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IONIC FLUXES AND PERMEABILITIES OF CELL MEMBRANES IN RAT LIVER

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SUMMARY

1. Intracellular ion concentrations, measured in rat liver perfused with saline solutions were, at steady state:

$[K]_i = 113$; $[Na]_i = 16{\cdot}4$; $[Cl]_i = 25{\cdot}5$ m-mole $l.^{-1}$ of cells.

2. Intracellular Cl concentration was measured when both $[Cl]_o$ and membrane potential were changed. The experimental values were close to the predicted ones by the Nernst equation, indicating a passive distribution of this ion across the cell membrane.

3. Fluxes were determined by means of radioactive tracers and had the following values:

$m_K = 6{\cdot}6$; $m_{Na} = 12{\cdot}4$ and $m_{Cl} = 8 \times 10^{-12}$ mole cm^{-2} sec^{-1}.

4. When Na was replaced by Li in the perfusing solutions, the Na efflux was decreased by $3{\cdot}3 \times 10^{-12}$ mole cm^{-2} sec^{-1}. This was attributed to a Na-for-Na exchange (exchange-diffusion).

5. A mathematical model was applied to the perfused liver. It allowed estimation of the actual fluxes across the membrane. Corrections resulting from the application of the model remain small.

6. The permeability coefficients were calculated from the passive fluxes and were:

$P_K = 7{\cdot}6$; $P_{Na} = 4{\cdot}0$; $P_{Cl} = 12{\cdot}3 \times 10^{-8}$ cm sec^{-1}, corresponding to relative permeabilities of $P_{Na}/P_K = 0{\cdot}52$ and $P_{Cl}/P_K = 1{\cdot}6$.

7. The membrane potential calculated from the Goldman equation was significantly different from the measured one. This may be accounted for by an electrogenic activity of the Na–K pump. Applying the Mullins & Noda equation, the ratio of active Na flux to active K flux becomes 3/2.

INTRODUCTION

In recent years, cell membranes potential (E_m) has been extensively studied in rat liver. Most data have pointed out the dependence of E_m, *in vivo* or *in vitro*, on endocrine state (Claret, Coraboeuf & Ehrhart, 1966), metabolic activity (Limberger, 1963; Caille & Schanne, 1967; Folke, 1971), or pharmacological actions (Toida, Tamai & Takeda, 1960; Haylett & Jenkinson, 1969; Williams, Withrow & Woodbury, 1971*a*, *b*; Friedman, Somlyo & Somlyo, 1971). However, in these studies there was no attempt to clearly determine the origin of membrane potential (E_m). Available electrophysiological studies on liver cells do not provide the required information on absolute ionic permeabilities to describe adequately membrane potential. This lack may be attributed to the dependence of E_m on more than two ionic permeabilities and, possibly, on electrogenic Na pump activity (Claret, Coraboeuf & Favier, 1970; Folke, 1971; Williams *et al.* 1971*a*, *b*).

The purpose of this study was to determine ionic permeabilities of liver cell membranes. To achieve this, we used the isolated perfused rat liver preparation, which proved to be a suitable technique for quantitative measurements of ionic movements. Tracer studies of unidirectional Na, K and Cl fluxes allowed to estimate permeabilities and membrane conductances. Thereafter, the measured membrane potential was compared to the theoretical one, calculated from the constant field theory (Goldman, 1943; Hodgkin & Katz, 1949) and to that predicted by the equation of Mullins & Noda (1963).

METHODS

Preparation and solutions

The rat liver was isolated and perfused as previously described (Claret *et al.* 1970). The physiological solutions used in these experiments contained (mM): Na, 130; K, 5, 6; Ca, 2, 8; Mg, 1, 0; Cl, 127; HCO_3, 12; H_2PO_4, 2, 0 and glucose 1 g.

To study the variations of intracellular chloride concentration ($[Cl]_i$) as function of membrane potential (E_m), the solutions were modified by replacement of NaCl with equimolar quantities of either KCl or Na-benzene-sulphonate (Claret *et al.* 1970). In other sort of experiments, NaCl was totally replaced by LiCl and bicarbonate-CO_2 buffer was replaced by Tham-maleate-NaOH buffer.

Flux measurements were performed by adding the radioactive isotopes directly to the perfusion fluids. Specific activities of 1–2 μ c/ml. (^{24}Na and ^{42}K, supplied by C. E. A., Saclay) and 0·01–0·05 μ c/ml. (^{36}Cl and ^{14}C inulin, supplied by Radiochemical Centre, Amersham) were achieved.

All the solutions were maintained at 38° C and pH 7·4 and were equilibrated with 97% O_2 and 3% CO_2, except Tham-maleate-NaOH buffer solutions which were equilibrated with 100% O_2.

Electrolyte determinations

Total water: after 30 min of equilibration, small pieces of liver were rapidly removed and blotted gently. They were weighed to determine the wet weight and were then placed in a drying oven at 109° C. After 24 hr of drying, the samples were reweighed to give the dry weight.

K chemical analysis: total tissue K content was estimated on small samples (a few milligrams), the cells of which were disrupted in liquid N. The residue was dissolved in 5 ml. distilled water and the ion content determined by flame photometry. Intracellular K concentrations were calculated from the total tissue K and the extracellular space (see below). Their values were expressed in m-mole.$l.^{-1}$ cells.

Extracellular space determination

Extracellular space was determined after a 30 min period of perfusion with [^{14}C]inulin. It has been shown previously (Claret & Favier, 1971) that this time is adequate to achieve equilibrium. Small samples were removed as indicated above and were dissolved in a scintillation vial using Soluene Solubilizer (Packard). A scintillation mixture (Chevallier & Maurice, 1961) was added to each vial and the total ^{14}C was counted in a liquid scintillation spectrometer (Intertechnique). Each sample was counted for at least 8 min and corrected for quenching, using the channels ratio method. [^{14}C]inulin space was expressed as ml. kg^{-1} wet wt.

Fluxes

Fluxes were measured as described by Casteels (1969). The effluxes (m_o) were determined from the equation developed by Keynes & Lewis (1951), $m_o = k.C.V/A$ where V/A is the volume/surface ratio of liver cells, C the intracellular ion concentration and k the rate constant of ionic loss into inactive solution. Livers were first loaded during 30 min with an isotope solution, then washed with an inactive solution. Hepatic flow-rate was continuously monitored and samples of effluent were taken every 2 min (Cl and Na) or 5 min (K). The activity of the liquid samples containing ^{24}Na or ^{42}K was counted directly in an autogamma spectrometer (Nuclear). Those containing ^{36}Cl were counted with Instagel Emulsifier (Packard), in a liquid scintillation spectrometer (Intertechnique); no correction was necessary for quenching. The radioactivity lost by the liver was expressed in counts.min^{-2} and the values were plotted semilogarithmically against time.

Na and Cl influx were calculated by extrapolation to zero time of the linear part of semilogarithmic efflux curves after a loading time with ^{24}Na or ^{36}Cl solution of 2–5 min. After complete isotopic equilibration (30 min), this technique allows an estimate of the intracellular concentration of Na (Casteels, 1969). The influx of K was estimated by measuring ^{42}K uptake into the tissue after 5 min loading. At the end of this period, the total liver was homogenized and 10 ml. aliquots of the homogenate were counted directly. Total tissue content of Cl was measured on samples of liver removed after 60 min of loading, a period which is adequate for equilibration. In two experiments, intracellular Cl was determined by extrapolation process like we did for Na.

Corrections on fluxes

In the influx experiments, since an appreciable fraction of intracellular ion was sometimes exchanged, the ionic uptake were corrected by the factor $kT/[1-\exp(-kt)]$ where k was the rate constant of efflux curves and T the loading time (Keynes, 1954). Other corrections were applied on influxes and effluxes as shown in the Appendix I.

Membrane potentials

Fixed micro-electrodes (from 8 to 30 MΩ) were used as previously described (Claret *et al.* 1970). The micro-electrode and indifferent electrode were connected to calomel half cells. Membrane potentials were recorded by means of a cathode follower (Medistor) and a pen recorder (Beckman).

Volume/surface ratio (V/A)

To obtain an estimate of ionic fluxes, it is necessary to know the volume/surface ratio of liver cells (Keynes & Lewis, 1951). Although the shape of most liver cells is polygonal, they were treated as roughly regular spheres; this approximation allows an easier estimation of V/A ratio, since in a regular sphere the ratio V/A is equal to 1/6 of the cell diameter. Cell diameter was measured in perfused rat liver from which slices were cut and applied on slides. The cells sticking to the glass were subsequently coloured with Giemsa liquid. From these measurements, the calculated V/A ratio was $2{\cdot}5\ \mu \pm 0{\cdot}3$ (70) (mean ± S.D. of an observation).

Since the actual cell shape is not a sphere and the staining may produce some shrinkage, the actual value of V/A ratio could be as much as 25 or 30% larger or smaller. The possible error thus introduced in flux measurements will be carried further in estimating ionic permeabilities (P_{Na}, P_K, P_{Cl}); however, it will cancel out whenever the ratios P_{Na}/P_K and P_{Cl}/P_K are used rather than absolute values of ionic permeabilities.

The results are expressed by their mean ± S.D. of an observation.

RESULTS

Intracellular K and Na concentration and [^{14}C]inulin space

To estimate intracellular ion concentrations in perfused liver, it is necessary to know the extracellular water content. We measured [^{14}C]-inulin space which is supposed to be close to extracellular space, since Parsons & van Rossum (1962) have shown that inulin gives a satisfactory measure of the extracellular water fraction of *in vitro* liver.

The [^{14}C]inulin space does not increase between the 15th and 60th min of incubation (Claret & Favier, 1971). The mean inulin space calculated after 30 min of equilibration was 250 ml. kg^{-1} wet wt. (Table 1).

Assuming that the density of liver is equal to that of the muscle, i.e. 1·05 $g.ml.^{-1}$, 1 kg of fresh liver will contain 702 ml. of cells. As the mean value of the V/A ratio is $2{\cdot}5\ \mu$, the surface of liver cells would be 2·8 $cm^2.mg^{-1}$.

Table 1 shows the mean values of ionic concentrations. $[Na]_i$ and $[K]_i$ are in good agreement with previously reported values in rat liver (Williams *et al.* 1971*a*, *b*). Since intracellular ionic concentrations remain constant over a period of at least 90 min (Claret & Favier, 1971), it may be concluded that the cells are in a steady state over that time.

TABLE 1. Total extracellular water (ml. kg^{-1}.wet wt.) and total ion content (K_T, Na_T, Cl_T) (m-mole.kg^{-1} wet wt.). Intracellular ion concentrations in perfused rat liver (m-mole.$l.^{-1}$ of cells) are calculated as mentioned in the text. The number of observations is given in brackets

Total water	720 ± 20 (27)
Extracellular water	250 ± 21 (20)
K_T	$80{\cdot}4 \pm 3{\cdot}2$ (30)
Na_T	$11{\cdot}5 \pm 3{\cdot}3$ (14)
Cl_T	$49{\cdot}5 \pm 4{\cdot}1$ (12)
$[K]_i$	113
$[Na]_i$	16·4
$[Cl]_i$	25·5

Intracellular Cl concentration

The intracellular Cl concentration was equal to 25·5 m-mole.$l.^{-1}$. cells or 36·4 m-mole $l.^{-1}$ cell water. This concentration is higher than that given by Williams *et al.* (1971*a*, *b*) for *in vivo* rat liver cells.

If Cl is passively distributed across liver cell membranes, as in most tissues, intracellular Cl concentration can be calculated by the Nernst equation from $[Cl]_o$ and E_m: $[Cl]_i = [Cl]_o.\exp.(E_m FRT)$. In liver perfused with control solutions, the measured E_m was -34 mV and $[Cl]_o$

TABLE 2. Intracellular Cl concentrations (m-mole.$l.^{-1}$ cell water) and membrane potential (mV) as a function of external K and Cl concentration (mM). The number of observations is given in brackets

External solution (mM)			
$[K]_o$	$[Cl]_o$	E_m (mV)	$[Cl]_i$ m-mole.$l.^{-1}$
5·6	127	$-34 \pm 0{\cdot}9$ (31)	$36{\cdot}4 \pm 3{\cdot}9$ (12)
56	127	$-28 \pm 1{\cdot}7$ (27)	$43{\cdot}8 \pm 2{\cdot}8$ (18)
112	127	$-11{\cdot}8 \pm 2{\cdot}2$ (26)	$95{\cdot}2 \pm 4{\cdot}0$ (5)
5·6	11	$-65 \pm 3{\cdot}3$ (24)	$0{\cdot}3 \pm 0{\cdot}7$ (20)

was 127 mM (Table 2). Thus, the predicted $[Cl]_i$ is 35·6 m-mole.1^{-1} of cell water; this value is not different from the measured one of 36·4 m-mole.$l.^{-1}$ of cell water. *In vivo* rat liver, Williams *et al.* (1971*a*, *b*) reported that Cl equilibrium potential (E_{Cl}) was most often higher than E_m. To interpret their results they suggested, as a possible mechanism, an active extrusion of chloride out of the cells. To test further whether Cl distribution can be adequately described by an active process or by a passive diffusion, the measured values of $[Cl]_i$ were compared to the predicted one over a wide range of concentrations. The latter was achieved by changing the product $[Cl]_o.\exp.(E_m F/RT)$ with ionic substitutions in the perfusing fluid: (i) Na with K ions at constant $[Cl]_o$, in order to depolarize the cell

membrane; (ii) chloride by benzene-sulphonate ions; this substitution alters both membrane potential and external Cl concentration (Claret *et al.* 1970).

Table 2 gives the measured values of internal Cl concentration (m-mole $l.^{-1}$ cell water) as a function of external Cl and membrane potential, when a new steady state was reached (30 min). $[Cl]_i$ varies from 0·3 to

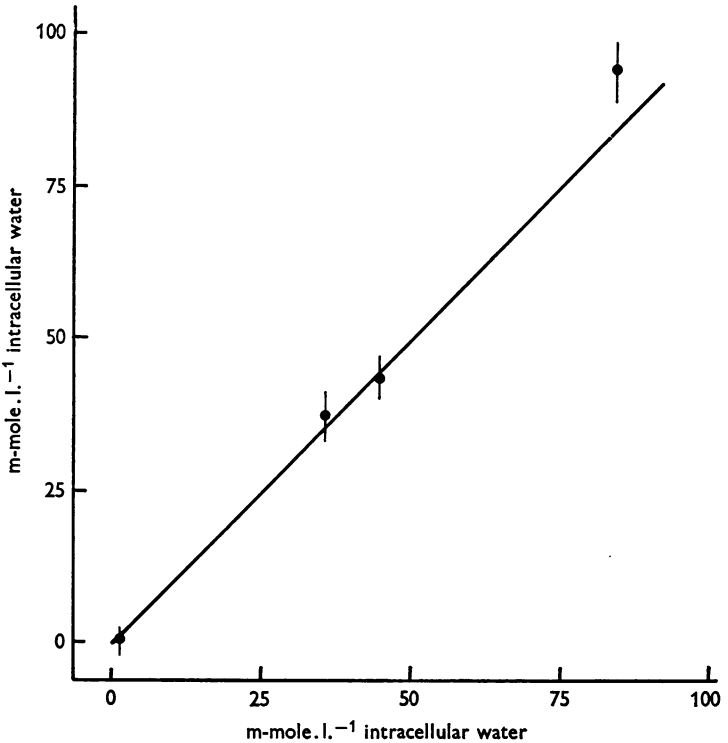

Fig. 1. Intracellular Cl concentrations (measured values expressed in m-mole/l. cell water) are plotted against predicted values: $[Cl]_o.\exp.(E_mF/RT)$. The straight line represents the slope given by the Nernst equation. Each point is the average of five to eighteen experiments (± S.D. of an observation).

95 mM as membrane potential changes from −65 to −12 mV, and $[Cl]_o$ increases from 11 to 127 mM. Fig. 1 shows the relationship between $[Cl]_i$ and $[Cl]_o.\exp.(E_mF/RT)$. It is apparent that the experimental points fall fairly well on the straight line defined by Nernst equation over a wide range of variations of $[Cl]_o.\exp.(E_mF/RT)$ product. The close relationship between measured and predicted values for $[Cl]_i$ support the view that chloride is passively distributed across the liver cell membrane.

K fluxes

Livers were perfused for 20 min in ordinary non-radioactive saline solution, and then transferred to a saline solution containing isotopes.

The uptake of ^{42}K by the tissue during a 5 min period was measured in an aliquot of homogenized total liver. As explained in the Appendix I and in Methods, corrections were made in K influx estimation. The uptake mean value was 1·07 m-mole kg^{-1} wet wt. min^{-1}. From this value and a cell surface of 2·8 cm^2 mg^{-1}, the calculated influx, after corrections, was $6{\cdot}7 \times 10^{-12}$ mole cm^{-2} sec^{-1}.

To measure the K efflux, the liver was loaded with ^{42}K for 30 min, then, the loss of activity into inactive solution was followed as a function of time. On a semilogarithmic plot, the experimental points after 10 min fit to an exponential loss of ^{42}K. Over a 2 hr period, the rate constant (k) was not altered. This observation supports the hypothesis that K ions are uniformly distributed in only one compartment. The average value of corrected k determined from the straight line drawn through the experimental points, was 0·842 hr^{-1}, corresponding to an efflux of $6{\cdot}6 \times 10^{-12}$ mole cm^{-2} sec^{-1}.

Na fluxes

When the liver was loaded for 30 min with ^{24}Na, then perfused with an inactive solution, again the washout curve indicates the presence of rapidly exchanging extracellular pool; the slow component of efflux may be ascribed to a loss of ^{24}Na from the cellular compartment. In some experiments, the results were difficult to interpret, because the efflux curves flatten with time. However, in most cases, it was possible to distinguish clearly between the intracellular and extracellular Na. The mean value of the smaller rate constant was 10·2 hr^{-1} and the corresponding calculated efflux was $11{\cdot}9 \times 10^{-12}$ mole cm^{-2} sec^{-1}.

Since the extracellular Na pool was three times greater than the intracellular one, it was impossible to use tissue uptake as a measure of influx into the cells, as we did with K. The uptake was obtained by integration of the slow exponential of the efflux curve after a 2 min loading with radioactive solution (Hodgkin & Keynes, 1955; Casteels, 1969). The mean rate of entry was 1·85 m-mole kg^{-1} min^{-1}. After corrections, the corresponding influx was $13{\cdot}4 \times 10^{-12}$ mole cm^{-2} sec^{-1}.

Exchange-diffusion

In frog sartorius muscle, Ussing (1949) suggested that a part of the observed Na efflux might be the result of a Na-for-Na exchange which does not require the expenditure of energy. Such a process was termed exchange-diffusion; to deduce its transport capacity, it was necessary to

stop it. Keynes & Swan (1959) have shown that this can be accomplished by replacing Na ions with a cation having a negligible affinity for this exchange-process. In order to test whether such a movement proceeds in liver cells, external Na was totally replaced by Li. Beaugé & Sjodin (1968) showed that the Na-pumping rate must be reduced to unmask any concomitant exchange-diffusion process. As some part of Na efflux might be considered to represent the active extrusion of cellular Na, in all cases,

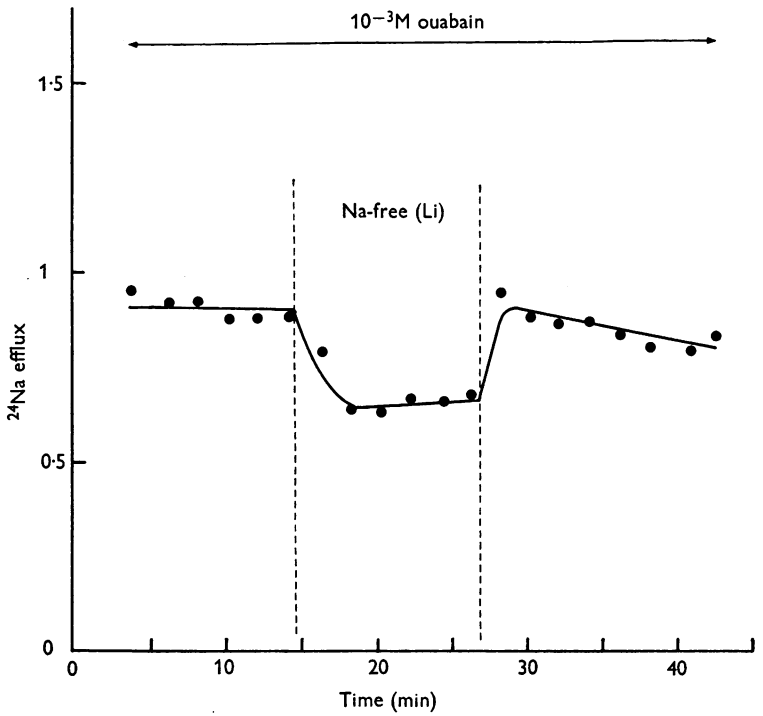

Fig. 2. Dependence of the ouabain insensitive Na-efflux on external Na ions. Na was totally replaced by Li in the presence of 10^{-3} M ouabain. Ordinate: instantaneous rate constant, i.e. the fraction of ^{24}Na in the liver lost per minute (data multiplied by 10). Abscissa, time in min.

ouabain was added to the washing perfusate to inhibit the Na pump and therefore suppress any direct Li-stimulating action on it (Beaugé & Sjodin, 1968).

The experiments were performed as follows: livers were loaded during 20 min with ^{24}Na, and the efflux into solutions containing 10^{-3} M ouabain was subsequently measured. First, the control solution was used, then livers were perfused with a Na-free Li medium for 12 min and finally, the initial solution was applied again. The results of a typical experiment are illustrated in Fig. 2, expressed as the instantaneous rate constant, i.e. the

fraction of the total tracer in the tissue lost per minute. Accurate estimates of changes in $[Na]_i$ and in Na efflux at each time during perfusion with Na-free solutions were calculated from the equations given by Hodgkin & Keynes (1955).

In all experiments the extrusion of Na ions was appreciably reduced by ouabain: the mean efflux value in six experiments was $5{\cdot}8 \times 10^{-12}$ mole cm^{-2} sec^{-1}. The substitution of Na by Li was followed by an immediate decrease in Na efflux, reaching within 2–4 min a minimum value of $2{\cdot}5 \times 10^{-12}$ mole cm^{-2} sec^{-1}. Thus, the inhibition subsequent to Na removal was $3{\cdot}3 \times 10^{-12}$ mole cm^{-2} sec^{-1} which corresponds to 28% of the total efflux. When Na solution was readmitted, the efflux did not return promptly towards the original value. The maximal recovery was $5{\cdot}3 \times 10^{-12}$ mole cm^{-2} sec^{-1}.

Cl fluxes

The Cl uptake by the tissue was estimated from the amount of tracer contained in liver samples taken after a 5 min period of soaking. The observed uptake was 1·10 m-mole kg^{-1} min^{-1}, which corresponds to a corrected influx of $7{\cdot}7 \times 10^{-12}$ mole cm^{-2} sec^{-1}.

As for K and Na efflux, the specific activity of ^{36}Cl in effluent samples decreases rapidly over 6–12 min and thereafter more slowly as a single exponential function of time. The mean rate constant of the slow component k, obtained from five experiments, was 4·6 hr^{-1}. The calculated efflux was $8{\cdot}3 \times 10^{-12}$ mole cm^{-2} sec^{-1}.

DISCUSSION

The isolated rat liver perfused by saline solutions appears to be a suitable preparation to study the ionic movements across cell membrane (Bristow & Kerly, 1964). This hypothesis is supported by the following results: (1) intracellular K and Na concentrations calculated on the basis of inulin-space are in good agreement with reported values for *in vivo* rat liver (Williams *et al.* 1971*a*, *b*); (2) in spite of the absence of erythrocytes in perfusing fluids the liver cells maintain a constant intracellular composition for at least 90 min (Claret & Favier 1971); (3) the magnitude of influx is quite close to the corresponding efflux for each ionic species; (4) the Na extrusion rate was reduced from $11{\cdot}9 \times 10^{-12}$ mole cm^{-2} sec^{-1} to $5{\cdot}8 \times 10^{-12}$-mole cm^{-2} sec^{-1} in livers treated by ouabain. Thus, it seems likely that the metabolic processes responsible for the transport of ions remain intact and that liver cells are in steady state.

The distribution of Cl is a more intricate subject. Williams *et al.* (1971*a*, *b*) reported that the calculated equilibrium potential (E_{Cl}) was slightly higher than the membrane potential (E_m), *in vivo*. They postulated that

differences between E_m and E_{Cl} were the consequence of an active chloride extrusion from the liver cells. In the present study, the large variations of $[Cl]_i$, consecutive to changes in membrane potential and/or $[Cl]_o$, were not different from that predicted by the Nernst equation, making E_{Cl} close to E_m in all circumstances. This finding rules out a systematic error during $[Cl]_i$ determination and is at best accounted for by a passive diffusion of Cl across the membrane. Therefore, the discrepancy between Williams's results and ours, as the possibility of experimental error or misinterpretation is rather unlikely, leads us to conclude that the liver looses its capacity to pump Cl out of the cells when it is isolated.

The liver is a complex structure made up of several cell types; on this basis ionic efflux curves for Na, K and Cl might be expected to be multi-exponential. However, after 10 min, the loss of labelled ions follows a mono-exponential decay and the integration of these curves give reasonable concentrations for Na and Cl ions. These arguments lead to assume, to a first approximation, that Na and Cl ions were free to exchange with labelled ions, in a homogeneous intracellular compartment. For K ions it has been reported (Heckmann & Parsons, 1959) that as much as a third of K could be bound into the liver cell. If so, the exchangeable K in the present results could be 79 m-mole $l.^{-1}$ of cells and corresponding efflux $4{\cdot}7 \times 10^{-12}$ mole cm^{-2} sec^{-1} instead of the observed 113 m-mole $l.^{-1}$ of cells and $6{\cdot}7 \times 10^{12}$ mole cm^{-2} sec^{-1}. However, the intracellular K-bound pool hypothesis is not consistent with the following observations: first, the efflux measured on the assumption of complete exchangeability is in good agreement with the observed influx; secondly, it has been previously found that external K concentration which makes the membrane potential zero, in the high K-permeable membrane of liver cells, is close to the intracellular K concentration (Claret *et al.* 1970). Therefore, if K ions are bound into the cells, it may be concluded that this fraction is rather small, provided that intra- and extracellular K activities are identical.

With regard to the particular setting of the hepatic circulatory system, the washout of extracellular space was studied. Since it is not instantaneous, any labelled ions moving out of the cell may re-enter before being carried away by the vascular stream. This will induce modifications of specific activity of ions in the micro-environment against the cell membrane. Using a theoretical model (see Appendix I), applied to our data, we were able to calculate to what extent the amount of labelled ions re-entering the cell from interstitium cuts down the actual efflux to its measured value. The results in the Appendix show that the required corrections were negligible for Na and Cl and relatively small for K.

If ions cross the membrane independently from one another, the permeability coefficients and the electrical conductances could be tentatively

calculated from the constant field theory (Goldman, 1943; Hodgkin & Katz, 1949). These parameters depend on the ease with which each ion can cross the membrane and are directly related to membrane potential, ionic concentrations and ionic passive fluxes. Passive K efflux was $6{\cdot}6 \times 10^{-12}$ mole cm^{-2} sec^{-1} and passive flux of Cl was estimated from the mean value of unidirectional fluxes to be 8×10^{-12} mole cm^{-2} sec^{-1}. Passive Na flux was more complex because it was partly composed of a Na-exchange-diffusion. Although this Na-for-Na exchange has not been extensively studied, its contribution to the total flux was estimated by the change brought about when it was suppressed: Na efflux decreased by $3{\cdot}3 \times 10^{-12}$ mole cm^{-2} sec^{-1}. The passive flux of Na along its electrochemical gradient may be calculated by subtracting the magnitude of Na exchange-diffusion from the measured influx. Estimated in this way, the mean value of the actual passive flux was $10{\cdot}1 \times 10^{-12}$ mole cm^{-2} sec^{-1}.

As the membrane potential of perfused rat liver was -34 mV, the calculated permeability coefficients have the following values:

$$P_K = 7{\cdot}6 \times 10^{-8} \text{ cm sec}^{-1},$$

$$P_{Na} = 4{\cdot}0 \times 10^{-8} \text{ cm sec}^{-1},$$

$$P_{Cl} = 12{\cdot}3 \times 10^{-8} \text{ cm sec}^{-1}.$$

It can be noticed that both relative Na and Cl permeabilities to K ($P_{Na}/P_K = 0{\cdot}52$; $P_{Cl}/P_K = 1{\cdot}6$) are in agreement with the results foreseen by others from electrophysiological studies *in vivo* and *in vitro* rat liver (Schanne & Coraboeuf, 1966; Biegelman & Schlosser, 1969; Claret *et al.* 1970; Williams *et al.* 1971*a*, *b*).

The membrane conductance may be calculated from the permeabilities, using the following equations:

$$G_K = P_K \frac{F^2}{RT} \frac{E_m}{E_K - E_m} \frac{[K]_o - [K]_i \exp(-E_m F/RT)}{1 - \exp(-E_m F/RT)},$$

$$G_{Na} = P_{Na} \frac{F^2}{RT} \frac{E_m}{E_{Na} - E_m} \frac{[Na]_o - [Na]_i \exp(-E_m F/RT)}{1 - \exp(-E_m F/RT)}.$$

Since $E_{Cl} = E_m$, the conductance to Cl ions can be calculated from a simplified form of these equations:

$$G_{Cl} = \frac{F^2}{RT} m_{oCl},$$

where m_{oCl} is the passive flux of Cl ions.

Applied to experimental results, the ionic conductances were:

$$G_K = 10\,\mu \text{ mho cm}^{-2},$$

$$G_{Na} = 12\,\mu \text{ mho cm}^{-2},$$

$$G_{Cl} = 30\,\mu \text{ mho cm}^{-2}.$$

Assuming that K, Na and Cl share the whole membrane current, total membrane conductance (G_m) is $52\,\mu$ mho cm^{-2}; the Cl contribution to G_m is 58 %.

If Cl is passively distributed across cell membrane and the algebraic sum of passive cationic currents is zero, the abbreviated form of Goldman equation can be used to calculate the theoretical value of membrane potential

$$E_m = \frac{RT}{F} \log \frac{[K]_i + \alpha[Na]_i}{[K]_o + \alpha[Na]_o}, \tag{1}$$

where α represents the P_{Na}/P_K ratio. Taking $\alpha = 0{\cdot}52$ and the ionic concentrations mentioned above, E_m was found to be $-23{\cdot}2$ mV; this value is significantly higher than the measured potential in perfused rat liver (-34 mV). This difference might be due to an over-estimation in P_{Na} or an underestimation in P_K. The main source of error in calculating absolute permeabilities originates from an approximate estimate of the volume/surface ratio; however, any error of this kind cancels out in determining α and would not affect the theoretical membrane potential. The difference between calculated and observed E_m could be accounted for by error higher than 60 % in Na influx or in K efflux: the similarity of the unidirectional fluxes makes it unlikely.

A similar situation has been described by Casteels (1969) in smooth muscle and was explained by an electrogenic Na pump, which increases membrane potential. The coupling between the active fluxes of Na and K is no longer neutral and the total passive current through the membrane deviates from zero. If so, constant field equation is no more appropriate to calculate membrane potential. Mullins & Noda (1963) have proposed a modified form of the above equation to account for the generated potential (E'_m). Assuming that intracellular ion concentrations are constant, i.e. that the algebraic sum of unidirectional fluxes for each ion is zero, net passive fluxes are related by: $m_{Na} = -rm_K$, where r is the coupling ratio or the number of Na carried out per K carried inwards. This leads to the following expression for membrane potential

$$E'_m = \frac{RT}{F} \log \frac{r[K]_i + \alpha[Na]_i}{r[K]_o + \alpha[Na]_o}. \tag{2}$$

If $r \neq 1$, eqn. (2) differs from eqn. (1) and allows to calculate a membrane potential E'_m, the difference of which from E_m is accounted for by the activity of the Na pump. Eqn. (2) can be also used in another way to estimate r from the measured value of potential. Using the data reported in this study r was found to be 1·64, which is compatible with an electrogenic Na-K pump: indeed, this value implies that 3Na ions are rejected from 2K ions pumped into the hepatic cell, in one cycle of the pump. The potential generated by such a pump should be inversely related to G_m (Rang & Ritchie, 1968). As G_{Cl} accounts for 58 % of G_m, it may be expected that removal of Cl will result in a 2·4 fold decrease in membrane conductance, giving rise to 16 mV in membrane potential. A 31 mV hyperpolarization was actually observed in liver perfused with low-Cl solutions (Claret *et al.* 1970). If Cl substitution by benzene-sulphonate suppresses completely anionic conductance, part of the observed hyperpolarization (actually 16 mV) may be the result of removing the short-circuiting effect of Cl; the remainder (15 mV) may be due to permeability changes (Claret *et al.* 1970).

In the present study are reported the first comprehensive data on flux measurements in liver cells; they allow to calculate directly ionic permeabilities and conductances. Comparison of measured membrane potential to that predicted by the Goldman equation is consistent with the concept of a Na-K electrogenic pump. Such a process was hinted by Claret *et al.* (1970) and Folke (1971) *in vitro* rat liver and discussed by Williams in *in vivo* studies. Further investigations are required to elicit the mechanism of the pump.

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APPENDIX

Since interstitial washout is not instantaneous, it slows down net labelled ionic exchanges (see Discussion). To estimate the actual fluxes the decrease in exchange rate of labelled ions through the membrane was studied in this appendix. A simplified model of Macey's treatment (1956) as applied to the liver by Thompson, Cavert, Lifson & Evans (1959) will be presented here. Several modifications have been introduced in this model to account for the suggestions of Goresky (1963).

It will be assumed that the liver is made up of elementary units; each one is composed of three compartments (Fig. 3):

(*a*) a sheet of hepatocytes (compartment 1);

(*b*) the interstitial space and the sinusoids irrigating it (compartment 2);

(c) the part (virtual) of the vascular bed providing fluid to the sinusoids of compartment 2 (compartment 3) as described by Zierler (1964). The flow is laminar.

Let for each unit: dv_1, dv_2, dv_3+dv_0 be respectively the volumes of compartment 1, 2 and 3: dv_0 being the part of compartment 3 due to the experimental device. Furthermore, it will be assumed that:

(1) compartments 1 and 2 are homogeneous for all ions studied, i.e. the vascular wall is not a diffusion barrier and the diffusion within these compartments is very fast with respect to other processes;

(2) at the entrance of compartment 3 the activity is that of the perfusing solution and, at the exit that of the compartment 2;

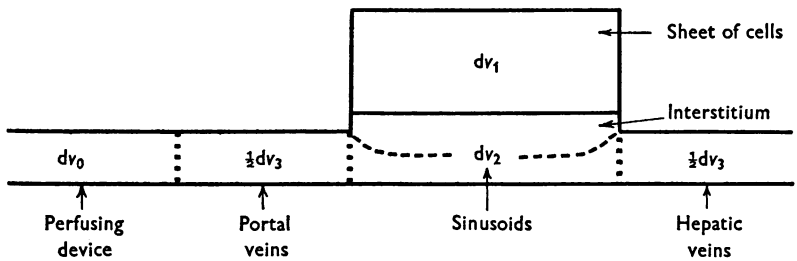

Fig. 3. A plane representation of an elementary unit of the model, each unit is composed of three compartments (volumes): Compartment 1: a sheet of cells (dv_1). Compartment 2: interstitium and sinusoids (dv_2). Compartment 3: perfusing device (dv_0) and portal and hepatic veins (dv_3); it was assumed that the portal and hepatic venous volumes are equal to $dv_{3/2}$. Furthermore, it was assumed that the variability in transfer time for individual particles through the liver was due to unequal lengths of compartment 3 from one unit to another.

(3) dv_1/dv_2 is constant throughout the liver;

(4) the rate constant (k) is also constant throughout the liver. This hypothesis is supported by the identity of the integrated value of the last exponential of a washout curve and the total intracellular activity;

(5) flow distribution is homogeneous, i.e. the saline tissue perfusion ratio is constant throughout the liver;

(6) the liver is in a steady state for the ions studied.

The equations describing the movement of radiotracers in one unit are:

$$\frac{1}{k_1}\frac{da_1}{dt}+a_1 = \frac{C_1}{C_2}a_2,$$

$$\frac{1}{k_2}\frac{da_2}{dt}+\left(1+\frac{k_1C_1}{k_2C_2}\frac{dv_1}{dv_2}\right)a_2 = a_0+\frac{k_1dv_1}{k_2dv_2}a_1,$$

where: C_1 and C_2 are the concentrations of the ions in the compartments 1 and 2;

a_0, a_1 and a_2 are the volumic activity of the tracer in the perfusing solution, the compartments 1 and 2 respectively;

dv_1 and dv_2 are the volumes of the compartments 1 and 2;

k_1 is the rate constant of equilibration of compartment 1;

k_2 is the rate constant of washing of compartment 2.

To solve out this system, it was necessary to admit that k_1 is negligible with respect to k_2.

From the above assumptions, it may be calculated several correcting factors which have the following values:

(*a*) rate constant:

$$k = \lambda \left(1+\frac{C_1}{C_2}\frac{v_1\lambda}{F}\right),$$

where

λ is the measured rate constant,

C_1 and C_2 the intracellular and extracellular concentrations of ions,

v_1 the intracellular volume,

F the total flow;

(*b*) uptake of Na, K, Cl.

Considering the transfer time through compartment 1, the ionic uptake is delayed by a factor t_0:

$$t_0 = \frac{1}{F}\left(2v_2+\frac{v_3}{2}+v_0\right);$$

(*c*) Na and Cl uptake.

As Na and Cl uptake are estimated by an extrapolation process, one more correction is needed

$$a_0 = a_e\left[1-\frac{\lambda(v_3+v_0)}{F}\right],$$

where

a_e is the extrapolated uptake of Na

a_0 the actual value of uptake.

Application. The above corrections were applied to each experiment. For instance, in a typical experiment, the liver weight was 6 g and the saline flow rate 20 ml. min^{-1}. From an extracellular space of 1·5 ml. and the data of Goresky (1963) the volumes v_2 and v_3 were estimated respectively to be 1·1 and 0·4 ml. The volume of the perfusion device was 1 ml.

Therefore, the rate constant is underestimated by 5·7 % for K and less than 1 % for Na and Cl. The value of intracellular Na and Cl uptake calculated by extrapolation is over-estimated by 1·1 %. The actual origin of the loading process is delayed by 10 sec.

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