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CORRELATION OF MICRO-ELECTRODE POTENTIAL RECORDINGS WITH HISTOLOGY OF RAT AND GUINEA-PIG THYROID GLANDS

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The thyroid gland removes iodine from the blood stream and concentrates it in the lumina several hundredfold (VanderLaan & VanderLaan, 1947; VanderLaan & Greer, 1950; VanderLaan, 1955; Halmi, 1957, 1961). To determine whether this uptake of iodine is an active process requires knowledge of the electrical potential difference between the cell or lumen and the plasma, because the iodine passing into the lumen is probably in ionized form (cf. Halmi, 1961). In addition, knowledge of the transmembrane potentials of thyroid cells permits calculation of chloride concentrations intracellularly and in the lumina, on the assumption that Cl^- is not actively transported. Furthermore, alterations of the potentials of thyroid cells produced by hormones and drugs may furnish important clues to the possible modes of action of these agents.

Differences in tissue potentials can be measured accurately with ultramicro-electrodes, but interpretation of such measured potentials is limited because the submicroscopic tip of the electrode cannot be visualized with certainty even in transparent media. Thus in semi-opaque materials such as thyroid tissue indirect means (e.g. correlation of potential-distance profiles with tissue structure) must be used to determine the location of the electrode at the time a shift in potential appears. The well known differences in the cellular and luminal dimensions of guinea-pig and rat thyroid follicles and the profound alteration of these dimensions by appropriate hormonal and drug treatment should permit an unequivocal interpretation of the sequence of potential changes observed when an electrode is advanced through the gland. In the rat thyroid cells are thick (10 μ) and the lumina small (35 μ); in the guinea-pig, thyroid cells are thin (5 μ) and the lumina large (80 μ). After chronic propylthiouracil treatment the cellular structure in the guinea-pig thyroid resembles that in the normal rat; chronic thyroxine treatment has opposite effects, so

that the rat thyroid resembles that of the normal guinea-pig. These histological differences suggest that the tip of a micro-electrode inserted into the thyroid of a normal rat and advanced through the tissue would be intracellular most of the time, whereas the most probable location of the electrode tip in a normal guinea-pig thyroid would be intraluminal.

The experiments reported here bear out these expectations and allow identification of the source of the potentials recordable from the thyroid. It will be shown that thyroid cells are normally about 50 mV, inside negative, with respect to the interstitial fluid and that the potentials in the lumina are within a few millivolts of the interstitial fluid potential.

METHODS

Dissection. Guinea-pigs and rats were anaesthetized with intraperitoneally injected pentobarbitone sodium, the initial dose being 25–30 mg/kg body weight; thyroxine-treated animals required only about half this dosage. The anaesthetic was supplemented as needed, usually once an hour. The trachea was transected well below the thyroid gland and a polyethylene cannula inserted. This transection was complete, to reduce transmission of respiratory movement to the thyroid gland. A small metal rod was inserted rostrally into the trachea and tied firmly to the trachea. The rod was then rigidly mounted on a supporting framework. This procedure greatly reduced but did not eliminate movement of the thyroid gland with respiratory excursions. The thyroid gland was exposed by excising the overlying muscles, while care was taken to avoid damage to the thyroid circulation. The skin edges were clipped to a stiff, horizontally mounted, two-pronged fork, to make a pool which was kept filled with mammalian Ringer's solution. Rectal temperature was maintained near 37° C by means of a manually controlled heat lamp.

Drug treatment. Propylthiouracil in alkaline solution was administered subcutaneously to rats and guinea-pigs; the dose was 20 mg/day for 5 days. Potential measurements were begun 6–12 hr after the last dose. Thyroxine was given subcutaneously to rats in the dose of 100 μg/day for 5 days and then 300 μg/day for 2 more days. Potential measurements were begun about 9 hr after the last injection. Hypophysectomized rats were obtained commercially.

Micro-electrode recording. Electrodes were pulled by hand and filled by being boiled gently for 1 hr in 3M-KCl. Electrode resistances were usually between 20 and 75 MΩ but were occasionally as high as 200 MΩ. Lower-resistance electrodes pulled by machine gave much less reproducible and lower values. Tip potentials (Adrian, 1956) were measured only on those occasions when the electrode broke while it was being advanced through the tissue; on these occasions the tip potentials were always less than 10 mV, usually less than 5 mV. The micro-electrodes were mounted rigidly, at an angle of about 60° to horizontal, on a Pfeiffer micromanipulator placed near the animal's head. A 360° low-torque potentiometer was attached to the micromanipulator advancement screw. The potential of the sliding arm was recorded on one channel of a dual-channel recorder. The potential of the micro-electrode with respect to an Ag-AgCl electrode in the pool was recorded with a low-drift (*ca.* 2 mV/hr) electrometer amplifier on the other channel. Calibration voltages were applied in series with the indifferent electrode.

Pulses of 50 msec duration and controllable amplitude were integrated and fed into the electrometer input via a 5 pF capacitor (Lettvin, Howland & Gesteland, 1958) 0·5 or 1 pulse per second. The pulse amplitude at the grid was proportional to electrode resistance (Fig. 1). The amplitude of the pulse was adjusted to give a deflexion of about 5 mm on the recorder when the micro-electrode was in the pool of Ringer's solution. The proportionality factor

was obtained by momentarily applying 10 V to the bottom of the $10^{10}\ \Omega$ grid resistor and measuring the resulting deflexion (1 mV deflexion equivalent to 1 MΩ electrode resistance).

Experience soon showed that tissue penetrations of more than 0·3 mm (*ca.* 1·5 mm electrode advancement) usually resulted in electrode breakage. Lack of tissue penetration and consequent bending of the electrode were recognized by a large rise in electrode resistance *pari passu* with advancement of the micromanipulator. The most constant and reproducible results were obtained by advancing the electrode a small distance (*ca.* 20–50 μ) and then waiting 10–30 sec for the thyroid to slip around the electrode and to reach a semi-stable configuration.

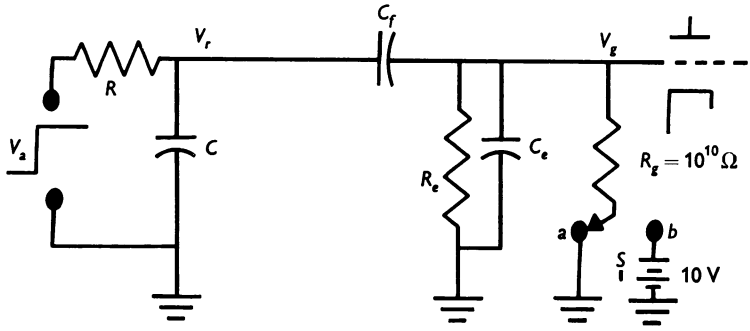

Fig. 1. Methods of measuring electrode resistance. Micro-electrode is represented by R_e and C_e. R_e can be measured directly in two ways.

(1) Moving S from position a to position b applies a current $I = 10\ \text{V}/10^{10}\ \Omega = 10^{-9}\text{A}$ to the electrode. In the steady state, the voltage (V_g) developed across R_e is $V_g = IR_e = 10^{-9}R_e$, i.e. electrode resistance (MΩ) = voltage developed (mV). This method is not suitable for measuring R_eC_e because of capacity across R_g (Brady & Woodbury, 1960).

(2) For $t \ll RC$, $V_r = V_at/RC$ and a current $I = C_f\text{d}V_r/\text{d}t = C_fV_a/RC$ is applied to the electrode. If $RC \gg t \gg R_e(C_e+C_f)$, $V_g = IR_e = V_aR_eC_f/RC$. Thus the final deflexion is proportional to R_e and is reached along an exponential path with time constant $= R_e(C_e+C_f)$. C_f should be small compared with C_e, to avoid excessive increase in effective input capacity. For $C_f = 2p\text{F}$, $V_a = 20$ V, RC must be 40 msec to produce a V_g change of 1 mV/MΩ. The change in V_g produced by V_a provides an accurate criterion for adjusting negative capacity feedback (Lettvin *et al.* 1958).

A single electrode track penetrated four or more follicles and took 15–30 min to complete. The base-line drift at the end of a penetration and withdrawal was usually less than 2 mV; but on the rare occasions when this drift was 5–10 mV linear interpolation in time was used to correct the measured potentials.

Two criteria were used in selecting points on the record at which estimates of transmembrane potentials would be made. Any abrupt negative deflexion which was maintained for 0·5 sec or more was considered to be an indication of electrode penetration into a cell and the peak deflexion (allowing for pen overshoot) was taken as the transmembrane potential (cf. Fig. 2). In some cases the abrupt negative deflexion was followed by a slow increase in negativity: in these cases the maximum negativity was selected as the best value of the transmembrane potential on the assumption that the slow change was attributable to an improvement in the seal of the membrane around the electrode. It is likely that the selected voltages included the lowered potentials of damaged cells but no objective criteria for rejecting such 'low' values could be formulated.

Histology. At the end of each experiment the thyroid was excised, fixed in 10% formol-saline, and prepared by standard histologic techniques (trichrome stain). Cell thickness and luminal diameters were measured in several follicles of most animals from which satisfactory potential records had been obtained (see Table 1). Since the measurements were for comparative purposes only, no attempt was made to correct the measurements for tissue shrinkage or non-equatorial section of the follicle. Thyroid measurements for the hypophysectomized rat were on a different animal than the one in which the thyroid potentials were measured.

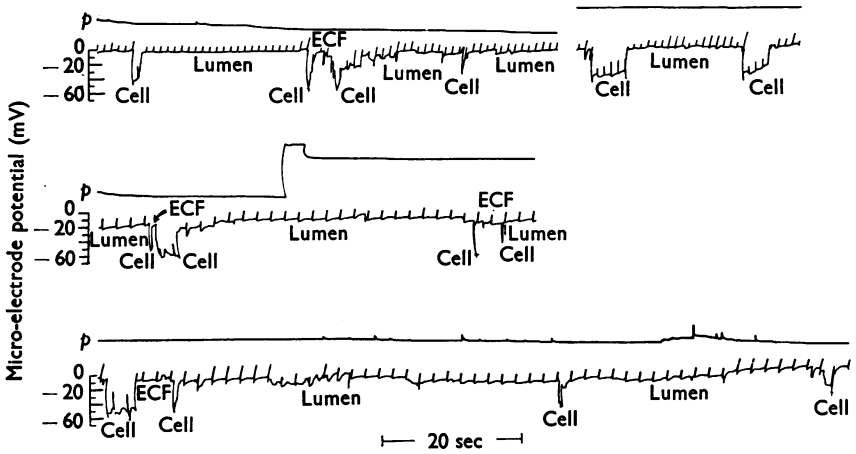

Fig. 2. Potentials recorded from guinea-pig thyroid. Ordinate, micro-electrode potential with respect to bathing fluid. Heights of regularly recurring pips are proportional to electrode resistance; in top record set these are 1 sec apart and in lower two sets 2 sec apart. *p*, ordinate proportional to electrode position; downward movement indicates electrode advancement. Sharp rise in middle set indicates completion of one revolution of micrometer head and an advancement of 500 μ. Pips on *p* trace are due to noisy spot on position potentiometer. Note that potential is zero at regions of record labelled 'Lumen'.

RESULTS

Electrode advancement–potential profiles

Advancement of an electrode through the thyroid gland resulted in a sequence of potential changes which were consonant with the histology of the tissue (Figs. 2–4). However, despite the rigid fixation of the electrode and the gland, impalements were poorly maintained; the voltage fell off with time after a penetration. Part of this drop in potential was undoubtedly due to injury wrought by the movement of the whole gland resulting from respiratory excursions. Another frequent cause of loss of potential was the way the tissue reacted to electrode advancement. Unless the advancement was slow, the shank of the conical electrode compressed the tissue; this compression appeared to be relieved by a series of 'jumps' of the tissue with respect to the electrode. These jumps caused both slow

changes in potential attributable to injury and abrupt changes attributable to membrane penetration. Potentials were much better maintained in rat (Fig. 3) than in guinea-pig (Fig. 2) thyroid cells, evidently because the follicles in the former are bigger and more surrounded by other cells.

Guinea-pig. Figure 2 shows some of the clearer records obtained from penetration of guinea-pig thyroid. In the upper left-hand record, the electrode was advanced steadily as shown by the slant of the upper line. The lower line shows that the electrode potential was usually zero, but occasional negative deflexions of short duration and rapid decline appeared. These deflexions were probably due to cell penetration and the zero potentials were due to luminal or interstitial tip locations. Our assessment of probable electrode locations is given beside the record. Although there seems little doubt that the electrode was in a lumen most of the time, there is much less certainty that the regions marked ECF (extracellular or interstitial fluid) are correctly labelled. Note that advancement of the electrode nearly always increased its resistance and that this increased resistance usually disappeared gradually after advancement was stopped (also see Figs. 3 and 4). This effect is thought to indicate bending of the electrode near its tip during advancement followed by gradual straightening as the electrode worked its way through the tissue, thereby reducing tissue compression.

Rat. Recordings from rat thyroid are shown in Fig. 3. The contrast with recordings from guinea-pig is dramatic. In the rat a negative potential was recorded most of the time and was considered to be intracellular. This interpretation conforms with the histology; rat thyroid is nearly all cells. As expected, the records suggest that the electrode sometimes moved from one cell to another without passing through interstitium or lumen; this is an unlikely occurrence in the normal guinea-pig (Fig. 2) but a frequent occurrence in propylthiouracil-treated guinea-pig (Fig. 4). Note that electrode resistance was 1·5 to 3 times higher in cells than in lumina, a nearly universal observation in rat thyroid even when no increase attributable to electrode bending was apparent. From this finding a near zero potential reading with a high electrode resistance could be interpreted as an electrode location in a badly injured cell, the high electrode resistance being due to the higher resistivity of the cell plasm.

Effects of propylthiouracil and thyroxine treatment. Figure 4 shows recordings from propylthiouracil-treated guinea-pigs. The records are indistinguishable from those obtained from normal rats. Similarly, a propylthiouracil-treated rat showed the same type of pattern, but with luminal penetrations being even more rare than in normal rats. The intracellular potentials shown in Fig. 4 were maintained much longer, and the second and third cells which were impaled without the electrode passing

through a lumen usually had higher potentials than the first. Note the transition from cell 3 to cell 4 on the top line and from cell 2 to cell 3 on the bottom line; the potential made a large jump, as though the electrode had passed from an injured to an uninjured cell.

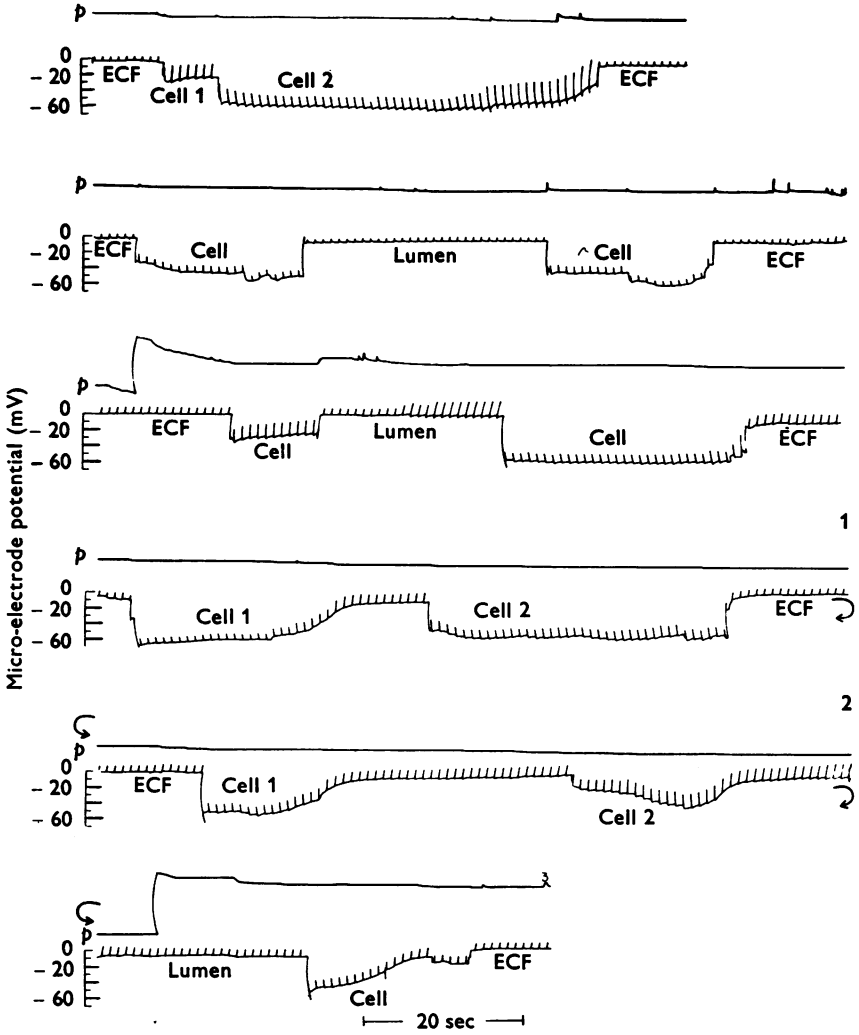

Fig. 3. Potentials recorded from rat thyroid; conventions as in Fig. 1. Electrode resistance pips are 1 sec apart. Curved arrows at end and start of lines indicate continuation of record.

In a thyroxine-treated rat the changes in potential profile were those expected from an increase in luminal size and a decrease in cell size, but were much less dramatic than the change from the normal in propyl-

thiouracil-treated guinea-pigs. Measurements in the thyroxine-treated rat were hampered by the tough connective tissue covering the gland. Good impalements were rare, and the cells seemed to be much more susceptible to injury. Guinea-pigs were not treated with thyroxine.

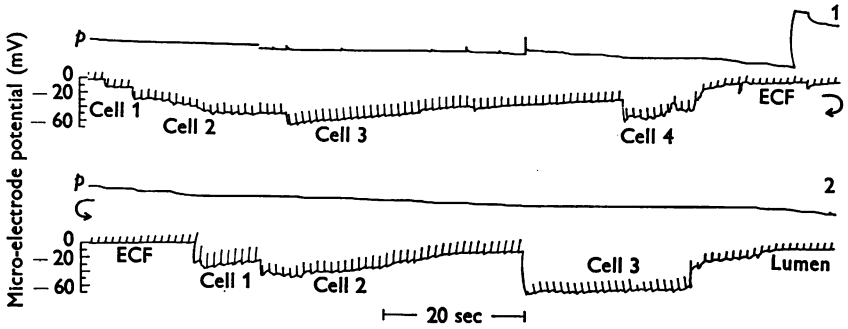

Fig. 4. Effect of chronic treatment with propylthiouracil on potentials recorded from guinea-pig thyroid; conventions as in Fig. 1. Electrode resistance pips are 1 sec apart.

Effects of topical 3M-*KCl.* In an attempt to determine whether the thyroid cell membrane has a high K^+:Na^+ permeability ratio, KCl solutions were applied locally. Ideally, the KCl should have been injected into the thyroid arterial circulation, but provision for such injections had not been made in the original preparation and improvisations failed owing to the necessity of injecting without dislodging the electrode from the cell. Possibly because of the high blood flow to the thyroid, topical application of isotonic KCl to the gland did not affect transmembrane potentials. One drop of 3M-KCl solution applied to the surface around the electrode produced a marked depolarization and a drop in electrode resistance, as is shown in Fig. 5. Some subsequent recovery of the potential and resistance toward normal occurred before the electrode became dislodged spontaneously at the end of the lower trace.

Mean potential values and drug treatment

Table 1 shows the mean values, standard deviations, and standard errors of the measured potentials of thyroid cells; the mean dimensions of the cell and lumina are also given, to facilitate comparison with the electrical phenomena. The mean potentials in untreated rats and guinea-pigs, and in thyroxine-treated rats were close to −50 mV. Propylthiouracil treatment reduced the potential to −38 mV; since the standard errors of the mean are about 2 mV, this reduction is highly significant. However, there seemed to be an increased amount of connective tissue

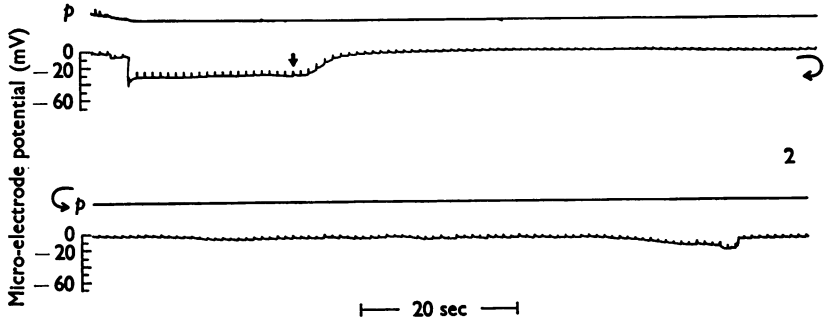

Fig. 5. Effects of KCl on transmembrane potential of a rat thyroid cell; conventions as in Fig. 1. Electrode resistance pips 1 sec apart. A cell was impaled at the left-hand end of the top record. The potential stayed constant for 20 sec after electrode advancement ceased. At the arrow 1 drop of 3M-KCl was dropped on the surface around the impaled region; note drop in resistance with voltage. Recovery was incomplete at the end of bottom record when the electrode left from the cell spontaneously.

TABLE 1. Transmembrane potentials and follicular dimensions of rat and guinea-pig thyroid

	Rat				
	Mean potential	S.D.	Mean follicle dimensions ±S.E. (μ)		
Treatment	±S.E. (mV)	(mV)	Follicle	Cells	Lumen
None (2 rats, 2 G-P)	−51·6±1·4 (118)	15·0	55·3±2·5 (20)	10·6±0·4	34·0±2·0
Propylthiouracil (1 rat, 2 G-P)	−37·8±2·1* (45)	13·8	69·6±6·3* (17)	13·7±2·1	42·1±3·9†
Thyroxine (1 rat)	−52·7±2·5 (39)	15·1	58·1±4·4 (12)	6·5±1·2*	45·0±4·5*
Hypophysectomy (1 rat)	−57·6±1·8* (48)	12·7	56·2±2·6 (30)	4·1±0·2*	47·9±2·4*
	Guinea-pig				
	Mean potential	S.D.	Mean follicle dimensions ±S.E. (μ)		
Treatment	±S.E. (mV)	(mV)	Follicle	Cells	Lumen
None (2 rats, 2 G-P)	−49·9±1·6 (91)	15·4	89·7±3·2 (28)	5·4±0·2	78·9±3·1
Propylthiouracil (1 rat, 2 G-P)	−37·9±1·7* (82)	15·6	98·3±5·8 (15)	9·5±0·6*	79·3±5·5
Thyroxine (1 rat)	—	—	—	—	—
Hypophysectomy (1 rat)	—	—	—	—	—
None (1 G-P)	−53·6±2·4 (23)	11·2	—	—	—
24 hr after TSH, 6 units (5 mg) (2 G-P)	−49·8±1·6† (60)	12·3	—	—	—
	−47·4±1·4* (82)	12·2	—	—	—

* Significantly different from untreated controls ($P < 0·05$); † $P = 0·1$–$0·05$.

around the thyroid and good impalements were difficult to obtain. The mean for a thyroxine-treated animal was somewhat higher than that for the untreated animals. Although the difference is not significant, the highest individual potentials, up to −90 mV, were obtained in this thyroxine-treated rat, and we have the definite impression that the actual transmembrane potentials were rather higher than those of untreated animals. The mean potential of a hypophysectomized rat was −57·6 mV, a significant increase. By way of contrast, the individual mean potentials obtained from two guinea-pigs 24 hr after injection of 5 mg TSH were −49·8 and −47·4 mV. The control animal, a cage-mate, gave a mean of −53·6 mV, rather higher than the control values shown in the upper part of the table. However, a year separated these two sets of control measurements. Accordingly, the −53·6 mV control value should be used for comparison, since results in Table 1 demonstrate that the potential depends on the hormonal state and the cage-mate control was more likely to be in the same hormonal state than the controls of the previous year.

Comparison of the relative sizes of cells and lumina in the various categories with the corresponding potential profiles shown in Figs. 2–4 provides strong evidence that these profiles are correlated with tissue structure. Neither age nor sex had detectable effects on the average potentials.

DISCUSSION

Correlation of potential profiles with histology

Despite the poor correlation between electrode advancement and actual tissue penetration and the absence *a priori* of information on cellular and luminal potentials of thyroid gland, the marked differences between the voltage–electrode advancement profiles of rat and guinea-pig thyroids leave little room for doubt that our interpretation of the results is substantially correct. This conclusion is further supported by the predictability of the changes in the profiles produced by the drug and hormone treatments. The interpretation of the records leads to the unequivocal conclusion that luminal fluid (LF) is near zero potential and that the interior of the thyroid cell is substantially negative to the interstitial space (IF) and hence also to LF.

No effort was made to estimate the deviations of luminal potentials from zero, since leakage current from damaged cells could easily have contributed a few millivolts. Since the composition of the LF is likely much the same as that of IF, tip potential errors in measurements of luminal potential would have been negligibly small. On the other hand, both injury and tip potentials could have appreciably reduced the measured

cellular potentials below their actual values. As mentioned above, tip potentials were measured only when the electrode broke during its advancement. On these occasions (which were progressively less frequent), tip potentials were 5 mV or less. Another, less objective, reason for believing that the tip potential errors were small is the 'feel' one gets after some experience for what the results should 'look' like on the recorder. Occasionally we tried several high-resistance electrodes before finding one that gave 'satisfactory' results. Some electrodes penetrated the tissue normally and gave sudden potential shifts, but these shifts were smaller than usual. The few measurements obtained from such electrodes were rejected on the basis that all known errors tend to reduce the observed potential. Thus, whether these low potentials resulted from use of broken, plugged electrodes or from use of high-tip-potential electrodes, they were rejected. In other words, selection of 'good' electrodes on this basis considerably reduced the variability of the results. The degree to which injury reduces the measured potential is difficult to assess. Injury should skew the frequency distribution of measured potentials to the low side. Such skewing is observed in some experimental results but no attempt was made to correct the measured values. Our impression is that the error is unlikely to exceed 5 mV.

Membrane potential and Na^+:K^+ permeability ratio, and membrane resistance

KCl depolarization. Figure 5 presents presumptive but not conclusive evidence that the membrane of the thyroid cell, like that of muscle and nerve cells, is much less permeable to Na^+ than to K^+. Clearly KCl, applied topically, depolarized the cell and some recovery occurred as the circulation reduced KCl concentration. However, the possibility that the depolarization resulted from the hypertonicity or from the blanching effect cannot be eliminated. Control experiments with equally hypertonic NaCl or sucrose solution were not attempted because of the great difficulty in maintaining a good impalement for even the length of time covered by the record in Fig. 5; it presents the only successful result of about 10 tries at the end of one regular experiment. (See note added in proof.)

The ratio of Na^+:K^+ permeability (p) can be estimated from the mean membrane potential on the basis of several assumptions: (1) The steady-state membrane potential is maintained by a one-for-one Na^+-for-K^+ exchange pump. (2) Active efflux of Na^+ is proportional to internal concentration of Na^+. (3) The pumping rate per unit concentration is much higher than passive Na^+ permeability (J. W. Woodbury, 1960). In this case p is given by $\exp(FV/RT) = ([K^+]_o + p[Na^+]_o)/[Na^+]_o$, where V is the transmembrane resting potential taken as a negative quantity and

the other terms have their usual meanings. Taking $V = -50$ mV $[Na^+]_o = 145$ and $[K^+]_o = 4{\cdot}14$ m-equiv/l. gives $p = 0{\cdot}12$ approximately, a value much higher than that for skeletal muscle and for nerve but commensurate with the lower resting potential.

More direct evidence is that $[K^+]_i \simeq 145$ m-equiv/l. cell water (D. M. Woodbury, unpublished observations) in thyroid. Assuming chloride is passively distributed (see below) and that Na^+ and K^+ are the only ions with appreciable transfer numbers, then the Goldman equation (Hodgkin & Katz, 1949) gives the identical answer for p, since $[K^+]_i \simeq [Na^+]_o$.

Maximum value of specific membrane resistance. It is common experience that electrode resistance increases when a cell is impaled. Most of this increased resistance is probably attributable to the higher specific resistivity of cell plasma, but in small cells some of the increase might be contributed by membrane resistance. A maximum value of membrane resistance can be calculated by assuming that the entire increase in electrode resistance is due to the cell membrane. From Table 1, the mean thickness of a normal rat thyroid cell is 10 μ; thus, the cell surface is no larger than a cube 10 μ on a side. At most, electrode resistance doubled. In a 50 MΩ electrode this would be an increase of 50 MΩ. The membrane specific resistance (R_m) is then, at most, $R_m = 50 \times 10^6 \Omega \times 6 \times 10^{-6}$ cm^2 = 300 Ω.cm^2. As the electrode resistance about doubled, regardless of its absolute value, when a cell was impaled (cf. Figs. 2–4), it seems likely that membrane resistance contributed little to the increase in electrode resistance. Thus, the true value of R_m is unlikely to be more than 0·1 of 300Ω.cm^2. Nevertheless, even the higher value is low compared with the values of nerve and skeletal muscle, 1000 to 10,000Ω.cm^2. Thus the permeability to at least one ion is much higher for thyroid cell membranes than for nerve or muscle cell membranes. The most likely possibility is a high permeability to monovalent anions in view of the active I^- transport function of the thyroid (see below).

Chloride distribution

Since Cl^- is distributed passively in skeletal muscle (Hodgkin & Horowicz, 1959) and motoneurones (Eccles, 1957) and is actively transported in gastric and intestinal mucosa (Hogben, 1955; Curran, 1960), the question how Cl^- is distributed in thyroid is open. The simplest assumption is that Cl^- is distributed passively, although it is quite possible that Cl^- has some affinity for the I^- pump. However, since these experiments have yielded an estimate of membrane potential, the passive distribution of Cl^- can be calculated. This calculated distribution can then be compared with the Cl^- distribution estimated from chemical measurements of the Cl^- and inulin spaces of the thyroid.

The mean potential in control rats (Table 1) is $-51{\cdot}6$ mV; hence the expected chloride ratio is $[Cl^-]_i:[Cl^-]_o = 10^{-51{\cdot}6/60} = 0{\cdot}138$. However, a more appropriate mean value is obtained by calculating the ratio for each potential measurement and then averaging the ratios. For control rats this average is 0·164, appreciably higher than the value obtained from the mean potential. The corresponding transmembrane potential is $-47{\cdot}2$ mV.

One of us (D.M.W.) has unpublished data on the chloride and inulin spaces of normal rat thyroid. Typical mean values are: $[Cl^-]_o = 115$ m-equiv/l.; fraction thyroid water = 0·751; inulin space = 0·304; total thyroid Cl^- = 43·4 m-equiv/kg wet tissue. Thus fraction cellular water is $0{\cdot}751 - 0{\cdot}304 = 0{\cdot}477$; interstitial Cl^- is $0{\cdot}304 \times 115 = 35{\cdot}1$ m-equiv/kg wet tissue; cellular Cl^- is $43{\cdot}4 - 35{\cdot}1 = 8{\cdot}3$ m-equiv/kg wet tissue; and $[Cl^-]_i$ is $8{\cdot}3/0{\cdot}477 = 18{\cdot}6$ m-equiv/l. The corresponding value from potential measurements is $[Cl^-]_i = 0{\cdot}164 \times 115 = 18{\cdot}9$ m-equiv/l. This extremely close agreement between the two independent estimates of $[Cl^-]_i$ must be regarded as fortuitous. Nevertheless, the quantitative agreement does constitute strong evidence that Cl^- is passively distributed in thyroid and that both the potential and chemical measurements are substantially correct. The alternative interpretation that Cl^- is actively transported into cells in appreciable amounts and that our measurements of potential are falsely and sufficiently low to compensate exactly for the extra Cl^- cannot be eliminated, but seems highly unlikely.

Thyroid potentials and iodine transport

Since the thyroid gland concentrates iodine and since thyroid potentials are either zero or negative, it can now be definitely concluded that iodine is actively transported from blood to thyroid no matter whether as I^-, as I_2 or in organic form. This conclusion is already widely accepted but complete proof has heretofore been lacking. Indeed, our results would have been suspect for a number of reasons if the measured transmembrane potentials in the thyroid had been internally positive, the condition necessary for passive accumulation of I^-.

It is of some interest to speculate on the site of the active I^- transport in the thyroid. Since transport is closely associated with membranes, there are the possibilities that the pumping membrane is facing the IF, or the LF, or both, and that I^- is either pumped into the cell interior and there converted to organic form, or is first pumped into the lumen and there converted. However, current ideas on thyroid iodine accumulation (Halmi, 1961), are difficult to reconcile with these and other data. If iodine is transported to the lumen as I^- before conversion, then one of the membranes should have an I^- pump and therefore should be practically

impermeable to I^-, and the other membrane should be highly permeable to I^-. This picture accords with our data indicating that R_m is small if it is assumed that the membrane permeable to I^- is also highly permeable to other monovalent anions, notably Cl^-. However, it is difficult to see what prevents I^-, as it is pumped into the lumen, from diffusing rapidly from LF to IF via the intercellular passages between circumluminal cells. These passages are only about 150 Å wide (Ekholm & Sjostrand, 1957), but zero luminal potential indicates that ions can flow through these passages. The large inulin space (30 %) suggests that these large molecules can penetrate into the lumen at least to some extent. Fixed negative charges on the walls of 150 Å water-filled passages could not prevent the diffusion of anions. It might be that I^- is bound near the luminal membrane as rapidly as it appears. However, the thyroid still accumulates I^- in the situation where propylthiouracil blocks incorporation of I^- into tyrosine (VanderLaan & VanderLaan, 1947). Another possibility is that the passages are filled with a negatively charged muco-polysaccharide which restricts anion but not cation movement.

The opposite view, that the whole membrane (interstitial and luminal sides) of the thyroid cell pumps I^- into the cell interior, is even more objectionable: the internal negativity opposes I^- accumulation, and in these circumstances the whole membrane should be highly impermeable to anions and R_m should be very high. The low R_m indicated by the experimental data could be attributed to high K^+ permeability, but this explanation is objectionable on the basis that a high K^+ permeability also implies a high Na^+ permeability. Consequently, there would be a large energy expenditure for Na^+ transport, an apparently needless waste even if the process were made useful by linkage of I^- transport to Na^+ transport.

Hormonal and drug effects on potentials

The data in Table 1 indicate that a change in circulating TSH causes a change of the opposite sign in the size of the membrane potentials. Thus the mean potential was $-57{\cdot}6$ mV in a hypophysectomized rat and $-37{\cdot}8$ mV in a propylthiouracil-treated rat, both means being significantly different from the mean of $-51{\cdot}6$ mV in normal rats. Similarly, 24 hr after TSH injection in two guinea-pigs (a time when the thyroid iodide-concentrating ability is maximum, D. M. Woodbury, unpublished observation), the mean thyroid potential was $-49{\cdot}8$ mV in one and $-47{\cdot}4$ mV in the other. The appropriate control value is $-53{\cdot}6$ mV. These experiments were done 1 year later than the previous ones. The reason for the higher control value is not known but TSH does affect voltage, and TSH levels probably vary.

Since iodine uptake varies directly with TSH level, the effect of TSH on

potential may give some further clues to the nature and location of the iodide pump and the factors controlling its rate. For example, if the pump is in the membrane facing the lumen, TSH could increase iodide uptake and lower potential simply by increasing the permeability of the outer membrane (facing the IF) to Na^+. The increased influx of Na^+ would lower potential and thus increase $[I^-]_l$. If the limiting factor in the pumping rate were $[I^-]_l$, then iodide uptake would increase. It is of interest in this connexion that Solomon (1961) has demonstrated an increased uptake of radio-sodium by the thyroid of the 1½-day-old chick under the influence of TSH. These data together with some unpublished observations (D.M.W.) suggest that iodide uptake is linked with Na^+ pumping.

SUMMARY

1. Cellular and luminal potentials of rat and guinea-pig thyroid glands have been measured with glass micro-electrodes. The location of the electrode tip was deduced by comparison of the variation in recorded potential as the electrode was advanced with cellular structure of the thyroid gland in the two species.

2. The luminal potential was zero with respect to the interstitial fluid. This relation was established from potential–electrode advancement profiles obtained in guinea-pig thyroids. These glands have large lumina surrounded by thin layers of cells. Consequently, the most likely tip location is luminal.

3. Cellular potentials average about 50 mV, inside negative with respect to the interstitial fluid. An intracellular tip location was established from profiles obtained from rat thyroids, in which the lumina are much smaller and the cells are much thicker than in guinea-pigs. Further confirmation came from measurements in propylthiouracil-treated guinea-pig thyroids, whose structure resembles that in normal rats.

4. Propylthiouracil treatment and TSH administration lowered the size of cellular potentials.

5. The potential measurements permit the definite conclusion that the uptake of iodine by the thyroid is active. If iodine is taken up as I^-, then no electrical work is required to transport it into the lumen. On the other hand, a considerable amount of electrical work must be done if I^- is concentrated inside the cells.

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Note added in proof. Preliminary unpublished observations by J. Williams and J. W. Woodbury on perfused rabbit thyroid demonstrate that elevated K concentrations in the perfusion fluid have a definite depolarizing effect on the measured transmembrane potentials. This supports the evidence presented on page 562 that local application of KCl causes depolarization of the thyroid cell membrane.

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