April, and June 1974. Each point of this figure represents at least 30 single data for heart rate and at least 5 single data for activity. Correlation of heart rate and activity rate. A clear connection between the two parameters can be seen (Figure 2a). High locomotor activity is mostly accompanied by a corresponding increase in heart rate. The coefficients of rank correlation (Spearman) are 0.46 for X 1973, 0,83 for I 1974, and 0.77 for IV and VI 1974. These data are significant (t-test, Student) with $\alpha < 0.1\%$. More recent simultaneous experiments with 3 single individuals gave the same results (Figure 2b). The correlation coefficients are 0.71 for III, and 0.83 for VI 1975 ($\alpha < 0.1\%$). We expect that the degree of correlation is a function of the sensitivity of the activity measuring system[10].

Circadian and circannual differences. All tests clearly show the synchronizing effect of the light-dark-cycle. The most pronounced changes of both parameters are to be seen after light-on and light-off.

There are clear seasonal differences in this circadian periodicity (Figures 2, a and b). Twice a year, in winter (I 1974, III 1975) and in summer (VI 1974 and 1975), the mean values of both swimming activity and heart rate are higher during light-time than during darkness. In spring (IV 1974) and autumn (X 1974) this relation is reversed. Thus a seasonal fluctuation of the daily mean heart rate under constant laboratory conditions can be seen. The daily mean heart rate shows a maximum in summer and a minimum in winter.

A multiple phase shift of locomotor activity is reported for some fish species (*Cottus poecilopus*[11], *Salmo trutta*[12]). Our results give a hint that this phenomenon could occur also in carp.

Regular daily changes of heart rate are known from experiments with *Lampetra fluviatilis*[4] and *Salmo gairdneri*[13]. CLARIDGE et al.[4] found a connection between heart rate and locomotion in relatively restrained *Lampetra*, but pointed out that the connection between breathing rate and locomotor activity is a closer one. Short term measurements in *Salmo gairdneri*[7,8] demonstrated that fish, swimming at varied velocities, show large changes of cardiac output. This results in a slight increase of heart rate and a large increase of stroke volume.

Our results, which demonstrate a close relation between locomotor activity and heart rate, complete these findings. So the heart rate of fish seems to be a more favourable and more sensitive parameter in recording the individual state than some visible behavioural patterns.

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Increased Erythrocyte Permeability to Li and Na in the Spontaneously Hypertensive Rat

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Summary. Red blood cells incubated in a physiological medium in which Li replaces Na (LiPSS) gain Li in exchange for Na and K. The rate of Li uptake is modestly but significantly increased in the spontaneously hypertensive rat (SHR) at 37 °C and at 22 °C. The slow rate of Na gain and K loss during cooling at 2 °C was about doubled in unmodified whole blood samples from the SHR.

Recent observations on ion distribution in experimental hypertension have provided new support for our general view that cell Na regulation plays a central role both in acute vasoconstriction and in the structural redesign of blood vessels characteristic of sustained hypertension[2,3]. We thus consider the bi-directional passive permeability of the vascular smooth muscle cell membrane, together with the transport protein available for actively sustaining the transmembrane Na gradient to be a totality ('net Na pumping activity'). Our present working hypothesis is that any derangement in this totality favouring accumulation of cell Na will, if sustained, be interpreted by the cell as an increase in its work load and a stimulus to increase its work capacity. It will respond by manufacturing new structural and transport protein which will in effect tend to mask the original defect.

It might be expected that in some forms of hypertension the original defect would be sufficiently generalized to affect all or most cell membranes and so be particularly apparent in the erythrocyte, which lacks the ability to synthesize new protein. An increase in red blood cell Na in hypertension in man has recently been reported[4] and BEN-ISHAY et al.[5] have noted enhanced Na efflux in erythrocytes from rats with one form of genetic hypertension. We have approached the problem by looking at the passive permeability of the membrane, using simple indicators which could then be applied clinically if warranted. Analytic rather than tracer methods were chosen for simplicity and movements of Li were compared with Na since passive processes should affect both ions and the larger hydrated size of Li would amplify marginal changes[6].

Materials and methods. Male rats with spontaneous hypertension (SHR-Carworth) and their matching controls (CFN) were used throughout. In each experiment, the animals were lightly anaesthetized with ether, blood

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Table I. The compositions of physiological salt solutions (mM), aerated with 95% O_2, 5% CO_2 (pH 7.4 ± 0.1 at 37 °C)

Solution	Na	K	Ca	Mg	Cl	HCO_3	HPO_4	Li	CO_3	Glucose	Sucrose
Normal (PSS)	141.2	5	1.7	1.2	123.4	25	1.2	—	—	11	—
K-free	141.2	—	1.7	1.2	118.4	25	1.2	—	—	11	10
Li subst. (LiPSS)	—	5	1.7	1.2	123.4	—	1.2	141.2	25	11	—
K-free Li subst.	1.2	—	1.7	1.2	117.9	—	1.2	141.2	25	11	7.6

pressure was measured directly from the femoral artery with a Sanborn P23A transducer and a 2 ml blood sample was taken through the same needle. This sample was centrifuged at 1475 g for 15 min at 2 °C, plasma and buffy coat were removed, and the red cells were then washed twice in 30 ml of normal Krebs medium and spun down as above. The normal medium was then replaced with test medium for incubation with occasional gentle mixing under specified conditions, the sample was spun down at 500 g for 5 min, the filtrate removed by aspiration, and 100 λ of erythrocyte lysed in 5 ml of distilled water for 10 min. To this, 5 ml of 10% trichloracetic acid was added and mixed, and the solution allowed to stand for 5 min before filtering with No. 42 Whatman paper. Na, K, and Li in the filtrate were measured by atomic absorption spectrophotometry. The incubation media are shown in Table I.

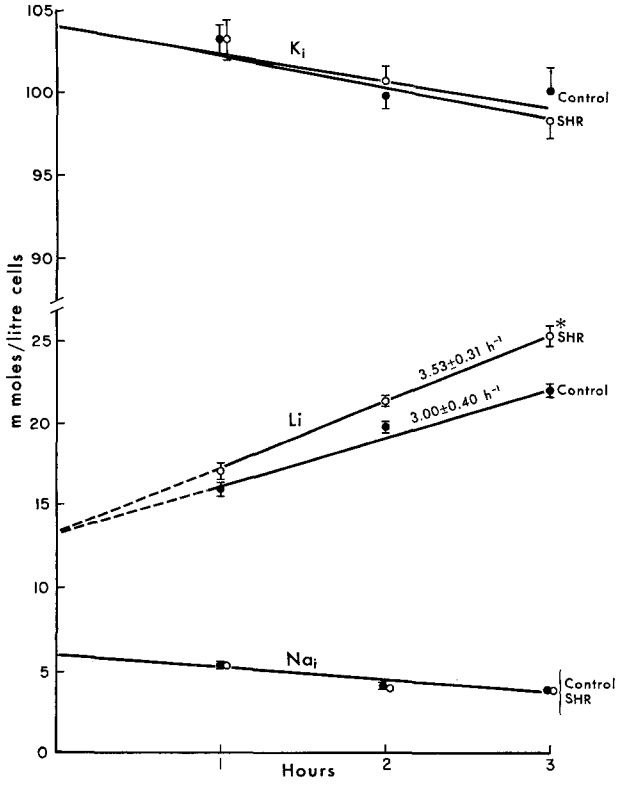

Fig. 1. Changes in cation content of a standard packed red cell sample following incubation at 22 °C in a normal medium in which Li replaces Na (LiPSS) for 1–3 h. Values for Li are uncorrected for Li in trapped extracellular medium indicated by zero intercept. Trapped fluid volume estimated from Li intercept as approximately 10% was used for calculating cell Na and K values. SE is indicated by vertical lines. Regression lines fitted by method of least squares. *$p < 0.02$.

Results. Li influx at 22 °C. This experiment was carried out with blood samples from 8 control and 8 hypertensive rats, 11 weeks old, and with blood pressures of 125 ± 2/77 ± 2 and 153 ± 3/86 ± 4 respectively. The net entry of Li into cells was estimated by incubating washed red cells in a medium in which Na was replaced by Li (LiPSS) for 1, 2 or 3 h at room temperature. The results are presented in Figure 1.

The Li content of the samples uncorrected for trapped fluid increased with time, indicating entrance into cells. The rate, described as a linear regression, was sufficiently faster in the SHR to result in a significant difference between groups at 3 h. The zero intercept was the same in both groups and indicated an extracellular trapped fluid volume of about 10%. This value was used for plotting the corrected values for cell Na and K. It is clear that the entry of Li into cells replaces mostly K, as expected, although the variance in this measurement is too great to permit separation of control and hypertensives.

Li influx at 37 °C. This experiment was modified from the foregoing to accelerate the rate of entrance of Li into cells and to improve on its estimation. Accordingly, the test medium was changed to K-free LiPSS and the incu-

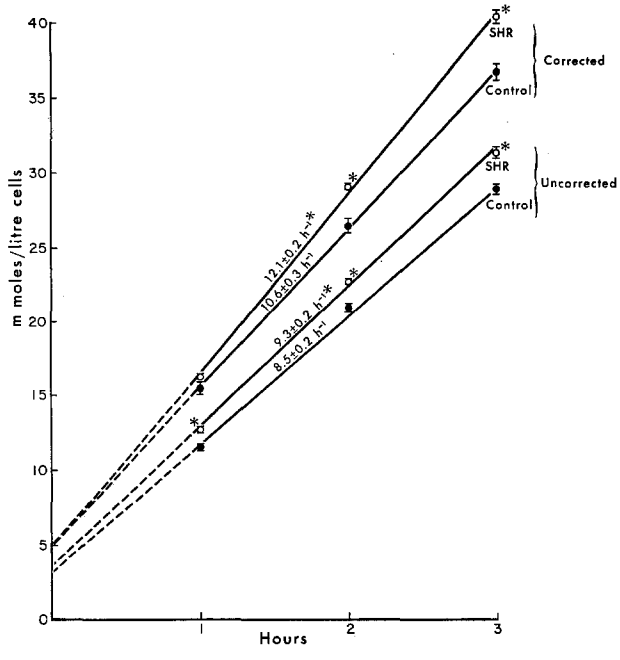

Fig. 2. Changes in Li content of a standard packed red cell sample following incubation at 37 °C in K-free LiPSS for 1–3 h. Extracellular Li was removed by a 30 min wash in cold (2 °C) PSS prior to sampling. Lower lines indicate cell Li uncorrected for trapped fluid volume. Upper lines indicate cell Li normalized by using the Na content of each sample as indicator of trapped fluid; this overestimates this volume and hence cell Li by a small constant amount.

Table II. Exchange of Na and K between erythrocytes and plasma during incubation at 2 °C for 23 h in late SHR (29 weeks) compared with normotensive controls

	Control	SHR
Blood pressure (mm Hg)		
Systolic	141 ± 3	196 ± 4[a]
Diastolic	87 ± 2	114 ± 3[a]
Plasma [Na] (mEq/l)		
Initial	138.1 ± 0.3	138.6 ± 0.3
Final	135.2 ± 0.5	131.5 ± 0.3[a]
Δ	− 2.9 ± 0.6	− 7.2 ± 0.3[a]
Plasma [K] (mEq/l)		
Initial	3.88 ± 0.07	3.34 ± 0.09[a]
Final	6.80 ± 0.17	9.89 ± 0.28[a]
Δ	+ 2.93 ± 0.16	+ 6.54 ± 0.31[a]
Hematocrit % (final)	47.2 ± 0.9	48.3 ± 0.09
Weight (g)	509 ± 3	320 ± 8[a]
No. of animals	12	12

± = Standard error of the mean. [a] $p < 0.02$.

bation temperature raised to 37 °C. Further, following incubation for 1, 2 or 3 h, the cells were spun down and washed in 50 volumes of K-free PSS at 2 °C for 30 min to remove extracellular Li before the final sampling for chemical analysis. The 8 control and 8 test rats were 17 weeks old, with blood pressures of 125 ± 3/81 ± 4 and 178 ± 4/108 ± 4 respectively. The results are presented in Figure 2.

The rate of entrance of Li into cells was about three-fold faster under the conditions of this experiment. Again, Li entered cells faster in the SHR and the difference was significant even after 1 h of incubation.

Na influx into cells at 2 °C. In parallel experiments involving the influx of Na induced either by ouabain or by an absence of K in the medium, we found the results too variable to permit firm conclusions. Exposure of the cells to low temperature, however, produced unequivocal results. A simple procedure in which whole blood samples were left undisturbed at 2 °C suffices to demonstrate SHR and control differences. In this case, glass electrodes were used to estimate plasma Na^+ and K^+, since this procedure is very precise and does not alter the sample[7]. The results are set out in Table II.

Initially, plasma Na^+ and K^+ values in control and SHR groups were within 0.5 mEq/l of each other. This difference is sufficient to establish that plasma K^+ is reduced slightly but significantly in the SHR but not to establish the converse for Na^+. After 23 h in the cold, the transmembrane Na and K gradients had run down sufficiently in the controls to lower plasma Na^+ and raise K^+ by about 3 mEq/l. This rate was more than doubled in the SHR.

Discussion. These experiments show an apparent increase in the passive cation permeability of the red cell. It is passive since it has been demonstrated with both Li and Na under conditions where the only driving force available to the ion movement is its transmembrane gradient. In interpreting their results, BEN-ISHAY et al.[5] suggested this possibility.

It has been shown that the red cell volume is decreased in the SHR[8] and it may be urged that our results can be explained on the simple basis that cell surface available to ion permeation is increased. In the case of the discoid red cell, however, there is no a priori direct relation between cell volume and cell surface unless it be assumed that cell shape is invariant. There is thus no simple direct way to test this possibility. It is unlikely, however, that the doubled rate of entrance of Na at 2 °C in the SHR can be thus simply explained. In fact, the modest increase in the rate of Li entrance at higher temperatures compared with the exaggeration of the differences between test and control groups at low temperature argues strongly for a conformational change in membrane structure. Since Na^+ and Li^+ are similarly affected, it is likely that the cation channels are affected. Further detailed study of this possibility is now under way. It is of interest that an apparent increase in cation permeability of the vascular smooth muscle cell membrane in both DOCA hypertension and the SHR has already been reported[9–11]. Furthermore, WESSELS et al.[4] have observed a significant positive correlation between sodium influx into red cells and blood pressure in a large series of normotensive and hypertensive subjects.

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Studies on Carbon Turnover in the Freshwater Snail *Ancylus fluviatilis* (Basommatophora)[1]

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Summary. Carbon turnover rates of young specimens of the freshwater snail *Ancylus fluviatilis* are nearly equal for all organs, whereas in adult specimens intestinal organs (midgut gland and others) show considerably higher turnover rates than the 'rest animal' (animal without organs of the pallial complex).

Only few studies exist of basic carbon balance sheets for different organs in invertebrates. Investigations were therefore run on incorporation and turnover of ^{14}C-labelled food in the patelliform pulmonate snail *Ancylus fluviatilis*, living as a primary consumer on stones of rivers and lakes. All the investigations were done with specimens of all size classes (1 mm–7mm aperture length of shell) and the value for individual length classes were calculated according to a regression line.

Diatoms (*Nitzschia actinastroides*) were labelled with $NaH^{14}CO_3$, filtered on millipore filters and offered to the snails within a conical flask at 22 °C. After 24 h, the snails were offered unlabelled food till the end of the experiment, 1 h to 16 days later. Then the animals were solubilized and measured in a liquid scintillation counter. All measurements were performed without the shell.

[1] Supported by the Deutsche Forschungsgemeinschaft.