Nucleic Acids of the Mammary Glands of Rats.* (20284)

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Two concepts of the relationship of nucleic acids to growth and activity of tissues suggested the present study of the growth and development of the mammary glands of rats. First of these is the concept that the desoxypentose nucleic acid (DNA) content of cell nuclei is constant for the various somatic cells of an organism(1,2). The second is that pentose nucleic acid (PNA) is closely related to protein synthesis(3,4).

The formation of the lobule-alveolar system in the mammary gland in early pregnancy is brought about by extension of the major ducts and rapid proliferation of the side branches and alveoli. The magnitude of cellular proliferation in this example of growth should be directly related to an increase in DNA in the total gland complex (mammary gland plus fatty pad). Analysis of PNA in the developing and lactating gland was of interest since the mammary gland is an organ highly specialized in the synthesis of fats, carbohydrates and proteins. Considering the DNA in the cells as constant then a survey of the ratio of PNA to DNA should give an evaluation of the condition of the cells in the growing and actively secreting mammary glands.

Materials and methods. Virgin, pregnant nulliparous and lactating primaparous albino rats were used in this study. The rats were bred at a weight of about 150-160 g. Some rats of this weight were killed and used as a virgin reference. Days of pregnancy in other rats were determined by examination of the size of the fetuses. Litters of lactating rats were reduced or increased to 5 in number. Young were removed from their mothers at 25 days of lactation and the latter killed 5 or more days later in order to obtain the gland undergoing involution. The animals were killed, rapidly skinned, and the 6 posterior glands (fatty pads) removed from the skin, pooled, weighed and frozen. The tissue from each rat was placed in a preweighed extraction thimble and lyophilized. The thimble and dried gland were weighed to determine the water content. The tissue was then extracted in a continuous extractor with hot alcohol for a period of 8-10 hours followed by extraction with ether for a similar period. The dry fat-extracted glands were weighed again to determine the fat-free weight. Each sample of glandular tissue representing the original 6 posterior glands of one female was ground to a fine powder in a Wiley mill. Fifty mg aliquots of the glandular powder were used for DNA and PNA determinations. Twenty-five mg aliquots were used for nitrogen determinations. DNA and PNA were extracted from the tissue sample by the procedure of Schneider(5) with the exclusion of the fat extraction step, the fat having already been extracted. The dry tissue powder was brought in contact with 2 ml of water in the cold for 2 hours in order to wet the tissue. The tissue was then extracted with 5 ml of cold 10% trichloroacetic acid (TCA), centrifuged and the supernatant discarded. The residue was extracted with 10 ml of hot 5% TCA twice for periods for 15 minutes each. This gave complete removal of substances giving color tests for DNA and PNA. The 5% TCA extracts were diluted to 25 ml and DNA determined on 3 ml aliquots by the diphenylamine reaction (6). PNA was determined on one ml aliquots by the orcinol reaction(7) modified by heating for one hour(8). Commercial DNA and PNA was repurified and used to prepare standard curves. The densities were read on a Beckman model DU Spectrophotometer. Nitrogen was determined by the micro-Kjeldahl procedure. No attempt was made to correct for the nitrogen content of milk present in the glands.

The values of DNA and PNA as determined were calculated for the total glandular ma-

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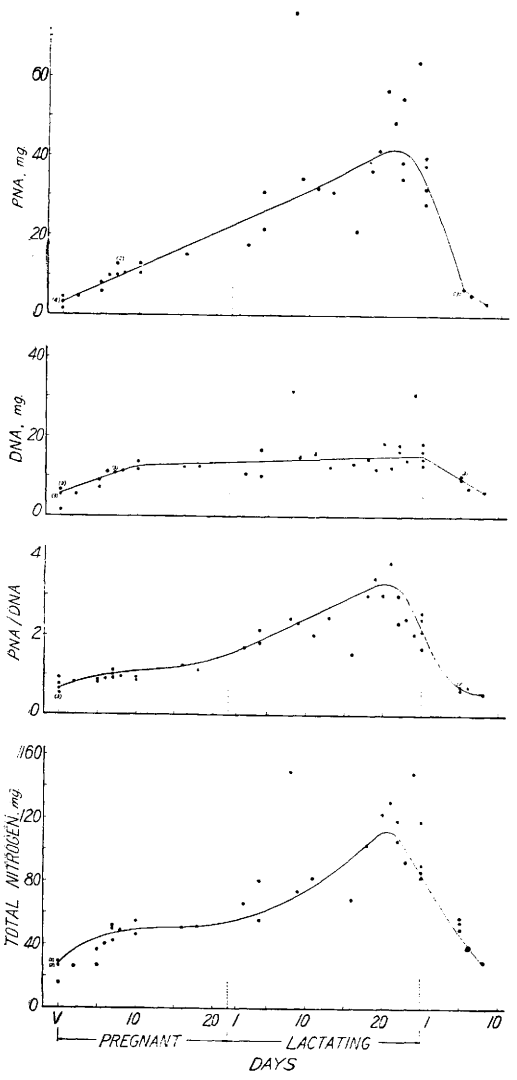

FIG. 1. PNA, DNA, PNA/DNA, and total nitrogen in the 6 posterior mammary glands of rats at different periods of pregnancy and lactation.

terial (fat free) taken from one female. The values obtained were plotted against days of pregnancy or lactation. The PNA-DNA ratio was calculated for each sample of glandular material and plotted as a function of the condition of the animal. Total nitrogen of the sample was calculated and plotted as above.

Experimental. In the virgin females the values of DNA, PNA, PNA/DNA and nitrogen were low signifying the undeveloped condition of the mammary glands. During the first half of pregnancy the DNA increased rapidly reaching a plateau at about mid pregnancy. From mid pregnancy to a point of maximum lactation the DNA in the glands increased only slightly.

The PNA rose parallel to DNA in the first half of pregnancy but continued to increase throughout lactation reaching a maximum value at 21-22 days. This increase in concentration of PNA above DNA is better shown by the increasing ratio of the 2 through pregnancy and lactation reaching a maximum at 21-22 days of lactation. Of interest here is the fact that glands represented by 8 and 24 days of lactation show values for DNA, PNA and nitrogen out of proportion to the other glands studied, yet the PNA-DNA ratio fits well with the values for the other glands.

The values for total nitrogen in general show the same trends as the PNA.

At involution the PNA sharply decreases in the mammary gland. The values of PNA, PNA-DNA and nitrogen are reduced in a manner approaching the values of the undeveloped gland.

Discussion. Reece and Warbritton(9) found that the mitotic activity of rat mammary glands was greatest at 10 days of pregnancy and low through lactation. Others (10,11) found that the epithelial tissue reached its maximum proliferation by mid pregnancy. The rise in DNA in the total gland in the first half of pregnancy agrees well with this histological data. The 2-fold increase in DNA from the virgin condition to mid pregnancy appears to be low for such a rapidly developing tissue. There are 2 points to be considered in this connection. One, that the increase in DNA represents a doubling of the total number of cells in the entire fatty pad. In the undeveloped gland the fat and connective tissue make up a major portion of the total fatty pad. Under the stimulus of pregnancy cell proliferation occurs predominantly in the epithelial tissues. A second consideration is that the virgin condition is represented by rats that may have been through several estrous cycles, hence the duct system of the mammary glands would be somewhat elaborated and the base level shown here

would be greater than a base level that may be shown by castrated animals.

An increase in the PNA content of regenerating liver has been shown by several authors(12,13). However, in a study of the relation of nucleic acids to protein formation in the pancreas, Daly and Mirsky(14) failed to show any significant change in PNA content during the cycle of secretion. These authors object to the use of the mammary gland for studying the relationship of nucleic acids to protein synthesis on the basis that protein synthesis in the mammary gland depends on the formation of new cells. This objection is without foundation for the scarcity of mitosis in the lactating gland(15-17) indicates that in milk secretion there is little turnover of cells.

In the present study it is seen that the PNA increases to a maximum at 21-22 days indicating an average increase in PNA concentration per cell. In relation to this, the data of Brody and Nisbet(18) showed that some rats appeared to reach maximum milk production at 18-19 days of lactation while others did not reach maximum production in the 21-day period studied. Thus the PNA level in the mammary gland closely parallels the synthetic activity of the gland.

Nitrogen values of the mammary glands increased with the height of lactation reaching a maximum value at 20-22 days. A considerable portion of this nitrogen is undoubtedly represented by milk nitrogen, however, a part of the increase must represent hypertrophy of the alveolar cells. Although no attempt was made to calculate the nitrogen of the gland contributed by milk this could probably be done on dry tissue similarly as it was done on wet tissue by Folley and Greenbaum(19).

McShan and coworkers(20) demonstrated a rapid rise in PNA in the pigeon crop gland tissue under the influence of administered lactogenic hormone. The DNA increased initially but reached a plateau while the PNA continued to increase. This is similar to the results reported here for the normal growth and activity of the mammary glands of rats.

The downward trend in PNA at 25 days of lactation may be the result of voluntary weaning. Forced weaning at the 25th day of lactation is followed by a rapid decline in the PNA and the PNA-DNA ratio. This decline in involution is like the rapid fall in enzyme activity of rat mammary glands on involution as reported by Folley and Greenbaum(19).

Factors influencing the growth and maintenance of the mammary gland in a secretory condition are under investigation.

Summary. A study of the nucleic acid concentrations (DNA and PNA) in the developing and functioning mammary glands of rats is reported. DNA increases in the early part of pregnancy and increases only slightly in the lactating gland. PNA increases throughout pregnancy and continues to increase in the lactating gland reaching a maximum value at 21-22 days of lactation. Following enforced weaning the PNA content of the gland falls rapidly. These findings are discussed in regard to growth and function of the glands.

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Inhibition of Aldehyde Oxidation by Adrenergic and Adrenolytic Substances.* (20285)

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The metabolic disposal of epinephrine and related amines is usually attributed, in part at least, to the monoamine oxidase system. This system oxidizes amines to the corresponding aldehydes which then will be converted to the acid. The studies reported in this paper show that the enzyme present in guinea pig liver which converts aromatic aldehydes into the corresponding acids is inhibited reversibly by adrenergic amines and irreversibly by adrenergic blocking agents.

Methods. Enzyme activity was measured by means of an ultraviolet spectrophotometric method. Fig. 1 shows that salicylaldehyde, salicylic acid and saligenin (salicylalcohol) have distinct absorption peaks in the ultraviolet. The molar extinction coefficient of salicylaldehyde is 2300 at 325 mμ (the peak at 260 mμ was not used because of its proximity to the absorption zone of trichloracetic acid), that of salicylic acid 4450 at 303 mμ. These values are valid only for the present experimental conditions since the spectra are very sensitive to pH changes(1). The substrate used in all experiments was salicylaldehyde (Eastman-Kodak). The enzyme was prepared from guinea pig liver homogenized in 0.1 M phosphate buffer at pH 7.4. The

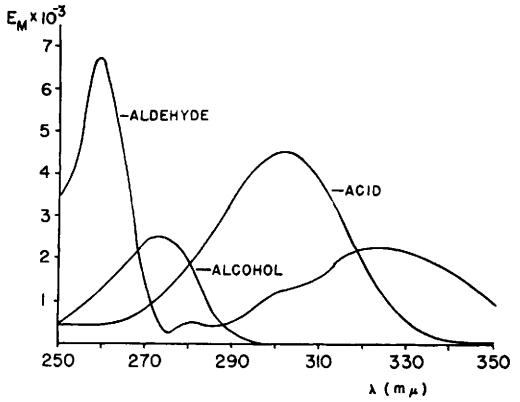

FIG. 1. Ultraviolet absorption spectrum of salicylaldehyde, salicylic acid and saligenin, in the presence of trichloracetic acid in proportions specified in the text. λ = wavelength in mμ; E_M = molar extinction coefficient.

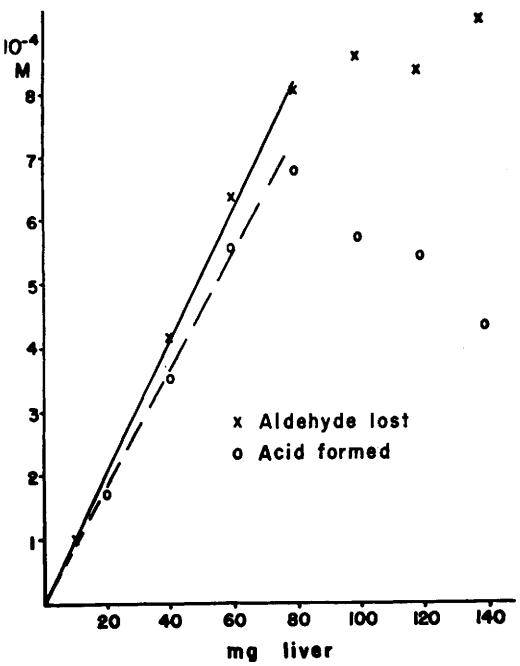

FIG. 2. Influence of enzyme concentration on disappearance of salicylaldehyde and formation of salicylic acid. Abscissa: enzyme concentration in mg of fresh liver per 3 ml of reaction mixture. Ordinate: aldehyde lost or acid formed in 10 min. The initial concentration of salicylaldehyde was 10^{-3} M in 3 ml.

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