal protein exhibited in agar gel electrophoresis a variable mobility that was dependent on concentration. The fetal protein reacted strongly with a nonspecific antiserum.

The presence of a serum globulin with properties differing from those of the adult globulins in the fetus and newborn of certain ruminants has been known for a number of years (*1*). A characteristic change in the serum proteins of tumor-bearing, pregnant, young, and fetal rats has been described (*2–4*).

Although it has been stated that this protein is associated with tissue growth (*3*), increased levels were also found in fasted rats (*3*), and rats on a biotin-deficient diet or with diarrhea (*4*). The concentration of this protein in the maternal rat serum is equal to or greater than that in the fetal serum at all stages of gestation (*3*). In those species where quantitative techniques have been used to study fetuin, its concentration in fetal serum is 25 to 50 times that of any similarly reacting adult protein (*5*).

Our report describes some of the properties of a protein in fetal rat serum which could not be detected in either normal adult or in maternal rat serum.

Immunoelectrophoresis was carried out in a system similar to that originally described (*6*) with modifications (*7*). Rabbit antisera to normal adult rat and pregnant rat serum proteins were produced by a complete Freund adjuvant technique (*8*). Antiserum diffusion was carried out at room temperature.

Comparisons were made of 25 matched maternal and fetal sera. At least three ratios of maternal and fetal

serum volumes were used in each case. All the fetal sera showed a precipitate that occurred in a position different from any maternal precipitate. When undiluted fetal serum was used the protein migrated at the same rate as the α_3 globulins of adult rat serum. This fetal serum protein showed an apparent increase in mobility on serial dilution; at a 1:10 dilution it migrated with the α_1 globulins. Prior dialysis of the fetal serum against the electrophoretic buffer did not affect this property. No other fetal or adult protein showed a change in mobility that was dependent on concentration. The position of the precipitate between the line of electrophoretic migration and the antiserum trough was also unusual. That the precipitate formed very close to the protein's electrophoretic position indicated minimum diffusion during the development of the immune precipitates with various antisera. The location of the precipitate changed only slightly, if at all, with variations of the ratio of antigen to antiserum, whereas all other fetal and maternal protein precipitates shifted in the expected fashion.

For undiluted fetal serum, the precipitation arc assumed a linear configuration similar to that of the major γ globulin component (*6*), but the cause of this linear shape in the case of the fetal protein is probably quite different from that of γ globulin. The material was found throughout the last 7 days of gestation, and the absolute concentration during this period, as judged by serial dilutions of fetal serum, did not change significantly. It could not be detected after the tenth day postpartum. In all 25 pairs of fetal and maternal sera the concentration of this protein in the fetal serum was at least 10 times that of any similar material in the maternal serum. When samples of maternal sera were concentrated (2.5 times) the fetal serum contained at least 25 times as much of this new protein. The fetal protein reacted equally with antisera to normal adult male rat serum and serum of pregnant rats. Therefore a similar antigenic determinant must be present in the nonpregnant adult.

No reactions were seen between normal rabbit serum and fetal or adult rat sera. An antiserum to normal human adult serum proteins (*9*) was used to develop several of the maternal and fetal rat immunoelectrophoretic plates. Weak reactions were seen between this antiserum and adult rat albumin and β globulin. With fetal rat serum a rather strong precipitate occurred which corresponded very closely in shape and position to the precipitate formed by the fetal protein and the specific antiserum to rat serum proteins. The time for precipitate development with the nonspecific antiserum was 4 to 6 days, whereas only 2 days were required with the specific antiserum. Such cross reactions between human and rat serum proteins as well as between various other species have been reported (*10*).

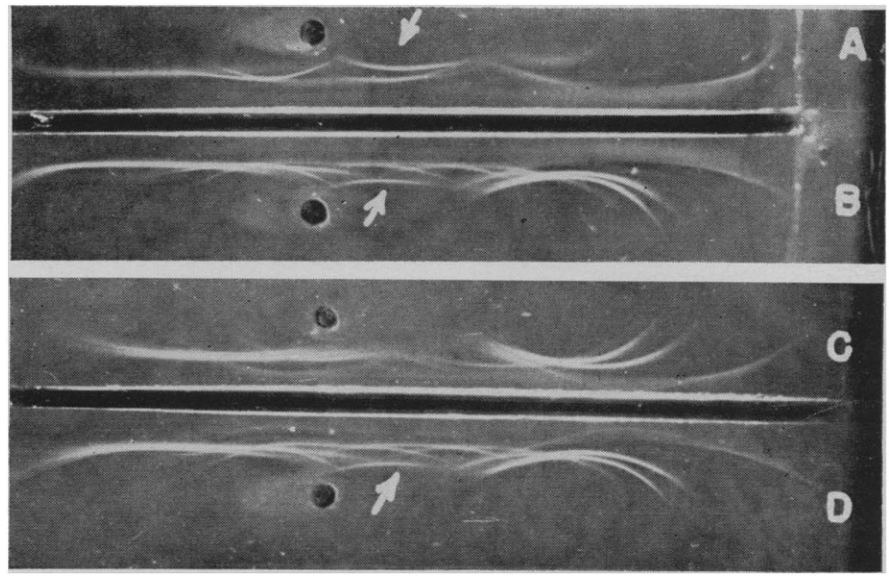

Fig. 1. Immunoelectrophoretic comparison of maternal (*C*), fetal (*A*), and mixed maternal and fetal serum (*B* and *D*). The arrows indicate the precipitate formed by the fetal serum protein. Gestation age was 20 days.

Rat fetal and maternal sera were mixed in a 1:1 ratio by volume and compared with the unmixed sera (Fig. 1). The fetal protein was not seen to form a reaction of identity with any adult protein. Neither the position nor shape of any of the adult protein precipitation arcs was altered by the admixture of fetal serum. This makes unlikely the possibility that an adult counterpart of this protein may be present, but in a different electrophoretic position, because of a concentration-dependent behavior similar to that of dilute fetal serum.

Staining of the electrophoretic patterns of maternal and fetal serum in agar again indicated the presence of a fraction in the fetal serum differing from the maternal proteins. The contrast was less marked than that obtained with immunoelectrophoresis. The protein did not show the characteristic color reaction of hemoglobin with benzidine reagent.

We have no explanation for the concentration-dependent electrophoretic properties of this material in agar gel. A physical or chemical interaction between it and the gel seems likely, however. At present the only demonstrated similarity of this protein to fetuin is the similar period in the life span of the respective animals in which they are present in the serum in high concentrations.

P. C. KELLEHER
C. A. VILLEE
Department of Biological Chemistry, Harvard Medical School, and *Research Laboratories, Boston Lying-in Hospital, Boston, Massachusetts*

References

1. K. O. Pederson, *Nature* **154**, 575 (1944); R. A. Kekwick, *Advan. in Protein Chem.* **14**, 231 (1959).
2. D. A. Darcy, *Nature* **176**, 643 (1955); ———, *Brit. J. Cancer* **11**, 137 (1957); P. N. Campbell, B. A. Kernot, I. M. Roitt, *Biochem. J.* **71**, 155 (1959); W. G. Heim, *Nature* **194**, 491 (1962).
3. D. A. Darcy, *Brit. J. Cancer* **14**, 524 (1960).
4. G. H. Beaton, A. E. Selby, M. J. Veen, A. M. Wright, *J. Biol. Chem.* **236**, 2005 (1961).
5. F. H. Bergman, L. Levine, R. G. Spiro, *Biochim. Biophys. Acta* **58**, 41 (1962).
6. P. Grabar and C. A. Williams, *ibid.* **10**, 193 (1953).
7. P. C. Kelleher and C. A. Villee, *ibid.* **59**, 252 (1962).
8. J. Freund and K. McDermott, *Proc. Soc. Exptl. Biol. Med.* **49**, 548 (1942).
9. Kindly supplied by Dr. J. A. MacLaren.
10. M. Kaminski, *Bull. Soc. Chim. Biol.* **39**, Suppl. 1, 85 (1957); M. Muller, G. Fontaine, P. Muller, *Lille Med.* **4**, 150 (1959); J. Clausen and J. Heremans, *J. Immunol.* **84**, 128 (1960).

20 September 1962