preparations were exposed at room temperature (15°C approximately) during preincubation (30 min), incubation with H^3NE and washout period. In 15 experiments carried out in summer, preparations were exposed at room temperature (20°C approximately) during preincubation, incubation with H^3NE and washout period.

The same table shows that there is no statistically significant difference ($P > 0.7$) between both series of experiments.

Discussion. The results of our experiments showed that there was no significant difference in the uptake of H^3NE, by isolated ventricle of frog when preparations were exposed at different temperatures, namely: 25°C, 35°C, and control (room temperature, 15°C approximately). We did not find significant difference either, between experiments performed in summer and winter (at room temperature).

These findings do not support the hypothesis that a change in catecholamines uptake, due to differences of temperature, could explain the discrepancy between the sensitivity of frog ventricle to catecholamines, reported by ERLIJ at al.[3], and that reported by SANCHEZ-GARCIA et al.[4]. On the other hand, seasonal variations in concentrations of catecholamines reported by DONOSO and SEGURA[5], IZQUIERDO et al.[6] could not be explained by a different ability to the uptake of the catecholamines produced by seasonal variations of temperature.

The evidence presented in this paper suggests that uptake system of norepinephrine (these results are not necessarily transferable to other catecholamines), in the frog heart remain unchanged, despite the different temperatures at which our experiments were exposed and different seasons in which the experiments were performed.

Resumen. La incorporación de H^3NE al ventriculo aislado de rana no varía, si las preparaciones son sometidas a 25°C, 35°C o temperatura ambiente (15°C, aproximadamente); asimismo tampoco existen diferencias significativas entre experimentos hechos en verano o invierno a temperatura ambiente.

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Effect of Vasopressin on the Renal Tubular Reabsorption and Cortico-Papillary Concentration Gradient of Phenacetin and its Metabolites

Analgesic nephropathy usually results from ingestion of a mixture of analgesic compounds. Which of them is responsible for causing the nephropathy is uncertain but suspicion has been focussed on phenacetin[1]. Analgesic nephropathy is more likely to develop in the presence of dehydration[2,3] when vasopressin activity is increased. One factor which may be important in determining whether a particular drug causes nephropathy or not, is the effect that vasopressin has on its reabsorption and that of its metabolites from the collecting duct. In the present study on the dog, we have examined the effect of vasopressin on 1. the reabsorption of phenacetin and its metabolites, acetaminophen (n-acetyl-*para*-aminophenol) and *p*-phenetidine (*p*-ethoxy-aniline) from the tubular fluid, and 2. the cortico-papillary concentration gradient of phenacetin and *p*-phenetidine.

The method used to assess the effect of vasopressin on the tubular handling of phenacetin and its metabolites was similar to that previously used to demonstrate the effect of this hormone on urea[4] and sodium reabsorption[5]. Experiments were performed on 7 greyhound bitches, lightly anaesthetized with approximately 125 mg/kg of chlorolase and given phenacetin 70–120 mg/kg dissolved in 25 ml warmed ethyl alcohol at the time anaesthesia. Following a loading dose of 60 mg/kg, creatinine was given by a constant infusion pump at 0.18 mg/kg/min. A brisk water diuresis was established by giving 2.5% dextrose, until a load of 40 ml/kg had been achieved and thereafter the water load was maintained constant by infusing fluid at a rate equal to urine flow. When urine flow was constant vasopressin in doses of 2.5 to 8 mU/kg was given i.v. The tubular handling of urea and phenacetin and its metabolites, during vasopressin activity, was assessed by comparing the U/P concentration changes of these substances during the control and vasopressin period, with that of the glomerular marker, exogenous creatinine. The U/P concentration during the control period was taken as the mean of the concentrations in the period immediately before giving vasopressin and in the period 90 to 130 min afterwards when urine flow had returned to within 20% of control, and during the vasopressin period, when urinary creatinine concentration was maximal. Unconjugated phenacetin, acetaminophen and *p*-phenetidine in plasma, urine and tissue samples were determined by the extraction and spectrophotometric assay of BRODIE and AXELROD[6,7]. Urea and creatinine were determined with an autoanalyser.

Results. The results are shown in Table I. If vasopressin had no effect on the tubular handling of a urinary solute then one would expect its U/P concentration change between the control and vasopressin periods to be similar to that of the 'glomerular substance', creatinine. It is seen that following administration of vasopressin the U/P concentration of phenacetin, acetaminophen and *p*-phenetidine along with that of urea rose significantly less than that of creatinine, indicating increased reabsorption during the period of vasopressin activity.

In 3 dogs the concentrations of phenacetin and *p*-phenetidine in the cortex and renal papilla were measured 1 h after the administration of 5 U of long acting vasopressin

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Table I. U/P concentrations and concentration ratios of urea, phenacetin, acetaminophen and *p*-phenetidine following vasopressin administration

Animal		U/P concentration $\frac{\text{vasopressin period 'v'}}{\text{control period 'c'}}$					U/P concentration ratios			
		creatinine	urea	phenacetin	acetaminophen	*p*-phenetidine	$\frac{\text{v/c urea}}{\text{v/c creatinine}}$	$\frac{\text{v/c phenacetin}}{\text{v/c creatinine}}$	$\frac{\text{v/c acetominophen}}{\text{v/c creatinine}}$	$\frac{\text{v/c phenetidine}}{\text{v/c creatinine}}$
D	1	3.59	2.90	1.18	3.44	1.70	0.81	0.33	0.96	0.47
	2	4.53	4.14	1.70	4.12	1.87	0.91	0.38	0.91	0.41
	3	6.29	4.77	5.10	4.16	2.90	0.76	0.81	0.66	0.46
A		5.56	5.42	4.13	4.62	3.98	0.97	0.74	0.83	0.64
B		15.25		10.11		11.66		0.66		0.76
E		6.49	5.29	2.03	1.84	2.41	0.82	0.31	0.28	0.37
J	1	5.11	4.27	4.59	4.53	3.09	0.84	0.90	0.89	0.60
	2	2.53	2.45	1.29	2.32	2.11	0.97	0.51	0.92	0.83
	3	5.28	3.63	2.48	4.30	1.76	0.69	0.47	0.81	0.33
[a] mean							0.84	0.53	0.74	0.52
99% confidence limits							0.73–0.97	0.39–0.83	0.56–0.97[b]	0.36–0.74

[a] Mean and confidence limits derived from the $\log_{10}$ distribution. [b] Confidence limits at the 90% level.

Table II. Phenacetin and *p*-phenetidine concentrations

Dog	Dose of phenacetin (mg/kg)	Phenacetin (μmoles/l)		*p*-phenetidine (μmoles/l)		Concentration papilla/cortex		
		Cortex	Papilla	Cortex	Papilla	Urea	Phenacetin	*p*-Phenetidine
L	70	556	1630	185	489	9.3	2.9	2.6
M	80	730	2701	193	876	13.1	3.7	4.5
O	100	664	4080	323	1680	9.7	6.1	5.2
Mean						10.7	4.2	4.1

tannate in oil. The kidneys were removed rapidly and frozen in a mixture of acetone and dry ice. A cross-section of the frozen kidney was cut by saw and samples of cortex and papilla were taken, ground with a pestle and mortar and extracted into benzene for assay of phenacetin and *p*-phenetidine. Tissue water content was assessed by drying to a constant weight in an oven at 103 °C. Cortical and papillary concentrations and the gradient between them are shown in Table II. The concentration of phenacetin and *p*-phenetidine in the papilla is seen to be considerably above that in the renal cortex. The concentration gradient between the papilla and cortex was on average about 40% that of urea which is comparable with that of acetaminophen found in the dog during antidiuresis[8].

The results indicate that vasopressin increases the reabsorption of phenacetin and its metabolites from the collecting duct and causes their sequestration at high concentration in the renal papilla. The ability of acetaminophen to augment vasopressin mediated water transport[9,10] may be a factor enhancing the concentration of these compounds in the renal papilla. Although acetaminophen is the major metabolite of phenacetin, any nephrotoxic effect of this drug is more likely to be due to *p*-phenetidine or its breakdown products, such as *p*-aminophenol. This may be related to the ability of these compounds to cause methaemoglobinaemia and/or to reduce the deformability of the red cell membrane. Methaemoglobin generation within the medulla would tend to reduce the availability of oxygen to the tissues both because of its consumption in the auto-oxidative processes within the red cell and by shifting the oxygen dissociation curve to the left and impairing oxygen release[11]. A reduction in the deformability of the red cell membrane may be a more important factor, as this would tend to impede the flow of blood through the vasa rectae and might even completely obstruct them.

Résumé. En comparant lex concentrations U/P de phénacétine et ses métabolites (l'acétaminophène, et la phénétidine avec créatinine) avant et après l'administration de la vasopressine, nous avons constaté que l'hormone augmente leur réabsorption tubulaire. De plus, l'hormone les maintient dans la papille du rein, ce qui peut être important dans la production de la néphropathie de type analgésique.

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