the changes found in similarly treated male rats, and to the changes produced by experimental cryptorchidism in rabbits.

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Lactation Activity, Chemical Composition, and *in vitro* Metabolism of Rat Mammary Tissue.

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During work on the metabolic effect of lactogenic hormone one of us observed that mammary tissue from a rat in full lactation consumed *in vitro* 2.7 ± 0.3 cmm of oxygen per mg of dry matter per hour. Mammary tissue from a rat that had suckled only one young, weaned 8 days before the test, consumed *in vitro* only 0.7 ± 0.2 cubic millimeter of oxygen per mg of dry matter per hour.

We have repeated and extended these *in vitro* measurements of the metabolic rate of mammary tissue because the work offered an opportunity to connect metabolic rate with "activity," a term often used rather vaguely.[1] The relation of metabolic rate to the amount of "active" tissue has a sound meaning only when the activity is defined and measured independently of the metabolic rate itself.* Lactation provides such an independent definition af activity. During the trials described here we noted that both lactation activity and metabolic rate are related to the nitrogen and water content of the mammary tissue.

Method. The rats used were bred in our colony from the Long-Evans strain and kept on our ordinary colony diet. On the day of the respiration trial they were killed by a blow on the head. Immediately the thorax was opened; the mammary gland was placed in oxygenated balanced ion glucose solution containing phosphate buffer;[2] and parts of the gland, excised with a forceps, were placed in buffered balanced-ion glucose solution and subjected to Warburg's standard procedure of micro respiration trial in a water-bath kept at 37°C. The time between killing the rat and installing the manometers in the waterbath averaged to 18 ± 1 minute and did not exceed 27 minutes. The amount of tissue used in each respiration chamber was measured by determining the nitrogen in the sample after the trial by the micro Kjeldahl method. In extra samples of the same tissue, the nitrogen : dry matter ratio and the water content were determined.

Results. Table I summarizes the results of these measurements with mammary tissue taken on the 20th day of pregnancy and tissue taken on the 21st day of lactation. The number of fetuses per pregnant rat ranged from 9 to 11. Each of the 8 lactating rats included in the means suckled 6 young. This is the size to which each larger litter in our colony is reduced at birth.

The lactating mammary tissue contained less than half as much dry matter as the mammary tissue from the pregnant rats. The mammary tissue of a rat that had suckled only 3 young contained, on the 21st day of lactation, 26% of dry matter—a figure higher than that for any rat with 6 young. The mammary dry-matter content of a rat with only 2 young was as high as 35%. Lactation, therefore, increases the water content of the mammary gland. The nitrogen content of the dry mammary tissue had the same trend as the water content of the fresh gland. The dry matter of the lactating mammary glands averaged over 7 times as much nitrogen per

[1] Benedict, F. G., *Vital Energetics*, 1938, 199.

* Otherwise one relates just two different expressions for only one variable.

[2] Kleiber, M., and Cole, H. H., *Am. J. Physiol.*, 1939, **125**, 747.

TABLE I.
Lactation Activity, Composition, and Metabolic Rate of Rat Mammary Tissue.

Condition of rat at time of microrespiration trial with mammary tissue		20th day of pregnancy	21st day of lactation
No. of rats included in means		5	8
Mean No. of fetuses per rat		10 ± 0.5	
Mean No. of young suckled per rat			6 ± 0
Dry-matter % of mammary tissue	%	59 ± 5	23 ± 1
Nitrogen % in dry mammary tissue	%	1.3 ± 0.5	9.5 ± 0.3
Oxygen consumption *in vitro* per hour:			
Per mg of moist tissue	mm^3	0.5 ± 0.1	0.6 ± 0.1
" " " dry tissue	mm^3	0.9 ± 0.2	2.9 ± 0.3
" " " nitrogen	mm^3	88 ± 17	31 ± 3

unit weight as the dry nonlactating mammary tissue. The 2 rats with less than 6 young apiece again confirm the general trend; the nitrogen content of their mammary dry matter was 7.8 and 5.7% respectively for the tissue from the rat with 3 and that with 2 offspring. Lactation thus increases the protein content of the dry matter in the mammary gland.

The metabolic rate *in vitro* per mg of fresh tissue is within the range of the standard deviation—the same for lactating and nonlactating mammary tissue. The ordinary Warburg quotient Q_{O_2} (cmm of oxygen consumed per hour per mg dry tissue) is, however, 3 times as high for the lactating mammary tissue as for the mammary tissue taken from the pregnant rats. This increasing effect of lactation on the metabolic rate per unit dry tissue is statistically highly significant. The metabolic rate per unit nitrogen is, in contrast to that per unit dry matter, only one-third as high in the lactating tissue as in the tissue from the pregnant rat.

The results of our micro respiration trials lead, accordingly, to the puzzling conclusion that lactation does not affect the metabolic

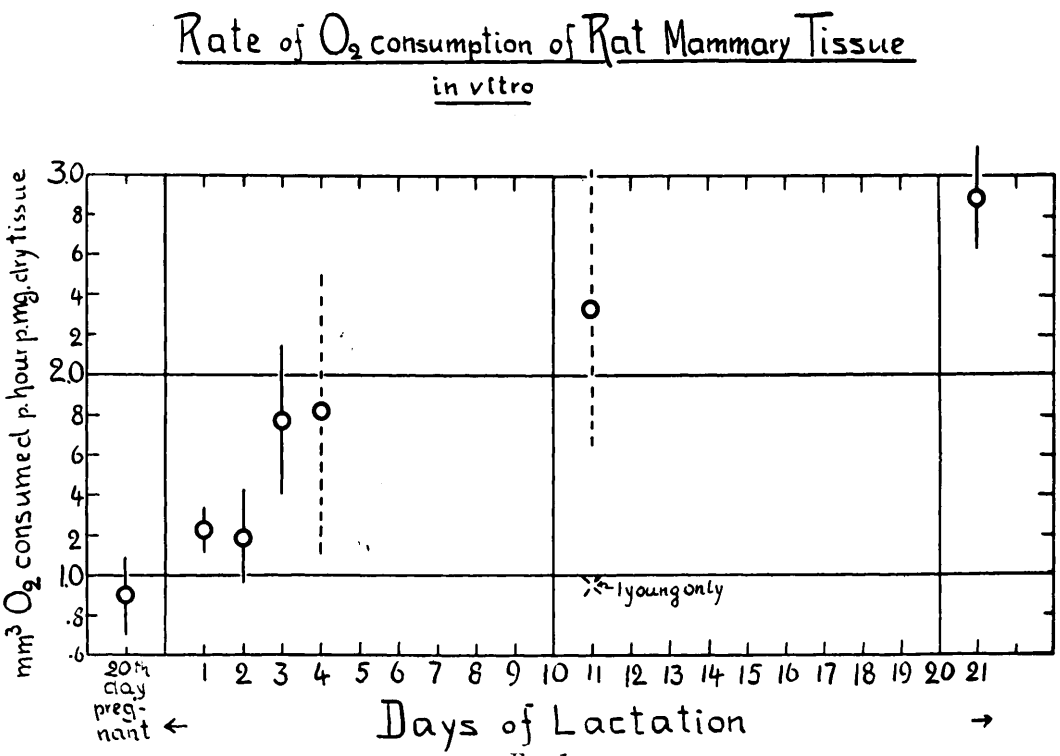

FIG. 1.
Rate of O_2 consumption of rat mammary tissue.

rate of the mammary gland, and yet increases it or decreases it, depending on whether one means the metabolic rate per mg of fresh tissue, per mg dry matter, or per mg nitrogen.

The nitrogen content, usually taken as "the most serviceable measure for the active protoplasmic tissue,"[1] may be misleading as such a basis for the metabolic rate of the mammary gland, because much of the nitrogen in the lactating tissue might be inert milk protein or a precursor of this substance.

Fig. 1 shows the trend of *in vitro* metabolic rate per unit of dry matter for the duration of lactation. The standard deviation of the means is indicated by the vertical lines connected to the circles. In two instances (namely, on the 4th and 11th day of lactation) when we had only the results from one rat each, the broken vertical line indicates the extent of the standard deviation for a single measurement as calculated from the data on the 3rd day.

The metabolic rate *in vitro* per unit of dry tissue increases up to the 21st day of lactation. The rate of milk production in rats in terms of milk energy per day also rises up to this time, according to Brody and Nisbet[3] (see Fig. C, page 13). Fig. 1, accordingly, indicates a positive correlation between metabolic rate per unit of dry matter and lactation activity through the lactation period. The low Warburg quotient at the 11th day (marked with a cross in Fig. 1) resulted from the mammary tissue of a rat that suckled only one young. This result also confirms the rule that lactation intensity and metabolic rate per unit of mammary dry matter are positively correlated.

Summary. Mammary tissue of rats at the end of pregnancy and at the height of lactation was analyzed for dry matter and nitrogen content, and the metabolic rate of this tissue was measured *in vitro*.

Lactation increased the water content of the mammary tissue and the protein content of the mammary dry matter.

Lactation did not affect the metabolic rate per unit of fresh tissue *in vitro;* it increased the metabolic rate per unit of dry matter and decreased the metabolic rate per unit of nitrogen in the tissue.

Observations were also made on rats that suckled only 1-3 instead of 6 young; and measurements were taken on rats with 6 young at earlier stages of lactation before full milk production was reached. These indicate a positive correlation between the intensity of lactation and the magnitude of the effect that lactation has on the composition and metabolic rate of mammary tissue.

The authors are glad to acknowledge that Robert E. Smith helped them effectively with the microrespiration trials.

[3] Brody, S., and Nisbet, Ruth, *Missouri Research Bul.*, 285, 1938.

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Effect of Estradiol on Urinary Excretion of Ascorbic Acid in the Dog.

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An investigation was made of the effect of the estrogen, estradiol-benzoate (Progynon-B, Schering†), on the urinary excretion of ascorbic acid by the dog, an animal capable of synthesizing ascorbic acid. Studies were made of the influence of estradiol on the 24-hour excretion and on the mechanism of ascorbic acid clearance. This was done to determine if changes in total excretion might be due to alteration of the normal renal mechanism, which consists of filtration at the glomerulus

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† Supplied by courtesy of Dr. Max Gilbert, Schering Corp.