TABLE II. Comparison of Estrone Effect by Groups.

	No. of mothers sectioned	No. intact fetuses obtained	No. of cleft palate				% of cleft palate	No. of absorbed fetuses	Absorbed fetuses / Intact fetuses
			Total	(L)	(M)	(S)			
							%		%
Group A	18	81	10	3	4	3	12.3	69	85.2
B	15	88	13	3	8	2	14.8	36	40.9
C	19	63	17	5	7	5	27.0	72	114.3
D	15	71	25	10	6	9	35.2	43	60.6
E	10	14	3			3	21.5	62	442.9

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Desoxyribonuclease (DNA'ase) Activity of Rat Mammary Gland During Pregnancy and Lactation.*† (23886)

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In previous publications(1,2), it was reported the content of desoxyribonucleic acid (DNA) of rat mammary gland epithelial cells decreased following parturition(1); and DNA-phosphorous of mammary gland also declined at day of parturition when determined on dry tissue(2). An explanation of these unusual findings was sought in determining DNA'ase activity of mammary gland during pregnancy and lactation to see if there was correlation between DNA and DNA'ase activity.

Methods. Data were obtained from primiparous albino rats during late pregnancy (18-20 days), day of parturition and during advanced lactation (20-21 day). The animals were killed, the 6 posterior mammary glands rapidly removed. These were placed in ice-cold sucrose or saline solution, diluted to 50 mg/ml, homogenized for 2½ minutes in a Waring Blendor, then centrifuged for 10 minutes at 2600 rpm. The supernatant was saved for the following procedure. Six individual samples were prepared by addition of one ml

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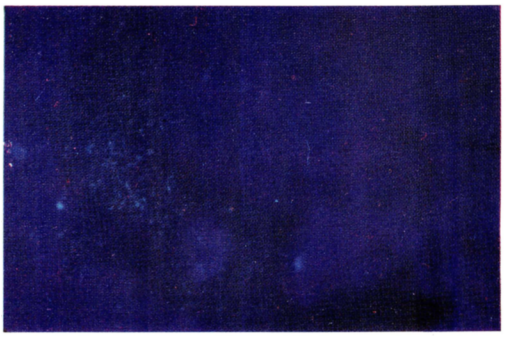

FIG. 1. Photomicrograph of section of normal rabbit spinal cord (white matter) treated with rabbit thyroid autoantiserum (R 515) and stained with fluorescein isocyanate conjugate of goat anti-rabbit globulin antiserum. Only the blue autofluorescence of fixed spinal cord tissue is apparent (negative staining reaction).

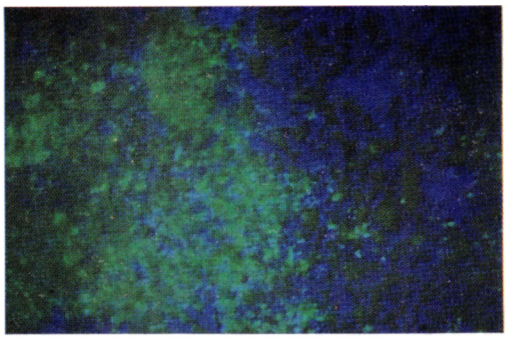

FIG. 2. Photomicrograph of section of normal rabbit spinal cord (white matter) treated with rabbit spinal cord autoantiserum (R 59) and stained as in Fig. 1. There is considerable amount of green fluorescence due to antibody-bound fluorescein; localization of green fluorescence corresponds to site of antigen-antibody reaction. Sites of reaction are suggestive of myelin sheaths (positive staining reaction).

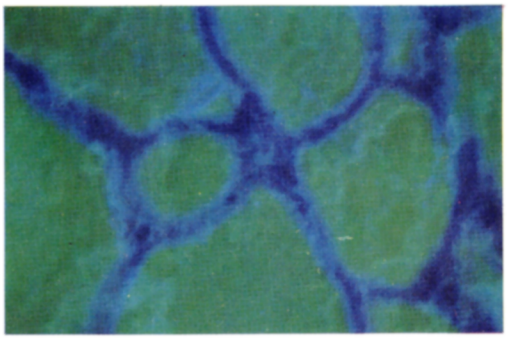

FIG. 3. Photomicrograph of section of normal rabbit thyroid treated with rabbit thyroid autoantiserum (R 515) and stained as for Fig. 1. Green staining of colloid indicates site of antigen-antibody reaction (positive staining reaction).

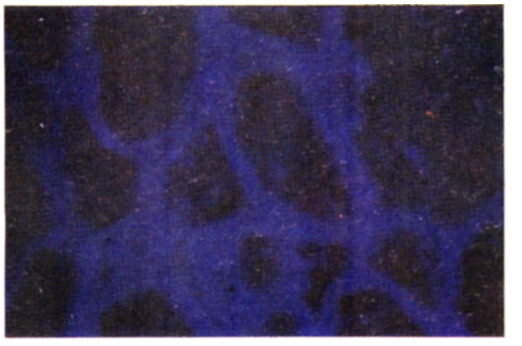

FIG. 4. Photomicrograph of section of normal rabbit thyroid treated with normal rabbit serum and stained as for Fig. 1. Only the blue autofluorescence of cellular elements is visible (negative staining reaction). The same result was obtained with rabbit thyroid sections treated with rabbit spinal cord autoantiserum (R 59).

All photomicrographs were made with dark field microscope and ultraviolet light source. Final magnification is 300 ×. We are indebted to Dr. J. Vazquez of University of Pittsburgh for taking the photographs.

TABLE I. DNA'ase Activity of Mammary Gland in Late Pregnancy and Early and Late Lactation.

	No. of animals	$A_2 - B_2$*	$B_1 - A_1$†
Pregnancy	12	Mean 277 ± 30.98	307 ± 30.78
Early lactation	11	Mean 588 ± 53.30	−47.3±124.45
Late lactation	12	Mean 377 ± 32.65	345 ± 35.75

* $A_2 - B_2$ = decrease in DNA after 1 hr incubation in μg.

† $B_1 - A_1$ = increase in acid-soluble nucleotides after incubation in μg.

of supernatant to substrate solution containing 2400 μg of DNA/ml and one ml of acetic acid, sodium acetate buffer(3,4). Three samples were used as controls to determine DNA (A_2 fraction) and level of acid-soluble nucleotides (A_1 fraction) before incubation. This was accomplished by addition of 5 ml of 10% TCA to 1 ml of supernatant. Remaining 3 samples were placed in water bath for one hour at 37°C, centrifuged and resulting precipitates separated from supernatant to determine DNA (B_2 fraction) and acid-soluble fraction (B_1 fraction). DNA'ase activity was estimated by decrease in DNA substrate as compared with control samples, or by increase in acid-soluble fraction as compared with control samples. Each method acted as a check on the other, a decrease in DNA causing a corresponding increase in the acid-soluble fraction. A_2–B_2 = decrease in DNA, B_1–A_1 = increase in acid-soluble nucleotides. Preliminary observations indicated enzyme activity of the complete homogenate demonstrated wide variance between enzyme activity and nucleotides, probably caused by unequal distribution of nuclei in the aliquots. The method of Webb and Levy(5) was used to determine DNA and acid-soluble fractions.

Results. DNA'ase activity of mammary glands in late pregnancy was lower than in either early or late lactation, while decrease in DNA during pregnancy was directly correlated with increase in acid-soluble nucleotides (Table I). The average decrease in DNA was 277 μg released/hour, while the increase in the acid-soluble fraction was 307 μg/hour. On the first day of lactation there was an abrupt increase of over 100% in enzyme activity and a marked decrease in acid-soluble fraction as compared with late pregnancy (B_1). There was a marked decrease over pregnancy. In half the cases this decrease ranged from −195 μg to −570 μg below controls, while in remaining fractions increase in acid-soluble nucleotides was not proportional to the decrease in DNA. Average enzyme activity for this stage of lactation was 588 μg/hour; the average increase in acid-soluble nucleotides was −47.3 μg/hour. By 20th day of lactation enzyme activity had decreased to average of 377 μg/hour and the increase in the acid-soluble fraction was again proportional to the decrease in DNA with average increase of 345 μg/hour.

There was a significant difference in DNA'ase activity in both pregnancy and late lactation as compared with early lactation ($P<.0025$). The difference between pregnancy and late lactation approached significance ($P>.05$).

Discussion. During pregnancy and late lactation enzyme activity is directly correlated with an increase in acid-soluble nucleotides. During these 2 periods DNA/cell remains within normal values. Increase in DNA'ase activity coupled with decrease in acid-soluble nucleotides on first day of lactation suggests that the decrease in DNA/cell is due to greater catabolism of cellular components reflecting increased need by animal for sugar and phosphate during milk secretion. DNA'ase activity was not compared on basis of protein nitrogen or dry weight during late pregnancy or lactation because the mammary gland is actively synthesizing milk, thus variable amounts of protein, fat and water would be present during these periods.

Summary. DNA'ase activity was determined in late pregnancy and in early and late lactation in conjunction with measurement of acid-soluble nucleotides. DNA'ase activity was correlated with increased acid-soluble nucleotides during pregnancy and latter stages of lactation. There was, however, on first day of lactation an increase in enzyme activity and a decrease in acid-soluble nucleotides suggesting an increased catabolism of these products in early lactation for use in secretory process.

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Effects of Saturated and Unsaturated Fat on Cholesterol Metabolism in the Rat. (23887)

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Studies in several laboratories have established the remarkable effects of unsaturated fats in lowering serum cholesterol levels in man, *e.g.*(1,2). The mechanism by which these fats act is not yet known. While effects in animals may be different in some respects from those in humans, the extra parameters that can be evaluated make animal studies worthy of exploration. Previous animal studies on effects of dietary fat have frequently been complicated by simultaneous feeding of cholesterol, of cholesterol-containing animal fat, or other atherogenic factors. In our studies a comparison is made of effects of 2 vegetable oils, corn oil and coconut oil, on rate of cholesterol biosynthesis and on distribution of cholesterol in body pools in rats on a cholesterol-free diet.

Methods. Adult male rats of Osborne-Mendel strain were fed *ad libitum* with ground Purina chow containing 20% by weight of corn oil or coconut oil. Both oil-containing diets were consumed at about same rate, supplying animals with same amount of calories. The rats given these diets had a caloric intake higher by about 35% than control rats on Purina chow alone. At an exactly defined time before sacrifice, animals were injected intraperitoneally with a tracer amount of labeled compound serving as cholesterol precursor. The rats were anesthetized with ether, killed by bleeding and livers were removed and chilled. Aliquots of liver, serum or whole adrenal glands were extracted with alcohol-acetone 1:1, saponified and the non-saponifiable fraction counted in the Packard scintillation counter. Free and esterified cholesterol in tissues were determined according to Sperry and Webb(3).

Results. Table I shows effect of diet on cholesterol levels in serum. Except in Exp. I, the shortest one, both coconut oil and corn oil fed rats had higher serum cholesterol levels than controls. Comparison of corn oil fed animals with coconut oil fed animals shows that in all experiments, irrespective of feeding period, total serum cholesterol of corn oil fed rats was lower. In 2 experiments the P value of this difference was between 0.05 and 0.1. However, the difference was never very large compared with that observed by others in man. Livers from these animals were analyzed for cholesterol content and here the effects of diet were marked (Table II). Cholesterol concentration in liver of corn oil fed animals was almost twice that in controls. The effect of feeding coconut oil was much smaller and not always significant. Of particular interest was the fact that almost all the increase was in the normally small cholesterol ester fraction. There were only small differences between groups in concentration of free cholesterol.

TABLE I. Effect of Diet on Cholesterol Concentration of Rat Serum.*

Exp.	Duration of feeding (days)	Serum cholesterol (mg %) Control diet	Coconut oil diet	Corn oil diet
I	17	68.5 ± 6.9†	77.6 ± 13.6	63.4 ± 3.2
II	20	75.1 ± 7.9	87.0 ± 10.0	82.4 ± 10.7
III	32	68.8	79.9	76.6
IV	80	60.1 ± 6.3	81.1 ± 10.9	67.7 ± 8.9

* Each figure represents mean value for 6 rats.
† ± stand. dev.