to electrophoresis. Six-hour embryos show only three peaks for DPN L-malic dehydrogenase, I, II, and IV. After 12 hours of incubation, peak V appears, and these four electrophoretically distinct DPN malic dehydrogenases persist until the embryo is 48 hours of age. Band three does not appear in embryo extracts. After the 48-hour stage of development peaks I and II are less distinctive; peak II is spread over more fractions and overshadows peak I. Peak V, usually present in a single 1-cm fraction, occupies three such fractions in the 48-hour embryos.

At each age there are six or seven L-malic dehydrogenase peaks for APDPN and three for TNDPN, with no marked changes as development proceeds. The ratio of APDPN/DPN malic dehydrogenase activity is 0.68 in the supernatant fraction from unfertilized eggs before electrophoresis, 0.56 after the first 12 hours of development, and 2.2 by 48 hours of development. This change with embryonic age is not due to a change in the properties of the protein with the major L-malic dehydrogenase activity, peak II, for the APDPN/DPN ratio of this peak remains relatively constant throughout the period studied. The malic dehydrogenases of the sea urchin differ markedly from those of vertebrate tissues—chick, rat, and man—in which only two electrophoretically distinct proteins have been observed (*6*, *8*).

RICHARD O. MOORE
CLAUDE A. VILLEE

Marine Biological Laboratory, Woods Hole, Massachusetts, and Department of Agricultural Biochemistry, Ohio State University, Columbus, and *Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts*

References and Notes

1. C. Markert and F. Møller, *Proc. Natl. Acad. Sci. U.S.* **45**, 753 (1959).
2. N. O. Kaplan, M. M. Ciotti, M. Hamolsky, R. E. Bieber, *Science* **131**, 392 (1960); C. A. Villee, *Biol. Bull.* **119**, 298 (1960); R. O. Moore and C. A. Villee, *ibid.* **121**, 398 (1961); F. Wroblewski, Ed., "Multiple Molecular Forms of Enzymes," *Ann. N.Y. Acad. Sci.* **94**, 655 (1961).
3. C. L. Markert and E. Appella, *Ann. N.Y. Acad. Sci.* **94**, 678 (1961).
4. R. D. Cahn, N. O. Kaplan, L. Levine, E. Zwilling, *Science* **136**, 962 (1962).
5. L. B. Flexner, J. B. Flexner, R. B. Roberts, G. De La Haba, *Develop. Biol.* **2**, 313 (1960).
6. B. Wiggert and C. A. Villee, *Science*, this issue.
7. O. Smithies, *Biochem. J.* **61**, 629 (1955).
8. This research was aided by grants A-4241 and C-2400, from the National Institutes of Health, and grants from the Charles and Marjorie King Fund and the Association for the Aid of Crippled Children.

20 September 1962

Lactic and Malic Dehydrogenases in Human Fetal Tissues

Abstract. The nonparticulate fraction of homogenates of heart, liver, kidney, lung, and cerebral cortex from human fetuses of varying gestational ages was subjected to electrophoresis on starch grains. Five lactic dehydrogenases and two malic dehydrogenases with characteristic rates with pyridine nucleotide analogs were found in all tissues at all ages. Quantitative differences in the relative proportions of these peaks were observed as gestation proceeded.

Multiple molecular forms of lactic dehydrogenase have been observed in human serum (*1*) and adult human heart and liver (*2*). Analyses of several adult human tissues revealed a total of five electrophoretically separable lactic dehydrogenases. Not every tissue had all five of these forms (*3*). Changes in the relative proportions of the molecular forms of lactic dehydrogenase during development have been observed in rat tissues (*4*) and guinea pig tissues (*5*).

Human fetal tissues were obtained at the time of operation during interruptions of pregnancy and were immediately frozen in dry ice and stored at −10°C until analyzed (*6*). In preparation for starch grain electrophoresis, purified potato starch was washed according to the method of Bloemendal (*7*). A starch block, 33 by 7 by 1 cm, provided with flannel wicks to connect with the electrode vessels was prepared, and both block and wicks were wrapped in thin plastic. A starch segment 1 cm wide was cut out of the block 11 cm from the cathode end. A one to one homogenate of 2 to 4 grams of fetal tissue was made in a glass homogenizer with barbital buffer (*p*H 8.6, ionic strength 0.1) that was also used for electrophoresis. The homogenate was centrifuged at 10,000*g* for 1 hour, and the clear supernatant fraction was mixed with dry, washed starch. The resulting paste was placed in the 1-cm slit in the block. Horizontal electrophoresis was continued for 36 hours at 250 v and 30 ma. Platinum electrodes were used; the electrode vessels were divided into two compartments with filter paper wicks to provide fluid bridges. During electrophoresis the starch block was cooled by circulating water at 1°C. After electrophoresis 1-cm fractions were cut and eluted with 0.15*M* NaCl. Lactic and malic dehydrogenase activities were measured in glycine-NaOH buffer at *p*H 10, with a Zeiss (PMQ II) spectrophotometer (*8*). Enzyme activity, calculated from the initial linear portion of the curve, was expressed as millimicromoles (mμmole) of reduced pyridine nucleotide.

Five electrophoretically separable forms of lactic dehydrogenase (LDH) and two forms of malic dehydrogenase (MDH) were found in human fetal heart, liver, lung, kidney, and cerebral cortex. In a few instances, one of the five peaks of LDH was not detected, but in none of the tissues examined was any one of the five peaks absent in all stages of development. The two

Table 1. Percentage of total enzyme activity associated with each electrophoretically separated fraction.

Age*	LDH activity (%) in peak					MDH activity (%) in peak	
	1	2	3	4	5	1	2
				Heart			
13.5	0.7	28.2	31.8	31.8	7.5	—	—
14.5	0.5	18.8	43.9	33.4	3.4	16.8	83.2
16.0	2.5	12.2	34.8	37.8	12.7	30.4	69.6
21.5	2.7	10.0	37.7	37.7	11.9	41.3	58.7
24.0	1.6	4.5	28.5	31.6	33.8	29.4	70.6
Term	7.8	3.9	29.8	40.3	18.2	40.8	59.2
				Lung			
10.0	2.2	36.5	27.4	23.9	10.0	27.4	72.6
11.0	2.2	3.3	44.9	28.5	21.0	51.5	48.5
14.5	1.1	1.4	27.9	49.3	20.3	47.2	52.8
16.0	2.9	0.0	24.6	53.0	19.5	52.5	47.5
Term	37.2	27.8	23.6	6.5	4.9	59.8	37.2
				Liver			
8.4	2.6	19.4	35.6	38.2	4.2	54.3	45.7
10.0	6.6	21.1	34.4	22.2	15.7	51.6	48.4
11.0	4.5	23.2	39.8	27.2	5.3	57.8	42.2
12.0	3.8	39.8	36.4	17.5	2.4	35.5	64.5
14.5	15.3	19.4	40.1	16.6	8.6	36.0	64.0
21.5	19.4	35.9	21.4	17.6	5.7	53.7	46.3
24.0	11.6	5.0	35.2	41.5	6.7	45.2	54.8
Term	4.5	9.9	37.1	37.4	11.1	51.6	48.4

* Crown-rump length in centimeters.

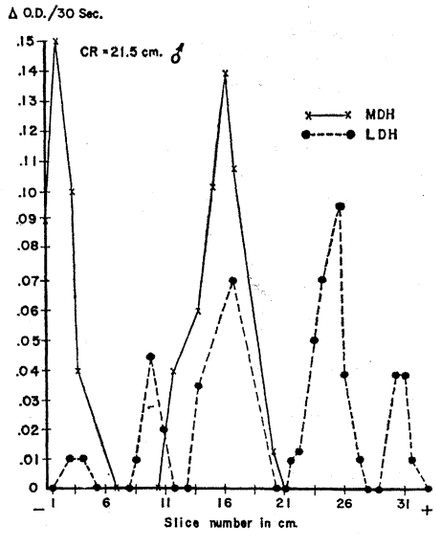

Fig. 1. Electrophoretic separation of lactic and malic dehydrogenases in the nonparticulate fraction of heart homogenate from a human fetal specimen measuring 21.5 cm from crown to rump.

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