Summary. 1. This is an early evaluation of a new, non-sulfonylurea hypoglycemic agent. 2. In this study of 20 cases, DBI, the hydrochloride of N[1]beta-phenethylformamidinylimino urea, appears to have definite blood sugar lowering properties. The mechanism of action is not yet known. 3. There are annoying side effects in some cases which are restricted to the gastrointestinal tract and are not related to the blood sugar levels. 4. No other annoying or toxic effects have been observed at this time. 5. The size of the effective blood sugar lowering dose is smaller than that reported for other oral hypoglycemic agents.

1. Camerini-Davalos, R., Root, H. F., and Marble, A., *Diabetes,* 1957, v6, 74.

2. Duncan, G. G., Goiner, C. L., and Lee, C. T., *Metabolism,* 1956, v5, 964.

3. Camerini-Davalos, R., Marble, A., and Root, H. F., *ibid.,* 1956, v5, 904.

4. Ungar, G., Freedman, L., and Shapiro, S. L., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 190.

5. Pomeranze, J., Fuiji, H., and Mouratoff, G. T., *ibid.,* 1957, v95, 193.

6. Nelson, N., *J. Biol. Chem.,* 1944, v153, 375.

Received March 26, 1957. P.S.E.B.M., 1957, v95.

Desoxyribonucleic Acid (DNA) Content of Mammary Gland During Pregnancy and Lactation.*† (23216)

DAVID R. GRIFFITH AND CHARLES W. TURNER
Department of Dairy Husbandry, University of Missouri, Columbia.

It has been postulated that for any given species the somatic cell nuclei contain a constant amount of desoxyribonucleic acid (DNA)(1). This being true, determination of DNA would provide an accurate estimation of the number of cells formed during the developmental phase of a tissue, gland or organ(2). Upon the basis of this theory, studies have been conducted to determine total DNA of mammary glands of rats(3), rabbits(4) and mice(5) during normal and experimental development of the mammary gland in order to measure extent of mammary gland growth.

It was thought necessary to provide a sound basis for the above studies(3,4,5) by determining the DNA content per cell of mammary gland during growth and lactation.

Methods. Virgin, pregnant and lactating primiparous albino rats were used. Pregnancy ranged from 15-20 days and was determined by examination of fetuses. Animals which were used for determination of DNA of early lactation were all taken on the day of parturition. The days of late lactation ranged from the 18th to the 21st. The animals were killed and the 6 posterior glands were quickly removed and placed in 30 ml of ice cold isotonic sucrose containing 1% citric acid. The kidney, spleen and mammary gland were treated in an identical manner.

Tissue was homogenized for 2 minutes in a Waring Blendor(6) and strained through cheesecloth, 2 and 4 layers in thickness. This filtered homogenate was then used for subsequent fractionations. Filtered homogenate was centrifuged 30 minutes at 2100 rpm, 1100 rpm for 15 minutes, 800 rpm for 10 minutes and 600 rpm for 10 minutes. Final sediment was suspended in 10 ml of homogenizing solution and nuclei were then counted on a hemocytometer. Tissue residue was extracted with ice cold 10% trichloracetic acid and DNA determined by a method recently published(7). DNA per cell was then determined by dividing total quantity of DNA by known number of nuclei in the original suspension.

Results. It was observed that DNA of the nucleus remained within reported literature values in the virgin and the pregnant animal. In the virgin animal DNA content of pooled

* Contribution from Department of Dairy Husbandry, Mo. Agr. Exp. Sta. Journal Series No. 1736. Approved by Director.

† This investigation was supported in part by grant from Am. Cancer Soc.

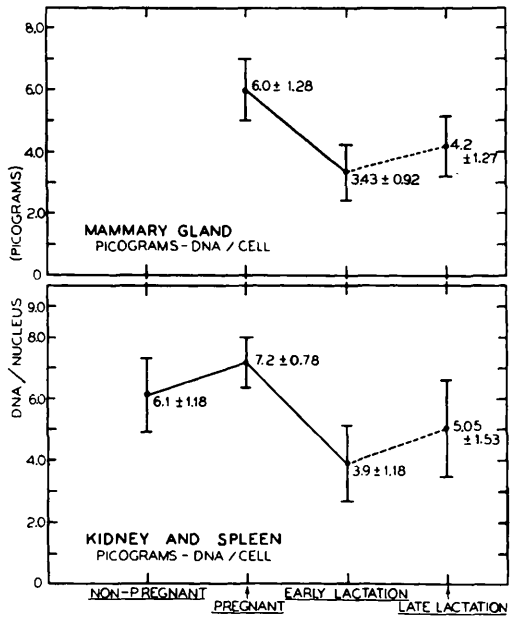

FIG. 1. DNA content per nucleus of mammary gland cells of rat during pregnancy and lactation compared to kidney and spleen cells. The marked decline in DNA per cell in early lactation has not been reported previously.

kidney and spleen only was determined due to insufficient development of the mammary gland at this stage. DNA per nucleus in virgin animals had a mean average of 6.1 picograms,† while the mean for pregnant animals was 7.2 for pooled tissue and 6 picograms for mammary gland.

On the first day of lactation, in both tissues there was an appreciable drop in nuclear DNA content. The mean value for pooled tissue was 3.9 picograms and for mammary gland 3.4 picograms. There was a statistical difference ($P<.0025$) between the pregnant animal and the animal in early lactation.

By the time of late lactation DNA had risen to the mean value of 5.05 picograms for pooled tissue and 4.2 for mammary gland. These values were not statistically different from those obtained during the non-pregnant and pregnant stages of this study.

Throughout the experiment there was no statistical difference among groups with the exception ($P<.05$) of pooled kidney and spleen and mammary gland during pregnancy.

A number of determinations were carried out on male mice with experimentally induced mammary gland growth. The values, an average of 5.1 picograms per nucleus, were within the reported value of other somatic tissues of the mouse (kidney and spleen) of 5 picograms per nucleus(8).

Discussion. The results of this experiment show that during the period of pregnancy or experimentally induced gland growth the estimation of DNA would be an accurate measurement of cell multiplication. The abrupt drop of DNA per cell at onset of lactation seems to be contrary to reports in other tissues. To the authors' knowledge, this is the first report of DNA content of cells during the physiological changes of pregnancy and lactation. The estimation of cell number during this period would be accurate only if a correction factor were applied for the change in DNA.

Summary. A study of the DNA concentration per nucleus was carried out on kidney and spleen and the developing and lactating mammary glands of rats. The DNA concentrations remained within normal values in the pregnant and non-pregnant stages. On the first day of lactation there was an abrupt drop in this value which returned approximately to normal by 18th day of lactation.

† 1 picogram (pg) $= 10^{-12}$ g.

1. Mirsky, A. E., and Ris, H., *Nature,* 1949, v163, 666.

2. Davidson, J. N., and Lesilie, I., *Cancer Res.,* 1950, v10, 567.

3. Kirkham, W. R., and Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 123.

4. Yamamoto, H., and Turner, C. W., *ibid.,* 1956, v92, 130.

5. Damm, H. C., and Turner, C. W., *ibid.,* in press.

6. Bass, A. D., McArdle, A. H., and Grisham, J. W., *Science,* 1956, v123, 372.

7. Webb, J. M., and Levy, H. B., *J. Biol. Chem.,* 1955, v213, 107.

8. Vendrely, R., and Vendrely, C., *Experientia,* 1949, v5, 327.

Received April 1, 1957. P.S.E.B.M., 1957, v95.