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.

and active tubular reabsorption.[1]

Methods. Control values of the maximal tubular reabsorptive capacity (Tm) for ascorbic acid of 3 female dogs were determined by repeated clearance studies within 34 days of the beginning of treatment. This was done by constant intravenous infusion of ascorbic acid and of creatinine, the clearance of the latter measuring glomerular filtration rate in the dog. Ascorbic acid was infused at a rate sufficient to give load levels in excess of that needed for tubular saturation. The clearance values of 3 or 4 successive urine collection periods were averaged in computing the data. The dogs were hydrated prior to the clearance to maintain adequate urine flows.

The creatinine determinations were made by the method of Folin and Wu,[2] after the plasmas had been precipitated by the $CdSO_4$ method.[3] The ascorbic acid of both plasma and urine was determined by a modification of the method of Mindlin and Butler.[4]

Two of the dogs were kept in metabolism cages so that 24-hour collections of urine could be made. Deterioration of the ascorbic acid was minimized by the addition of sulphuric acid to the collection vessels.

Daily injections of 1.66 mg of estradiol-benzoate in sesame oil were given intramuscularly for periods of 10 to 14 days. During this time, 24-hour collections of urine were continued and further clearance determinations of the ascorbic acid Tm were made. To allow the plasma levels to return to normal values the urine collections were resumed 18 to 24 hours after infusion of the ascorbic acid. Fasting plasma levels of ascorbic acid were determined before and during estradiol treatment.

Results. Although a renal threshold exists, some ascorbic acid is lost in the urine at low plasma levels. Dog A had an average daily control excretion of 83.0 mg (range: 64-89 mg); dog B, 112 mg (range: 101-125 mg). The control fasting plasma levels of ascorbic acid in dog A averaged 0.49 mg%; in dog B, 0.43 mg%. The average control ascorbic acid Tm values (mg/min) were as follows: dog A, 0.581; dog B, 0.531; dog C, 0.560.

The effects of the injection of estradiol became apparent in 3 or 4 days by a rise in the daily excretion of ascorbic acid, by a decrease in tubular reabsorption, and by a rise in the ascorbic acid clearance/creatinine clearance ratio. The 24-hour excretion rose to an average of 112.0 mg (range: 97-129) in dog

TABLE I.
Effect of Estradiol on Clearance of Ascorbic Acid in the Dog.

		Dog A: 19 kg .087 mg Progynon/kg		Dog B: 18 kg .092 mg Progynon/kg		Dog C: 13.5 kg .123 mg Progynon/kg	
		Control	Treated	Control	Treated	Control	Treated
No. of clearance exper.*		7	6	7	6	6	7
Load:	range:	1.16-2.35	1.14-2.36	1.43-2.69	1.66-2.88	1.42-1.94	1.08-1.65
	(mg/min) avg:	1.67	1.71	2.04	2.12	1.61	1.47
Tm:	range:	.489-.661	.408-.537	.410-.628	.063-.351	.461-.671	.150-.420
	(mg/min) avg:	.581	.480	.531	.224	.560	.306
$\frac{C_{aa}}{C_{cr}}$ †	range:	.540-.725	.600-.773	.638-.776	.812-.974	.608-.695	.667-.890
	avg:	.636	.704	.733	.900	.659	.790

* Each clearance experiment consists of 3 or 4 urine collection periods.

† $\frac{C_{aa}}{C_{cr}} = \frac{\text{Ascorbic acid clearance}}{\text{Creatinine clearance}}$

[1] Sherry, S., Friedman, G. J., Paley, K., Berkman, J., and Ralli, E. P., *Am. J. Physiol.*, 1940, **130**, 276.

[2] Folin, O., and Wu, H., *J. Biol. Chem.*, 1919, **38**, 81.

[3] Fujita, A., and Iwatake, D., *Biochem. Z.*, 1931, **242**, 43.

[4] Mindlin, R. L., and Butler, A. M., *J. Biol. Chem.*, 1938, **122**, 673.

A and to 186 mg (range: 154-243) in dog B. The fasting plasma levels of ascorbic acid decreased to 0.32 mg% in dog A and to 0.34 mg% in dog B. This was accompanied by an average decrease in reabsorptive Tm of 17.5% in dog A, 58% in dog B, and 45.3% in dog C. The clearance ratio rose 11, 23, and 20% respectively.

Conclusions. Estradiol increases the clearance of ascorbic acid in the dog by reducing tubular reabsorption, the clearance approaching that of creatinine in some cases. As a result, the fasting plasma levels of ascorbic acid fall during the treatment.

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A Test Paper for the Rapid Estimation of the Level of Sulfonamide in Serum.

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The reaction of Ehrlich's reagent, *p*-dimethylaminobenzaldehyde, with aromatic amines in acid solution has been made the basis of a colorimetric determination of sulfonamides by Werner[1] and by Morris.[2] Fuller[3] has employed this substance in test papers for estimating the level of sulfonamide in laked blood after precipitation of proteins with an acid reagent, comparing the yellow color produced with standard color strips. The present paper describes the preparation of acid-containing test papers with which the level of sulfonamide can be estimated directly in serum without the addition of precipitating or acidifying reagents.

One gram of *p*-dimethylaminobenzaldehyde (straw-colored material is usable) is dissolved in 2 cc of concentrated hydrochloric acid and to the solution is added 0.8 cc of syrupy phosphoric acid (sp. gr. about 1.7) and water to make 100 cc. Large pieces of a good grade of absorbent paper (such as is used in making litmus paper) are soaked in the solution and immediately hung up to drain. When superficially dry, the papers are stored in the dark in covered glass jars for from 5 to 10 days, during which time most of the hydrochloric acid evaporates. The edges of the paper, which are usually yellow, are then trimmed off and the paper is cut into small strips and preserved in suitable vials. The strips are almost colorless, but become discolored if they come in contact with the skin at any time during preparation or use. The phosphoric acid remaining in the papers is sufficient to give a pH of approximately 3 when they are moistened with serum.

Estimation of the sulfonamide level is made by spreading evenly the serum to be tested over one end of an impregnated strip by means of a fine dropper, care being taken that unabsorbed fluid does not remain. In this way, the serum is spread over the paper by the movement of the dropper rather than by the capillarity of the fibers, and the reagents are consequently not leached out of areas which are oversaturated. Within 10 seconds a maximum development of color occurs. To avoid translucence of the paper interfering with the reading, the wet strip is held close to a white background or placed on white paper or white porcelain. The color is then judged in comparison with a graded series of dry standard color strips. The color ranges from a pale violet with normal human serum to a bright yellow with serum containing 15-20 mg sulfadiazine per 100 cc. A comparison of values obtained by the test-paper method with those secured by the method of Bratton and Marshall[4] are set forth in Table I.

1 Werner, A. E. A., *Lancet*, 1939, **1**, 18.

2 Morris, C. J., *Biochem. J.*, 1941, **35**, 952.

3 Fuller, A. T., *Lancet*, 1942, **1**, 760.

4 Bratton, A. C., Marshall, E. K., Babbitt, D., and Hendrickson, A. R., *J. Biol. Chem.*, 1939, **128**, 537.