this group were the same as in the first series, except that localized tail heating was omitted. Erythrocyte and leukocyte counts were made with the usual pipets and diluting fluids; differential counts were made on smears stained with Wright's stain, 100 cells on each of 2 slides being employed for each analysis. Results are summarized in Table 1.

A significant difference was observed in the total WBC and lymphocyte count of the peripheral blood of rats, when blood specimens were taken following total body heating, as distinct from localized tail heating. In the latter group, the data for total WBC and lymphocytes in peripheral blood were comparable to those reported by other investigators for the rat (*2*); animals subjected to total body heating, however, had a significant reduction in the total WBC and lymphocyte count. The reduction was apparently not due to hemodilution, since little, if any, decrease occurred in the erythrocyte count. A reduction was also noted in the number of granulocytes per cubic mm of peripheral blood following total body heating. This decrease, however, was less marked than the reduction in lymphocyte count. Similar findings were obtained on both experimental rations. Determinations were also made of the leukocyte count of peripheral blood after localized tail heating and 20 min later on the same animals after total body heating. A reduction in the peripheral leukocyte count after total body heating was observed in these animals of the same order of magnitude as that reported above.

It has been established that the white cell count of peripheral blood is significantly greater than that of heart blood (*5–8*). This condition is caused, according to Quimby and Goff (*6*), by the "damming up" of white cells in the peripheral areas of the vascular system as a result of the normal resistance of the arterioles and capillaries to the flow of the blood. When rats were anesthetized with ether, the difference in white cell count between heart and peripheral blood was abolished, presumably because "ether and other substances which relax the contractile elements of the blood vessels and increase their lumen diameter reduce the resistance offered by the peripheral vascular bed to the flow of blood cells and result in a more even distribution of the leukocytes between the large and small vessels" (*6*). In the present experiment, total body heating similarly resulted in a significant reduction in the leukocyte count of peripheral blood to values comparable to that of heart blood (*5–6*). It would seem likely that an increase in circulation rate and small vessel dilatation (*9–11*) resulting from total body heating were responsible, at least in part, for the observed effects.

Total body heating has the following advantages over tail heating as an experimental procedure for obtaining blood specimens in the rat: (1) less handling of the animal is required in this procedure, since localized warming of the tail is omitted, and as a result, blood specimens may be obtained more quickly (once the animal is wrapped in the towel) with a minimum of restraint to the animal; (2) alterations in the blood count due to the stressor effects of warming the tail in hot water and the longer period of restraint in animals subjected to localized tail heating are minimized when total body heating is employed (this advantage would be particularly marked when multiple counts are made over an extended period of time); (3) the flow of blood on severing the tip of the tail, after total body heating, is more profuse than that generally obtained after localized tail heating. Total body heating would appear to have particular value, therefore, in obtaining blood specimens from nutritionally deficient or "toxic" animals from whom adequate amounts of free-flowing tail blood are difficult to obtain following localized tail heating.

References

1. DONALDSON, H. H. *The Rat.* Memoirs of the Wistar Institute, Philadelphia (1924).
2. SCARBOROUGH, R. A. *Yale J. Biol. & Med.*, **3**, 267 (1931).
3. JORDAN, H. E. *Handbook of Hematology.* Vol. II, 703. H. Downey, Editor. New York: Hoeber (1938).
4. CRESKOFF, A. J., FITZHUGH, T., and FARRIS, E. J. In: Griffith and Farris: *The Rat in Laboratory Investigation,* 351. Philadelphia (1942).
5. QUIMBY, F. H., SAXON, P. A., and GOFF, L. G. *Science,* **107**, 447 (1948).
6. QUIMBY, F. H., and GOFF, L. G. *Am. J. Physiol.*, **170**, 196 (1952).
7. KLIENEBERGER, C., and CARL, W. *Die Blut-Morphologie der Laboratoriums-Tiere.* Leipzig: Barth (1912).
8. ROOFE, P. G., LATIMER, H. B., MADISON, M., MAFFET, M., and WILKINSON, P. *Science,* **111**, 337 (1950).
9. GOODMAN, L., and GILMAN, A. *The Pharmacological Basis of Therapeutics,* 67. New York: Macmillan (1941).
10. MANN, F. C., ESSEX, H. E., HERRICK, J. F., and BALDES, E. J. *West. J. Surg.*, **43**, 177 (1935).
11. FOREGGER, R. *Anesthesiology,* **4**, 392 (1943).

Manuscript received November 13, 1952.

Estrogen Excretion in Women with Mammary Cancer before and after Adrenalectomy[1]

Thomas L-Y. Dao

Ben May Laboratory for Cancer Research, University of Chicago, Chicago

This paper presents data on the excretion of estrogen in the urine of women with cancer of the breast, before and after both adrenal glands were excised. Estrogens are known to be importantly involved in the genesis of mammary neoplasms in mice (*1*). It is known (*2*) that ovariectomy sometimes causes a remission of this disease in women. It has been further established that bilateral adrenalectomy (*3*), with maintenance on cortisone acetate, causes a significant regression of mammary cancer in ovariectomized women in certain cases.

It has been shown (*4*) that estrogen is produced in the adrenals of certain strains of mice in which the gonads were excised in early life. Also, estrone has

[1] This investigation was supported by grants from the American Cancer Society, upon recommendation of the Committee on Growth of the National Research Council and from the Jane Coffin Childs Memorial Fund for Medical Research.

TABLE 1

ESTROGEN EXCRETION IN URINE OF HUMAN FEMALES
I.U./24 hr.

Groups*	Number	Age (years)	Range (I.U.)	Mean (I.U.)
1. Normal, premenopausal	8	21–41	13.8–55.6	33.0
2. Normal, postmenopausal	4	45–77	0.3– 9.1	4.7
3. Cancer, premenopausal	4	38–51	13.4–53.8	28.2
4. Cancer, x-ray castration	6	43–58	4.7–20.1	14.7
5. Cancer, surgical ovariectomy	6	38–57	0 –38.1	16.6
6. Cancer, ovariectomy and adrenalectomy	12	38–58	0	0

* Groups 3–6, widespread mammary cancer.

been extracted from adrenal gland concentrates (*5*). It is known that in the human male (*6, 7*), adrenal neoplasms which produce feminization cause a slightly increased excretion of estrogen.

Estrogen assays were performed on multiple collections of urine, each of 24 hr, from 12 women with breast cancer, and from 12 normal noncancerous women as controls. Estrogen was extracted from the urine after acid hydrolysis by a slight modification of the method of Pincus and Pearlman (*8*). Quantitative determinations of estrogen in the extracts were made by bioassay using the uterine weight method of Evans, Varney, and Koch (*9*).

Thus, confirming previous studies (*8, 10, 11*), the urinary excretion of estrogen in premenopausal women was in the same range regardless of the presence or absence of breast cancer. Elimination of ovarian function by roentgen irradiation or ovariectomy led to similar lowered values of estrogen excreted in urine; the amount was reduced by one-half (Table 1). When adrenalectomy was performed after elimination of ovarian function, estrogen was no longer detectable in the urine. Nine of 12 patients with mammary cancer subjected to adrenalectomy in this series, showed an actual measurable decrease in the extent of neoplastic involvement after the operation. This regression was observed in one or more of the following areas: local recurrent carcinoma, osseous metastases, pleural involvement.

The results show: first, patients with breast cancer who had had eradication of ovarian function by irradiation or surgical excision, still excreted estrogen in significant amount; secondly, adrenalectomy eliminates estrogen from the urine of those cases. It is evident that the adrenals are the only significant source of estrogen in women with breast cancer who do not have active ovarian function.

The 3 patients with mammary cancer who did not have a regression of the neoplasm after adrenalectomy, excreted 9.6, 12.3, and 20.1 I.U. *per diem* preoperatively. Two of these values are below the mean titer for ovariectomized women (Table 1). It is impossible, however, to state from this study that the reduction of estrogen was the critical factor in the remission of the disease.

References

1. LACASSAGNE, A. *Compt. rend.*, **195**, 630 (1932).
2. BEATSON, G. T. *Lancet*, **2**, 104, 162 (1896).
3. HUGGINS, C., and BERGENSTAL, D. M. *Cancer Research*, **12**, 134 (1952).
4. WOOLEY, G., FEKETE, E., and LITTLE, C. C. *Proc. Natl. Acad. Sci. U. S.*, **25**, 277 (1939).
5. BEALL, D. *Nature*, **144**, 76 (1939).
6. WILKINS, L. *J. Clin. Endocrinol.*, **8**, 111 (1948).
7. HUGGINS, C., and BERGENSTAL, D. M. *J. Am. Med. Assoc.*, **147**, 101 (1951).
8. PINCUS, G., and PEARLMAN, W. H. *Cancer Research*, **1**, 970 (1941).
9. EVANS, J. S., VARNEY, R. F., and KOCH, F. C. *Endocrinology*, **28**, 747 (1941).
10. TAYLOR, H. C., MECKE, F. E., and TWOMBLY, G. H. *Cancer Research*, **3**, 180 (1943).
11. NATHANSON, I. T. *Cancer Research*, **3**, 132 (1943).

Manuscript received December 29, 1952.

A Metal-Filled Microelectrode[1]

Robert M. Dowben[2] and Jerzy E. Rose

Departments of Medicine and Environmental Medicine, Physiology and Psychiatry, The Johns Hopkins University, Baltimore, Maryland

Microelectrodes are finding a steadily increasing area of application in biological research. Glass microcapillaries filled with an electrolyte solution are in common use (*1–5*). Such electrodes record single-unit activity with a low signal-to-background ratio if their tips are large. Electrodes with small tips have a high impedance making necessary the use of a special high-input-impedance amplifier (*5*). The microelectrodes of Weale (*6*) and Svaetichin (*7*) are recent efforts to fill the need for an electrode of low impedance. The Svaetichin electrode is difficult to prepare, and we have been unable to make a satisfactory electrode following Weale's directions. A new low-impedance-microelectrode is described here in which a glass "wetting" metal is used to fill a glass microcapillary. This microelectrode is sturdier than saline-filled electrodes of comparable size, and it is relatively easy to prepare.

Gallium and indium[3] and many of their alloys possess the unusual property of "wetting" glass. An alloy of 50% In—50% Sn, m.p. 110° C, or pure indium, m.p. 156° C, is used in the preparation of the microelectrodes.

Precision drawn Pyrex capillary tubing, 0.8 mm O.D., 0.5–0.6 mm I.D., is thoroughly cleaned, dried, and cut into 4–5 in. lengths. The metal is melted on

[1] Aided by a contract between the Office of Naval Research, Department of the Navy, and the Johns Hopkins University (NR 113–241) and by a grant (B-17) from the Research Grants Division, National Institutes of Health, USPHS.

[2] Present address: Department of Medicine, Hospital of the University of Pennsylvania, Philadelphia.

[3] The gallium and indium used in this work were generously supplied by the Aluminum Corporation of America and the Indium Corporation of America respectively.