in cancer sera in a gradient and at high titers, no other indication could be found to show that this may be an antigen-antibody reaction, as could have been expected if the bentonite particles became coated with either excess antigen or antibody present in the sera. Also, addition of guinea pig complement did not restore the reaction in sera heated to 56°C for 30 minutes. Since *in vivo* antigen-antibody interaction has been considered to take place in some acute or chronic diseases such as systemic lupus erythematosus and glomerulonephritis, this theory should not be discarded.

Absorption of the a_2 globulin by the bentonite suggests an ionic type of reaction with some component of this fraction. Immunoelectrophoretic studies using anti-a_2 globulin sera and bentonite may clarify this point.

The low percentage of reactivity of normal and other sera as compared with cancer sera suggests the possibility of a test based on the effect of bentonite on specific components of the a_2 globulin.

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Placental Transfer of Free Fatty Acids in the Pregnant Rat. (29263)

ZEEV KOREN AND ELEAZAR SHAFRIR (Introduced by Felix G. Sulman)
Departments of Biochemistry and Obstetrics and Gynecology, Hebrew University-Hadassah Medical School and Hadassah University Hospital, Jerusalem, Israel

The transfer of maternal plasma lipids to the fetus has been investigated with isotopic technics by Goldwater and Stetten(1) and by Popjak and collaborators(2), who demonstrated that the placenta is relatively impermeable to cholesterol and phospholipids (PL). These lipids, as well as triglyceride esters of fatty acids (TG) circulate as constituents of lipoproteins of large molecular size, which seems to preclude their direct passage through the placental barrier. Free fatty acids (FFA) are known to be available for the metabolism of body tissues, where they are assimilated by esterification into TG or PL. Albumin, the main carrier protein of FFA has been shown to enter the fetal circulation of man and rabbit to a small degree(3,4). The sheep and rabbit placental membranes were reported to be somewhat permeable to palmitate-1-C^{14}(5,6). The crossing through rat placenta from the maternal or fetal side, of labeled palmitic, stearic and linoleic acids, was presently studied.

Experimental. FFA labeled with C^{14} at 1-position were purchased from Amersham (Great Britain). After conversion to their respective sodium salts the FFA were complexed to a 1% solution of bovine albumin (Pentex, U.S.A.) in phosphate buffer pH 7.4, and the solution filtered before use. Albino rats of a local strain were kept on a stock diet and used during 17-19th day of pregnancy. The abdomen was opened under light ether anesthesia and either 0.5 ml of the FFA solution injected into the lower abdominal aorta, or doses of 0.1 ml injected into each of the amniotic sacs. Intrafetal injections were effected by entering through the uterine wall into the peritoneum of each fetus. On the average 8 fetuses were present (range 6-11) and total radioactivity introduced per animal was within 2-3 μc. The rats were then closed and sutured and at various times again anesthetized and bled through the abdominal aorta. The tissues were promptly excised, weighed, rinsed and extracted thoroughly by homogenization in a large volume of ethanol-ether 3:1. Aliquots of the extracts were counted after evaporation *in vacuo* in a Packard Tricarb Scintillation Spectrometer. Other aliquots were used for separation of PL by precipitation with cold

TABLE I. Percentage of Injected Radioactivity Recovered in Maternal and Fetal Tissues 2 Hours After Administration of FFA.

Method of inj:	Intra-aortic			Intra-amniotic			Intra-fetal		
1-C^{14}-labeled FFA:	Palmitate	Stearate	Linoleate	Palmitate	Stearate	Linoleate	Palmitate	Stearate	Linoleate
Maternal tissues*	18.1	21.9	17.8	.7	.6	2.2	.4	.5	.8
Placenta	1.2	1.0	2.6	10.5	6.7	5.5	1.3	1.1	2.0
Fetal tissues†	1.2	.7	1.8	9.7	8.1	10.1	37.0	41.0	34.2

Values in Table are averages of 3 exp.

* Liver, heart, lungs, kidneys and spleen. † Liver, heart, lungs, kidney, spleen and carcass.

acetone in presence of $MgCl_2$. TG were recovered from the acetone supernatant into heptane, which was then washed with alkaline ethanol. Correlation of the counting efficiencies of the various lipids was obtained through the use of internal standards.

Results. Table I comprises representative data on the distribution of radioactivity recovered in several selected maternal tissues, as compared with that found in the pooled placentas and fetuses of the same animal. Two hours after administration of 3 different FFA into the maternal aorta, the radioactivity trapped in the placental tissue or recovered on the fetal side amounted to only a small fraction of the injected material. In numerous experiments values in the pooled placentas were always below 3%, and in the fetuses less than 1.5% of the injected radioactivity. Only small variations were seen with the different FFA employed. It should be pointed out that this small percentage was obtained with the intra-aortic method of FFA administration, which was chosen in order to introduce the label close to the placental circulation and to diminish the effect of pool reduction through rapid FFA uptake by other tissues upon intravenous administration. The low penetrability through the maternal-fetal barrier was also evident when FFA were administered into the amniotic cavity or injected into the fetal peritoneum. Following intra-amniotic injection, the placenta and fetal tissues became strongly labeled, whereas only a small proportion reached the maternal tissues. Although the fetal tissues were capable of assimilating most of the FFA, an appreciable portion of the radioactivity was, however, detected in the amniotic fluid in the form of FFA 2 hours after injection. The exact amount of this residual activity could not be determined because of the difficulty of quantitative collection of the amniotic fluid. Similarly, after injection into fetal peritoneum only a small fraction reached the placenta and the maternal tissues. Some of the FFA radioactivity appeared to remain unassimilated in the peritoneal cavity of the fetuses at time of sacrifice.

Table II presents a typical time-course of distribution of radioactivity after FFA injection. Upon intra-aortic administration, the radioactivity in the selected maternal tissues decreased, probably due to utilization or egress toward adipose and peripheral tissues. On the other hand, radioactivity in the placentas and fetuses increased somewhat between 15 minutes and 5 hours, then appeared to decrease to show little change up to 18

TABLE II. Changes with Time in Proportion of Palmitate-1-C^{14} Radioactivity Retained by Fetal and Maternal Tissues.

Method of inj:	Intra-aortic				Intra-amniotic			
Hr after inj:	¼	1	5	18	¼	1	3	16
Maternal tissues*	35.7	26.9	14.0	9.5	.3	.6	1.5	1.8
Placenta	1.0	1.4	1.6	1.1	5.2	6.1	16.4	21.1
Fetal tissues†	.8	1.6	2.4	1.9	9.1	—	19.4	34.3

Values in Table are averages of 3 exp.

* Liver, heart, lungs, kidneys and spleen. † Liver, heart, lungs, kidneys, spleen and carcass.

TABLE III. Distribution of Radioactivity Among Maternal and Fetal Tissues and Incorporation into Tissue TG and PL.*

Method of inj:	Intra-aortic				Intra-amniotic				Intra-fetal			
Assimilation of 1-C^{14}-labeled FFA:	Palmitate		Linoleate		Palmitate		Linoleate		Palmitate		Linoleate	
	%	TG/PL	%	TG/PL	%	TG/PL	%	TG/PL	%	TG/PL	%	TG/PL
Maternal tissues												
Liver	32.0	2.5	26.8	2.0	.54	2.2	1.20	1.0	.39	2.0	.60	1.4
Heart	1.9	1.1	4.1	.9	.03	†	.37	.5	.02	†	.04	.4
Lungs	1.8	.4	1.9	.5	.04	†	.18	.4	.04	†	.05	.3
Kidneys	1.1	.8	2.7	.7	.04	†	.10	.6	.03	†	.05	.5
Spleen	.4	.9	1.1	.8	.01	†	.04	.7	.01	†	.01	.4
Placenta	1.0	.6	2.2	.6	5.6	2.2	6.3	1.8	1.1	1.6	1.8	1.0
Fetal tissues												
Liver	.3	.4	.4	.5	1.3	.7	3.3	.5	9.2	.6	8.9	.8
Heart					.3	1.2	.7	1.0	2.0	1.0	.6	1.4
Lung					1.5	.6	2.4	.5	1.6	.4	4.5	.3
Kidneys	.7	.6	.6	.5	.1	.8	.3	.5	.5	.4	.3	.6
Spleen					.2	.7	.2	.9	.6	.8	.9	1.1
Carcass	.3	.9	.2	.8	6.6	1.5	7.5	1.3	13.0	1.7	18.1	1.6

* Expressed as percentage of total radioactivity injected, recovered in lipid extract of tissues removed 30 min after injection.
† Values too low for reliable assessment of TG/PL ratio.

hours. The small rise could not be attributed to the continuing direct passage of radioactive FFA through the maternal-fetal barrier since after 15 minutes only negligible amounts were found in the maternal plasma. Radioactivity in the circulating TG and PL was also small but at one hour exceeded that of FFA, conforming to previous observations in rats(7,8). After intra-amniotic administration of palmitate-1-C^{14} crossing in the opposite direction was also very limited, since at 16 hours the proportion of radioactivity reaching the maternal side rose to about 2% at most. In this case a direct transfer of FFA might have been possible, since even at 16 hours the presence of FFA radioactivity could still be demonstrated in the amnion. This was apparently the reason for the continued marked rise in radioactivity assimilated by fetal tissues and placentas which remained in direct contact with the FFA of the amniotic fluid.

Table III includes the ratio of radioactivity incorporated into tissue TG *vs* that assimilated into the PL. Thirty minutes after intra-aortic administration of palmitate or linoleate about 30% of the injected radioactivity was recovered in the maternal liver, a proportion similar to the results of other investigators after intravenous administration to male rats (7-9). The liver contained far more radioactivity than other maternal viscera combined. On the other hand, of the radioactivity which crossed the placenta, the fetal liver appeared to contain less than the corresponding fetal viscera taken together. The ratio of the incorporated label TG/PL was higher in the maternal than in the fetal liver, indicating that in the former the larger FFA uptake was associated with preferential deposition as TG. The TG/PL ratios in other maternal or fetal tissues were low and did not appear to show consistent differences. Upon intra-amniotic administration of FFA, the distribution of the small proportion of radioactivity reaching the maternal side resembled that after intra-aortic injection. On the fetal side the distribution of radioactivity was affected by the route of FFA introduction. Following intra-amniotic injection the radioactivity in the liver was smaller than in the other fetal viscera combined. The amount of

radioactivity assimilated by the fetal lungs was as high as that assimilated by the liver, which may have been caused by direct contact of fetal lungs with amniotic fluid containing the radioactive FFA. The TG/PL ratio of radioactivity taken up by the viscera generally paralleled that on the maternal side, with the possible exception of the fetal heart, which exhibited a higher ratio than other viscera. After intra-fetal introduction the liver and the carcass assimilated a larger proportion of radioactivity perhaps by virtue of the direct contact with the FFA in the peritoneal cavity.

Discussion. The results indicate that the maternal circulation is not an important source of direct transfer of fetal FFA. Since the transfer of albumin has also been demonstrated to occur to a limited extent only(3,4), it may be inferred that whatever passage takes place, it is not by exchange of FFA between the maternal and fetal side but by a small seepage of FFA with the original carrier protein. As an additional tributary of FFA to the fetal circulation placental lipolysis may be considered. The FFA (or fatty esters) initially retained as TG or PL may be released on the fetal side after the cleavage of the ester bonds. Such a mode of transfer is compatible with the continuing, though small, rise in fetal radioactivity observed at periods when virtually no labeled FFA were present in the maternal circulation (Table II). It may also account for the reported changes in saturation induced in fetal fat by alteration of maternal dietary intake or for the passage of rare fatty acids when fat such as elaidin was fed to pregnant rats(10). Most of the fetal lipids seem to be derived then by independent synthesis and adequate mechanisms for this function are probably available(2,11). The contribution of preformed fatty acid molecules appears to be of minor importance to the fetus with the possible exception of essential fatty acids.

Summary. Direct placental transfer of albumin-bound, labeled palmitate, stearate or linoleate administered to albino rats was very small, whether introduced by intra-aortic, intra-amniotic or intra-fetal injection. Time-course experiments indicated a small indirect transfer of radioactivity initially assimilated by the tissues as TG or PL. The distribution of the assimilated FFA between tissue TG and PL was generally similar on fetal and maternal side.

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Effect of Protein Nutrition on Leukocyte Mobilization. (29264)

IRVING GRAY (With technical assistance of Sara Willard)
U. S. Army Medical Unit, Fort Detrick, Md.

The interaction between nutrition and resistance to infectious disease has been well established(1). While many reviews have summarized the current status of this problem(2), more specifically Dubos and Schaedler have demonstrated the effect of lysine deficiency on resistance to infectious disease(3). We(4) have related the lowered resistance to