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Esterification oleic acid
lipids small intestine

Uptake, Activation, and Esterification of Fatty Acids in the Small Intestine of the Suckling Rat

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Extract

The small intestinal mucosal phase of fatty acid absorption was studied in suckling and adult rats. Fatty acid binding protein (FABP) is present in the cytosol of jejunal mucosa of 6-day-old rats in amounts equivalent to that found in mucosal cytosol of adult rats (16.4% and 15.0%, respectively). The percentage of oleic acid binding to FABP is the same in 6-day-old and adult rats (13.9% and 10.2%, respectively). The specific activity of jejunal microsomal oleoyl-CoA synthetase is high in the fetus, falls abruptly after birth, but increased by the third day of life to remain constant thereafter into adult life. In contrast the specific activity of acyl-CoA:monoglyceride acyltransferase is low in the fetal jejunum, gradually increases, and is significantly higher in the 6- and 12-day-old rat than in the adult. Uptake of oleic acid by jejunal slices of 6- and 11-day-old animals is three- to fivefold higher than uptake by jejunal slices prepared from adults rats. The rate of esterification of oleic acid is higher in jejunal slices from 6- and 11-day-old rats, reflecting the enhanced uptake of oleic acid.

Speculation

In the suckling rat, the increased intestinal mucosal epithelial cell capacity for fatty acid esterification coincides with a diminished lipolytic activity within the lumen. This paradox suggests that the fatty acid esterification process in the small intestine of the suckling

rat may be involved with aspects of lipid metabolism other than that of fatty acid absorption. Extrapolating these observations on experimental animals to the human neonate, we suggest as working hypothesis that inefficient fat absorption should not be attributed to diminished mucosal epithelial cell function.

Extensive studies, especially in the last decade, have delineated the mechanisms for fat absorption in adult mammals, including man. In comparison our knowledge of these same processes during the early perinatal period is relatively meager. A more complete understanding of the mechanism of fat absorption in the newborn is needed as fat is an important nutrient during this particular period of life. The high fat content of natural milk and commercially prepared formulas subjects the human infant to an ingested fat load which exceeds that encountered by the mature individual.

Balance studies, performed in infants during the first 3 months of life, show a lower coefficient of fat absorption, especially in those infants on cow milk-based formulas (1, 8, 10, 32, 37, 39). Many factors may contribute to the relative inefficiency of absorption of lipid by the newborn. It has been shown recently that the pool size of conjugated bile acids is smaller in infants than in adults (36), and is further diminished in low birth weight infants (35). However, the balance studies performed on newborns have repeatedly been interpreted as indicating the existence of diminished lipolytic activity in the gastrointestinal contents of young infants (8, 10, 34, 37). Recent studies by Zoppi and coworkers (38) have firmly established the validity of this observation by measuring pancreatic lipase output after intravenous pancreozymin-secretin stimulation. In addition to these studies on human infants, previous studies in animals have shown that low lipolytic activity of gastrointestinal secretions is a general phenomenon and not limited to the human infant (4, 5, 13, 14, 26, 30). Thus, although the clinical and animal studies have concentrated on the luminal phase of fat absorption, the mucosal phase has been practically neglected. In the studies presented in this report, we evaluated the mucosal phase of lipid absorption during the perinatal period. The suckling rat was chosen for several reasons. Suitable data of fat digestion and absorption have been accumulated from the studies with the adult rat, and both man and rat are known to have a low lipase activity during the immediate period after birth. In addition, the rat is born relatively immature and offers the opportunity to study the processes of lipid digestion and absorption during the different phases of perinatal development.

The mucosal phase of fat absorption was evaluated during the perinatal period by studying both the activity of the microsomal enzymes involved in the esterification of free fatty acids and the esterification capacity of the total intestinal tissue. Studies also included the evaluation of FABP in suckling and adult rats. Preliminary reports were presented elsewhere (7, 43).

MATERIALS AND METHODS

Pregnant Charles River females shipped as 12-day pregnant animals gave birth in our own animal house. The litter size was reduced to 8–9 animals at the third day after birth. Suckling rats were taken directly from the mother and killed by decapitation at 9 AM. Microsomes were prepared from the jejunum (first third of the small intestine) and the ileum (last third of the small intestine) according to the methods described by Rodgers, *et al.* (28). The entire intestinal wall of rat fetuses, 1- and 3-day-old rats was used at the initial starting tissue for microsomal preparations. In older rats, mucosal scrapings were obtained and used as the initial starting material. The specimens from one litter were pooled and treated as one sample; small intestine from fed 3-month-old male rats was used singularly as samples of adult intestine. Contamination by other cell fractions was evaluated by the appropriate enzymatic and biochemical markers: the presence of microvilli was monitored by assay for neutral β-galactosidase (15), lysosomes by acid β-galactosidase (15), mitochondria by succinodehydrogenase (24), and nuclei by DNA (11). Protein was determined by the Folin-phenol reaction (19).

The determination of oleoyl-CoA synthetase activity was based on the hydroxamic acid trapping method of Kornberg and Pricer (18), as modified for the small intestine by Rodgers *et al.* (29). Oleic acid was homogenized in 0.27% lecithin so that the final concentrations of oleic acid and lecithin were 1.95 and 8.5 mM, respectively. Acyl-CoA:monoglyceride acyltransferase (EC. 2.3.1) was assayed as described previously (7) according to the method of Rodgers (27), using palmitoyl-CoA and 1-mono-olein as substrates at final concentrations of 0.17 and 0.09 mM, respectively. The sulfhydryl trapping agent, 5,5′-dithiobis-(2-nitrobenzoic acid) was used to measure the rate of CoA liberated by the reaction. The activity of both enzymes was assayed under linear conditions with respect to time and protein concentration. Under standard conditions 0.1 mg microsomal protein was used for both assays.

Uptake and esterification of fatty acid were studied *in vitro* using jejunal slices according to the method described by Clark (2). Three slices of jejunal mucosa, weighing between 10 and 20 mg each from either 6-day-old, 11-day-old, or adult rats were incubated in flasks containing 2ml Krebs-Ringer phosphate buffer (pH 7.4) without added calcium or magnesium, containing 1 mM [1-^{14}C]oleic acid (specific activity 0.3 μCi/mM) (40) and 10 mM sodium taurocholate (41). After 15 min of preincubation at 0.5–2° (cold), the flasks were transferred to 37° water bath for given periods of time. Slices were then removed, rinsed twice in nonradioactive media, blotted on a moist filter paper, weighed, and plunged into test tubes containing 1 ml 0.1 N HCl. Lipids were extracted from the homogenized slices by standard methods (9) and separated by Silica Gel G (42) thin layer chromatography. The areas corresponding to the mono, di-, and triglycerides and fatty acid standards were scraped into the scintillation vials containing 10 ml toluene containing 3% Liquiflor (40) and the radioactivity counted at 85% efficiency. Using the external standarization methods, there was no significant quenching of any of the samples analyzed.

The FABP in jejunal mucosal cytosol was determined according to the method of Ockner *et al.* (22). [1-^{14}C]Oleic acid (220 μmol) (40) was added to 30–40 mg cytosol protein, incubated on ice for 30 min with intermittent mixing, applied to a Sephadex G-75 column, and eluted in 4-ml fractions. Fractions 24–28 contained FABP and were assayed for protein and radioactivity.

RESULTS

To evaluate the esterification capacity of the small intestine during the perinatal development two approaches were chosen. The first one involved determination of the activity of the two microsomal enzymes involved in the esterification pathway. These studies using isolated microsomes were paralleled by a second group of experiments in which the esterification capacity of the small intestine was evaluated by studying the uptake and esterification of fatty acids by intestinal slices incubated *in vitro*.

PERINATAL CHANGES OF ACTIVITY OF OLEOYL-CoA SYNTHETASE AND ACYL-CoA: MONOGLYCERIDE/ACYLTRANSFERASE IN JEJUNAL AND ILEAL MUCOSA

Activity of these enzymes was determined in isolated microsomal fractions of intestinal mucosa. Before the developmental studies were started, we evaluated the quality of the isolated microsomal fraction obtained from small intestinal mucosa of rats of various ages. As seen in Table 1, the recovery of protein in the microsomal fraction from mucosa of rats of all ages is fairly similar. DNA and succinodehydrogenase activity was present in the microsomal fractions in all age groups, but the amount was always less than 0.5% of the amount in the initial homogenate. The contamination by microvilli as judged by the amount of neutral β-galactosidase activity in the microsomal fraction never exceeded

Table 1. *Comparison of microsomal preparations from jejunum and ileum of developing rats*

		Protein[1]	Neutral β-galactosidase[1]	Acid β-galactosidase[1]
Fetus	Jejunum	9.5 ± 2.7 (3)[2]	8.4 ± 5.5 (3)	2.1 ± 0.5 (3)
	Ileum	8.0 ± 2.0 (3)	7.6 ± 2.3 (3)	3.8 ± 0.1 (3)
3-day-old	Jejunum	6.8 (1)	10.7 ± 2.3 (3)	2.3 ± 0.5 (3)
	Ileum	4.1 ± 0.5 (3)	11.6 ± 3.5 (3)	8.1 ± 2.6 (3)
6-day-old	Jejunum	7.0 ± 1.8 (4)	9.9 ± 1.2 (4)	5.7 ± 2.1 (4)
	Ileum	4.4 ± 0.4 (4)	9.0 ± 4.0 (3)	6.6 ± 2.9 (3)
12-day-old	Jejunum	7.0 ± 1.9 (4)	12.1 ± 2.7 (3)	7.6 ± 1.4 (6)
	Ileum	4.8 ± 0.7 (6)	16.9 ± 2.5 (3)	10.0 ± 1.7 (6)
Adult	Jejunum	7.5 ± 0.6 (13)	12.3 ± 2.8 (12)	5.9 ± 1.1 (13)
	Ileum	6.6 ± 0.5 (11)	11.2 ± 2.3 (10)	8.7 ± 2.3 (11)

[1] Percentage of amount present in total homogenate.
[2] Mean ± SE (number of determinations).

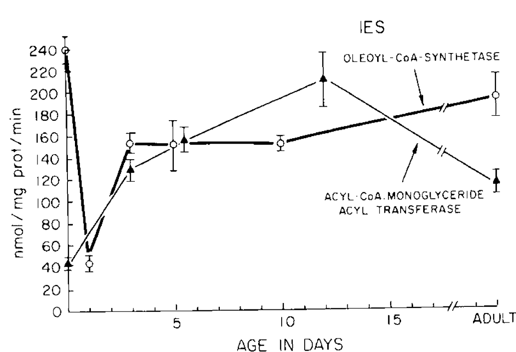

Fig. 1. Developmental pattern of microsomal oleoyl-CoA synthetase and acyl CoA:monoglyceride acyltransferase activity in rat jejunum. Results are expressed as nanomoles of product formed per mg of protein per min. Each point represents the mean ± SE of at least four litters for the suckling rats and 13 adult animals. There is no difference in oleoyl-CoA synthetase activity among 3-, 5-, 10-day-old, and adult values. Fetal and adult values of acyltransferase activity are significantly lower than the values of either the 6- or the 12-day-old animals ($P < 0.01$).

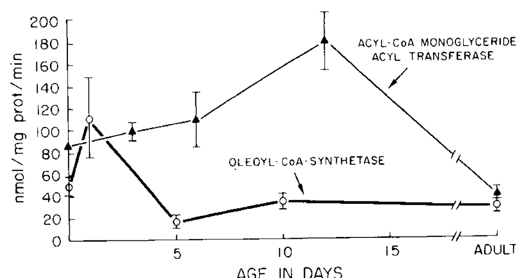

Fig. 2. Developmental pattern of microsomal oleoyl-CoA synthetase and acyl-CoA:monoglyceride acyltransferase activity in rat ileum. Results are expressed as in Figure 1. There is no difference in oleoyl-CoA synthetase activity among the 5-, 10-day-old, or adult values. Acyltransferase activity in the adult is significantly lower than in the 6-day-old ($P < 0.05$) and in the 12-day-old animal ($P < 0.001$).

17% of the total homogenate and is similar to that reported by others for microsomes prepared from the mucosa of adult rats (3, 12). The amount of acid β-galactosidase acitivity varied in jejunal preparations from 2 to 8%, in ileal preparations from 4 to 10% of the total homogenate activity. As these microsomal preparations were judged to be satisfactory for further experiments, perinatal changes of the above mentioned microsomal enzymes were followed in the jejunum and ileum separately.

Jejunum. The highest activity of oleoyl-CoA synthetase was found in rat fetal jejunum. The activity falls rapidly after birth, but rises again sharply by the third day of life to remain constant thereafter into adult life (Fig. 1). With the exception of the change in activity during the first 2 days of life, the activity of this enzyme in jejunal microsomes does not differ during the suckling period from that found in the intestinal mucosal microsomes prepared from adults rats. The activity of acyl-CoA monoglyceride acyltransferase is low in the rat fetus, gradually increases, and is highest in 6- and 12-day-old rats. These values are significantly higher than those found in microsomes prepared from either fetal or adult intestine.

Ileum. Changes of activity of these enzymes in the ileum are depicted in Figure 2. In contrast to the high specific activity of oleoyl-CoA synthetase in the jejunum, low values were found in microsomes from the ileum of the fetal rat. This activity remains low thereafter into adult life. A proximal-distal gradient of synthetase activity exists during the fetal life, and changes little throughout the life of a rat. The activity of acyl-CoA:monoglyceride acyltransferase in the ileum follows a pattern similar to that seen in the jejunum. The specific activity increases slowly from fetal values to a peak by 12 days of age. Thereafter a gradual decrease in activities is seen. A proximal-distal gradient of acyltransferase activity is not observed in the perinatal period; a gradient in adult rats is observed as reported by Rodgers *et al.* (28, 29).

RATE OF UPTAKE AND ESTERIFICATION OF OLEIC ACID BY INTESTINAL SLICES FROM SUCKLING AND ADULT RATS

In these experiments, the esterification capacity was evaluated using intact intestinal slices. Based upon the previous experiments we have limited the experiments to the jejunum and have used only 6- or 11-day-old suckling rats and adults. Since during the esterification process at least two steps are involved, namely, uptake and esterification, the first experiments were designed to evaluate uptake alone.

Uptake. Uptake was studied at 0.5–2° (cold) since esterification was found to be inhibited at this temperature (20). In agreement with this observation, we have found that esterified fatty acids accounted for less than 10% for the total tissue radioactivity at all periods of incubation studied. A faster rate of oleic acid uptake was observed in jejunal slices obtained from the suckling rats than from adult rats (Fig. 3).

Esterification. In the next set of experiments the esterification rate of the accumulated oleic acid was studied. After 15 min of incubation in the cold, the flasks were transferred to a 37° water bath for varying periods of incubation. The results of these studies are depicted in Figure 4. Esterification is expressed as nanomoles of oleic acid converted to triglyceride. In all of these experiments, esterified oleic acid, either as monoglyceride or diglyceride, accounted for less than 5% of the total esterified oleic acid. As shown, the oleic acid is esterified to triglycerides at faster rates in jejunal slices from the suckling rats than in slices from adult rats.

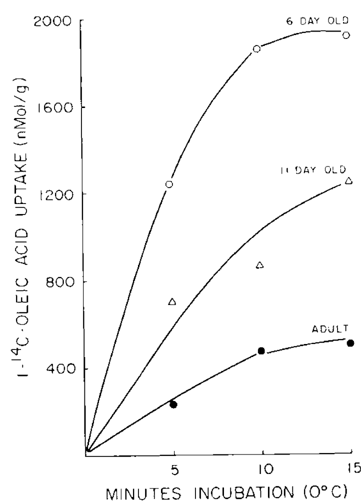

Fig. 3. Uptake of [1-^{14}C]oleic acid by jejunal slices from 6-, 11-day-old, and adult rats. Incubations were performed in triplicate; two litters of each age group and three adults were studied. The symbols represent the mean value of all flasks for each time point. Results are expressed as nanomoles of oleic acid per g of wet tissue weight.

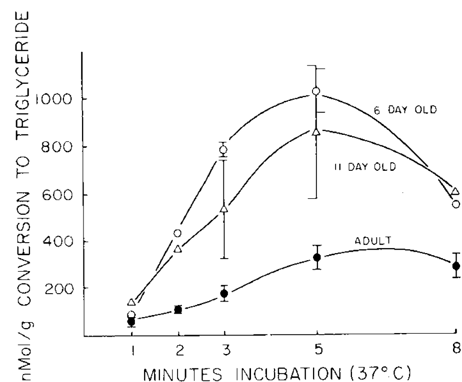

Fig. 4. Esterification of [1-^{14}C]oleic acid by jejunal slices from suckling and adult rats. Each point represents the mean ± SE of four litters of suckling or seven adult rats; each time point for each experiment was performed in triplicate. Results are expressed as nanomoles of oleic acid converted to triglyceride per g of wet tissue weight.

When the ratio of esterified fatty acid after incubation at 37° to free fatty acid present at the end of the incubation in the cold is calculated, no difference between the three age groups is seen (Fig. 5). Therefore, the higher rate of esterification in suckling rats appears to be directly proportional to the higher rate of uptake of oleic acid during the cold preincubation.

Among the various possibilities explaining the higher uptake of oleic acid of suckling rat slices, we have considered the role of FABP, known to be present in adult rat mucosa and found to be increased in the adult rat by high fat diet (23). Fatty acid binding protein is present in cytosol of jejunal mucosa from 6-day-old rats. Neither the amount of FABP nor the percentage of oleic acid binding in a 6-day-old rat jejunal mucosal cytosol is different from that found in adult rat (Table 2). It appears as though an additional factor other than the function of FABP accounts for the increased uptake of oleic acid in jejunal slices from suckling rats.

DISCUSSION

There are two aspects of our present work that we want to discuss. As we have studied the activity of two of the three enzymes which constitute the triglyceride synthetase complex and the esterification capacity of the entire slice, the first aspect deals with the discrepancy of the rate of these reactions as disclosed in our experiments. The second aspect is concerned with the functional projections of our data for the more general problem of lipid digestion and absorption by the gastrointestinal tract during the early postnatal period in mammals, including man.

The process of triglyceride absorption is divided customarily into three main phases: luminal, mucosal, and transport phases. We have, in this series of experiments, studied several aspects of the mucosal phase. The esterification capacity of the small intestinal mucosa was evaluated both by studying the activity of two microsomal enzymes as well as by determination of the capability of the entire intestinal slice *in vitro* to convert oleic acid to triglyceride. Although the determination of enzyme activity showed only minor differences between adult and suckling rats (in favor of the latter), the rate of esterification by the jejunal slices *in vitro* from suckling rats exceeded by several times the rate found in preparations from adults. This difference, as judged from the data derived from the two different types of experiments, can be interpreted as indicating that the activity of the enzymes is not the limiting factor, but that the process of esterification is regulated by other factors as well. We are aware that the present data, obtained under *in vitro* conditions, are limited by this experimental approach and have to be extended by future experiments *in vivo;* however, we can conclude that the availability of free fatty acid on, or in, the intestinal tissue is one factor responsible for the increased rate of esterification. This conclusion is based on the close correlation between the rate of oleic acid uptake and esterification rate in different age groups. The reason for the higher uptake in suckling rats remains obscure: data from the FABP experiments suggests that the amount of FABP does not cause this difference. Recent data of Marrubio *et al.* (20) offer another explanation for this observation. These authors found that intestinal slices from

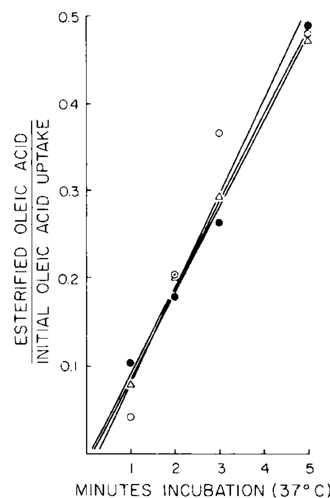

Fig. 5. Ratio of esterified oleic acid to initial oleic acid taken up by jejunal slices preincubated in the cold for 15 min in 6-day-old (O), 11-day-old (Δ), and adult rats (●).

Table 2. *Fatty acid binding protein in jejunal mucosa of suckling rats*

	% Total protein[1]	% Oleic acid binding[2]
6-day-old	16.4 ± 0.9 (4)	13.9 ± 4.0
Adult	15.0 ± 2.1 (5)	10.2 ± 1.0

[1] Expressed as percentage of total supernate protein; number of animals in parentheses.

[2] Expressed as percentage of total amount of [^{14}C]oleic acid added to cytoplasmic supernate.

adults, if preloaded *in vitro* with monoglyceride, accumulated fatty acids at a faster rate than control slices. Thus we can speculate about the possible effect of an increased tissue pool of monoglyceride in the intestine of suckling rats. Both the verification of a similar mechanism in suckling rats as well as its manifestation after peroral intake remain to be shown.

Although the data from the experiments delineating the developmental pattern of the activity of the microsomal enzymes show little difference between the suckling and adult rat, another aspect is interesting. As individual separation of the enzymes comprising the triglyceride synthetase complex has not been achieved (25), the finding of nonparallel behavior of oleoyl-CoA synthetase and monoglyceride acyltransferase activity during development (high synthetase and low acyltransferase activity in the fetus) raises some questions concerning the metabolic regulation of this complex. Whereas McManus and Isselbacher (21) have shown an increase in palmitoyl-CoA synthetase activity in microsomes of adult rats fed a high fat diet and a decrease in rats on a fat-free diet, our findings of high fetal activity and the absence of a decrease after the weaning when the fat intake falls suggest that regulation acyl-CoA synthetase activity in the suckling rat may not reflect the influence of the exogenous fat load as much as in the adult. Other factors during the suckling period, such as hormonal factors, may be more influential than the effect of the dietary load. Further studies are underway exploring these possibilities. Another consideration is that our data were obtained using oleic acid. Although the amount of oleic acid in milk is substantial, both human and rat milk contain a relatively large amount of medium chain length fatty acids (6, 16, 17). Thus it is obvious that corresponding studies in which these shorter chain fatty acids are used as substrates in experiments with suckling animals are needed.

The second aspect of these studies centers around the general picture of lipid digestion and absorption in suckling animals and whether it differs substantially from that in the adult animal. Before our present data were obtained a paradoxic situation existed: fat intake is high in the suckling animal when the lipolytic activity of the gastrointestinal tract is low (16, 17). With our data we can extend this paradox by comparing the low lipolytic activity with the higher rate of esterification within the epithelial cell. There are several possible explanations for this second paradox. In sucklings the high re-esterification rate is only a reflection of the high uptake of fatty acids. This high uptake (if proven *in vivo*) is logical in the light of the high fat intake. A similar relationship has been shown in adult rats placed on a high fat diet (31). The question to be answered is whether the high uptake of fatty acids is paralleled also by a high uptake of all lipid components. If this observation is proven then the low lipolytic activity accompanied by high uptake of all the forms of luminal lipid would suggest that the gastrointestinal tract of the suckling rat is capable of handling lipid in a manner analogous to the manner in which it handles other macromolecules such as proteins during this stage of life (33). Another possibility to explain the high esterification of absorbed free fatty acids may be more involved in the transesterification of glycerides, especially those from the circulation. This would place the gastrointestinal tract of the suckling animal in a more significant and central role in the regulation of lipid metabolism of the organism as a whole.

SUMMARY

Several processes of the mucosal phase of fatty acid absorption from the small intestine of the suckling rat were compared with those of the adult rat. *In vitro* studies showed that the uptake of oleic acid by jejunal slices from suckling rats is three- to fivefold greater than is observed from adult rats. The rate of esterification of oleic acid was correspondingly higher. The activity of acyl-CoA:monoglyceride acyltransferase in isolated mucosal microsomes is higher (about 50%) in microsomes prepared from the jejunum of suckling rats than of the adult rat. The activity of oleoyl-CoA synthetase is similar to that found in adults. The amount of fatty acid binding protein and the percentage of oleic acid binding is similar in intestinal cytosol of 6-day-old and adult rats.

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Bilirubin kernicterus ganglioside mitochondria

Bilirubin Interaction with Ganglioside: Possible Mechanism in Kernicterus

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Extract

Reaction of bilirubin with increasing amounts of ganglioside purified from neonatal brain significantly alters the spectral absorption of bilirubin in proportion to the quantity of ganglioside added. Increments in absorbance occur at 353 nm with a prompt but transient increase at 486 nm. A decrease in absorbance occurs which is most marked at 447 nm. When gangliosides are added to bilirubin (9.1 μg/ml or 0.016 μM/ml), the decrease in absorbance is essentially linear up to the highest concentration of purified ganglioside tested (182 μg/ml or 0.097 μM/ml), which represents a molar ratio of 6.1:1. The asymptotic nature of the bilirubin-ganglioside reaction as measured by the decrease in absorbance with time suggests a stoichiometric relationship between the two substances. An isosbestic point was demonstrated at 405 nm. Observations reported here suggest bilirubin reaction with ganglioside is at least a two-step process.

Speculation

Bilirubin cytotoxicity may be related in part to plasma membrane effects which involve bilirubin interaction with ganglioside at concentrations which do not disturb mitochondrial metabolism. The difference between the ganglioside composition of infant and adult gray matter may in part explain the marked cytotoxicity of unconjugated bilirubin for the infant nervous system.

Kernicterus, a bilirubin encephalopathy, results from the accumulation of nonconjugated, non-albumin bound bilirubin which leads to well described changes in the nervous system (6). In most experiments, anoxia (1, 9, 18), or hypoglycemia (17), in association with hyperbilirubinemia, produces more profound lesions in the experimental model (1, 9, 18) than nonanoxic hyperbilirubinemia. Neurons in such lesions demonstrate cytoplasm with myelin figures and dense bodies thought to be a pigment-lipid complex (10). Cytoplasmic membranous bodies have been described in enlarged Purkinje cell mitochondria of Gunn rats although none were present in astrocytes or oligodendrogliocytes (19). The enhanced susceptibility of specific regions of the nervous system to bilirubin toxicity as well as the increased susceptibility of the infantile nervous tissue has not been explained adequately.

Metabolic studies suggest that bilirubin pigment exerts four effects in mitochondrial reactions: (*1*) stimulation or inhibition of respiration depending on concentration of bilirubin, (*2*) abolition of respiratory control, (*3*) uncoupling of oxidative phosphorylation, and (*4*) induction of energy-requiring swelling. Levels of total bilirubin in the range of 10–40 μmol/liter increase oxygen consumption of mitochondria (2, 11). Concentrations necessary to initiate uncoupling of oxidative phosphorylation within adult or infant rat liver and brain mitochondria *in vitro* are much higher than those found in the brains of adult Gunn rats with experimental bilirubin encephalopathy (3, 5). Furthermore, brain mitochondria of newborn guinea pigs with the clinical features of severe bilirubin encephalopathy fail to demonstrate uncoupling of oxidative phosphorylation (4). Bilirubin inhibition of oxygen uptake is greater for whole brain homogenate from newborn rats than from adult rats (21). Mitochondria from whole brain or cerebellum of newborn guinea pigs with bilirubin encephalopathy fail to exhibit