

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

± = Standard error of the mean. *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 sample⁷. 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 SHR⁸ 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 reported⁹⁻¹¹. 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.

⁷ S. M. FRIEDMAN, in *Glass Electrodes for Hydrogen and Other Cations* (Ed. G. EISENMAN; Marcel Dekker, New York 1967), p. 442.

⁸ S. SEN, G. C. HOFFMAN, N. T. STOWE, R. R. SMEBY and F. M. BUMPUS, *J. clin. Invest.* 51, 710 (1972).

⁹ S. M. FRIEDMAN, *Circulation Res.* 34, 1-123 (1974).

¹⁰ A. W. JONES, *Circulation Res.* 34, 1-117 (1974).

¹¹ A. W. JONES and R. G. HART, *Circulation Res.* 37, 333 (1975).

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 ¹⁴C-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 NaH¹⁴CO₃, 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.

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Table I. Incorporation of carbon into the organs of *Ancylus fluviatilis* at 22°C

Whole animal ^b	Incorporation (ng/d)		Incorporation (%)		Significance ^a	Specific incorporation (ng C/μg C)	
	2 mm	6 mm	2 mm	6 mm		2 mm	6 mm
	1675	30997	100.4	104.5	-	40.0	40.0
Midgut gland	399	9525	23.8	30.7	*	51.8	95.3
Gut	89	2266	5.3	7.3	*	43.4	86.0
Stomach	92	1500	5.5	4.8	NS	43.1	53.8
Gonad	19	794	1.1	2.6	*	—	44.6
Albumen gland	—	2238	0	7.2	*	—	47.4
Rest of female genital tract	—	3044	0	9.8	*	—	51.7
Rest animal	1084	13037	64.7	42.1	*	36.4	26.3

^aSignificance of the difference between the percentage incorporation of young and adult animals. NS, not significant. * Significant at the 95% level. ^b Values for the whole animal are not exactly the sum of the individual organs, because of independent measurements.

Single organs could be measured by putting the animals into a Bouin mixture, dissecting them at a magnification of 10–40 and dissolving the organs in a solubilizer.

The possible limitations of calculating turnover rates in animals have been outlined several times, but at least a rough approximation can be obtained^{2,3}.

It was shown elsewhere that absolute values of carbon incorporation into the whole animal depend on animal size, temperature and season⁴. Incorporation into the organs is shown in Table I for young specimens of 2 mm in length (corresponding to 41.9 μg C soft tissue as an average) and mature specimens of 6 mm in length (774.9 μg C). These animals had been fed labelled food for 24 h and unlabelled food for 96 h. Incorporation rates into the organs were adjusted to a value of 40 ng C/μg C · day for the whole animal, which represents approximately the maximum incorporation rate at 22°C in spring for adult as well as for young specimens⁴.

The first 2 columns indicate the difference in absolute values. Percentage incorporation into the organs reveal differences between young and adult (significance at the 95% level indicated by an asterisk): Young individuals incorporate more carbon into the rest animal (= animal without organs of the pallial complex), whereas mature ones incorporate it preferentially into the organs of the pallial sac. Specific incorporation (last 2 columns) indicates that in young individuals incorporation rates are nearly the same in all organs (range: 36.4–51.8 ng/

μg C), but differ markedly in adults (26.3–95.3 ng/μC). It is an interesting fact that the reproductive organs show relatively low specific incorporation rates. It has been shown elsewhere that we must assume that material for egg capsule production is stored to a great extent in other parts of the body, especially the midgut gland, and then mobilized if needed⁴.

There seem to be fundamental different metabolic strategies in young and adult specimens: Young show an equally anabolic activity in the whole body and a high mortality rate in nature⁵. Adult individuals, on the other hand, grow at a reduced rate, but store a large pool of carbon, which enables them to lay egg capsules at intervals and also to endure periods of hunger for several days.

Half-life times and turnover rates were calculated by measuring the exponentially declining radioactivity within the organs for different times after the labelling period (1 h, 6 h, 1 day, 4 days, 16 days). A second method was used also, taking specific incorporation rates as a base for calculation. The range of results obtained by the 2 methods is presented in Table II, column 1. In the second column, average percentage turnover rates per day

² D. L. BUCHANAN, Arch. Biochem. Biophys. 94, 500 (1961).
³ U. SPECK and K. URICH, Z. vergl. Physiol. 63, 405 (1969).
⁴ B. STREIT, Arch. Hydrobiol., Suppl. 48, 1 (1975).
⁵ B. STREIT, Oecologia, Berl., 22, 261 (1976).

Table II. Half-life time and daily turnover rate of carbon in *Ancylus fluviatilis* at 22°C

Whole animal	Half-life time (days)	Average daily turnover rate in % of carbon	Respiration of <i>Helix pomatia</i> (μg O ₂ /mg dry weight · h)
	14	4.8	—
Ganglia	—	—	2.53–4.00
Midgut gland	7– 8	9.7	1.39–2.78
Gut	7– 8	9.1	2.56–2.70
Kidney	—	—	2.05–2.24
Stomach	6–13	8.5	—
Radula	9–12	7.8	—
Gonad	7–16	6.7	—
Rest of female genital tract	12–14	5.1	ca. 1.03
Albumen gland	13–15	4.8	1.17–1.20
Rest animal	24–27	2.7	ca. 0.85

Tissue respiration rates of *Helix pomatia* according to KERRUT and LAVERACK⁶ at 28°C. All the specimens were mature.

are calculated. We again see that turnover rates are approximately 3 times as high in midgut gland, gut and stomach compared to the rest animal.

For comparison, respiration rates for different organs for the terrestrial pulmonate snail *Helix pomatia*, as presented by other authors⁶, are summarized also in Table II, column 3. Respiration rate obviously goes more or less parallel to carbon turnover rate. Organs of the pallial complex show about 3 times higher a respiration rate than the rest animal and reproductive organs show again a rather low value. The incorporation and turnover rates of the organs correspond to some extent to the results for rats and mice: the highest being in liver, then kidney, spleen, lung, muscle, and the lowest in the brain. In crustaceans (*Orconectes limosus*), the succession: gut, midgut gland, stomach, muscle was found, when ¹⁴C-glucose was injected into the hemolymph⁷.

If half-life times of the midgut gland, stomach and gut of *Ancylus fluviatilis* are converted to mammal tempera-

ture of 38°C by assuming a Q_{10} of 2, values of 2.3–5.3 days are found, which nearly equal values for mice and for *Orconectes* when converted⁸. On the other hand, respiration rates are about 2500 $\mu\text{l O}_2/\text{g} \cdot \text{h}$ for mice, 40 $\mu\text{l O}_2/\text{g} \cdot \text{h}$ for *Orconectes*³ and 200 $\mu\text{l O}_2/\text{g} \cdot \text{h}$ for *Ancylus fluviatilis*⁸, the latter both at 18°C. Assuming again a Q_{10} of 2, mice have a respiration rate about 15 times as high as *Orconectes* and about 3 times as high as *Ancylus*. Such calculations have to be regarded with caution, as neither invertebrate can exist at 38°C and any assumed value of Q_{10} is arbitrary. But nevertheless it now seems to be a general phenomenon that invertebrates, although showing lower respiration rates than mammals, have quite comparable incorporation and carbon turnover rates, if converted to the same temperature.

⁶ G. A. KERKUT and M. S. LAVERACK, J. exp. Biol. 34, 97 (1957).

⁷ K. GRASZYNSKI, Z. vergl. Physiol. 59, 110 (1968).

⁸ K. BERG, Hydrobiologia 4, 225 (1952).

Carotid Chemoreceptor Influence on the Cardiac Sympathetic Nerve Discharge

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Summary. Pure stimulation of carotid chemoreceptors induces, as a primary effect, the general spontaneous activation of both the cardioexcitatory and the cardioinhibitory medullary centres. These effects have been shown by keeping constant those parameters which, when modified, produced secondary effects hiding the investigated primary ones.

Chemoreceptor stimulation of carotid glomus provokes in spontaneously breathing animals an increased activation of the respiratory centres, hypertension and tachycardia^{1,2}. When the animals are thoracotomized or curarized and artificially ventilated, the same chemoreceptor stimulus results in bradycardia^{3–5}. It is generally accepted that the tachycardia observed in the first experimental condition is ascribable to the increase of ventilation provoked by the chemoreceptor stimulus, which is to say that the tachycardia is a secondary response to the increase of pulmonary stretch receptor activity^{4,6,7}. The primary effect of carotid chemoreceptor stimulation on the heart should consist in a decrease of its frequency: such a bradycardic effect appears unmasked if the animal is not allowed to increase its ventilation.

As far as the mechanisms of this bradycardia are concerned, beside the well defined increase of the vagal activity^{8–11}, there is still some disagreement as to whether there is any participation of the cardiac sympathetic activity in this response. Among others, DOWNING and SIEGEL¹² affirm that the cardiac sympathetic nerve does not show any contribution, while ALANIS et al.¹³ have described experiments in which a decrease, an increase or no change in the cardiac sympathetic activity occurred.

The present experiments have been undertaken in order to analyze the role of the cardiac sympathetic nerve in this reflex bradycardia.

Methods. The experiments were performed on 20 cats anaesthetized with urethane (250 mg/kg) and chloralose (30 mg/kg), thoracotomized along the midline and artificially ventilated. The arterial blood pressure was recorded from right subclavian artery. The electric activity from the central cut end of the inferior cardiac nerve and of the phrenic nerve was recorded by means of an AC pre-amplifier and an integrator following the technique of ALANIS et al.¹³. Tracheal $\text{CO}_2\%$ was continuously monitored and the ventilation was adjusted as to keep the end-tidal $\text{CO}_2\%$ at the control level throughout the

experiment. When required, the cat's arterial blood pressure was kept constant by connecting the abdominal aorta (at the bifurcation) to a large plastic tube which led to a big reservoir containing buffered saline solution. The pressure in the reservoir was fixed at the same level as the mean blood pressure the animal exhibited just before each trial. The heart was paced, when necessary, by electrical impulses at a suitable frequency, delivered through a couple of ring-shaped platinum electrodes fixed to the anterior wall of the right atrium. NaCN solution at a concentration of 50 $\mu\text{g/ml}$ was injected by means of an infusion pump, through the cannulated thyroid artery.

Results and discussion. The NaCN injection in thoracotomized-artificially ventilated cats (Figure A) evoked the usual increase in phrenic discharge. At the same time the sympathetic discharge exhibited an abrupt, short-lasting increase followed by modifications that were synchronous

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