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The Passive Transport of Potassium in Rat Liver Cells

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Summary. The effect of high-K solutions on the intracellular concentrations and fluxes of K, Na and Cl have been studied in rat liver perfused with high-K solutions. External K induces a sigmoid increase in unidirectional K fluxes. This transport of K cannot be interpreted as a response to membrane depolarization or to a change in internal K concentration, but may be explained by a 12-fold increase in potassium permeability. Na and Cl permeabilities are not significantly altered. After a 10-min exposure of the liver to high-K solutions a sudden hyperpolarization develops when normal medium is restored. The good fit between the amplitude of this measured hyperpolarization and that predicted from K fluxes indicates that K transport, as induced by external K obeys the independence principle. The relation between passive fluxes and external K can be accounted for if it is assumed that K can cross the membrane combined with a carrier molecule. Three models are proposed to explain the K dependence of the carrier concentration.

Previous experimental studies have shown that the membrane potential of hepatic cells *in vitro* is less dependent on external K than on external Na or Cl (Schanne & Coraboeuf, 1966). This is illustrated by the very low slope of the relation between internal potential and external K found in these cells. This finding indicates that K ions make a minimal contribution to membrane potential. However, more recently, it has been pointed out that the membrane becomes increasingly sensitive to K if the cells are equilibrated in high-K solutions (Claret, Favier & Coraboeuf, 1970). After 10 min of soaking, the membrane potential behaves roughly like a potassium electrode. This large increase in the slope of the relation $Em - [K]_o$ suggests that K ions then carry most of the membrane current. Such a change in the membrane sensitivity to K was interpreted by assuming that the relative K permeability of liver cells was somehow related to external K and/or to the magnitude of the driving force as described in several excitable tissues (Hodgkin & Horowicz, 1959; Carmeliet, 1961; Adrian & Freygang, 1962; Müller, 1965; Noble, 1965; Sjodin, 1965).

The present experiments were designed to determine some properties of the process underlying the increased membrane sensitivity to K. To achieve this, the flux dependance on external K was tested for K, Na and Cl. According to the laws of diffusion, the passive movement of an ion through the membrane is related to permeability, to concentrations, and to potential by the following equations:

$$\Phi_o = PC_i f(Em) \tag{1}$$

$$\Phi_i = PC_e f'(Em) \tag{2}$$

where Φ refers to unidirectional fluxes, C to aqueous concentrations, $f(Em)$ and $f'(Em)$ to factors representing the effect of the electrical field on ions crossing the membrane, and subscripts o and i outside and inside, respectively. If the movements of K obey Eqs. (1) and (2), according to the independence principle (Ussing, 1949), an increase in K permeability at constant membrane potential should increase both the K fluxes, but have no effect on the movements of the other ions present.

The data demonstrate that the external K induces large movements of K through the membrane. The good fit between the changes in membrane potential as predicted from measured fluxes and those actually observed leads to the conclusion that K transport occurs by diffusion of ions along their electrochemical gradient. This transport is highly selective and rises as a sigmoid function of external K concentration. Such a dependance of diffusional fluxes on K is interpreted here by assuming that external K controls the membrane concentration of a passive carrier. This carrier, the movement of which is limited to the membrane, provides for the K transport (Horowicz, Gage & Eisenberg, 1968).

A preliminary account of the present results has already appeared (Claret, Claret & Mazet, 1974).

Materials and Methods

The general methods used here for the determination of fluxes, for cation analysis, for membrane potential measurements, and for perfusion of isolated liver are the same as those reported in detail in a previous study (Claret & Mazet, 1972).

Experiments were performed on rat livers perfused with saline solutions, at constant pressure (13 ml H_2O). A standard Tyrode's solution of the following composition was used (mM): Na, 144; K, 5.6; Ca, 2.2; Mg, 1.2; Cl, 141; HCO_3, 15; H_2PO_4, 1.2; glucose, 11.5. In two experiments, NaCl was replaced by LiCl. Livers were equilibrated in these solutions for at least 30 min before the experiments began. Changes in external K concentration were made by replacing NaCl (or LiCl) with equimolar quantities of KCl. Radioactive solutions were prepared to obtain specific activities of 1 to 2 $\mu C\,ml^{-1}$ (^{24}Na and ^{42}K supplied by C.E.A., Saclay) and 0.01 to 0.05 $\mu C\,ml^{-1}$ (^{36}Cl and [^{14}C] inulin, supplied by the Radiochemical Centre, Amersham). Radioactive counting was

carried out as before with an autogamma spectrometer, or with a liquid scintillation spectrometer. All the solutions were aerated with 97% O_2 and 3% CO_2. The pH was maintained at 7.4, and the temperature at 35 °C.

Total ion concentrations were determined from small pieces of perfused liver after 20 min of equilibration in K-rich solutions ($[K]_o = 56$ and 112 mM). K and Na contents were measured by flame photometry and Cl content was determined by measuring the activity of tissue samples after complete equilibration with ^{36}Cl, as in the previous study. Total water was obtained from the wet weight and the dry weight, and extracellular water estimated from the distribution space of [^{14}C]inulin. Ion and water concentrations of control livers perfused with 5.6 mM K, taken from a previous study (Claret & Mazet, 1972), are set forth in Table 1 for comparison purposes.

Effluxes of K and Cl (Φ_{oK} and Φ_{oCl}) were calculated by using the equation: $\Phi_o = k C_i V/A$ (Keynes, 1954), where k is the rate constant of ionic loss into the inactive solution, C_i is the amount of intracellular ions expressed in cellular volume unit, and V/A the volume/surface ratio of liver cells. To determine the dependance of Φ_{oK} and Φ_{oCl} on external K, the livers, after 30 min of loading with ^{42}K and ^{36}Cl, were first washed with Tyrode's solution, then for 20 min with an inactive solution containing a higher concentration of K (22, 33, 56 or 112 mM). The radioactivity lost by the tissues was calculated from the effluent activity and hepatic flow rate. The slope of the semi-logarithmically plotted activity of the effluent was taken to represent the value of the rate constant.

The K influx was obtained independent of the cellular entry of ^{42}K after a 10-min loading period. In experiments in which the pumped fraction of total K uptake was not negligible (5.6, 20 and 33 mM, *see* Results), the K influx was determined in the presence of ouabain. Livers were first perfused with a solution containing a given K concentration (5.6, 20 or 33 mM). Ouabain was then added to the medium and the cells re-equilibrated for 5 min. The ^{42}K influx was then measured over a 10-min period.

Since, in loading experiments, an appreciable fraction of intracellular ions was sometimes exchanged with labeled ions, the apparent ion uptake of K and Na had to be corrected by the factor: $kT/[1-\exp(-kT)]$ where k is the rate constant of efflux at a given K concentration and T the loading time (Keynes, 1954).

In high-K solutions or in the presence of ouabain, the perfusion flow rate was altered as a result of salt and water entries into the cells and/or of vascular smooth muscle contracture. Within the framework of the diffusion model recently described (Claret & Mazet, 1972), the correction applied to each experiment ranged from 2 to 7 per cent. However, this model assumes that the concentration throughout the capillary bed was the same as that of the effluent and therefore does not take into account the presence of an eventual gradient of concentration of ^{42}K along the capillary bed. Since such a simplification could become inconvenient were the perfusion flow rate to be significantly lowered, the relationship between the rate constant of K efflux and the perfusion flow rate was studied to determine if the model needed to be improved. The results show that there is no relationship between these two variables: $r = -0.2$ ($n = 19$); $r = -0.32$ ($n = 10$); $r = 0.17$ ($n = 6$), respectively, for 5.6, 56 and 112 mM K. It could thus be concluded that, in the range of experimental values, the exchange rate constants do not depend on the flow rate and that, as a first approximation, the model previously described was appropriate.

In experiments reported here, K and Cl permeabilities were calculated from Eq. (1) and Na permeability from Eq. (2) (Goldman, 1943; Hodgkin & Katz, 1949) where:

$$f(Em) = (Em F/RT)/[1-\exp(-Em F/RT]$$

$$f'(Em) = (Em F/RT)/[\exp(Em F/RT)-1].$$

Em is the membrane potential inside the cell, and *F*, *R*, and *T* have their usual significance.

The volume/surface ratio needed to estimate fluxes calculated from histological sections of liver cells was found equal to 2.50 μ in livers perfused with standard solutions (Claret & Mazet, 1972). However, the result of the increase in $[K]_o$ at constant $[Cl]_o$ is that the cells gain KCl and water. This cell swelling could be estimated from the relative variations of intra- and extracellular space. *V/A* ratios were found to be 2.77 μ and 3.28 μ for 56 and 112 mM K, respectively.

All the results are expressed as mean ± SD of an observation.

Results

Effects of K on Intracellular Ion Concentrations

The values obtained from chemical and radioactive analysis are given in Table 1. Total and extracellular water, total ion content and membrane potential measured at steady state (i.e., after a 20-min equilibration in high-K solutions) are presented as a function of external K concentrations. The intracellular water, the concentration and equilibrium potentials of ions were then calculated from these experimental values.

Table 1. The effect of K on water and ion content and on membrane potential of liver cells

$[K]_o$ (mM)	5.6	56	112
Total water (ml kg^{-1} wet wt)	720 ± 20	749 ± 13	782 ± 16
Extracellular water (ml kg^{-1} wet wt)	250 ± 21	231 ± 29	222 ± 30
K_T (mmoles kg^{-1} wet wt)	80.4 ± 3.2	95.0 ± 7.3	106 ± 14.0
Na_T (mmoles kg^{-1} wet wt)	11.5 ± 3.3 [b]	24.0 ± 8.0	4.6 ± 1.3
Cl_T (mmoles kg^{-1} wet wt)	49.5 ± 4.1	53.3 ± 1.6	77.5 ± 4.0
Em (mV)	−32.8 ± 1.5	−25.2 ± 2.1	−10.4 ± 4.1
Intracellular water (ml kg^{-1} wet wt)	471	518	560
$[K]_i$ (mM)	164	158	145
$[K]_c$ (mmoles · l^{-1} cells)	111	113	111
$[Na]_i$ (mM)	24	11	1.1
$[Cl]_i$ (mM)	38	46	88
E_K (mV) [a]	−90.2	−27.7	−6.9
E_{Cl} (mV) [a]	−32.2	−27.1	−9.8
V/A (μ)	2.50	2.77	3.28
A (cm^{-2} mg^{-1})	2.82	2.62	2.25

Values in the first column were taken from a recent study and are given for comparison (*see* Materials and Methods). Intracellular water and ion concentrations, the volume/surface (*V/A*) and the surface (*S*) of the cells were estimated as indicated in the Methods section.

[a] Equilibrium potentials were calculated from Nernst's equation.

[b] The Na concentration in 5.6 mM K was determined by integrating in the ^{24}Na efflux curve. So, this value does not include the extracellular Na pool.

The most patent effect of liver equilibration in 56 mM K is to induce a net movement of water between the extra- and intracellular compartments. Total water increases while the fraction of extracellular water decreases. These effects probably reflect the cell swelling due to a cellular uptake of water. At the same time, a decrease in internal Na concentration is observed, probably due to the replacement of external Na by K. The slight increase in chloride concentration that occurs in the same solution can be attributed to the passive redistribution of this ion throughout the cell membrane of the hepatocyte (Claret & Mazet, 1972). This point is illustrated by the maintenance of the equality of chloride equilibrium potential and membrane potential.

All the effects on liver perfused with 56 mM K described above were systematically enhanced when external K concentration was increased to 112 mM. It must be noted, however, that despite these changes the 10 and even the 20-fold increase in $[K]_o$ does not significantly alter the internal K concentration.

It seems likely that the cell swelling which develops in 56 and 112 mM K without $[K]_i$ alteration results from a net passive entry of KCl and water through a mechanism similar to the one described in frog muscle fiber (Boyle & Conway, 1941). The result of these increases in $[K]_o$ is that K and Cl enter the cells at the same rate to preserve electroneutrality, and, together with water, to maintain osmotic equilibrium. This movement continues until a new steady state is reached at which the K and Cl equilibrium and membrane potentials approach each other ($E_K = E_{Cl} = Em$). Assuming that only the K and Cl ions participate in the establishment of this equilibrium, the data reported in Table 1 make it possible to estimate that cells gain about 14 and 33 mM of KCl, and 98 and 218 ml of water per Kg of liver in 56 and 112 mM K, respectively.

Effect of K on K Efflux

The effects of $[K]_o$ increase on K efflux are summarized in Table 2. The values of rate constants of ^{42}K efflux (k) were measured at apparent steady state after 10 min of equilibration in each solution. The transitory variations in the rate constant for the on-and-off effects are not reported here. Table 2 shows that the rate constant is markedly increased by external K. This effect was always reversible when returning to normal medium. Between 5.6 and 33 mM K, Em remains constant. If Eq. (1) is valid, remembering that $[K]_i$ remains unaltered, the variation in Φ_{oK} is the consequence of a proportionate alteration in the permeability coefficient. In high-K solutions, the ionic

Table 2. Effect of K on K efflux and on membrane potential

$[K]_o$ (mM)	k_K [a] (hr^{-1})	Em (mV)	Φ_{oK} (pmoles cm^{-2} sec^{-1})	P_K (10^{-8} cm sec^{-1})
5.6	0.84 ± 0.14	-32.8 ± 1.5	6.6	7.9
22	2.13 ± 0.39	-34 ± 3.2	16.5 [b]	20.5 [b]
33	3.60 ± 1.22	-32 ± 1.8	28.1 [b]	33.1 [b]
56	7.55 ± 1.86	-25.2 ± 2.1	68	69
112	11.2 ± 1.7	-10.4 ± 4.1	127	91

[a] Rate constants of K efflux were corrected for diffusion delay of ions in the interstitial space.

[b] Values of Φ_{oK} and P_K in 22 and 33 mM K were calculated based on the assumption that intracellular K concentration and volume/surface ratio are the same as those found in 5.6 mM K.

strength was maintained constant by lowering Na concentration. The question therefore arises whether the rise in K efflux was not induced by the decrease in $[Na]_o$. To make certain that Na was not acting on K efflux, additional tests were made with solutions in which total NaCl was replaced by an equimolar quantity of LiCl. A 10-fold increase in $[K]_o$ still results in a similar rise in K efflux. It was therefore concluded that Na ions do not directly participate in the phenomenon.

In 112 mM K, the effect on rate constant was still more marked. The data show that K efflux grows by a factor of 19. The absolute value of membrane potential declines from 33 to 10 mV on the average. Within the framework of the ionic diffusion theory, it is possible to predict a consequent increase of 1.5 in the factor $f(Em)$ as estimated by Eq. (1). Assuming the observed K efflux to be entirely passive, it may be calculated that apparent potassium permeability increases 12-fold when $[K]_o$ is changed from 5.6 to 112 mM K.

Effect of K on K Influx

Measured influx values as compared to efflux values, make it possible to test whether the K transport induced by external K obeys the independence principle or not (Ussing, 1949). If some interaction between passive K fluxes occurs, the direction of deviation of the flux ratio from the independence law enables us to determine whether K facilitates the movement of K ions going in the same direction, as described in nerve and muscle fibers (Hodgkin & Keynes, 1955; Horowicz *et al.*, 1968), or in the opposite direction as, for instance, in K–K exchange-diffusion (Sjodin, 1965). These points were examined by measuring the effects of changes in external K concentration on ^{42}K uptake.

Table 3. Effect of K on K influx

$[K]_o$ (mM)	Ouabain (mM)	Qc [a] (mmoles kg^{-1} min^{-1})	Φ_{iK} (pmoles cm^{-2} sec^{-1})	Φ_{iK}/Φ_{oK} [c]	
				measured	theoretical
5.6	1	0.35±0.06	2.10	0.31	0.12
20	1	1.44±0.26	8.50 [b]	0.52	0.47
33	1	2.67±0.41	15.6 [b]	0.56	0.66
56	0	12.0±1.2	76		
112	0	18.7±1.5	138		

[a] Cellular uptake of K, Qc, was measured in the presence (5.6 to 33 mM K) or absence of ouabain (56, 112 mM K). The rate of entry of ^{42}K was corrected for ^{42}K loss and for diffusion delay of ions in the interstitial space as indicated in the Methods section.

[b] The value of K influxes in 20 and 33 mM K were obtained by assuming that the cellular membrane area is the same as that found in 5.6 mM K.

[c] The flux ratios were given only for experiments in which the K driving force was different from zero. The theoretical ratios were calculated from Ussing's equation: $\Phi_i/\Phi_o = [K]_o/[K]_i \exp(-EmF/RT)$.

As reported in previous studies (Leaf, 1956; McLean, 1960; Elshove & Van Rossum, 1963; Claret, Claret & Mazet, 1973), the movement of actively pumped K into the liver cells accounts for most of the total K uptake. Inhibition of the pump was therefore required to adequately test the dependance of the passive influx on external K. Therefore, ouabain at a concentration of 10^{-3} M, was added to all perfusion solutions except those containing 56 and 112 mM K. At these latter concentrations, we did not try to inhibit transport with ouabain, since high-K concentration would necessarily antagonize to some extent the inhibitory effect of the glycoside.

K influxes, as determined from the initial linear slope of ^{42}K uptake, are given in Table 3. It can be seen that the K influx increases 65 times, from 2.1 to 138 pmoles cm^{-2} sec^{-1}, between 5.6 and 112 mM K, and that over 33 mM K, it becomes relatively close to efflux. For experiments in which the K driving force is not nul, Table 3 also gives the values of measured flux ratios as well as those calculated from Ussing's equation. The measured values do not coincide very well with predictions based on the independence principle. An interaction of the single-file type should normally lead to flux ratios which deviate more from unity than the independence equation predicts; whereas, an interaction of the exchange-diffusion type should bring the flux ratios closer to unity. In the present experiments, since discrepancies from the calculated ratios were observed in both directions, we were inclined to attribute them to imperfections in the methods of determining the K influx. In 5.6 mM K, for example, the very much larger than expected flux ratio suggests that a nonnegligible fraction of the small K influx measured in the

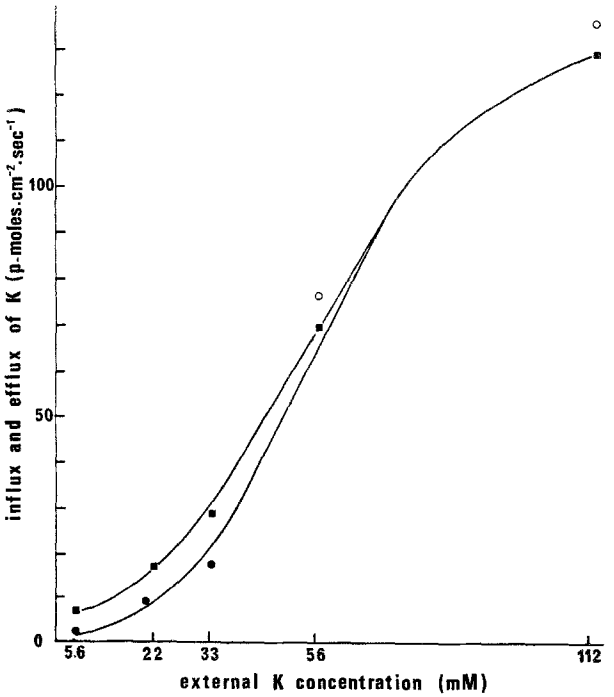

Fig. 1. The change in the influx (●, ○) and efflux (■) of K (pmoles cm^{-2} sec^{-1}) as induced by the external K concentration. Influxes were measured in the presence (●) or in the absence (○) of 10^{-3} M ouabain. The curves through the experimental points are drawn according to Eqs. (4), (5) and (6) of the theoretical model. *See text* for details of measurements and calculations

presence of ouabain was independent of the driving force. The value of this flux was attributed to a passive entry of K. Such a method supposes that pump activity can be totally blocked by ouabain. However, as discussed in a recent work (Claret *et al.*, 1973), 10^{-3} M ouabain does not completely block the Na-K pump, so that a small component of active inward K movement might well be maintained under these conditions. It seems likely that this pump residue led to some inaccuracies in determining the passive K influx and therefore made an accurate estimate of the actual value of the flux ratio impossible.

For experiments performed in 56 and 112 mM K, there was no point in calculating the flux ratios. In these solutions, the driving force for net K movement is close to zero. Under these conditions, regardless of the nature of the interactions between the ions moving within the membrane, the ratio of the undirectional K fluxes tends to assume a value of 1.0. The only conclusion we may draw from the relative equality of measured K fluxes shown in Tables 2 and 3 is that the liver is in a steady state and that, unlike

the control experiments, the fraction of the total influx due to active uptake is negligible.

It is clear, based on the arguments developed above, that Ussing's equation cannot be adequately tested in the present experiments. However, other arguments in favor of the diffusional nature of the K transport will be presented in the Discussion which follows.

Fig. 1 illustrates the kinetic relationship between the K fluxes and the external K concentration. It shows that influx, as well as efflux, increases slowly up to 22 mM, steeply from 22 to 80 mM; above this concentration, the fluxes behave as if they had a saturable component. This similarity in the change in K fluxes is consistent with the hypothesis of a permeability dependence on K.

Effect of K on Na and Cl Permeabilities

To test whether external K regulates membrane permeability to other ions, passive Na influx and Cl efflux were estimated in livers equilibrated in 56 mM K.

The Na influx was determined by integration of the linear part of the efflux curves. The mean value of Na uptake (3 experiments) was 1.36 ± 0.14 mmoles kg^{-1} min^{-1} which corresponds after corrections (*see* Materials and Methods) to an influx of 10.3 pmoles cm^{-2} sec^{-1}. The Na permeability could not be estimated directly from this passive influx, the Na fluxes of liver cells being partly composed of a Na-for-Na exchange which accounts for 28% of the total fluxes or 3.3 pmoles cm^{-2} sec^{-1} in control experiments (Claret & Mazet, 1972). Because of the difficulty in estimating it accurately in K-rich solutions, the Na exchange was not measured here. However, assuming its activity to be proportional to the Na concentration, a value of 2.1 pmoles cm^{-2} sec^{-1} could be predicted for its contribution in 56 mM K, making the actual diffusional Na influx roughly equal to 8.2 pmoles cm^{-2} sec^{-1}. The rate constant of Cl efflux was estimated directly from the loss of labeled ions. The average value of the three rate constants was 3.85 ± 1.30 hr^{-1} which amounts to acorrected efflux of 9.1 pmoles cm^{-2} sec^{-1}.

The Na and Cl permeabilities, calculated from diffusional fluxes and based on a membrane potential of -25.2 mV (Table 1), were 6.6×10^{-8} cm sec^{-1} and 12.7×10^{-8} cm sec^{-1}, respectively. The values of these coefficients differ little from those determined under normal conditions (Claret & Mazet, 1972). These results demonstrate that external K does not directly affect the Na and Cl permeabilities and that the K transport induced by K through the membrane is therefore selective.

Discussion

The results reported in the first portion of this study show that the substitution for external Na of 56 and 112 mM of K leads to a rapid redistribution of K, Cl and water between extra- and intracellular compartments. After about 10 min of equilibration in high-K solutions, the relative equality of K unidirectional fluxes indicates that the liver has reached a new steady state. It has been shown in the Results section that the establishment of this equilibrium results in a gain of K, Cl and water which causes the cells to swell, $[Cl]_i$ to increase and $[K]_i$ to remain unchanged. Since internal K concentration as measured in hepatocytes accounts for the virtual totality of positive ions, and internal Cl for a mere fraction of the negative ions, it seems obvious that the isotonic entry of KCl predicted on the basis of Boyle and Conway's theory must alter not $[K]_i$, but $[Cl]_i$.

The establishment of this equilibrium is associated with a large acceleration of unidirectional movements through the hepatocyte membrane. This K transport presents the four following main features:

(1) K dependence. It is dependent on K since it is induced by the increase in external K concentration and not by the simultaneous decrease in external Na concentration. Moreover, it cannot be interpreted as a response of the membrane to depolarization, since the membrane potential is not altered until 33 mM K (Table 1), whereas the K transport is already appreciably operative at this concentration.

(2) Selectivity. The transport of K is selective. It occurs with no concomitant changes in sodium and chloride permeabilities. This finding suggests that K ions cross the membrane through a specific channel.

(3) Independence principle. As considered in the Results section, although the analysis of flux ratios failed to test the independence principle, the parallel rise between fluxes might be interpreted as an increase in K permeability. However, another explanation which would also account for the larger than expected flux ratios found in Table 3, is the activation of a K-for-K exchange (Sjodin, 1967). But, since such a transport is unable to carry electrical currents under any conditions, it cannot account for the effects of high-K solutions on membrane potential that have been previously reported (Claret *et al.*, 1970) and which are reiterated in Fig. 2*a*. When a solution containing 56 mM K has been applied for 10 min, the membrane potential falls slowly from its original value of 34 mV to a stable value of 27.4 mV. Upon restoration of standard solution, a sudden hyperpolarization develops within 1 min; the potential then drifts back to its original level within 6 to 10 min.

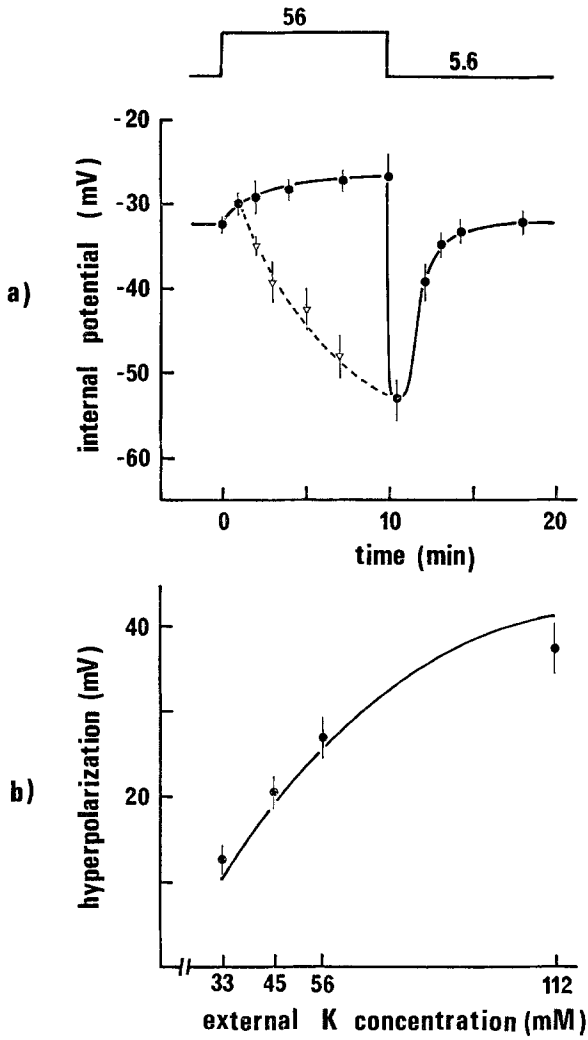

Fig. 2. Effect of K on membrane potential. (*a*) Record illustrating the effect on membrane potential of the equilibration time of a solution containing 56 mM K (•). Dashed curve (▽) joins the tops of hyperpolarizations occurring when normal medium is restored after 1, 2, 3, 5, 7 and 10 min in high K solutions, respectively. This curve may reflect the time course of the permeabilization induced by K. (*b*) Relation between the maximum size of hyperpolarization and the external K concentration of the test solution (•). The solid line is drawn according to Eq. (3)

This hyperpolarization displays patterns similar to those of the fluxes: its amplitude is maximal after 10 min of equilibration and it is dependent on the external K concentration. This similarity makes it obvious that at least a part of the K transport must be related to a diffusional process. To further test the extent of participation of diffusional fluxes in K fluxes, we

compared the amplitude of the measured hyperpolarization to the one calculated from the fluxes (assuming the K transport to be entirely diffusional). If all ions cross the membrane independently of one another, the value of the hyperpolarization (ΔEm) is given by the following equation:

$$\Delta Em = E'm - Em = RT/F \ln \frac{P'_K[K]'_o + P'_{Na}[Na]'_o + P'_{Cl}[Cl]'_i}{P'_K[K]_o + P'_{Na}[Na]_o + P'_{Cl}[Cl]'_i} \quad (3)$$

where primed symbols refer to the stable values in test solutions, the unprimed ones to the values at the peak of hyperpolarization. Under these conditions, the sum of the passive ionic currents through the membrane is zero. This equation was derived from Patlak's equation for diffusion potential (Patlak, 1960). It was assumed, as a first approximation, that permeabilities and internal Na and Cl concentrations do not change within the minute following the restoration of the normal solution. It must be noted that the permeability coefficient P used here has a purely phenomenological meaning, which does not involve any limiting hypothesis about ionic transport process. Eq. (3) applies to free diffusion as well as to carrier-mediated diffusion.

Theoretically, the value defined by Eq. (3) should be corrected for the electrogenic effect of the Na–K pump (Claret *et al.*, 1973). The potential generated by this pump reaches about 6 mV under control conditions. However, especially between 33 and 112 mM K, the membrane is shunted by the K conductance, so that the pump potential may be considered negligible despite the increased external K concentration. The curve in Fig. 2*b* was drawn according to Eq. (3) in which permeabilities and concentrations have the values given in the Results section: the good correspondence of the measured hyperpolarization to computed curve was the main argument making any interaction between K fluxes (exchange-diffusion or single-file diffusion) unlikely. It provides indirect evidence for the hypothesis that K induces a diffusion pathway through the membrane and that the K transport obeys the independence principle.

(4) Activation time. The ^{42}K-measured fluxes as well as the peak hyperpolarization described in paragraph (3) need 10 min to reach their maximal values. The time of activation is not due to the diffusion delay of K throughout the extracellular space, since the mean transfer time of ions through this compartment is 10 sec (Claret & Mazet, 1972). This statement is illustrated by the existence of a hyperpolarization which develops when normal medium is restored (Fig. 2*a*). Indeed, no hyperpolarization should be observed if the changes in K concentration close to the membrane are barely more rapid than the changes in membrane K permeability. This

indicates that the time of activation is, in fact, an intrinsic property of the permeabilization mechanism of the hepatocyte membrane.

Interpretation of Data

The specificity of the diffusion pathway for K may be most easily accounted for by assuming that K combines with a specific carrier to cross the membrane (Horowicz *et al.*, 1968; Adrian, 1969). The equations of fluxes of carrier-combined ions, derived by these authors have been simplified here. We presumed the carrier to be neutral, and, since our results proved the absence of interaction between fluxes, attributed to this carrier one site able to carry one K, so that the order of the reaction between the site and K is one. The fluxes were therefore defined by:

$$\Phi_{oK}=\frac{k_{-1}[T][K]_i}{([K]_o+[K]_i+2K_s)\{1+\exp(-EmF/RT)\}} \tag{4}$$

$$\Phi_{iK}=\frac{k_{-1}[T][K]_o}{([K]_o+[K]_i+2K_s)\{1+\exp(EmF/RT)\}} \tag{5}$$

where k_{-1} and K_s are the rate and equilibrium constants, respectively, for the dissociation of the complex TK, and $[T]$ refers to the concentration of carrier sites available for the translocation.

Since the internal K concentration, K efflux, and membrane potential have been measured as a function of external K concentration (Tables 1 and 2), it is of interest to estimate from relation (4) the K dependence of the carrier concentration. Fig. 3 shows the limit values of the relative carrier concentration when the dissociation constant K_s is nul (curve *a*) or infinite (curve *b*). Curve *a* as well as curve *b* clearly displays a sigmoid kinetic. This latter finding indicates that K ions result in an increase of $[T]$ through a more complex mechanism than a single reaction of order one. The permeabilization induced by K must therefore be differentiated from the translocation of K, i.e. K ions participate in two different mechanisms: first, the transport through the membrane described above, and second a particular reaction of carrier T activation. Three models which describe, in a more or less satisfying way the activation of the K transport may be proposed:

First model. According to Changeux, Blumenthal, Kasai & Podleski (1970), the carrier molecule T may be considered as an ionophore which exists in two conformations in equilibrium: one (R) in which the ionophore is not permeant, the other (S) in which the ionophore is permeant. A K ion can be bound to ionophore T in the S-state, shifting the equilibrium towards

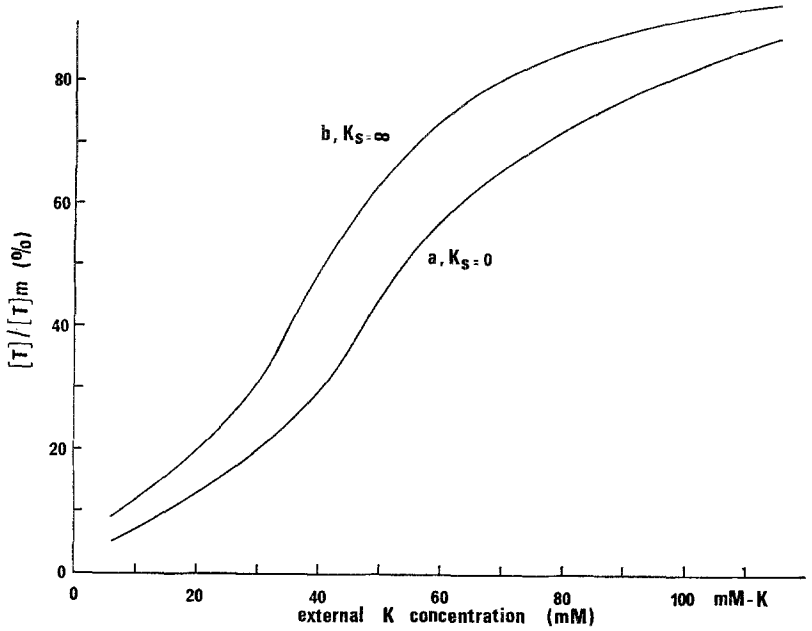

Fig. 3. Fraction of ionophores T available for K transport as a function of external K concentration. The curves, drawn from measured efflux using Eq. (4), give the limit range of the variation of this fraction when K_s is varied

this state. N ionophores are gathered in a polymer, the main property of which is that all its ionophores are in the same state (R or S). Monod, Wyman and Changeux (1965), have shown that the fraction of ionophores T in the S-state is:

$$\frac{[T]}{[T]_m} = \frac{(1+[\mathrm{K}]_o/\mathrm{K}_s)^n}{L+(1+[\mathrm{K}]_o/K_s)^n} \tag{6}$$

where K_s is the dissociation constant for one activation site, L the equilibrium constant of transition between the states R and S, and $[T]_m$ the total concentration of ionophores.

The number of ionophores gathered in a polymer was fixed at n = 4, a common value in cooperative kinetics. A reasonable fit of the data was obtained taking $k_{-1}[T]_m = 780$ pmoles cm^{-2} sec^{-1}. It is worth noting that this value has the same order of magnitude found by Horowicz *et al.* (1968) in muscle cells (650 pmoles cm^{-2} sec^{-1}). The coefficients K_s and L, as estimated by UNIVAC 1110 computation, were found equal to 37 mM and 33, respectively. This cooperative model describes simply and adequately the sigmoid type of flux concentration curves (Fig. 1). However, it does not fully account for all the situations which have been experimentally scruti-

nized, such as the rather long activation time of the K transport or its high sensitivity to temperature and to metabolic inhibitors as recently observed *(unpublished observations)*.

Second model. This last observation suggests that the K transport is not a process limited to the membrane, but also relates to cellular metabolism. The carrier molecules T would thus be continuously synthesized and broken down by the cell; we can then assume that the binding of K ions prevents these molecules from breaking down and hence increases the total number of available carrier molecules without modifying their rate of synthesis. In this perspective, the activation of the K transport would reflect the turnover rate of the T molecules.

Third model. Another hypothesis is that the carrier molecules T are released from a pool which consists of a precursor of these molecules. The release of T would be catalyzed by external K concentration through a membrane receptor located at the external edge of the membrane. The concentration of T would be limited either by its degradation and/or by a feedback control of the release reaction. The existence of a chain of chemical reactions would account for the S-shaped relation between $[K]_o$ and $[T]$, and the rate of release of the molecules T would account for the time of activation. The latter two models would also provide an explanation for the high sensitivity of the K transport to temperature and metabolic inhibitors.

The increased K movements presented here seem to differ from the one reported in liver cells by Frimmer and Kroker (1973). These authors described a paradoxical net loss of K when $[K]_o$ was increased. This loss of K occurred after 60 to 300 min of soaking in high-K solutions, and was completely inhibited by ouabain. In contrast with these results, the K-transport as induced by K in our experiments results in a net entry of K and is not altered by ouabain. However, further analysis is needed to determine whether or not these mechanisms actually differ in origin.

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