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.

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A Metal-Filled Microelectrode[1]

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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

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[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.

a hot plate or in an oil bath. Excessive heating will cause the formation of yellow oxides of indium. By means of gentle suction, metal is drawn up the glass capillary in a continuous column to about the midpoint and allowed to solidify (Fig. 1a). To prevent the contamination of capillaries, any scum which forms on the surface of the melt should be removed frequently.

At a point about 2 mm beyond the end of the metal column, the glass capillary is heated and drawn out into a micro-tip of the desired size and shape (Fig. 1b). The use of a Livingston micropipette puller (*8*) enables rapid preparation of large numbers of electrodes of uniform tip dimensions. After the tip has been drawn, the capillary is heated on a hot plate until the metal column just melts. Using a snugly fitting sewing needle inserted into the open end of the capillary as a piston, the metal column is gently pushed to fill the tip. Metal is extruded from the tip to form a tiny drop 30–50 μ in diameter. The electrode is cooled and the metal ball gently blown off. An undesirable cuff of metal may be formed on the outer surface of the glass if the metal ball is too large. The steel needle remains in place; its free end is used for electrical contact.

A layer of gold is electrodeposited on the tip of the microelectrode immediately after the filling operation. A gold cyanide solution (0.2% Au) is used for plating, with a 1.5 v dry cell in series with a 5–10 megohm resistor as a source of current. The microelectrode is connected to the negative pole. For an electrode with a 2–4 μ tip, the plating time is 30–45 sec. On the chemically inert and impervious gold base, platinum black is electrodeposited from a bath of 0.4% platinum chloride (Fig. 1c). The plating time and current are the same as those used for gold plating. The microelectrodes are examined under the microscope for defects at each stage in their preparation.

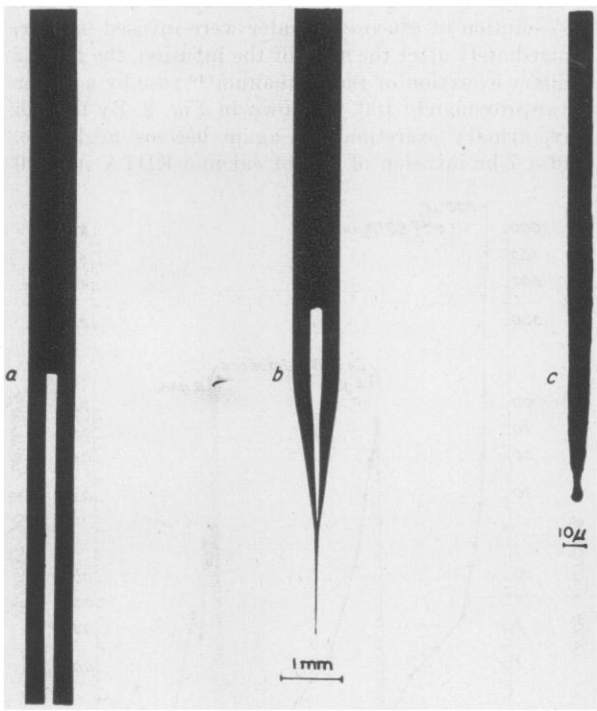

FIG. 1. Three stages in preparation of the microelectrode. (a) capillary tubing filled half-way with metal: (b) the capillary after the tip has been drawn and before the metal was pushed to fill it completely: (c) the end of the electrode filled with metal. Note the platinum-black deposit visible as a small ball. Magnification in (a) and (b) is indicated by the line representing 1 mm: magnification in (c) is shown by the line representing 10 μ. The shadows cast during photography make the walls of the capillary in (a) and (b) appear to be thicker than 0.2 mm.

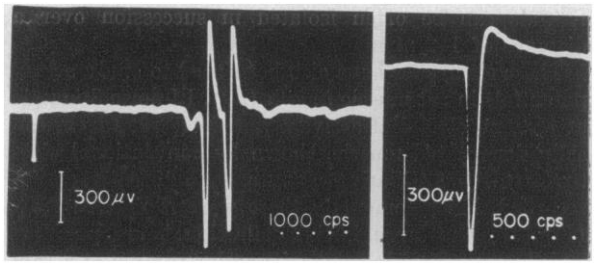

FIG. 2. Tracing on left: Single-unit discharge in the tactile region of the thalamus resulting from electrical stimulation of a small twig of a cutaneous nerve in the skin of the forearm. Cat. Nembutal anesthesia. Note that the unit discharges twice. The deflection on extreme left is the stimulus artefact. Tracing on right: Spontaneous potential recorded from fibrillating rat muscle. Anterior gracilis muscle denervated 12 days. Downward deflection is positive in both records.

The electrode conductivity is tested in the following manner. A platinum wire, insulated except for a 1-mm length near its midpoint, is connected across the output of a 1000-cycle oscillator. The wire, submerged in a tumbler full of physiological saline solution, acts as a source of current. A steel-needle-recording electrode is immersed in the saline bath a few cm from the current source and connected to an amplifier of 0.5 megohm input impedance. A grounded wire is submerged in the bath to complete the circuit. The signal amplitude is noted on a cathode-ray oscilloscope. The steel needle is now replaced by the microelectrode to be tested and the amplitude of the signal is compared with that previously recorded. Microelectrodes with excessively large exposed metal surfaces will record signals 80% or more as high as the control. Signals of 30–60% height are obtained from satisfactory electrodes of 2–4 μ tip diameter.

The electrodes described have been used by Mountcastle and Rose (*9*) to record potentials in the somatic-sensory group of nuclei in the thalamus. In this region, almost continual unitary activity initially negative in sign is recordable with electrodes of 2–4 μ. Usually, however, more than one such potential is recorded at the same time and the discharges tend to decrease in amplitude with time. Occasionally, a potential representing activity of a single unit, initially positive, is recorded within the nuclear region (Fig. 2). The discharge is, as a rule, recorded in complete isolation, and it often tends to persist underneath the electrode for several hours without much change. In the tactile thalamic region several initially positive unitary po-

tentials can be often isolated in succession over a distance of 1–2 mm.

These electrodes have also been used to study action potentials in rat muscle *in situ* (*10*). In normal muscle at rest, no electrical activity was detected. In chronically denervated muscle, shown by conventional electrodes to be fibrillating, spontaneous potentials were recorded. Most commonly these were diphasic spikes (Fig. 2), the large initial deflection being positive, and they recurred in totally irregular rhythm at a rate averaging about 6/sec.

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Modification of the Distribution and Excretion of Radioisotopes by Chelating Agents[1]

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When the sodium salt and calcium chelate of ethylenediamine tetraacetic acid (EDTA) are administered intravenously to man, an equivalent amount of calcium is rapidly excreted by the kidneys (*1, 2*). Lead-EDTA and yttrium EDTA have been demonstrated to leave the body quite rapidly when injected intravenously (*3, 4*). Furthermore, when carbon^{14}-labeled EDTA is injected into animals, 99% is excreted in 48 hr (*5*). It was, therefore, rather unexpected to find in the course of our work on the distribution and excretion of lanthanum (*6*) in man, that less than 10% of the lanthanum is excreted through the kidneys when lanthanum-EDTA is intravenously administered, and it was decided to investigate this effect further.

A tracer dose of 200 μc lanthanum^{140} as the EDTA complex was injected intravenously. The patient was catheterized to facilitate accurate urine collections. Urine and blood samples were collected at frequent intervals until the activity was no longer measurable. After 48 hr, the urinary excretion of lanthanum^{140} had become negligible. However, only about 5% of the injected lanthanum^{140} dose had been excreted up to that time, as indicated in the lower curve of Fig. 1.

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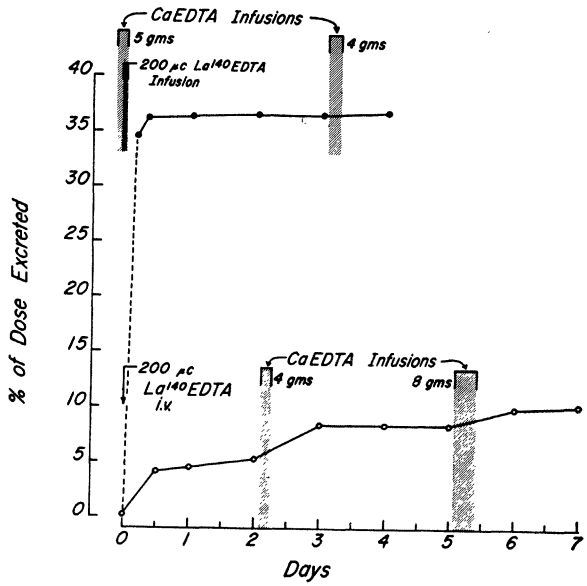

FIG. 1. Effect of calcium-EDTA upon the cumulative urinary excretion of lanthanum^{140}-EDTA. The upper graph illustrates the effect of preceding and *simultaneous* administration, and the lower graph that of *subsequent* infusions of calcium-EDTA.

At this time, 4 g of calcium-EDTA in 500 ml of a 5% solution of glucose in water were infused in 3 hr. Immediately after the start of the infusion, the rate of urinary excretion of the lanthanum^{140} rose by a factor of approximately 100, as shown in Fig. 2. By the 5th day, urinary excretion had again become negligible, and a 7-hr infusion of 8 g of calcium-EDTA in 1000

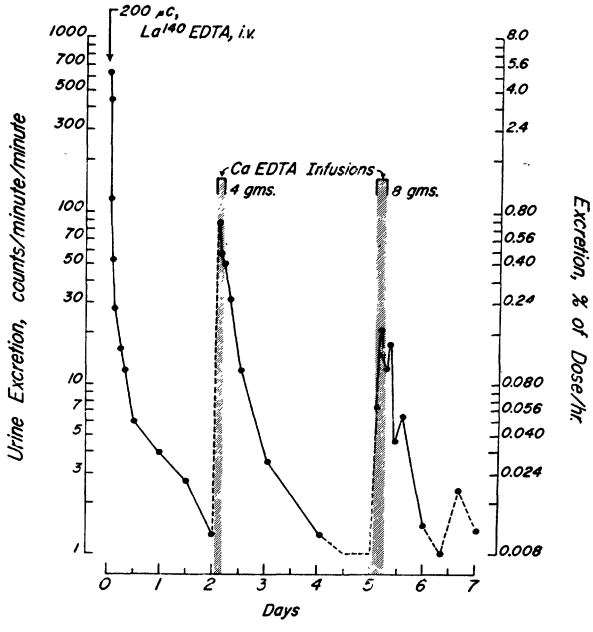

FIG. 2. Influence of calcium-EDTA upon the rate of urinary excretion of lanthanum^{140}-EDTA, expressed in terms of measured radioactivity and of the proportion of the total dose excreted per hour.