output than the heart rate. For example, between June and November 1970, cardiac output increased by 69%, stroke volume by 53%, but heart rate by only 12%. Arterial blood pressure, on the other hand, did not coincide with the seasonal variations of cardiac output (Figure 2). Thus, the changes of cardiac output were compensated by reversal changes of peripheral resistance.

What is the cause of these seasonal variations? Cardiac output is mainly regulated by the blood demands of peripheral tissues[6]. Exercise and motility on the one hand, thermal regulation on the other hand are the most important factors demanding a higher body-blood supply. Both can be excluded as causes of the variations in cardiac output seen in our experiments. Firstly, the cardiovascular investigations were done in anesthetized rats which were not able to move. Secondly, the rats were housed at a constant environmental temperature of 23 °C before the experiments, and body temperature was kept at 36.5 °C during the experiments.

We suppose that the thyroid gland is involved in seasonal variations of cardiac output. It was shown[7] that the thyroid gland of rats is at a higher level of activity during the winter than during the summer season. These changes occurred even though the animals were maintained under conditions of constant temperature (22 to 25 °C) and light. Seasonal variations in thyroid function (higher serum thyroxine levels during the winter period than during the summer period) were also seen in new-born children[8]. Thyroid hormones increase the metabolic rate and moreover have a direct positive inotropic and chronotropic effect on the heart[9–11]. Both actions are capable of increasing cardiac output.

Since cardiac output, heart rate, stroke volume, and peripheral resistance of rats are subject to seasonal variations, results obtained at different times of the year cannot be compared. Therefore, cardiovascular research in rats always requires simultaneous control experiments.

Summary. Cardiac output of rats shows seasonal variations with low values in spring and summer and high ones in autumn and winter. The stroke volume was much more implicated in these changes than the heart rate. The seasonal changes of cardiac output are probably due to changes of thyroid function.

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Effect of Furosemide on the Permeability to Cl of the Isolated Skin of *Leptodactylus ocellatus*

Furosemide is known to be a potent inhibitor of active chloride transport in the epithelia. Evidence of this effect was provided by the abolition of the potential difference (PD) (positive inside) in the thick ascending limb of Henle's loop perfused with chloride-containing solutions[1], and by the decrease in short circuit current (SCC) in the frog cornea which only transports Cl^-[2]. More recently, furosemide was found to decrease Cl^- influx in selected, low voltage, short-circuited skins of the European *Rana temporaria*[3]. Since in this preparation Cl^- influx slightly excedes Cl^- efflux, the results were thought to represent a specific inhibitory effect on active Cl^- transport.

The possibility of additional effect of furosemide on passive Cl movements is still controversial and largely supported by indirect evidence: 1. According to BURG et al.[1] furosemide inhibits passive Cl^- influx into the lumen of the perfused ascending limb more than can be accounted for by changes in PD; 2. EIGLER et al.[4] reported that furosemide increases the PD of the toad skin with little change in the SCC which was considered to be indirect evidence of decreased permeability to anions. Contrasting with these findings, LOTE[3] reported that furosemide has no effect on passive Cl^- efflux in the skin of *Rana temporaria*. It is possible that this discrepancy is due to low permeability to Cl^- of the preparations used in those studies.

The aim of this study was to investigate the effect of furosemide on passive Cl fluxes in a preparation which is highly permeable to Cl. For this purpose the isolated skin of the South American frog (*Leptodactylus ocellatus*) was chosen because its active and passive Cl fluxes are 10 times faster than those of *Rana temporaria*, and passive Cl effluxes 10 times faster than passive Na fluxes[5,6]. In this respect it compares favorably with the thick ascending limb where the ratio between the 2 passive fluxes is only 0.5[7]. Also the possibility of eliminating electrical gradients by short-circuiting the isolated skin makes it possible to detect permeability changes without any correction for PD changes.

The results indicate that, in addition to its known inhibitory effect on active Cl transport, furosemide also decreases passive Cl fluxes in the isolated skin of *Leptodactylus ocellatus*. Extension of this effect to the thin ascending limb of Henle's loop may bear some relationship to the natriuretic properties of the drug.

Methods. The isolated abdominal skins of *Leptodactylus ocellatus* were used in all experiments. After dissection the skins were mounted in lucite chambers as described by USSING and ZERAHN[8] covering 3.14 cm^2 of skin. Both chambers were filled with 5 ml of Ringer solution (NaCl 115.5 m*M*; $NaHCO_3$ 2.4 m*M*; KCl 2.0 m*M* and Ca gluconate 1.0 m*M*) and bubbled with air. PD was measured through agar-Ringer bridges and calomel electrodes with a Keithley 200 B electrometer and the SCC with the external circuit described by USSING and ZERHAN[8]. Tissue conductance was calculated as SCC/PD.

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Chloride ion fluxes. Influx and efflux of Cl^- were measured in different skins. No attempt to use halves of the same skin for simultaneous determinations was made, owing to the large difference in electrical parameters observed in pieces of the same skin. ^{36}Cl was placed on the appropriate side of the skin, 1 ml samples were drawn from the cold side at 15 min intervals and the volume kept constant by addition of non-radioactive solution. In each experiment, the chloride flux was measured during 8 periods in the short circuit condition, except when the circuit was opened for 2 sec at the end of each period for PD measurements. Furosemide was added to the solution bathing the external side of the skin until a final concentration of 10^{-3} *M* at the end of the 4th interval. The radioactive samples were added to 10 ml of dioxane-naphthalene mixture and counted in a liquid scintillation counter. Specific activity of chloride on the 'hot' side was determined and the unidirectional flux calculated in terms of $\mu Eq \cdot h^{-1} \cdot cm^{-2}$.

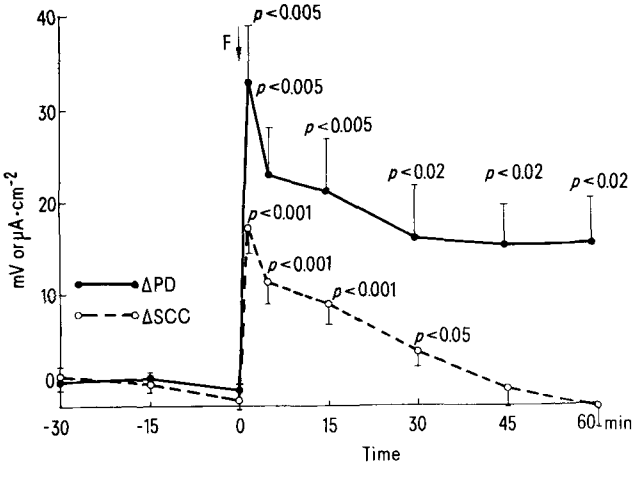

Fig. 1. Changes in potential difference (PD) and short-circuit current (SCC), of the skin of *Leptodactylus ocellatus* produced by addition of furosemide (F) 10^{-3} *M* to the solution bathing the external side. Values are means ± SE of 13 to 16 observations. Only significant differences are shown.

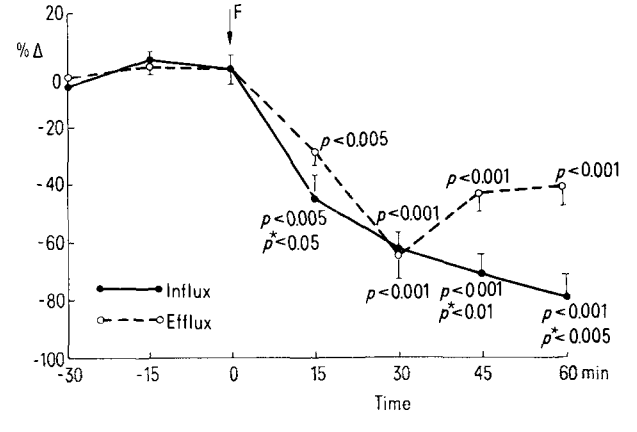

Fig. 2. Percent changes in Cl influx and efflux of the skin of *Leptodactylus ocellatus* produced by addition of furosemide (F) 10^{-3} *M* to the solution bathing the external side. Values are means ± SE of 6 and 7 experiments respectively. Significance of changes (p) and significance of differences between influx and efflux changes (p^*) are shown.

Results. Control values. Mean values of SCC and PD were 59.1 ± 10.7 $\mu A\ cm^{-2}$ ($n = 16$) and 16.2 ± 3.8 mV ($n = 14$). Calculated conductance was 4.56 ± 0.55 mho · $cm^{-2} \cdot 10^{-3}$ ($n = 14$). Chloride influx and efflux values were 3.54 ± 1.35 ($n = 6$) and 3.71 ± 0.54 ($n = 7$) $\mu Eq.\ h^{-1} \cdot cm^{-2}$. Although these values are not significantly different, this does not rule out active chloride transport, since influx and efflux measurements were made on different skins.

Effect of furosemide. Within 2 min after adding furosemide to the external solution, the SCC increased 17.1 $\mu A\ cm^{-2}$ and PD 32.9 mV (Figure 1). During the subsequent hour SCC returned to control values while PD stabilized at a level which was twice as high as control values. Tissue conductance decreased within the first 2 min by 2.96 ± 0.5 ($n = 6$); $p < 0.005$) and remained between 2.29 and 2.71 mho · $cm^{-2} \cdot 10^{-3}$ during the rest of the experiment. Chloride influx continuously dropped till the end of the experiment. By averaging the values obtained during the 1st h at 15 min interval, a mean decrease of −2.30 ± 0.42 $\mu Eq \cdot h^{-1} \cdot cm^{-2}$ was observed, representing a 64.6 ± 4.98% inhibition ($p < 0.001$). Chloride efflux also significantly dropped, reaching a minimum at 30 min. During the subsequent 30 min it stabilized at a level of 2.10 ± $\mu Eq \cdot h^{-1} \cdot cm^{-2}$ (Figure 2). The mean 1 h decrease in chloride efflux produced by furosemide was −1.66 ± 0.29 $\mu Eq \cdot h^{-1} \cdot cm^{-2}$ representing a 37.2 ± 13.8 inhibition ($p < 0.001$), which is significantly lower than the influx inhibition ($p < 0.005$).

Discussion. The results indicate that furosemide (10^{-3} *M*) added to the external side of the skin simultaneoulsy increases SCC and PD, decreases the conductance of the tissue and inhibits both Cl influx and efflux. Since Cl influx was inhibited to a greater extent than Cl efflux, it is reasonable to conclude that furosemide not only decreased the permeability of the skin to Cl^- but also inhibited active Cl^- transport.

The increase in SCC may be due either to stimulation of active Na transport and/or inhibition of active Cl transport. During the first 15 min of maximal furosemide stimulation, Cl^- influx was inhibited by 1.67 $\mu Eq \cdot h^{-1} \cdot cm^{-2}$ and Cl^- efflux by 1.0 $\mu Eq \cdot h^{-1} \cdot cm^{-2}$, resulting in a net flux inhibition of 0.67 $\mu Eq \cdot h^{-1} \cdot cm^{-2}$. This would correspond to a SCC stimulation of 18 $\mu A \cdot cm^{-2}$ which is very close to the peak value of 17.1 $\mu A \cdot cm^{-2}$ found experimentally 2 min after furosemide administration. This finding certainly does not exclude the possibility of a transient stimulating effect of furosemide on Na^+ transport but suggests that its main action was the inhibition of active and passive Cl^- movements. Further steady decline of SCC in the presence of continuously inhibited Cl^- fluxes precluded this type of calculation at later stages.

In conclusion, furosemide was shown to markedly decrease passive Cl movements in the skin of *Leptodactylus ocellatus*. This finding may have pharmacological implications. It has been suggested that the natriuretic properties of the drug can be accounted for by the inhibition of an active Cl transport in the thick ascending limb of Henle's loop[1]. However, in spite of the fact that ouabain completely inhibited this active process in the perfused thick ascending limb, full inhibitory doses of the drug infused into the renal artery in vivo failed to prevent the natriuretic response to furosemide[9]. This fact made it necessary to postulate additional mechanisms,

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and it is possible that permeability changes play a role. It has been shown recently that the thin ascending limb of Henle's loop lacks any active transport mechanism but has a selective high permeability to Cl. As a result, NaCl difuses from the lumen of this tubular segment to the interstitium following its concentration gradient and a hypotonic tubular fluid is thus created by pure physical forces[10]. Inhibition of these passive movements by furosemide would be expected to contribute to its natriuretic effect. This possibility, which still awaits experimental verification, certainly merits investigation[11].

Summary. Furosemide added to the Ringer solution bathing the external side of the isolated skin of *Leptodactylus ocellatus* increased the PD and SCC and inhibited both active chloride influx and passive chloride efflux. The action on chloride permeability is thought to contribute to the diuretic effect of the drug.

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Increased Sensitivity to Pentobarbital in Rats Fed a Diet Lacking Flavonoids

It has been shown that feeding rats a diet lacking flavonoids gives rise to cerebral oedema[1], due to the increased permeability of the blood-brain-barrier[2]. Similarly, it has been demonstrated that their deficiency in the diet greatly increases lymphoedema of the face and neck after cervical lymphatic obstruction[3]. In rats fed a diet lacking flavonoids (which had supplementary vitamin C) definite fine structural alterations were found in blood capillaries and tissues. These were quite different from those reported in C-avitaminosis[4].

Composition of the flavonoid-free diet (for 100 kg)

Isolated soya protein	22.96	kg
Maize starch	33.62	kg
Saccharose	10.00	kg
Soya oil	3.97	kg
Cellulose powder	5.16	kg
"Hostalen PP"	1.86	kg
$CaCO_3$	13.70	g
$CaHPO_4$	2831.20	g
NaCl	457.30	g
K_2SO_4	1113.00	g
MgO	90.30	g
Ferrous fumarate	34.00	g
MnO	6.40	g
$CuSO_4$	1.30	g
ZnO	7.60	g
$NaMoO_4$	0.025	g
KJ	0.650	g
NaF	0.11	g
Vitamin A	5.20	g
D_3	0.25	g
E	1.00	g
K_3	5.30	g
B_1	5.00	g
B_2	5.00	g
B_6	7.00	g
B_{12}	24.00	g
Panthothenic acid	10.0	g
Nicotinic acid	10.0	g
Folic acid	2.5	g
Biotin	5.0	g
Inosite	10.0	g
Vitamin C	25.0	g
Choline	100.0	g
Methionine	384.0	g
Lysine	120.0	g

In the course of these studies, it has been observed that rats fed a diet lacking flavonoids when anesthetized with pentobarbital sodium[5] slept longer than those fed a normal diet. The present paper deals with this phenomenon.

Materials and methods. In December 1974, Pentobarbital – induced sleeping time was estimated in 10 ♂ Sprague-Dawley rats (body weight 470 ± 18 g) fed for 118 days a diet lacking flavonoids[6] (Table) and in 10 ♂ rats of the same species (body weight 415 ± 9 g) fed a normal diet (Herilan® RM 204). Pentobarbital sodium50 mg/kg was injected i.p. The interval elapsing until the animal, lying on his back, was able to hold its head up was regarded as sleeping time.

In June 1975, the same study was performed, using 16 ♂ Sprague-Dawley rats (body weight 480 ± 36 g) fed for 160 days the diet lacking flavonoids and 16 ♂ rats (body weight 462 ± 13) of the same species fed Herilan® R/M 204. Statistical analysis was performed by the *t*-test[7].

Results. In the study performed in winter, sleeping time in control rats amounted to 4487 ± 777″, in rats fed the diet lacking flavonoids 6386 ± 859″. The difference is highly significant ($p \ll 0.0001$). In the study performed in summer, control rats slept 4247 ± 1192″, rats fed the diet lacking flavonoids 5539 ± 1239″. This difference is also highly significant ($p < 0.005$).

Discussion. Since BENTSÁTH, RUSZNYÁK and SZENT-GYÖRGYI[8] first described vitamin P, it has been the subject of considerable debate[9]. The results described in this

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