Biochimica et Biophysica Acta, 291 (1973) 116–126

BBA 76053

THE EFFECT OF K^+ ON THE MEMBRANE POTENTIAL IN HeLa CELLS

YASUNOBU OKADA[a], MASAHARU OGAWA[a,*] NATSUE AOKI[b] AND KOSAKU IZUTSU[b]

[a]*Department of Physiology, Kyoto University School of Medicine, Kyoto (Japan) and* [b]*Department of Pathology, Mie Prefectural University School of Medicine, Tsu (Japan)*

(Received March 30th, 1972)
(Revised manuscript received July 3rd, 1972)

SUMMARY

1. The membrane potential in HeLa cells was determined with the use of a microelectrode under visual control with a phase contrast microscope at a high magnification.

2. The mean value of the potential for 158 cells in culture media at 37 °C was -48.1 ± 0.95 mV (SE), interior negative, while the mean for 297 cells in the control Dulbecco's phosphate buffer solution was -48.2 ± 0.68 mV.

3. When chloride in the medium is partially replaced with sulphate at a fixed external K^+ concentration, $[K^+]_0$, the membrane potential was scarcely affected, whereas marked changes in the potential were observed with varying $[K^+]_0$ under a constant $[Cl^-]_0$.

4. Such a change in the membrane potential caused by varying $[K^+]_0$ obeys Nernst's equation in the range of $[K^+]_0$ higher than 20 mM, but deviates largely from the equation at lower K^+ concentrations. The potential increases with decreasing $[K^+]_0$.

5. Using Wickson-Ginzburg and Solomon's data (*J. Gen. Physiol.*, 46 (1963) 1303) on the ionic composition of HeLa cells, the value of P_{Na}/P_K in the range of $[K^+]_0$ less than 20 mM was estimated from Goldman's equation (*J. Gen. Physiol.*, 27 (1943) 37) under the assumption that Cl^- never contributes to the membrane potential. The ratio of the permeability constants markedly increases with decreasing $[K^+]_0$. Such an increase in the ratio P_{Na}/P_K, was discussed from the viewpoint of permeability of non-excitable cell membranes.

INTRODUCTION

It is of great importance to study the membrane potential of cancer cells in culture, not only to know properties of the cancer cell membrane but also to elucidate the ion transport mechanism of the cell membrane *per se* by comparing such an inexcitable membrane with excitable ones. There have been reported, however, only a few measurements of the membrane potential of cells in culture[1–4], probably because of technical difficulties. Recently Borle and Loveday[5] as well as Kunishima and

* Present address: Department of Pharmacology, Faculty of Pharmaceutical Sciences, Okayama University, Okayama, Japan.

Izutsu[6] reported on the electrical potential difference across the membrane of HeLa cells in culture. To compare the ion permeability of an inexcitable membrane to that of excitable one, however, these studies still seem not so satisfactory. We attempted, therefore, to measure the membrane potential of these cells in a wider range of external K^+ and Cl^- concentrations.

Our results are significantly different from those reported hitherto, demonstrating that the Nernst equation applies to such an inexcitable membrane as Hela cell membrane, though to a limited extent. This paper is chiefly concerned with this point.

MATERIALS AND METHODS

Cell culture

HeLa cells maintained uncloned were used exclusively. They were cultured in YLD medium[7] *plus* 20% bovine serum, 1 ml of penicillin–streptomycin mixture (10000 units and 10000 μg, respectively) being added per 100 ml. This culture medium is composed of Dulbecco's phosphate buffer saline[8], glucose, yeast extract, lactalbumin, and phenol red. Routine subcultures were made every 3.5 days in milk dilution bottles.

Membrane potentials were measured on the cells growing as monolayers on the bottom of hollow chambers. These were made by boring a 20 × 32 mm hole in a 30 mm × 76 mm glass slide 5 mm thick; a glass coverslip was then placed under the hole and sealed to the slide with an acetone solution of polyvinyl acetate; the acetone was allowed to evaporate before sterilization of the slide by dry heat.

HeLa cells were detached from the culture bottles by trypsinization and resuspended in the culture medium at a concentration of $1.5 \cdot 10^5$ cells/ml and 2.5 ml each of the cell suspension was poured in the well of the slide. The upper opening was then covered by another coverslip which was sealed with paraffin. These cells were cultured for 2 days at 37 °C as a monlayer on a coverslip constituting the bottom of a chamber.

Apparatus

Microscope

The measurements were made on an inverted microscope (Chiyoda Type T-2) equipped with phase contrast, long-working condenser and objective. To maintain the temperature of a cell chamber mounted on the microscope stage at 37 ± 0.5 °C, the temperature of the stage was controlled by circulating warm water with a pump.

Electrodes

Glass micropipettes were employed as recording electrodes filled with 3 M KCl, which were bent at an angle of 100° at a point about 7 mm apart from the tip by using a platinum filament as a heat source, their resistance ranging from 12–40 MΩ. Since Adrian[9] reported that the measured potential was seriously affected when the tip potential of a microelectrode was higher than 14 mV, we used only electrodes, whose tip potential was definitely less than 13 mV (on an average 5 mV). The measurement of the tip potential was made by the method described by Adrian[9] with slight modification. Reference electrodes were micropipettes filled with agar–3 M KCl whose tip had been broken off. Selecting an appropriate pair of Ag–AgCl electrodes for leading to a preamplifier, the greatest care was taken to minimize the effect of their liquid junction potentials by cancelling out each other.

Arrangement of apparatus

Experimental arrangement for the measurement of membrane potential is schematically illustrated in Fig. 1. Two Leitz micromanipulators were used to effect movement of these electrodes, which were led to the input of a high-input impedance preamplifier(Nihon Kohden, MZ-3B). For further amplification and recording of membrane potential an electronic polyrecorder (Toa Electronics, EPR-2T) was used.

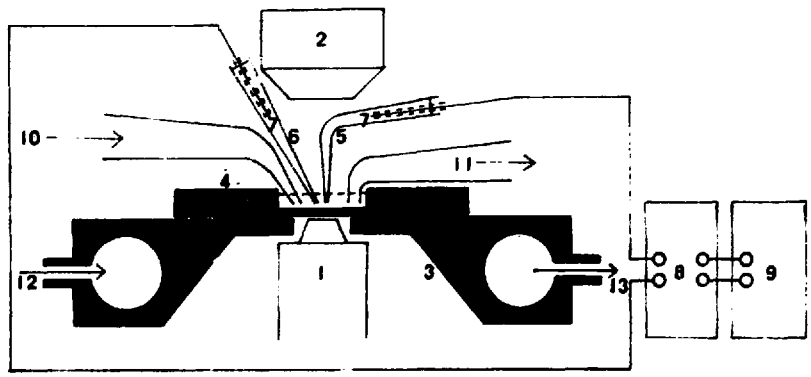

Fig. 1. Diagram of the experimental arrangement for the measurement of membrane potential in HeLa cells. (1) Long-working distance objective; (2) long-working distance condenser; (3) microscope stage; (4) cell chamber; (5) recording microelectrode; (6) reference microelectrode; (7) Ag–AgCl wire; (8) preamplifier; (9) penrecorder with amplifier; (10) inlet of solution; (11) suction of solution; (12) inlet of circulating water; (13) outlet of circulating water.

Solutions

As the control medium for HeLa cells under study, Dulbecco's phosphate buffer solution was used throughout the present study. In some occasions, however, Hanks' salt solution was employed.

In order to change K^+ and Cl^- concentrations in the external fluid, modified Dulbecco's solutions were prepared to have the same osmolarity and ionic strength as the standard Dulbecco's solution. Composition of these test solutions as well as the control one is illustrated in Table I.

TABLE I

COMPOSITION OF SOLUTIONS USED AS THE MEDIUM FOR MEMBRANE POTENTIAL MEASUREMENTS

A is Dulbecco's phosphate buffer solution. B–J are solutions of the same pH, tonicity and ionic strength as A but with different $[K^+]$ or $[Cl^-]$ Concentrations are expressed in mM.

Solution	K^+	Cl^-	Na^+	HPO_4^{2-}	$H_2PO_4^-$	Mg^{2+}	Ca^{2+}	SO_4^{2-}	*Sucrose*
A	4.2	142.5	153.0	8.0	1.5	0.5	0.9	—	—
B	4.2	14.3	110.7	8.0	1.5	0.5	0.9	42.9	127.0
C	4.2	1.4	109.0	8.0	1.5	0.5	0.9	47.2	140.0
D	10.5	142.5	146.7	8.0	1.5	0.5	0.9	—	—
E	22.8	142.5	134.4	8.0	1.5	0.5	0.9	—	—
F	41.5	142.5	115.7	8.0	1.5	0.5	0.9	—	—
G	100.6	143.0	56.1	8.0	1.5	1.0	0.9	—	—
H	3.1	142.5	154.1	8.0	1.5	0.5	0.9	—	—
I	2.1	142.5	155.1	8.0	1.5	0.5	0.9	—	—
J	0	142.5	157.2	8.0	1.5	0.5	0.9	—	—

Measurements

After removing the coverslip of the cell chamber at 48 h in culture, the procedure of measurement was carried out in the following manner.

The medium surrounding the cells was electrically connected with the reference electrode. As the shaft of the recording electrode was held by a micromanipulator at an angle of about 10 degrees from the horizontal plane with the stem end up, its tip approached the cell almost vertically from the top. Under direct microscopic observation, the electrode was inserted into a cell kept at 37 °C and the potential of the cytoplasm was recorded, care being taken not to impale the nucleus.

The criteria for a successful puncture were, (1) a sharp jump to peak voltage when the electrode penetrated into the cell; (2) an abrupt drop to a stable baseline when it was removed from the cell (see Fig. 2a). If the potential did not return to its original baseline on withdrawal from the cell (Fig. 2b), the data were rejected whatever their magnitude. All data included in this report were collected from the values of the initial peak thus obtained.

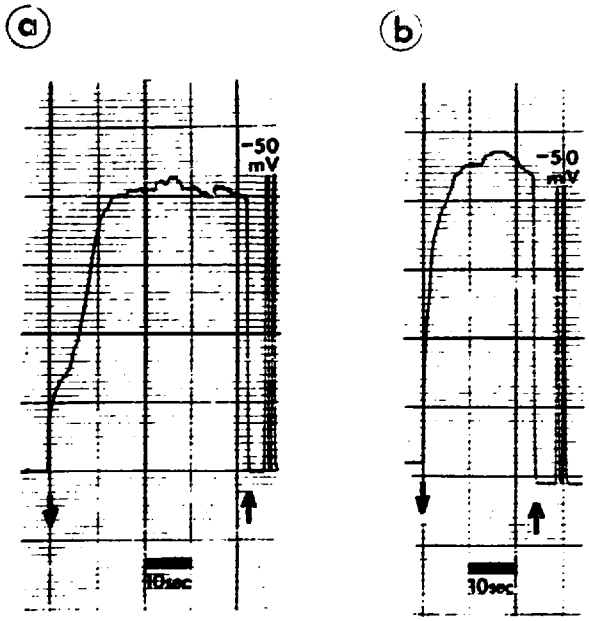

Fig. 2. Recording of the membrane potential in HeLa cells. Upward and downward arrows indicate the time of entry and exit of the recording microelectrode, respectively. (a) An example of a successful puncture; (b) an example of an unsuccessful puncture.

In order to avoid effects of differences in mitotic phases and in layer formation, HeLa cells used in the present study were only those in the interphase and those forming monolayer.

Exchange of a solution in the cell chamber with another test solution could be made almost instantaneously through the inlet and outlet tubes working under suction. When a test solution such as a potassium-rich one was applied, it was replaced by the standard Dulbecco's phosphate buffer solution within 15 min. Otherwise the cells were often found to deteriorate after an exposure to abnormal media for a long duration.

Since the initial peak was taken as a measure of the membrane potential, we could not continuously follow up changes in the potential in a test solution on

one and the same cell. All the data presented are, therefore, values obtained in different cells with successive punctures. Such a procedure resulted in a large fluctuation of data, so statistical analyses were applied.

RESULTS

The membrane potential in physiological media

The membrane potential of HeLa cells measured on 158 cells in YLD medium (supplemented with bovine serum) ranged, as shown in Fig. 3a, from −24 mV to −84 mV; their mean value being −48.1 ± 0.95 mV (S.E.).

In the control Dulbecco's phosphate buffer solution (Solution A in Table I), the membrane potential showed a quite similar frequency distribution (Fig. 3b) and its average was −48.2 ± 0.68 mV (n = 297), a value in good agreement with that in the culture medium. When the solution in the cell chamber was replaced by new solution at 30-min intervals to keep the medium fresh, the potential remained almost unaltered for at least 3 h; the initial membrane potential was −51.8 ± 2.00 mV (n = 9), after about 1 h −51.6 ± 0.82 mV (n = 18) and after about 3 h −50.5 ± 0.98 mV (n = 7). The differences between these values have statistically no significance ($P \gg 0.05$).

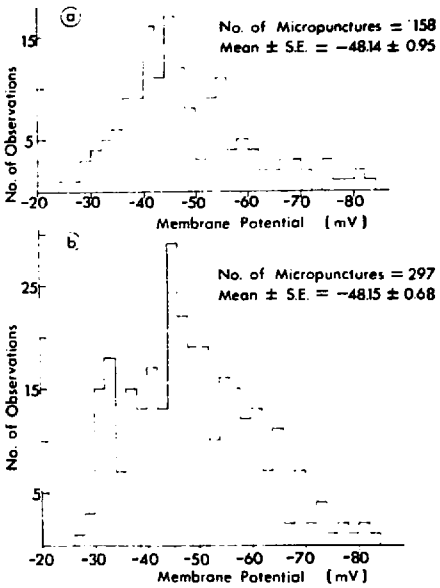

Fig. 3. The distribution of membrane potentials in HeLa cells at 37 °C. (a) In YLD medium; (b) in Dulbecco's phosphate buffer solution.

In Hanks' salt solution, HeLa cells also showed stable membrane potential. After the membrane potential in YLD medium was measured, the medium was replaced by Hank's salt solution. The potential measured 7 min after the replacement was −52.1 ± 3.06 mV (n = 16), while that in YLD medium was −51.4 ± 3.32 mV (n = 10). The difference between both values is not statistically significant ($P \gg 0.25$)

These results clearly demonstrate that the membrane potential of HeLa cells under nearly physiological conditions is stable and around −50 mV, a value significantly higher than that reported hitherto[5,6].

Effects of variation of $[Cl^-]_0$ *at constant* $[K^+]_0$.

In order to observe effects of reducing the external concentration of Cl^- on the membrane potential, test solutions B and C indicated in Table I, were employed, in which Cl^- in the control solution was partially replaced by SO_4^{2-} and the appropriate amount of sucrose was added to maintain the total tonicity as isoosmotic. The membrane potential in Solution B was -54.3 ± 1.54 mV ($n = 35$) and that in C -55.2 ± 1.32 mV ($n = 32$). In these solutions, the concentration of K^+ was maintained at 4.2 mM but the Na^+ concentration was reduced, so that the means value thus obtained cannot be compared to that in the control medium, but it is not so apart from the control value of about -50 mV. Studying the membrane potential of single muscle fibres, Hodgkin and Horowicz[10] reported that abrupt transient depolarization of about 20 mV occurred by suddenly reducing the concentration of Cl^-, but the potential drifted back to its original level with a time constant of 4 min. Because of technical difficulty we could not follow up changes in the potential immediately after replacing the medium solution. However several successful punctures with 1–2 min after the replacement never showed transient depolarization of such a magnitude as reported by these investigators. It might be said, therefore, that Cl^- as well as SO_4^{2-} made practically no significant contribution to the membrane potential of HeLa cells, at least for $[K^+]_0 = 4.2$ mM.

Now the constant-field equation derived by Goldman[11] and by Hodgkin and Katz[12] for the membrane potential of HeLa cells can be reduced to the following form by neglecting the term of the Cl^- concentration, if the extracellular and intracellular fluids are assumed to be nearly ideal solutions,

$$E = \frac{RT}{F} \ln \frac{[K^+]_0 + P_{Na}/P_K[Na^+]_0}{[K^+]_i + P_{Na}/P_K[Na^+]_i} \tag{1}$$

where P_i (i = Na or K) is the "permeability constant" of species i, $[K^+]_i$ and $[Na^+]_i$ the concentration of respective ion within the cell. Wickson-Ginzburg and Solomon[13] have already reported on the ion composition of HeLa cells. Substituting $[K^+]_i$ and $[Na^+]_i$ with the values reported by these investigators, Eq. 1 can be written as

$$E = 61.5 \log \frac{[K^+]_0 + \alpha[Na^+]_0}{140 + 50\alpha} \tag{2}$$

where α stands for the ratio, P_{Na}/P_K. Putting $E = -48.2$ mV, the average E of 297 cells in the control medium, α is calculated to be 0.13, a value far greater than that found for single muscle fibres[10]. Using this value for α, the membrane potential in Solutions B and C are predicted as -55.1 and -55.5 mV, respectively, which values are in fairly good agreement with those observed. Such a result seems to provide support for our assumption that the Cl^- term is safely omitted from Goldman's equation.

Effects of high $[K^+]_0$ *at constant* $[Cl^-]_0$

The effects of high K^+ concentrations on the membrane potential were studied with test Solutions D, E, F and G in Table I, in which Na^+ in the standard Dulbecco's solution (A in Table I) was partially replaced with K^+ but the Cl^- concentration was kept constant at 142.5 mM. As an example, the effect of suddenly increasing $[K^+]_0$

from 4.2 mM (Solution A) to 100.6 mM (Solution G) is illustrated in Fig. 4. When this solution was applied the potential of the cells immediately fell from their average level of −53.0 mV to a new level of −16.2 mV and remained at this level. On restoring the control Solution A, its recovery nearly to the original level occurred immediately. Hence such a change in the membrane potential induced by high $[K^+]_0$ seems to be reversible at least for about 10 min. With other test solutions, the results obtained were quite similar, the only difference being that the membrane potential level in depolarized states depends on $[K^+]_0$ applied.

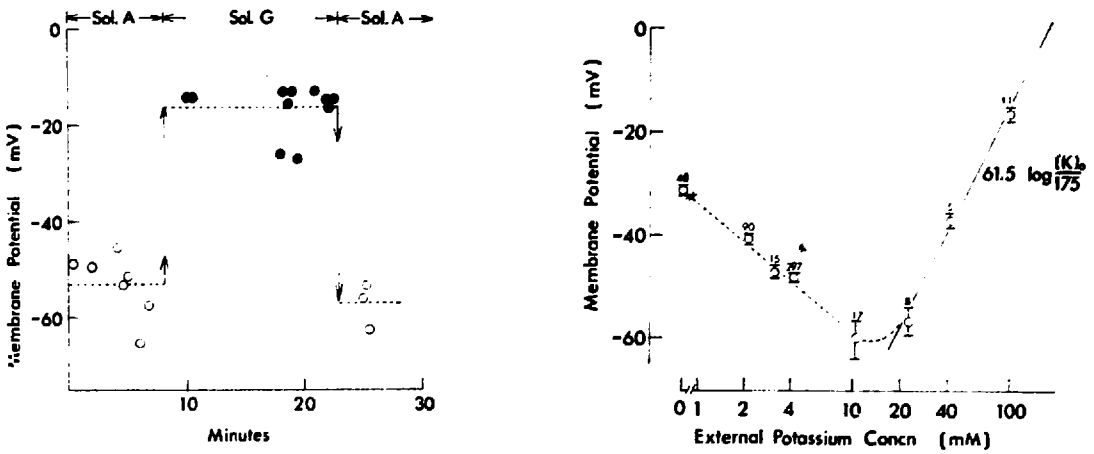

Fig. 4. The effect on membrane potential of changing $[K^-]_0$ from 4.2 to 100.6 mM at constant $[Cl^-]_0$. ○, with 4.2 mM K^+; ●, with 100.6 mM K^+. The dotted lines indicate the means levels of the potential observed at $[K^+]_0 = 4.2$ and 100.6 mM.

Fig. 5. The relation between membrane potential and log $[K^+]_0$. ○, the averages of membrane potentials. Vertical bars represent standard errors on either side of averages for number of measurements indicated. The solid line is drawn according to Eqn 3 in the text.

When such a potential level in depolarized states due to high $[K^+]_0$ was plotted against log $[K^+]_0$, a linear relation was obtained in the range of $[K^+]_0$ higher than 20 mM, which agreed well with the Nernst equation for a potassium electrode (as seen in Fig. 5) and was expressed as

$$E = 61.5 \log \frac{[K^+]_0}{175} \tag{3}$$

According to Wickson-Ginzburg and Solomon[13], $[K^+]_i$ and $[Na^+]_i$ remained nearly constant at 175 mM and 35 mM, respectively, when HeLa cells were equilibrated for 1 h with media containing more than 20 mM K^+. Our results indicate that Eqn 1 with $\alpha = 0$ is applicable by substituting $[K^+]_i$ and $[Na^+]_i$ with the values reported by these investigators. In our experiments, such an equilibrium of ions between the inside and outside of the cells may not yet have been attained and so an objection is raised to the application of the values for internal ion concentration observed at the equilibrium. But if the exchange of ions is considerably rapid, our results are not so unexpected. Our results also suggest that α is practically zero in high-K^+ media.

Effects of low $[K^+]_0$ *at constant* $[Cl^-]_0$

The effects of low K^+ concentrations were studied with test Solutions H, I and J as shown in Table I, in which KCl in the control Dulbecco's Solution A was appro-

priately replaced with NaCl. Fig. 6 illustrates the results obtained on applying these test solutions successively with an interval allowing recovery in the control medium. Large scattering of observed values resulted from the nature of the present experiment and, in view of the large variation in the membrane potential under nearly physiological conditions, it seems to be unavoidable. Statistical analyses show, however, that not only the differences between the means potential levels in three test solutions from each other are significant with $P < 0.05$, but also they significantly ($P < 0.05$) differ from the average of the potential observed in the control solution (-51.0 ± 0.27 mV, $n = 24$). Contrary to our expectation from the effects of high K^+ concentrations, therefore, the membrane of HeLa cells is rather depolarized at low K^+ concentrations. Moreover, Fig. 6 shows that such a depolarization is reversible except for the K^+-free medium, its immediate recovery being observed on restoring the control Dulbecco's solution. After exposure to the K^+-free medium lasting over 20 min, HeLa cells appear to be deteriorated; the membrane potential never returned up to the original level, but decreased progressively. Such a tendency is also seen in the figure.

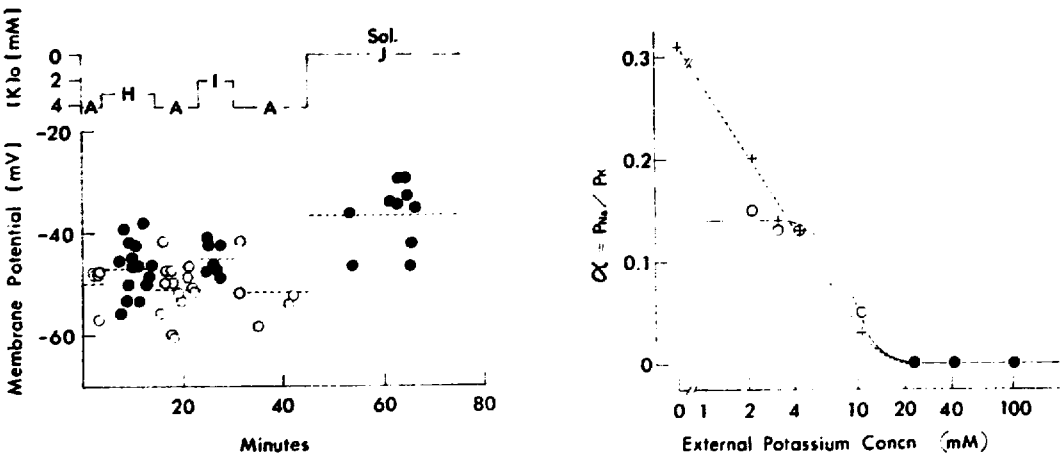

Fig. 6. The effect on membrane potential of lowering $[K^+]_0$ from 4.2 to 3.1, 2.1 and 0 mM successively at constant $[Cl^-]_0$. ○, observations with 4.2 mM K^+; ●, observations with lower K^+. The dotted lines indicate the average levels of the membrane potentials. The solid line indicates the external K^+ level.

Fig. 7. The relation between α values, P_{Na}/P_K and log $[K^+]_0$. Closed circles are the α values in the range of higner $[K^+]_0$ than 20 mM, so they indicate that the changes of the membrane potential agree with the Nernst equation in this region. +, the α values estimated by applyinge Eqn 2 at lower $[K^+]_0$. ○, the α values modified from the data of Wickson-Ginzburg and Solomon[13] at lower $[K^+]_0$.

The relation between the membrane potential and log $[K^+]_0$ is included in Fig. 5, which shows that the results observed deviate considerably from the Nernst line but the potential varies almost linearly with log $[K^+]_0$. If the constant-field equation in the form of Eqn 1 is still applicable at low K^+ concentrations, such a log-linear relation between $[K^+]_0$ and E would be explained by changes in the α value related to $[K^+]_0$. Using the observed values of the membrane potential and Eqn 2, the value of α can be calculated for a given $[K^+]_0$ and $[Na^+]_0$. In Fig. 7, the α values thus estimated ($\times$---$\times$) are plotted against log $[K^+]_0$, which shows that α increases almost linearly with decreasing log $[K^+]_0$. On the other hand, as mentioned above, α should be practically zero for $[K^+]_0 > 20$ mM.

DISCUSSION

One of the present authors has already reported on the membrane potential of HeLa cells[6]. Since the earlier work seemed unsatisfactory from the technical point of view, however, the present study was attempted. The results presented above demonstrate that the membrane potential of these cells equilibrated with their culture medium as well as with the normal Dulbecco's phosphate buffer solution is considerably high (about −50 mV) and obeys the Nernst equation as a potassium electrode at high external K^+ concentrations, being in a sharp contrast with the results reported by Borle and Loveday[5].

As a culture medium, these investigators employed a minimal essential medium and Hanks' salt solution, whereas YLD medium was used in the present study. As stated above, however, our results demonstrate hardly any significant difference between the mean potential levels in both culture media. The cells were cultured for 2 days in our experiments, but for 3 days in those of Borle and Loveday. Even if Eqn 1 is only approximately applicable, it would be expected from the data of Wickson-Ginzburg and Solomon[13] on the ionic composition of HeLa cells that the potential of these cells cultured for 3 days would be higher than that for 2 days. Neither difference in the culture media used nor that in the period of culture, therefore, seem to provide any convincing explanation for such a discrepancy as found on the membrane potential levels. The third point to be taken into account is the difference in the criteria for a successful impalement of a recording electrode into a cell. For the membrane potential of HeLa cells, Borle and Loveday[5] used the potential level which maintained a stable plateau for at least 5 s, while the initial peak value employed in the present study was as Aull[2] made on Ehrlich mouse ascites tumor cells.

Since puncture with a microelectrode injures the cell membrane and results in some leak from cell interior, a lower potential and the deviation from the Nernst equation are expected from Borle and Loveday's[5] procedure. Moreover, these investigators made no mention of the liquid junction potential and the tip potential of their electrodes which considerably affected the potential measured. On the other hand, the membrane potential of HeLa cells reported here obeys the Nernst relation in the high $[K^+]_0$ region and deviates from it at lower K^+ concentrations, a pattern quite similar to that of the resting potential in many other excitable membranes. At present, therefore, our results appear to be more reasonable.

For K^+ concentrations less than 20 mM, deviation from the Nernst equation is quite remarkable; the membrane of HeLa cells is more depolarized with decreasing $[K^+]_0$. This finding forms a marked contrast to the result of experiments on many excitable membranes. Fig. 6 suggests that the deviation is not due to some irreversible damage of the membrane so that, from the viewpoint of "ionic theory", other factor(s) such as an increase in the permeability to some ion species other than K^+ should be taken into consideration.

According to studies by Hempling and his group[14–17], Cl^- appears to be only passively distributed across ascites tumor cell membranes. This we assumed to be similar in HeLa cell membranes. This assumption is of a speculative nature until measurements of fluxes of Na^+, K^+ and Cl^- are made on HeLa cells.

But our observations on the effect of varying $[Cl^-]_0$ upon the membrane potential suggest that this assumption is probably reasonable. It seems probable, therefore,

that Cl^- has no bearing on the above-stated deviation in low $[K^+]_0$. Furthermore, such a reasoning provides a support for the use of the constant-field equation in the form of Eqns 1 and 2.

The values of α, the ratio of P_{Na} to P_K, estimated from the potential at low external K^+ concentrations by applying Eqn 2 range from 0.13 to 0.31 and increase exponentially with decreasing $[K^+]_0$ as shown in Fig. 7 ($\times \cdots \times$).

The internal K^+ and Na^+ concentration of HeLa cells bathing in a medium of extremely low K^+ concentration could not be regarded as the same as those under physiologically normal conditions and so a straightforward application of Eqn 2 may be doubtful. Indeed, if Eqn 2 is used to estimate α in the range of high external K^+ concentrations, large negative values are obtained, whereas, as stated in the previous section, the use of the values of $[K^+]_i$ and $[Na^+]_i$ on the cells equilibrated with the media provided a reasonable explanation for the E–log $[K^+]_0$ relation (Eqn 3), which conformed with the Nernst equation as a potassium electrode in high K^+ media. Such a fact appears to suggest that exchange of K^+ and Na^+ across HeLa cell membranes is not so slow such that $[K^+]_i$ and $[Na^+]_i$ in the media of various ratios of $[K^+]_0/[Na^+]_0$ rapidly reach the levels not so far from those at the equilibrated state. It may be more reasonable for considering changes in α for the entire range of $[K^+]_0$, therefore, to apply also here the results of Wickson-Ginzburg and Solomon[13], from which $[K^+]_i$ and $[Na^+]_i$ of the cells equilibrated for 1 h with any low-K^+ media could be estimated by interpolation. The α values thus calculated are presented in Fig. 7 (o—o). As seen in the figure ,the α values abruptly increase from zero to about 0.13 at $[K^+]_0$ = 5–10 mM. This finding suggests an abrupt change in membrane properties.

Irrespective of which is the real case — a gradual increase in α with decreasing $[K^+]_0$ or an abrupt increase in α around $[K^+]_0$ = 10 mM — it seems worthy to note here that the ratios of P_{Na} to P_K estimated from the potential at low K^+ concentrations (including the physiological region) are much higher by far than that of many excitable membranes. For instance, the α values roughly estimated by applying Eqn 1 to the membrane potential of some excitable tissues at low K^+ concentrations are as follows; 0.04 for squid giant axon[18], 0.03 for sepia giant axon[19], 0.05 for myelinated nerve fibre[20] and 0.01 for muscle fibre of frog[10].

A high value of α results either from high P_{Na} or from low P_K or from both. Indeed, high permeability to Na^+ in Ehrlich mouse ascites tumor cells has been reported by several investigators[2,21,22]. Schanne and Coraboeuf[23] suggested that high Na^+ permeability may be one of the characteristics of inexcitable cells. Such a high permeability to Na^+ shared with other tumor cells and other inexcitable cells seems to afford an explanation for our findings that, in low $[K^+]_0$ media, the membrane potential of HeLa cells is lower and their α values higher than those of excitable cells. But it has still remained unsettled how to explain the fact that α decreases nearly to zero at $[K^+]_0$ of about 20 mM, giving the potential which obeys the Nernst equation.

The above-stated discussions are based on the assumption that not only the activity coefficients of Na^+ and K^+ bathing solutions but also those within the cell are identical and so cancel in the Goldman equation. Recent experimental studies on toad oocytes have shown, however, that the activity coefficient of Na^+ within the cell is only 0.36, whereas that of K^+ is 0.73 (ref. 24). Similar results have been also reported on some excitable cells[25–30]. In the absence of any experimental evidence with

respect to the HeLa cells, our assumption might be allowable. Moreover, it was found that our qualitative conclusions were not altered even if the values of the activity coefficients cited above were applied.

So far as we discuss the problem from the viewpoint of so-called ionic theory and apply the Goldman–Hodgkin–Katz equation, it is difficult to afford a substantial explanation; for all the changes in the membrane phenomena are attributed to changes in P_i values, operational parameters. Further detailed studies, such as on ion flux or electrogenic pump of the cell, are needed to elucidate the problem.

ACKNOWLEDGEMENTS

We wish to express our thanks to Prof. A. Inouye of the department of Physiology, Kyoto University School of Medicine, for much helpful advice and for the revision of this manuscript. We also wish to thank Dr. K. Kamino for much helpful discussion. We are greatly indebted to Mr K. Yamato who kindly made the equipment used in the present experiments.

REFERENCES

1 H. G. Hempling, *J. Cell. Comp. Physiol.*, 60 (1962) 181.
2 F. Aull, *J. Cell. Physiol.*, 69 (1967) 21.
3 K. Redmann, Ch. Stolte and D. Luders, *Naturwissenschaften*, 255 (1967) 54.
4 A. Prieto, P. L. Kornblith and D. A. Pollen, *Science*, 157 (1967) 1185.
5 A. B. Borle and J. Loveday, *Cancer Res.*, 28 (1968) 2401.
6 N. Kunishima and K. Izutsu, *Symp. Cell. Biol.*, 21 (1970) 279 (in Japanese).
7 M. Taki, *Mie Med. J.*, 19 (1970) 245.
8 D. J. Merchant, R. H. Kahn and W. H. Murphy, Jr, *Handbook of Cell and Organ Culture*, Burgess Publishing Co., Minneapolis, U.S.A. 1960.
9 R. H. Adrian, *J. Physiol.*, 133 (1956) 631.
10 A. L. Hodgkin and P. Horowicz, *J. Physiol.*, 148 (1959) 127.
11 D. E. Goldman, *J. Gen. Physiol.*, 27 (1943) 37.
12 A. L. Hodgkin and B. Katz, *J. Physiol.*, 108 (1949) 37.
13 M. Wickson-Ginzburg and A. K. Solomon, *J. Gen. Physiol.*, 46 (1963) 1303.
14 H. G. Hempling, *J. Gen. Physiol.*, 41 (1958) 565.
15 H. G. Hempling, *Fed. Proc.*, 18 (1959) 67.
16 H. Grobecker, H. Kromphardt, H. Mariani and E. Heinz, *Biochem. Z.*, 337 (1963) 462
17 H. G. Hempling and H. Kromphardt, *Fed. Proc.*, 24 (1965) 709.
18 H. J. Curtis and K. S. Cole, *J. Cell. Comp. Physiol.*, 19 (1942) 135.
19 A. L. Hodgkin and R. D. Keynes, *J. Physiol.*, 128 (1955) 61.
20 A. L. Hodgkin and R. Stämpfli, *J. Physiol.*, 112 (1951) 496.
21 B. M. Johnstone, *Nature*, 183 (1959) 411.
22 F. Aull and H. G. Hempling, *Am. J. Physiol.*, 204 (1963) 789.
23 O. Schanne and E. Coraboeuf, *Nature*, 210 (1966) 1390.
24 D. A. T. Dick and S. G. A. McLaughlin, *J. Physiol.*, 205 (1969) 61.
25 J. A. M. Hinke, *Nature*, 184 (1959) 1257.
26 J. A. M. Hinke, *J. Physiol.*, 156 (1961) 314.
27 A. A. Lev, *Nature*, 201 (1964) 1132.
28 S. G. A. McLaughlin and J. A. M. Hinke, *Can. J. Physiol. Pharmacol.*, 44 (1966) 837.
29 J. A. M. Hinke and S. G. A. McLaughlin, *Can J. Physiol. Pharmacol.*, 45 (1967) 655.
30 S. G. A. McLaughlin and J. A. M. Hinke, *Can. J. Physiol. Pharmacol.*, 46 (1968) 247.