Fraction V (albumin) and of Fraction IV-I (α-globulin). The similarity of these results and those of the present study demonstrate yet another link between the properties of DMBA-^{3}H and those of steroids.

Summary. The appearance of tritiated 7, 12-dimethylbenz(a)anthracene in blood after oral administration to rats was determined and its distribution in plasma investigated. Initially the radioactivity was associated with neutral fat but after some hours the hydrocarbon migrated on electrophoresis with the albumin fraction.

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Effect of Prenatal Diet on Serum Cholesteryl Ester Fatty Acids in Newborn and Adult Rats.* (29984)

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Comparison of maternal and fetal plasma lipids has shown that in man and many other mammals the blood of the newborn contains lower concentrations of lipids than does the blood of the mother or the non-pregnant adult(1,2). Muldrey *et al*(3) found what appeared to be a characteristic "fetal" cholesteryl ester fatty acid (CEFA) pattern in human cord blood serum and in that of the newborn. This "fetal" serum CEFA pattern differs from what was then thought to be the typical "adult" serum pattern in that oleate was the predominant cholesteryl ester in fetal and neonatal blood, in contrast to the predominance of cholesteryl linoleate in adult blood serum. They reported that the concentrations of cholesteryl palmitate, oleate and arachidonate in cord serum were each approximately one-half of the corresponding values in the maternal serum, but that the cholesteryl linoleate concentration in cord serum was only one-tenth that of maternal serum. Thus, these differences must arise as the result of an "active process" which involves selective metabolic fates for the individual fatty acid esters of cholesterol. Zollner *et al*(4) have substantiated these findings of a human "fetal" and "adult" pattern of serum CEFA. A similar elevation of the cholesteryl oleate : linoleate ratio (O/L) in the serum of fetal and newborn animals of a number of species has been observed by Muldrey (unpublished observations). In the light of the evidence cited, experiments were designed in the present study to test the effect of certain variations in the fatty acid composition of prenatal diets on rat "fetal" and "adult" serum CEFA patterns and especially on the ratio of concentration of cholesteryl oleate : cholesteryl linoleate (O/L).

Furthermore, Okey and Lyman(5) suggested that a lipotropic effect of protein is most evident in a rapidly growing animal, in which a diet low in protein rapidly leads to fatty liver. Olson *et al*(6) showed that protein intake in man must be reduced to deficiency levels before the serum cholesterol level is depressed, and Moyer *et al*(7) reported similar findings in rats. Robinson and

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TABLE I. Percentage Composition of Purified Diets Used During Experiments.

Dietary group	Casein	Gluten	Cottonseed oil	Butter	Glyceryl mono-oleate	Glyceryl mono-linoleate
	(g %)					
A	18	—	5	—	—	—
B	—	12	5	—	—	—
C	18	—	20	—	—	—
D	18	—	—	20	—	—
E	18	—	—	—	20	—
F	18	—	—	—	—	20

In addition to the above, the synthetic diets contained the following (in g %): 4% salts mixture(9), 1% water-soluble vitamin mix(9), 2% fat-soluble vitamin mix(9), 0.5% cystine, 0.2% choline chloride, 4% cellulose powder (cellu-flour, Chicago Dietetic Supply House) and sucrose sufficient to make 100%.

Seakins(8) found that puromycin injections into rats produced fatty liver and decreased serum lipids by inhibiting lipoprotein synthesis by the liver. Because of the numerous possible sites in both the mother and the fetus where proteins are probably involved in the synthesis and transport of lipids, the present study was designed to include a group of rats fed an inadequate amount of a protein of poor quality.

Materials and methods. CFN (Carworth Farms) pregnant rats weighing 170-200 g were divided into several groups upon their arrival at the animal house and each group was started on a different diet within 2 or 3 days after mating. The rats were kept in individual cages and were given food and water *ad libitum* throughout the experiments. The composition of the 6 different purified diets which were prepared and used in these experiments is indicated in Table I. The distinguishing features which characterized the diets were as follows: A: 18 g % casein and 5 g % cottonseed oil (CSO); B: 12 g % gluten and 5 g % CSO; C: 20 g % CSO; D: 20 g % butter; E: 20 g % glyceryl mono-oleate; F: 20 g % glyceryl monolinoleate. Thus, "fat" furnished approximately 15% of the calories in diets A and B, and 37% of the calories in diets C through F. Each synthetic diet contained a generous supply of vitamins and minerals in the form of 1 g % of water-soluble vitamin mix(9), 4 g % of salts mix(9), and 2 g % of fat-soluble vitamin mix(9) made up in corn oil for diets A, B, C and F and in butter for diets D and E. All diets were stored in the cold room between feedings.

In the first trial 4 groups each containing 6 pregnant rats were placed on diets A, B, C and D. Blood was obtained from each of the mother rats by tail bleeding on the day on which she littered, and the blood of one pup from each mother was obtained by jugular incision within a few minutes after birth. In the second trial six groups each containing ten pregnant rats were placed on diets A through F listed above. Blood from the mother rats was obtained by orbital bleeding(10) the day before littering was anticipated, and blood from the pups was obtained by jugular incision sometime during the first 24 hours after birth.

Serum CEFA patterns were determined by a microassay(11) based on chromatography on silica gel-impregnated glass paper(12) and densitometry of the charred spots produced with sulfuric acid and heat(13,14). The esters are separated by this technique into a series of spots which contain, in order of decreasing R_f value, esters of long chain saturated fatty acids, monoenoic acids, dienoic acids and so forth. The ester groups are designated by the predominant component fatty acid within each group: cholesteryl "palmitate," "oleate," "linoleate" and "arachidonate," respectively. Aliquots of 2 μl and 5 μl of serum were applied directly to the gel-paper on which appropriate standards were also spotted. Lipid extracts of the serum samples were then prepared directly on the gel-paper, which was then chromatographed, charred and read in the densitometer. This technique is the method of choice for working with small animals such as newborn rats, from which only a few microliters of serum

TABLE II. Influence of Prenatal Diet on Concentration of Various Esters of Cholesterol in Serum of Mother Rats and Newborn Pups (Within a Few Minutes After Birth).

Dietary group	Animal	Serum cholesteryl esters (mg per 100 ml of serum) † Palmitate	Oleate	Linoleate	Arachidonate	O/L ratio
A	Mother (6)*	7 ± 1.3‡	12 ± 1.0	20 ± 1.7	45 ± 2.2	.6
	Pup (6)*	15 ± 2.2	31 ± 2.3	12 ± 1.0	26 ± 4.2	2.6
B	Mother (3)	5 ± .3	5 ± .3	28 ± .6	32 ± 1.1	.2
	Pup (4)	11 ± .8	12 ± .6	7 ± .2	14 ± .8	1.7
C	Mother (4)	5 ± .9	11 ± 2.1	23 ± 2.5	30 ± 1.9	.5
	Pup (4)	21 ± 1.3	48 ± 3.2	11 ± 1.9	18 ± .7	4.3
D	Mother (5)	14 ± .7	26 ± 1.6	12 ± .7	34 ± 1.2N.S.‡	2.2
	Pup (4)	23 ± 2.3	45 ± 4.2	26 ± 1.2	31 ± 2.6N.S.‡	1.7

* No. of animals.
† Expressed as mean ± standard error of mean.
‡ Differences between paired values are significant at $p < 0.01$, except for those marked N.S., which are not significant at $p = 0.05$.

can be obtained. Statistical analyses were made on all results by calculating the mean and the standard error of the mean and by evaluating the significance of difference with Student's "t" test. Differences are reported and discussed only when they were significant at $p<0.05$.

Results. The serum CEFA concentration values obtained in the first trial are shown in Table II, together with an evaluation of the statistical significance of the paired mother-pup differences. In each dietary group the difference between mean values of cholesteryl oleate in the serum of the rat pups and mothers was significant at $p<0.01$; this was also found to be true for cholesteryl linoleate. Therefore, the comparison of O/L ratios of mothers and pups is a valid as well as a useful means of comparing the effects of prenatal diets on these transplacental differences, particularly when a major variant among the diets is the ratio of oleate to linoleate in the dietary fat. It is apparent that in each of the dietary groups except group D the O/L ratio was less than unity (0.2-0.6) in the maternal serum and greater than one (1.7-4.3) in the pups.

In most of the dietary groups the concentrations of cholesteryl palmitate (P) and oleate (O) were higher and linoleate (L) and arachidonate (Ar) were lower in the neonatal than in the maternal blood serum. From these data it is obvious that there is a CEFA pattern which is typical of the mothers and a different CEFA pattern which is typical of the newborn pups.

The effect of dietary proteins on serum CEFA is apparent upon comparison of groups A and B; mothers receiving diet B had a lower concentration of O, L and Ar than mothers receiving diet A, and the pups in group B had a significantly lower concentration of all 4 cholesteryl esters than the pups in group A. In each of several trials it was observed that the number of pups per litter in the gluten-fed group was only about a third of the number per litter in the other dietary groups. Pups in group B were smaller in size than those of any other group and many pups appeared sick at birth or were stillborn. The mothers in this group seemed to experience a difficulty which resembled uterine inertia; the actual parturition process for one pup was frequently observed to extend over a prolonged period.

Mother rats in group C had lower P and O but higher L than mothers in group D. Pups in group C had higher L and Ar than those in group D, which was not anticipated. The pups in group C had a higher O/L ratio than pups in groups A, B or D; the explanation of this is not immediately obvious, but may be related to partial deficiency of vit E and selenium as indicated by preliminary studies by one of us (Lopez)

Comparison of groups A and C uncovered a significant difference in maternal arachidonate levels, which were higher in the mothers in group A. Pups in group A had lower palmitate and oleate than pups in group C.

Values obtained in the second trial, in

TABLE III. Influence of Prenatal Diet on Concentration of Various Esters of Cholesterol in Serum of Mother Rats and Newborn Pups (Within 24 Hours After Birth).

Dietary group	Animal	Serum cholesteryl esters (mg per 100 ml of serum) Palmitate	Oleate	Linoleate	Arachidonate	O/L ratio
A	Mother (10)*	7 ± 1.0†	24 ± 2.6	42 ± 2.1	80 ± 4.1	.6
	Pup (10)*	16‡	36	7	62	5.2
B	Mother (10)	5 ± .9	36 ± 1.0	31 ± 1.7	55 ± 2.3	.2
	Pup (8)	11	17	10	17	1.7
C	Mother (10)	8 ± 1.2	16 ± 1.7	55 ± 1.9	83 ± 3.8	.3
	Pup (8)	28	50	23	26	2.2
D	Mother (10)	10 ± 1.1	50 ± 2.8	20 ± 1.1	56 ± 3.2	2.5
	Pup (8)	21	44	24	27	1.8
E	Mother (10)	29 ± 1.2	47 ± 2.1	29 ± 2.5	32 ± 3.6	1.6
	Pup (7)	33	50	34	33	1.5
F	Mother (10)	13 ± .7	26 ± 2.2	60 ± 2.7	87 ± 2.6	.4
	Pup (8)	34	25	40	30	.7

* No. of animals sampled.
† Expressed as mean ± standard error of mean.
‡ Average value determined on pooled serum (equal volumes from each pup were pooled for analysis).

which single-fatty-acid diets were included, are shown in Table III. Although mean values in the second trial were higher than those observed in the earlier trial, the results of the two trials are in substantial agreement with respect to diets A through D. As already noted, the groups receiving gluten experienced the same difficulties in several trials. In agreement with the results of the first trial, cholesteryl palmitate and oleate were again generally higher, and linoleate and arachidonate were generally lower in the pups than in the mothers. Exceptions were found in groups D and E, where the relative excess of oleate and deficiency of linoleate appeared to upset this generalization. Also it was observed that the oleate levels were the same in the pups in group F as in the mothers in this group. It is obvious from the results of this second trial, that characteristic "maternal" and "fetal" serum CEFA patterns were maintained under certain variations in diet, but were altered under the stress of extreme variations in fatty acid composition of certain diets.

Comparisons of the results obtained in groups C and D in the second trial agreed with those of the first trial, except that the unanticipated relationships observed in the first trial with respect to linoleate and arachidonate were not substantiated in the second trial.

When groups E and F were compared, mothers in group E had higher P and O, and lower L and Ar values than mothers in group F. Pups in group E had higher O and lower L than pups in group F. Thus, the results obtained with diets E and F, which were dietary exaggerations of the oleate : linoleate composition of diets C and D, appeared to confirm the observation made on diets C and D, and to extend these observations in a manner which seemed understandable. For example, the pups in group F were the only pups in either trial to show an O/L ratio below 1.5 (O/L = 0.7). In this group of pregnant rats fed purified diets in which glyceryl monolinoleate was the sole lipid, cholesteryl linoleate became the major cholesteryl ester in the serum of the newborn rats. This reversal of the characteristic fetal serum CEFA pattern, which was observed only in this extreme dietary regimen, certainly suggests direct placental transfer of at least the linoleate moiety of the maternal serum lipids. Also when glyceryl mono-oleate was the sole dietary "fat," the O/L ratios in both mother rats and pups were similar to those observed when butter (O/L approximately 10) was the dietary fat.

In each trial, in addition to the dietary groups described, a group of pregnant rats was carried on a diet of Purina Rat Chow for the purpose of general evaluation of

weight gain and litter size, as compared with the groups on the various purified diets. The general progress, litter size, etc., were essentially the same in the groups fed chow as in the experimental dietary groups, and the typical maternal and fetal serum CEFA patterns were observed in the rats fed chow. The cholesteryl ester values are not reported, however, because the variability of composition of chow and the lack of information concerning its composition preclude rational comparisons of results obtained in the chow-fed animals with results obtained in groups of animals fed purified diets of accurately known composition.

Discussion. The present work establishes the existence of a typical "adult" serum CEFA pattern in pregnant rats on ordinary chow or casein-containing diets, which is suggestive of a homeostatic control over the composition of this serum lipid fraction. This control mechanism can maintain a serum CEFA pattern within a certain range of fatty acid composition in spite of the challenge of a different fatty acid composition of the food. That such a control mechanism is not without limitations can be seen from the "reversal" of the "normal" adult pattern in mother rats fed diets containing 20 g % of butter or mono-oleate (37% of the calories).

Hamilton *et al*(15) observed that normal human adults were able to maintain the typical adult human serum CEFA pattern even when fed butter or corn oil at 40% of the calories for alternating periods of 3 weeks each. However, Muldrey *et al*(16) found that cirrhotic patients, subjected to this same alternation of dietary regimens, altered their serum CEFA pattern within a week to reflect the dietary changes. These observations point to a regulatory mechanism probably located in the normal human liver. However, it must be remembered that adult serum CEFA patterns, often quite unlike the "normal" adult pattern of healthy adults from Western Europe and the United States(17,18), have been observed by Scott *et al*(19) in East Africa, by Muldrey(20) in Bolivia, and in Colombia by Miller (unpublished results on sera collected at random from adults during the 1961 ICNND Nutrition Survey in Colombia, South America). Whether this is a function of the level of dietary fat intake, of protein intake, or other factors remains to be established.

From the present work it is also obvious that there is a typical "fetal" serum CEFA pattern which is characteristically different from that of the mother, and which appears to be less susceptible to dietary variation than is the CEFA pattern of the mother rat. It is apparent that some active process(es) is responsible for the maintenance of these transplacental differences in serum CEFA patterns. Although early reports by Goldwater and Stetten(21) and by Popjak(22) indicated that fetal lipids are formed primarily by fetal synthesis, more recent work by Van Duyne(23) has shown that C^{14}-palmitate is transferred across the placenta. While the mechanism(s) of normal transfer of lipids across the placenta is unknown, it is certain that the placenta is more than a simple barrier; it consists of tissues capable of a variety of metabolic activities(24,25) including the synthesis of fatty acids(26). Preliminary evidence obtained in this laboratory suggests that placental tissue is able to carry out both the hydrolysis and the synthesis of cholesteryl esters.

Summary. Experiments were conducted to study the effect of prenatal diet on the serum CEFA pattern of newborn rats and their mothers. Evidence was obtained confirming the existence of a typical "fetal" CEFA pattern in the newborn rats characterized by a high oleate : linoleate (O/L) ratio (1.5 to 5.0), and of a typical "adult" CEFA pattern in the mother characterized by an O/L ratio of less than unity (0.2 to 0.6). These characteristic "adult" and "fetal" CEFA patterns were found to be maintained in spite of moderate alterations in the fatty acid composition of the prenatal diets, but could not be maintained in the face of the exaggerated imbalance of diets supplying only single fatty acids. Based on the data obtained, it was postulated that homeostatic mechanisms exist which function so as to maintain the characteristic adult and fetal CEFA patterns in the face of stresses consisting of variations in the dietary fatty acid composition.

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Dehydrogenases of Purine Metabolism in *Tetrahymena pyriformis*. (29985)

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Either guanine, guanosine or guanylic acid is required for the growth of *Tetrahymena pyriformis* due to its inability to synthetize the purine ring(1,2). Adenosine deaminase and purine nucleoside phosphorylase have been reported in *Tetrahymena*(3), but guanase and xanthine oxidase were considered to be absent from this ciliate by Kidder on the basis of nutritional studies(1). This was confirmed by Eichel with enzymatic experiments(3). Recently, however, Seaman showed that extracts from sonicated *Tetrahymena* contain adenase, xanthine oxidase, guanase, uricase and adenosine deaminase(4). We investigated the presence of guanase, xanthine oxidase and uricase as well as some effects of purines on the endogenous respiration of this ciliate in an effort to resolve these discrepancies.

In studying anaerobic reduction of triphenyltetrazolium chloride (TPTC) with some purines as substrates we found a marked activity with cell suspensions and homogenates of this organism. Incubation in air with these substrates showed that the activity producing uric acid was somewhat lower. These findings indicate that *Tetrahymena* is able to dehydrogenate actively