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Received February 20, 1964. P.S.E.B.M., 1964, v116.

Uptake of Ethionine-Ethyl-H^3 by the Rat Conceptus.* (29317)

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Recent studies have shown that ethionine is transported across the placenta of rats and mice and that this amino acid analogue is incorporated into the proteins of the fetus (1-3). Our studies(3) have indicated that the maximum concentration of free ethionine in the fetus and amniotic fluid occurs some time after that of the placenta and the plasma. The purpose of this study is to determine the relative rates of movement of labeled ethionine into the free amino acid pools and into the proteins of the placenta, fetus, and amniotic fluid of the pregnant rat.

Materials and methods. Intraperitoneal injections of 200 mg DL-ethionine (Nutritional Biochemicals) were given to eight 12-day pregnant rats (Sprague-Dawley). The rats were anesthetized 6 hours later and were injected intravenously with 250 μc (1.5 mg) L-ethionine-ethyl-H^3 (New England Nuclear Corp.). The urethra was clamped off at time of injection. At 5, 15, 30 and 120 minute intervals after injection of the tracer, blood was drawn from the vena cava inferior, the bladder was removed, and amniotic fluid, fetuses and placentae were collected.

Four pregnant rats were injected with 200 mg DL-ethionine and with 250 μc of L-ethionine-ethyl-H^3 intraperitoneally 12 and 72 hours prior to autopsy on day 12 of pregnancy. The rats were placed in metabolic cages and the urine was collected at 12 hour intervals. Blood and tissues were obtained as before.

Absolute ethanol was added to the plasma, amniotic fluid, and to homogenates of 8 fetuses and 2 placentae from each animal to bring the final concentration to 80% ethanol. The precipitates were separated by centrifugation and washed twice with 80% ethanol. The supernatant from each tissue or fluid was combined with the washes and constituted the soluble activity. Fat was extracted from the precipitates with ethanol-ether and ethanol-chloroform. The precipitates were hydrolyzed in 6N HCl at 110°C for 18 to 24 hours, evaporated to dryness, and dissolved in water to yield the protein-bound amino acids. The protein samples were decolorized with norit. All samples were evap-

* Supported by a grant from Nat. Inst. of General Med. Sciences, N.I.H., P.H.S., and a grant from the Fluid Research Committee, Univ. of Colorado Med. Center.

TABLE I. Soluble and Protein-bound Labeled Ethionine; Expressed as μg Ethionine-ethyl-$H^3 \times 10^{-3}$/fetus,/placenta, or /0.1 ml Amniotic Fluid or Plasma. Urine values are for total labeled ethionine excreted.

Time after inj	Plasma		Placenta		Fetus		Amniotic fluid*	Urine
	Soluble	Protein	Soluble	Protein	Soluble	Protein		
5 min.	1169.2	10.0	318.2	3.5	16.8	.1	69.0	2810
(2)	1109.4	17.5	209.8	3.2	37.9	.1	43.0	2462
15 min.	741.8	5.8	579.4	6.0	51.7	.2	164.4	5760
(2)	1044.3	7.1	466.6	4.1	90.9	.2	135.0	—
30 min.	748.4	4.8	414.2	1.6	79.6	.1	141.4	14131
(2)	669.0	2.4	369.0	1.2	64.0	.1	157.1	40501
2 hr	600.0	4.6	574.0	3.5	169.3	.8	451.8	49437
(2)	526.0	.4	430.3	2.0	117.1	.3	334.5	61084
12 hr	146.4	33.0	243.8	15.1	96.9	6.9	305.8	182400
(2)	198.4	21.3	240.2	18.8	106.8	10.1	378.5	98500
72 hr	1.7	.8	16.9	4.1	1.3	.3	3.2	178205

* 0.1 ml amniotic fluid represents the amount related to approximately 1¾ fetuses.

orated to dryness and diluted to volume with 10% isopropanol.

Urine samples were filtered through glass wool and diluted to a constant volume with distilled water. The collections from the metabolic cages were combined to determine the total excretion of the label.

Aliquots of each sample were evaporated to dryness in counting vials and dissolved in hydroxide of hyamine 10× (Packard Instrument Co.). A phosphor solution of 1,4-bis-2(5-phenyloxazolyl)-benzene and 2,5-diphenyl-oxazole in toluene was added and the samples were counted with the Packard Tri-Carb liquid scintillation system. Samples of known amounts of L-ethionine-ethyl-H^3 were counted with each group of samples. Internal standards were used to determine quenching in each sample.

The ethionine-ethyl-H^3 in the samples was identified by ascending paper chromatography using Whatman No. 1 paper and a solvent system consisting of butanol-acetic acid-water, 4:1:1. The chromatogram of each sample was cut into strips and the substance on each strip was eluted into a counting vial with 80% ethanol. These samples were prepared and counted in the same manner as the previous samples. A standard of ethionine-ethyl-H^3 was treated in the same fashion. The migration rates of the labeled samples were compared with those of unlabeled ethionine, methionine, ethionine sulfoxide, and methionine sulfoxide(3).

For autoradiography, pieces of liver, kidney and 2 conceptuses from each animal were fixed in alcoholic formalin, embedded in paraffin, and sectioned at 2 μ. The sections were dipped in Eastman NTB-3 emulsion, incubated at 3°C, and developed after 1 to 7 months of exposure. The sections were then stained with nuclear fast red.

Results and discussion. Other workers have shown a minimal amount of radioactivity in the proteins of the mouse fetus following a single injection of labeled ethionine(1). Our previous work with unlabeled ethionine has shown that a high concentration of ethionine is necessary in the maternal serum in order that small amounts of the compound could be detected in the fetal proteins(3). Therefore, we felt that the addition of carrier ethionine might bring the overall amount of ethionine (labeled and unlabeled) to a sufficient concentration to induce the uptake of the analogue by the fetal proteins. The 6-hour interval between the injection of the carrier and the label was chosen because following the initial ethionine-induced elevation of free ethionine and methionine in the serum the amounts of both compounds have tended to stabilize(3).

The amount of label present in the soluble fraction and in the proteins of the various tissues and fluids are presented in Table I. One animal injected 72 hours prior to autopsy had completely resorbed. In the sample of debris obtained from the uterus of this rat,

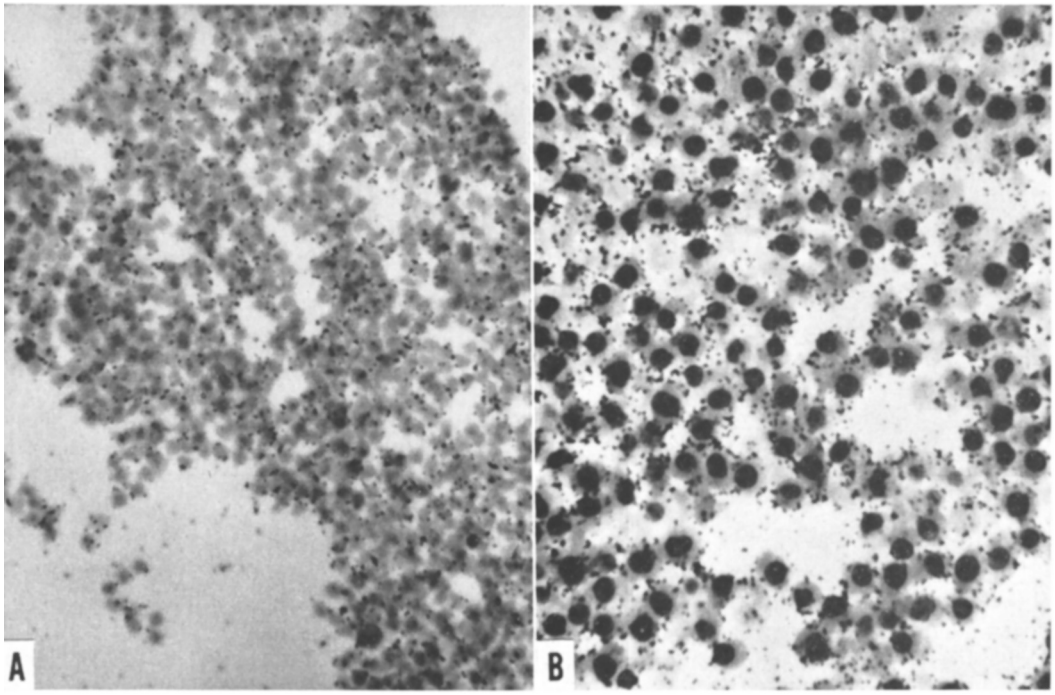

FIG. 1. Ethionine-ethyl-H^3 autoradiographs: (A) maternal erythrocytes in a maternal blood space of 12-day pregnant rat placenta (× 500); (B) fetal erythrocytes in an allantoic capillary of placental labyrinth. (× 375)

the count was 4 times higher in the protein-bound fraction than in the soluble fraction. This is in contrast to the higher count for soluble activity in the non-resorbed animals.

The data illustrate that in the placenta the highest concentration of label is present in the soluble fraction within 15 minutes after the injection. This amount appears to remain stable through 2 hours. In the fetus and amniotic fluid, the highest concentration was not reached until after 2 hours following injection. This is consistent with previous observations(3) that the highest rate of incorporation of ethionine across the placenta into the fetus and amniotic fluid is 6 hours after the injection of unlabeled ethionine. The combined data indicate that the placenta presents a barrier to the movement of ethionine to the fetus.

The maximum uptake of labeled ethionine into the protein of all tissues is observed at 12 hours after injecton while little was found in the proteins 2 hours after injection. This is in contrast to our observations with unlabeled ethionine(3) in which the highest concentration of ethionine was found in placental proteins 2 hours after injection and in the fetal proteins 6 hours after injection. Since the carrier ethionine was injected 6 hours prior to injection of the label, this may have sufficiently saturated the proteins with ethionine or diluted the tracer so that its appearance in the proteins was delayed. The different sites of injection may also be a factor in the uptake of the label by the protein in that label administered intraperitoneally might be retained in the body longer than that administered intravenously.

Chromatography showed the major radioactivity to be in the areas corresponding to ethionine and ethionine sulfoxide.

No significant counts were found in the fat samples from any of the tissues.

The autoradiography study showed a diffuse distribution of the label throughout the fetus, which has been previously shown(2), and throughout the placenta at all times after injection. There is an indication of a concentration of the label in or adhering to the maternal and fetal erythrocytes (Fig. 1). The

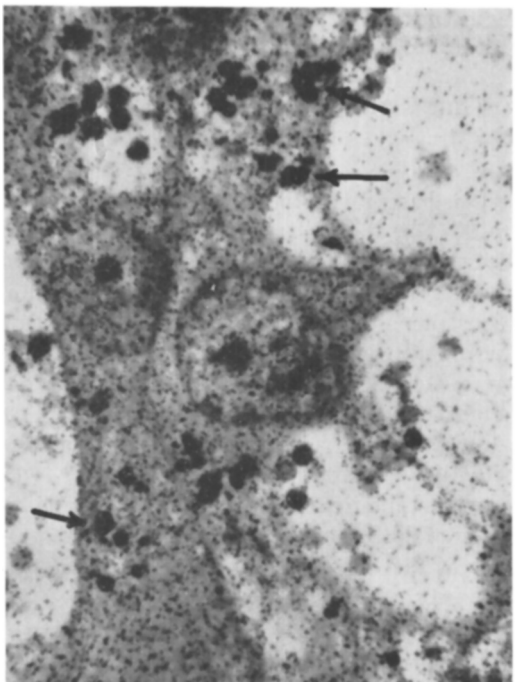

FIG. 2. Ethionine-ethyl-H^3 autoradiograph, giant cells in junctional zone between placental labyrinth and decidua basalis. Arrows indicate areas of concentration of label. (× 375)

concentration of label in or around the maternal erythrocytes was also seen in sections of the liver and kidney and in smears of washed maternal blood. In the placenta, there was a concentration of label in the giant cells (Fig. 2) only in animals autopsied 2 hours after injection of ethionine. Since the radioactivity detected by autoradiography has been shown to be due to newly synthesized protein(4), these observations indicate that ethionine is taken into the proteins of the fetus and placenta within 5 minutes after injection of the radioactive compound and either persists in the protein and/or continues to be incorporated through a 72-hour period.

Our observations on sections of the liver and kidney are similar to those previously reported(2,5), *i.e.* a diffuse distribution of the label in the liver and a concentration in the proximal convoluted tubules of the kidney.

Summary. Radioactive ethionine is present in the soluble fraction and in the proteins of the rat fetus and placenta within 5 minutes after injection on day 12 of pregnancy. The maximum amount of soluble activity is found in the placenta within 15 minutes after injection and not until 2 hours in the fetus and amniotic fluid. The maximum amount of protein-bound label is present in the conceptus 12 hours after injection. Autoradiography studies show the presence of diffuse radioactivity throughout the conceptus within 5 minutes after injection of ethionine, which persists up to 72 hours after injection. There is a possible concentration of the label in or adhering to the maternal and fetal erythrocytes at all times after injection of labeled ethionine.

The authors wish to express their appreciation to Dr. C. A. Woolever for use of the Packard Tri-Carb liquid scintillation system, to Mrs. Barbara D. Fleischaker and Miss Juanita Graves for technical assistance, and to Dr. S. W. Smith for suggestions on autoradiography.

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Received February 24, 1964. P.S.E.B.M., 1964, v116.