Role of Liver-Cell Potassium Ions in Secretion of Serum Albumin and Lipoproteins

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1. The incorporation of L-[1-¹⁴C]leucine into the proteins of liver slices and into the serum albumin and lipoproteins transported by these slices was investigated. 2. Transport rates were found to be dependent on the K⁺ content of the slices. 3. The effect of K⁺ on transport of serum albumin and of serum lipoprotein can be separated from any effect on synthesis by altering K⁺ concentrations after inhibition of protein synthesis by cycloheximide or puromycin. 4. The effect of low K⁺ concentrations is reversible. 5. There is linear relationship between the K⁺ content of the slices and the transport of protein. A simple method is described for maintaining various steady concentrations of K⁺ in the liver slices. 6. K⁺ may be replaced by Rb⁺. Cs⁺ is partly effective, but NH₄⁺ and Li⁺ are no more effective than Na⁺. 7. We found evidence that K⁺ content rather than the flux rates of K⁺ or Na⁺ is important in this effect. 8. These results are probably important in ethionine and carbon tetrachloride poisoning in the rat, and may be significant in liver transplantation.

The transport of serum albumin and of lipoproteins by rat liver has attracted attention in recent years (Campbell & Stone, 1957; Campbell, 1961; Campbell & Kernot, 1962; Gordon & Humphrey, 1961; Radding & Steinberg, 1960; Bruni & Porter, 1965). The possibility that cellular K⁺ might influence the secretion of these proteins occurred to us because it has been observed that ethionine intoxication causes a failure in Na⁺ and K⁺ transport induced by low ATP concentrations (Judah, Ahmed, McLean & Christie, 1966). Ethionine is thought to block incorporation of ¹⁴C-labelled amino acids into the plasma proteins by inhibition of protein synthesis (Robinson & Harris, 1961; Farber Lombardi & Castillo, 1963), but the effect could be due to diminished secretion. We investigated the possibility that decrease in univalent cation transport might diminish the ability to secrete the serum proteins.

METHODS AND MATERIALS

Animals. Female Sprague-Dawley rats obtained from Carworth Farms were kept in our animal house until they weighed about 200 g, fed on MRC 41B diet supplemented by α -tocopherol (McLean, 1963). The rats were not starved before use.

The rats were killed under light ether anaesthesia and bled out. The livers were removed to Ringer solution at 31°C and cut into slices with a Stadie-Riggs slicer (Arthur

Thomas Co., Philadelphia, Pa., U.S.A.). The slices were about 100–120 mg wet wt.

Composition of Ringer solution. The Ringer solution contained NaCl (140 mm), KCl (7.7 mm unless specified otherwise), MgSO₄ (1.3 mm), CaCl₂ (1.86 mm), Na₂HPO₄ (1.3 mm) (adjusted to pH 7.4 with HCl) and tris-HCl buffer (30 mm), pH 7.40, adjusted for temperature of incubation. With no added K⁺, about 0.08 mm was detectable in 'K⁺-free Ringer solution'. The gas phase was O₂. Incubations were made at 31°C. The Ringer solution was fortified with 0.1 mm final concentration of each of the 20 common amino acids, including L-leucine.

Maintenance of slice K⁺ content. In each experiment one slice (30–40 mg dry wt.) was added to 5.0 ml of Ringer solution. The K⁺ content of the slice can be held relatively constant at several desired values by three methods:

(1) Slice K⁺ content can be decreased to the lowest values by incubating slices in Ringer solution at 0°C for 30 min: K⁺ content falls to about 90 μ mol/g dry wt. If the slices are transferred to a K⁺-free Ringer solution (at 31°C) their K⁺ content falls slowly to about 80 μ mol/g dry wt. If the Ringer solution contains 7.7 mm-K⁺ the slice K⁺ content rises to about 270 μ mol/g dry wt. in about 60 min and is maintained for some hours.

(2) Slices placed directly in K⁺-free Ringer solution (at 31°C) contain 145 ± 10 μ mol of K⁺/g dry wt. at the end of 60 min incubation. This increases slowly (in 2 h or so) to 200 ± 20 μ mol/g dry wt. as the slices reabsorb K⁺ lost to the medium. If the Ringer solution contains 7.7 mm-K⁺ the slice K⁺ content will rise to 270 ± 25 μ mol/g dry wt. in about 60 min and stay constant thereafter.

(3) To obtain steady concentrations of K⁺ throughout

the experiment, slices are incubated in K^+ -free Ringer solution for 15 min at $31^\circ C$. They are then transferred to fresh Ringer solution and allowed to settle down for a further 60 min, when they attain steady states characterized by the external K^+ concentration. With no addition of K^+ the content was 99 ± 7.0 , with $1.0 \text{ mM-}K^+$ it was 175 ± 2.0 and with $7.7 \text{ mM-}K^+$ it was $282 \pm 5 \mu\text{mol/g dry wt.}$

In some experiments a number of slices were incubated together in a single vessel for repeated sampling of the Ringer solution. Unless the rate of shaking was kept to 80 strokes/min and glass beakers were used in place of the polyethylene vessels usually used, the slices tended to rub each other to pieces.

Determination of dry weight and water content and the extraction of Na^+ and K^+ from slices. These procedures were carried out by the method of McLean (1963), except that 0.1 M-HCl was used to leach out cations. Analysis was by atomic-absorption spectrophotometry. Incubation was in a Dubnoff shaker (Labline Instruments Inc., Chicago, Ill., U.S.A.).

Radioactive materials. $L-[1-^{14}C]$ Leucine and $[^3H]H_2O$ were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Preparation of proteins for determination of radioactivity. Slices were homogenized in 4 ml of ice-cold water, trichloroacetic acid was added to 5% (w/v) and the precipitate was centrifuged off and washed three times in 5% trichloroacetic acid containing 10 mM carrier leucine, after which the procedure of Schmidt & Thannhauser (1945) was followed. The protein residue was dissolved in $M-NaOH$ for measurement of radioactivity.

Serum albumin secreted into the Ringer solution was separated by antibody precipitation after addition of a small amount of rat serum albumin ($100 \mu\text{g/ml}$). Before precipitation the slice was removed and the Ringer solution centrifuged at 1200g for 10 min to remove any debris. Antibodies were prepared by the method of Gordon & Humphrey (1961). The antigen was rat serum albumin extracted from rat serum by the method of Debro, Tarver & Korner (1957). On immunoelectrophoresis against antibody prepared to whole rat serum, the antigen gave a single precipitin line. The purity of the antibody was confirmed by immunoelectrophoresis against rat serum, when a single precipitin line corresponding to serum albumin was observed. These immunological methods may not separate radiochemically pure serum albumin, and all references to serum albumin in this work mean 'serum albumin fraction'.

Chromatography on Sephadex G-200 was performed by the method of Killander (1964) after addition of 50 mg of rat serum albumin to the Ringer solution and concentration to a small volume by filtration dialysis against 0.15 M-NaCl .

Electrophoresis was on starch gel as described by Smith (1968) with borate buffer at pH 8.6, after addition to the Ringer solution of 0.10 ml of rat serum and concentration to about 0.5 ml by filtration-dialysis against 0.15 M-NaCl .

Serum lipoproteins were separated from the extracellular phase by flotation on KBr (Radding & Steinberg, 1960).

Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951).

The rates of transport of labelled protein were obtained graphically from the linear portions of the plots of radioactivity against time.

Radioactivity was determined by scintillation spectrometry, and the no. of disintegrations/min was corrected for quenching by using internal standards and automatic external standardization. The error of counting was never more than 2.5%.

The uptake of $L-[1-^{14}C]$ leucine by liver slices was measured by incubation of slices in Ringer solution containing $[^3H]H_2O$. The slices were removed, blotted carefully and homogenized. Protein was precipitated and the $^{14}C/^3H$ ratio determined in the extract and in the Ringer solution. Isotopic equilibrium was reached when the ratios were the same in both. ATP and ADP were determined enzymically by the method of Kalckar (1947).

RESULTS

Effect of slice K^+ content on incorporation of leucine into proteins. If slices are depleted of K^+ , the incorporation of $L-[1-^{14}C]$ leucine into slice proteins and secreted serum albumin is much diminished (Fig. 1). Labelled protein appears in the extracellular phase after a characteristic time-lag of about 20 min (transit time), in agreement with Peters (1962a,b) and Radding & Steinberg (1960).

Fig. 1. Effect of K^+ depletion on labelling of slice protein and on the secretion of serum albumin. Slices ($30\text{--}40 \text{ mg dry wt.}$) were depleted in ice-cold Ringer solution for 30 min. They were transferred individually to Ringer solutions containing $7.7 \text{ mM-}K^+$ or no K^+ , as described in the Methods and Materials section. Incubation was at $38^\circ C$ and the gas phase was O_2 . After 30 min incubation, $0.05 \mu\text{Ci}$ of $L-[1-^{14}C]$ Leucine (specified radioactivity $1 \mu\text{Ci}/\mu\text{mol}$) was added. Serum albumin was precipitated by antibody and slices were prepared for measurement of radioactivity as described in the Methods and Materials section. Points represent the means of two experiments. ○, $7.7 \text{ mM-}K^+$; ●, no K^+ . —, Slice protein; ----, serum albumin. In this and in subsequent figures the time of addition of labelled leucine is taken as zero time.

Table 1. *Identification of extracellular proteins*

The method of Debro *et al.* (1957) was applied to the Ringer solution after addition of 0.1 ml of rat serum. The other methods were as described in the Methods and Materials section. The results are given as means $\pm$ s.d.

Methods of analysis	No. of experiments	% of label appearing in	
		Serum albumin	Lipoproteins
Starch-gel electrophoresis	5	64 $\pm$ 12	13.4 $\pm$ 7.7
Method of Debro, Tarver & Korner (1957)	6	65 $\pm$ 7.9	—
Flotation	13	—	4.5 $\pm$ 1.0
Gel filtration	5	67 $\pm$ 2.0	—
Antibody precipitation	10	64 $\pm$ 6.6	—

Fig. 2. Reversibility of the effect of low K⁺ content on the labelling of slice protein and secretion of serum albumin. All details were as given in Fig. 1, except that the slices were all transferred to a K⁺-free Ringer solution. [1-¹⁴C]-Leucine was added at 0 min, and 7.7 mM-KCl at the arrow, 30 min later. ○, 7.7 mM-K⁺; ●, no K⁺. —, Slice protein; ----, serum albumin.

Table 1 shows that serum albumin accounts for about 65% and lipoproteins for about 5–10% of the labelled protein secreted by the slices.

The effect of low K⁺ content in slices is reversed by the addition of K⁺ (Fig. 2). There is a time-lag of 30 min before the effect of added K⁺ is apparent. In four experiments K⁺ was added at different times before and after the L-[1-¹⁴C]leucine. In each the result was identical and in each there was a time-lag of 30 min before the effect of K⁺ was seen. This is about the time required for the uptake of K⁺ and the attainment of a new steady state.

We lowered the temperature of incubation to see whether we could prolong the time taken for the appearance of labelled protein in the Ringer solution and so make it easier to observe effects of K⁺ on secretion. At 31°C the transit time increased from about 20 min to 60–80 min, and all subsequent experiments were at this temperature. Table 2 shows the effect of slice K⁺ content on the labelling of slice protein and on the secretion of serum albumin and of lipoproteins. Fig. 3 shows the effect of four fixed concentrations of slice K⁺ on the secretion of serum albumin. The three experiments show that the amount of serum albumin secreted appears to vary linearly with the K⁺ content of the slices.

The eight experiments of Table 2 and Fig. 3 invariably showed large effects of K⁺, but the variation from experiment to experiment was considerable. This may be because the physiological state of the rats varied.

Specificity of K⁺. We examined the effects of Li⁺, Cs⁺, Rb⁺ and NH₄⁺ as substitutes for K⁺. Slices were depleted of K⁺ by incubation in ice-cold Ringer solution for 30 min and then transferred to Ringer solutions at 31°C containing the various ions at 7.7 mM. Fig. 4 shows that only Rb⁺ replaced K⁺ in its effect on slice labelling and on the secretion of serum albumin. Cs⁺ had a modest effect on the secretion of serum albumin, and Li⁺ and NH₄⁺ were

Table 2. *Effect of K⁺ on serum albumin and lipoprotein secretion and slice labelling*

Slices were placed individually in beakers containing 5.0 ml of Ringer solution, either with 7.7 mM-K⁺ or without K⁺, as described in the Methods and Materials section (maintenance of slice K⁺ content at desired values, method 2). In the lipoprotein experiments labelling was started by addition of 0.5 μ Ci of [¹⁴C]-leucine (specific radioactivity 10 μ Ci/ μ mol), in the others by addition of 0.1 μ Ci (specific radioactivity 1 μ Ci/ μ mole). Rates were determined and other conditions were as described in the Methods and Materials section. The total time of incubation was 180 min. Incubation was at 31°C. The results are given as means $\pm$ s.d.

Protein fraction	Rate of secretion or of labelling (d.p.m./mg of slice protein/h)		Increase in labelled protein (K ⁺ /no K ⁺)	No. of experiments
	0.0 mM-K ⁺ in Ringer solution	7.7 mM-K ⁺ in Ringer solution		
Albumin	1.8 $\pm$ 1	5.3 $\pm$ 2.5	2.4 $\pm$ 0.4	5
Lipoprotein	1.34	2.5	1.7	2
Slice protein	103 $\pm$ 44	184 $\pm$ 75	1.8 $\pm$ 0.5	5

Fig. 3. Effect of K^+ content on secretion of serum albumin. Slices were brought to four different steady concentrations of K^+ as described in the Methods and Materials section. Incubation was at $31^\circ C$ and the gas phase was O_2 . The time of incubation was 210 min. Labelling was started by the addition of $0.5 \mu Ci$ of $L-[1-^{14}C]$ leucine (specific radioactivity $0.9 \mu Ci/\mu mol$). The lowest K^+ content was obtained by procedure 1, with further incubation in K^+ -free Ringer solution. The three higher K^+ contents were obtained by procedure 2 and further incubation in Ringer solutions containing no added K^+ , $1 mM-K^+$ or $7.7 mM-K^+$. The slice K^+ contents were ($\mu mol/g$ dry wt., means $\pm$ s.d.) 83 ± 7 , 99 ± 7 , 175 ± 2 and 280 ± 5 respectively. The three curves show three separate experiments (●, □ and Δ).

indistinguishable from Na^+ . This experiment was repeated four times with the same qualitative results.

Relationship to Na^+ efflux. The rate of Na^+ loss from the slices was measured in the systems described in the preceding section. K^+ , Rb^+ and NH_4^+ all promoted efflux of Na^+ (at a rate of 3.5 – $4.9 \mu mol/g$ dry wt./min). Cs^+ was less effective (rate of Na^+ loss $2.9 \mu mol/g$ dry wt./min), and Na^+ and Li^+ were indistinguishable ($1.9 \mu mol/g$ dry wt./min).

Cation composition of slices. When first placed in Ringer solution, slices tend to lose K^+ and to gain Na^+ and water. However, in time, they lose Na^+ and water and gain K^+ . The extent of the gain of K^+ and the rate of loss of Na^+ and water depend on the external K^+ concentration. After 60 min at $31^\circ C$ our slices reached the same water content, regardless of external K^+ , and differed only in the proportions of Na^+ and K^+ that they contained. Thus the sum concentration of these ions in slice water is constant (Table 3).

ATP and ADP concentrations. These are unaffected by K^+ content of the slices under the conditions of these experiments. Mean values of $3.2 \mu mol/g$ dry wt. for ATP and of $1.9 \mu mol/g$ dry wt. for ADP were obtained in eight experiments,

Fig. 4. Effect of several univalent cations on (a) labelling of slice protein and (b) secretion of serum albumin. Slices (30 – $40 mg$ dry wt.) were depleted of K^+ in ice-cold Ringer solution for 30 min and then transferred to fresh Ringer solution (at $31^\circ C$, gas phase O_2) containing $7.7 mM-KCl$ (●), $RbCl$ (○), $CsCl$ (■), or $LiCl$, NH_4Cl or $NaCl$ (all □). Labelling was started by addition of $0.5 \mu Ci$ of $L-[1-^{14}C]$ leucine (specific radioactivity $0.9 \mu Ci/\mu mol$).

whether the slices were depleted of K^+ or contained a normal amount of K^+ .

Uptake of leucine. The uptake of $L-[1-^{14}C]$ leucine was unchanged when slices depleted of K^+ were

Table 3. *Cation composition of slices*

Slices were placed in individual beakers containing Ringer solution as specified. The results are given as means $\pm$ S.D. of six experiments. Incubation was at 31°C, and the gas phase was O₂.

Incubation time (min)	Concn. of K ⁺ in Ringer solution (mM)	K ⁺ (μ mol/g dry wt.)	Na ⁺ (μ mol/g dry wt.)	Water (g/g dry wt.)	Concn. of Na ⁺ + K ⁺ in slice water (μ mol/g)
60	0.0	144 $\pm$ 19	299 $\pm$ 58	2.4 $\pm$ 0.1	189 $\pm$ 26
60	7.7	224 $\pm$ 28	217 $\pm$ 40	2.3 $\pm$ 0.1	192 $\pm$ 10
120	0.0	187 $\pm$ 19	253 $\pm$ 21	2.4 $\pm$ 0.1	185 $\pm$ 10
120	7.7	273 $\pm$ 17	186 $\pm$ 15	2.4 $\pm$ 0.1	193 $\pm$ 25

Table 4. *Effect of K⁺ on secretion of serum albumin and of lipoprotein after cessation of protein synthesis*

All details were as given in Fig. 5, except that in the lipoprotein experiments labelling was with 20 μ Ci of L-[¹⁴C]leucine (specific radioactivity 4.5 μ Ci/ μ mol). The results are given as means $\pm$ S.D. of five experiments.

Protein	K ⁺ content of slices (μ mol/g dry wt.)	Rate of secretion (d.p.m./h per mg slice dry wt.)	Increase in labelled protein (high K ⁺ /low K ⁺)
Albumin	166 $\pm$ 13	4.6 $\pm$ 0.61	—
	303 $\pm$ 26	9.5 $\pm$ 0.56	1.7 $\pm$ 0.2
Lipoprotein	139 $\pm$ 17	0.35 $\pm$ 0.13	—
	270 $\pm$ 30	0.51 $\pm$ 0.06	1.4 $\pm$ 0.1

compared with those with a normal K⁺ content. No effect of external K⁺ concentration was observed at various leucine concentrations (0.1mM–1.0mM). In addition, the velocity of exchange between unlabelled and L-[¹⁴C]leucine was determined in the presence or absence of external K⁺ after pre-loading the slices with L-[¹⁴C]leucine. No effect was observed on the exchange.

Amino acid pools. These were determined on extracts of slices with an automatic amino acid analyser. There was no qualitative or quantitative difference between slices containing low, moderate or high K⁺ contents, under the conditions of our experiments, which were carried out at 31°C after a preliminary settling down period of 60min and involved sampling at three times, corresponding to the start, the middle and the end of labelling.

Effect of actinomycin D and of inhibitors of protein synthesis. Actinomycin D, even at 5 μ g/ml, did not prevent the effect of K⁺ on labelling of slice protein or on the secretion of serum albumin. Cycloheximide and puromycin both inhibited the incorporation of L-[¹⁴C]leucine into slice protein, serum albumin and lipoproteins. The effects were seen in the presence or absence of external K⁺ and regardless of slice K⁺ content. To obtain at least 95% inhibition 50 μ M-cycloheximide or 100 μ M-puromycin was needed.

Separation of protein secretion from synthesis. Slices were brought to a steady K⁺ content of about 175 μ mol/g dry wt., and they were then labelled with

L-[¹⁴C]leucine for 15min and labelling was stopped with 50 μ M-cycloheximide. The slices were transferred 10min later to fresh Ringer solution containing 50 μ M-cycloheximide and either no K⁺ or 7.7mM-K⁺. Sampling of the two sets of Ringer solutions began 50min later and continued for a further 100min. Table 4 compares the K⁺ contents of the slices, the rates of secretion of serum albumin and of lipoprotein and the total amount of the proteins secreted. Though K⁺ invariably increased the rate and amount of protein secretion, there was a considerable variation in the pattern of responses. Fig. 5 illustrates the two extremes in the series of five experiments.

The same results were obtained when 100 μ M-puromycin was used to stop protein synthesis, or when labelling was stopped by isotopic dilution or by a combination of isotopic dilution and inhibition.

Effect of ouabain. The method described in the preceding section was used to test the effect of ouabain at 0.5mM, which greatly decreases the uptake of K⁺ by liver slices in the presence of 7.7mM-K⁺ (Judah & Ahmed, 1964). We found that when 0.5mM-ouabain was added at the moment when slices were transferred from K⁺-free Ringer solution to Ringer solution containing 7.7mM-K⁺ the secretion of serum albumin was greatly decreased. When ouabain was added after the K⁺ content had risen (50min after transfer of slices) its effect was small. This suggests that K⁺ content of the slices rather than K⁺ flux is important for the secretion of

Fig. 5. Effect of K^+ on secretion of serum albumin after inhibition of protein synthesis. Slices were incubated together in K^+ -free Ringer (20 slices/100 ml) in two 250 ml beakers for 60 min. Incubation was at $31^\circ C$ and the gas phase was O_2 . They were labelled for 20 min by addition of $10 \mu Ci$ of $L-[1-^{14}C]$ leucine (specific radioactivity $1.7 \mu Ci/\mu mol$). Cycloheximide ($50 \mu M$) was then added. Then 10 min later ten slices (350 mg dry wt.) were transferred to each of two fresh beakers containing 50 ml of Ringer solution containing $50 \mu M$ -cycloheximide and either $7.7 mM$ - K^+ or no K^+ . Samples of the Ringer solution were analysed at intervals. K^+ contents at the end of the experiment were 166 ± 13 or $303 \pm 26 \mu mol/g$ dry wt. for the two systems. Two experiments are illustrated: —, Expt. 1; ----, Expt. 2. $\circ$, $7.7 mM$ - K^+ ; $\bullet$, no K^+ .

protein. Fig. 6 illustrates one experiment of four that all gave identical results.

Effect of 2,4-dinitrophenol. Dinitrophenol at $0.05 mM$ causes a fall in slice ATP content within 5 min. It decreases the rate of secretion of serum albumin, but it also causes a fall in K^+ content of the slices. Because this makes interpretation difficult we have not further studied the action of metabolic inhibitors.

DISCUSSION

There are two aspects of the present work that require discussion: the effect of K^+ on secretion of serum albumin and lipoproteins, and the effect of K^+ on synthesis of these proteins.

The present work suggests that cell K^+ can have a

Fig. 6. Effect of ouabain on secretion of serum albumin. Twentyone slices were labelled together in K^+ -free Ringer solutions (details as in Fig. 5). After 15 min $50 \mu M$ -cycloheximide was added and 10 min later the slices were divided between three beakers, all containing Ringer solution containing $7.7 mM$ - K^+ , $50 \mu M$ -cycloheximide and one also containing $0.5 mM$ -ouabain. The K^+ contents 50 min later were $110 \mu mol/g$ dry wt. in the presence of ouabain and $215 \mu mol/g$ dry wt. in its absence. Ouabain ($0.5 mM$) was then added to a second beaker and samples of Ringer solution were taken at the times shown. $\bullet$, Ouabain from the start; $\circ$, ouabain at 50 min; $\blacksquare$, no ouabain.

profound effect on the rate of secretion and on the amount of secretion of the serum proteins. The results also support the view that it is the cell K^+ content that is important and not the rates of flux of K^+ or of Na^+ across the cell membrane. We cannot distinguish with absolute certainty between an 'activating' effect of K^+ on secretion and an 'inhibitory' effect of Na^+ , since under the conditions of these experiments Na^+ and K^+ complement each other within the cell. However, the fact that NH_4^+ promotes Na^+ efflux but has no effect on the secretion of protein does suggest that K^+ content determines protein secretion. The same experiments also illustrate the relative specificity of the K^+ effect. Only Rb^+ of the ions tested can replace K^+ fully. The secretion of serum albumin is a complex process (Campbell & Stone, 1957; Campbell, Greengard & Kernot, 1960; Peters, 1962b; Bruni & Porter, 1965), and the effect of K^+ could be exerted at any point between the ribosomes and the external boundary of the cell. The great increase in transit time of the

proteins when the temperature is lowered by 7°C suggests that some kind of metabolically dependent 'transport' system is involved, similar to that suggested for the exocrine pancreas (Jamieson & Palade, 1968a,b).

It seems unlikely that the physiological regulation of serum albumin and of lipoprotein secretion are mediated by alterations in liver-cell K^+ , but the effects observed by us could be very important in pathological conditions. After ethionine poisoning in female rats the liver-cell K^+ content falls to about $190\ \mu\text{mol/g}$ dry wt. within 3 h (Judah *et al.* 1966) and is associated with a marked decrease in the incorporation of labelled amino acid into serum albumin and lipoproteins (Robinson & Harris, 1961; Farber *et al.* 1963). These results could well be due to a failure in the secretion of the proteins brought about by low K^+ content. After poisoning with carbon tetrachloride a similar failure to label serum proteins is observed (Seakins & Robinson, 1963). Here there is no change in cell K^+ content, but the cell Ca^{2+} content increases (Reynolds, Thiers & Vallee, 1962). The incoming Ca^{2+} could act as a competitor for K^+ . Cell K^+ might also influence events in transplanted liver where initial cooling will probably decrease the cell K^+ content to low values and is worth investigation.

Experiments on slices that have been cooled before use for the study of incorporation of labelled amino acids into proteins may be vitiated by unknown fluctuations of cell K^+ content. For instance, Hultin, Arrhenius, Löw & Magee (1960) observed that incorporation of ^{14}C -labelled amino acid into the proteins of their cooled slices was low at first when they were placed in Ringer solution at 37°C , but increased after 60 min incubation at 37°C . This effect is probably due to depletion of cell K^+ .

Our results may bear on the physiological control of protein secretion or of intracellular movement of proteins in tissues other than the liver, e.g. the movement of proteins along nerve axons (Ochs, Sabri & Johnson, 1969) and secretion by the exocrine pancreas or by the salivary glands (Schramm, 1967).

The influence of cell K^+ on synthesis of serum albumin and of lipoproteins has not been discussed. The present work produced no evidence for or against it. However, Lubin (1967) showed that low K^+ concentrations induced in tissue culture cells by the application of amphotereticin B decrease the synthesis of cell protein. This effect can be reversed by the addition of $100\ \text{mM-}K^+$ to the tissue culture medium. Since the synthesis of nucleic acids is also decreased, the point of action of K^+ in this system may differ from that in ours. Actinomycin D does

not influence the action of K^+ . Therefore the effect of K^+ cannot be mediated by mRNA synthesis.

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