not have to mobilize the needed resources (for example, nitrogen) or divert needed nutrients from the leaves thereby killing the plant. The prevention of the internally programmed degeneration in soybeans through chemical treatment is not only of theoretical interest but may eventually open a way to yield improvement.

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21. Supported in part by research grant 416-15-79 from the U.S. Department of Agriculture Cooperative State Research Service under PL89-106. We thank R. Crotz and the University of Michigan Botanical Gardens for handling these plants during the first 6 weeks.

4 May 1979; revised 24 July 1979

Fluidity of the Rat Liver Microsomal Membrane: Increase at Birth

Abstract. *The lipid apparent microviscosity of the rat liver microsomal membrane on the first day after birth was found to be half of that observed on the last day of fetal life. This remarkable perinatal fluidization of the membrane resulted from a marked increase in the molar ratio of phospholipids to cholesterol.*

Among the dramatic events connected with the birth process there are sudden changes in the activity levels of many critical cellular proteins, such as enzymes and transport proteins. Studies in both laboratory animals and man have shown significant perinatal increases in the activity of some enzymes, while other enzymatic activities decrease from fetal life to adulthood (*1*). These developmental changes in enzyme activity are connected with changes in de novo protein synthesis. Since many of these enzymes are intimately associated with membranes it is to be expected that their activity will also depend on the physical properties of the membrane. A close dependence of uridine diphosphate glucuronosyltransferase (E.C. 2.4.1.17; UDPGT) activity on the fluidity of the lipids in guinea pig liver microsomal membranes has, in fact, been demonstrated by electron paramagnetic resonance analysis (*2*). It was therefore of interest to determine whether the perinatal changes in the activity of microsomal enzymes are accompanied by changes in membrane fluidity. We report here that at birth there occurs a remarkable increase in fluidity of the rat liver microsomal membrane.

Sabra rats were obtained from the Animal Facility of the Hebrew University and given free access to a diet of Purina Chow. Fetuses obtained from rats on the last day of pregnancy, and newborn rats on their first day of life, were decapitated and livers from each litter were excised and pooled together. Livers from adult rats (3 months of age) were examined individually. Homogenates (25 percent, weight to volume) were prepared in 1.15 percent KCl and microsomes were fractionated (*3*) and resuspended in 1.15 percent KCl. Microsomal protein was determined by the method of Lowry *et al.* (*4*). The apparent microviscosity ($\bar{\eta}$) of lipids was determined by measurement of fluorescence polarization with the use of the fluorophore 1,6-diphenyl-1,3,5-hexatriene (1 m*M* in tetrahydrofuran) (*5*). One microliter of this solution was added to 1 ml of a suspension containing 0.25 mg of microsomal protein and the mixture was incubated at room temperature for 1 hour. Fluorescence polarization was measured at 37°C by means of an instrument previously described (*6*). Excitation and emission wavelengths were 365 and 430 nm, respectively. Lipids were extracted from the microsomal membrane into a mixture of $CHCl_3$ and methanol (2 : 1 by volume) as described by Folch *et al.* (*7*). Total phospholipids were determined by phosphorus analysis (*8*) and cholesterol was determined by a cholesterol oxidase method (*9*).

The apparent microviscosity of the lipids of the rat liver microsomal membrane decreased by 48 percent between the last day of intrauterine life and the first day of extrauterine life (Table 1). A further small, but statistically significant, decrease in viscosity (15 percent) was observed at 3 months of age (Table 1). No significant differences in apparent microviscosity were found between male and female adult rats. The decrease in lipid apparent microviscosity observed in the perinatal period was paralleled by an increase in the microsomal phospholipid content of the liver while there was no change in its cholesterol content (Table 1). These results indicate that during the perinatal development of the smooth endoplasmic reticulum of rat liver, qualitative changes in lipid composition occurred which gave rise to increased phospholipid to cholesterol molar ratios

Table 1. Lipid composition and apparent microviscosity ($\bar{\eta}$) of rat liver microsomal membrane. Fetuses were studied on the last day of pregnancy and newborns during the 24-hour period following birth. Adult rats were 3 months old. Numbers in parentheses indicate number of litters, each litter consisting of 6 to 13 fetuses or newborn rats, or number of individual adult rats. The $\bar{\eta}$ values are expressed as means ± standard error. Protein, phospholipid, and cholesterol content were determined in composite pools from all the microsomal preparations used for measurement of $\bar{\eta}$ in each of the three groups.

Microsomes	$\bar{\eta}$ (poise)	Protein (mg/g liver)	Phospholipid (μmole/g liver)	Cholesterol (μmole/g liver)	Phospholipid/cholesterol molar ratio
Fetus	2.33 ± 0.13* (9)	14.0	3.86	1.12	3.45
Newborn	1.22 ± 0.05*† (7)	20.9	6.05	1.14	5.31
Adult	1.04 ± 0.02† (21)	22.3	8.05	1.07	7.52

*Significant at $P < .001$. †Significant at $P < .005$.

0036-8075/79/1116-0843$00.50/0

(Table 1). These changes accompanied the increased de novo formation of the protein and lipid components of the microsomal membrane.

It is well known that the apparent microviscosity of the lipid bilayer of model and biological membranes diminishes by increasing the phospholipid to cholesterol molar ratio (*10*). Increases in the degree of unsaturation of the phospholipid acyl chains, or in the molar ratio of phosphatidylcholine to sphingomyelin, may also decrease the membrane apparent microviscosity (*10*). In this respect Feuer (*11*) recently reported a significant perinatal increase in the production of liver microsomal phosphatidylcholine with an increasingly higher proportion of unsaturated fatty acids. The changes observed at birth in the apparent microviscosity of the microsomal membrane may be important in the perinatal regulation of biological activities that are associated with this membrane. The activity ratio of "native" (nonactivated) to detergent-activated UDPGT in fetal liver is higher than in adult liver (*12*). This could possibly be explained by an increased exposure of the enzyme in the more rigid fetal membrane (*13*). Results from our laboratory indicate that fluidization of fetal rat liver microsomal membranes by phosphatidylcholine enrichment reduces the activity of "native" UDPGT.

The present findings may also be relevant to other membrane-bound enzyme systems and membrane proteins, in liver microsomes or in other hepatic or extrahepatic organelles, the action of which is affected by the membrane fluidity. Recently, a significant increase in membrane fluidity similar to that observed in the present study was also reported for the plasma membrane of chick heart between the last day of embryonic life and adulthood (*14*). It was suggested (*14*) that the changes in membrane fluidity may contribute to developmental changes in the uptake of sugars, amino acids, and urea by the heart cells.

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10 April 1979; revised 2 July 1979

Tumor Location Detected with Radioactively Labeled Monoclonal Antibody and External Scintigraphy

Abstract. *Murine teratocarcinomas were located in mice by external gamma-ray scintigraphy with an iodine-125-labeled monoclonal antibody specific to the tumors. The specificity of the method was increased by subtracting the radiation produced by an iodine-125-labeled indifferent monoclonal antibody of the same immunoglobulin class as the tumor-specific antibody.*

The use of specific antibodies for tumor localization and treatment has been suggested and attempted for some time (*1*, *2*), with little success until recently. The main difficulties have been in the preparation and purification of specific antibodies to tumors and the suppression of background caused by unbound antibody or circulating antibody-antigen complexes.

Belitsky *et al.* (*3*) have successfully imaged both primary tumors and metastases in man by using ^{131}I-labeled immunoglobulin G (IgG) from absorbed goat antiserums to human renal carcinomas; these authors noted problems caused by the presence of a radioactive background due to a large excess of radioactively labeled nonspecific antibody. Goldenberg *et al.* (*2*) minimized some of these problems by combining ^{131}I-labeled affinity-purified goat antiserum to carcinoembryonic antigen (CEA) with a second radiolabel that remained in the general circulation (metastable technitium system: $^{99m}TcO_4$ and ^{99m}Tc-labeled serum albumin). The second radiolabel was distinguishable from the first by two-channel γ-ray scintigraphy; its image could therefore be subtracted from that of the radiolabeled tumor-specific antibody to provide a correction for unbound or metabolized antibody. Images of several human tumor types were obtained. Their results strongly support the possibility of using radiolabeled antibody for the detection of tumors and metastases, even those located deep within the body.

A more incisive approach is made possible by the recent development of monoclonal tumor-specific antibodies (*4*–*7*). Such antibodies, derived from lymphocyte hybridomas (*8*), are homogenous, require little labor for purification, and can be reproducibly prepared

Table 1. Accumulation of tumor antibodies in teratocarcinoma and selected organs. Five BALB/c mice, previously innoculated with MH-15 teratocarcinomas, were injected with ^{131}I-labeled tumor-specific antibody as described in the legend to Fig. 1. Lugol's solution (Lyne; two drops per 100 ml in drinking water) was administered to prevent accumulation of free iodine. After 48 hours the mice were killed and examined. Inspection revealed no metastases. Listed tissues were removed, weighed, and their radioactivity was determined. Specific activities represent means (± S.E.) of organs from separate mice; tumor to tissue ratios (means ± S.E.) were determined for each mouse, then averaged.

Tissue	Specific activity (cpm/mg)	Tumor to tissue ratio [(cpm/mg in tumor)/(cpm/mg in tissue)]*
MH-15 teratocarcinoma	41.7 ± 5.6	1.0
Thymus	8.1 ± 0.98	5.8 ± 1.6
Spleen	5.9 ± 1.2	5.1 ± 0.85
Liver	16.1 ± 1.7	2.55 ± 0.16
Heart	6.0 ± 0.76	7.2 ± 0.96
Lung	11.0 ± 1.6	3.8 ± 0.55
Forelimb muscle	3.3 ± 1.5	24. ± 9.4
Blood	31.7 ± 3.0	1.3 ± 0.21
Brain	1.16 ± 0.15	39.8 ± 9.3
Kidney	9.6 ± 1.5	4.7 ± 0.88

*In a separate experiment, two mice bearing P3-X63-Ag8 myelomas and MH-15 teratocarcinomas of equal size were similarly injected and killed. The tumor to tissue ratios of special interest were, respectively, MH-15/P3 = 1.92, 4.43; MH-15/liver = 1.00, 0.81; MH-15/spleen = 1.48, 1.41; and MH-15/muscle = 14, 21. That is, P3 myeloma tissue accumulates about as much antibody as other highly vascularized tissues.

0036-8075/79/1116-0844$00.50/0