scutellum and postnotum. 0.3 mm × 3/4″ hypodermic needles with a short sharp point were used. And, although injection was made with great care, activity measurements made immediately after injection suggested that not more than 20% of the activity was recovered from the interior of the thorax while the remaining 80% was recovered from the thoracic cuticle. This is due to the spontaneous uptake of insecticide by the cuticle from the tip of the needle at the start of injection. This difficulty appears to have been overcome in the technique of GEROLT[3] in which insecticide crystals were introduced into the haemocoele by means of a metal loop through an incision made in the ventral abdomen. The following technique however, is thought to be more satisfactory: The DDT solution from a 100 µl Hamilton Syringe mounted on a gear-controlled micrometer head is brought to the tip of the needle. It is then withdrawn by a volume of 0.1 µl. The tip of the needle is wiped clean of insecticide with tissue paper wetted with acetone. The needle is then inserted and injection of the 0.1 µl of air followed by the required dose is made. In this method, varying amounts of insecticide not exceeding 10% of the internal dose can nevertheless be recovered from the thoracic cuticle immediately after injection. It is certain that the latter resulted from contact of the cuticle with the tip of needle when the latter was withdrawn. Symptoms of poisoning appeared 15 min after injection and, no doubt, toxicity was caused by the internal rather than the topical dose.

Fate of Intra-thoracic DDT. Flies were killed instantly by placing on Cardic at 0 and 6 h after injection. They were separated into heads, thoraces and abdomens and these were homogenized and assayed for activity.

DDT poisoning leads to a considerable loss of body fluid through vomiting and anal excretion. It was therefore difficult to collect blood samples exceeding 0.05 µl from anyone fly. A total of 0.7 µl of blood collected from a large number of flies at various stages of poisoning showed no traces of radio-activity. Frozen sections of thoraces 2 h after injection were autoradiographed. These show that the injected DDT was largely localised at the site of injection from which gradual uptake by the surrounding thoracic muscles takes place. Uniform distribution indicating the exhaustion of the reservoir of insecticide was not yet reached in 12-h-old material. In all sections studied there was a concentration gradient of insecticide across the thorax.

Thoraces 9 h after injection were cut with scissors and homogenized in carrier-saturated 0.35 *M* sucrose. Cuticle and debris were separated by straining through a gauze pad. The remainder was fractionated in an MSC Model 18 ultracentrifuge into myofibrils (150–200 rpm), nuclei (1,000 rpm), mitochondria (6,000 rpm), microsomes (18,000) and supernatant. These fractions showed the following percentage activities, respectively: 90, 2, 3, 3 and 2%.

The use of ion exchange resin in the determination of blood volume in houseflies[4] showed that exchange equilibrium was reached within seconds, indicating that a whole circulation of the haemolymph is completed in a very short time. One would expect that the injected DDT will form a stable suspension and that activity in all parts of the body will be detected shortly after injection. Our results, however, indicate that an intrathoracic dose of DDT of the order of 2.5 µg becomes localised and that it diffuses slowly into the neighbouring thoracic muscles and nerves setting up a concentration gradiant across the thorax. This results in the increase in the concentration of DDT in the thoracic cuticle of older material as indicated by the results in the experiment, in which the DDT in the thoracic cuticle rose from 10 to 30% in a few hours. The concentration gradient will no doubt extend from the thoracic to both cephalic and abdominal cuticle accounting for the rise in their DDT content. It appears, therefore, that the housefly haemolymph doesn't play a significant role in the transport of internal doses of DDT, perhaps as a result of a response mechanism whereby substances foreign to the circulation are confined locally. The results of the fractionation experiment suggest that DDT toxicity can be exaggerated by the remarkable affinity of the insecticide to myofibrils. Its absence from supernatant and microsomes is expected since this strain lacks both aldrin epoxidase and DDT-ase.

Résumé. Des doses de C^{14}DDT de l'ordre de 2.5 γ injectées dans le thorax de mouches domestiques – témoins restèrent localisées. Des auto-radiographes du thorax montrent que la courbe de concentration de l'insecticide décroît vers la périphérie. On peut donc présumer que chez l'insecte la circulation joue un rôle mineur dans le transport du DDT vers son site d'action.

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Time (h)	Activity (%)				
	Thoracic cuticle	Thoracic muscles	Abdominal cuticle	Internal viscera	Head
0	10	87	2	0	1
6	30	60	7	7	1.5

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Influence of Temperature and Seasons on H^3-Norepinephrine Uptake by Isolated Strip Ventricle of Frog

The order of potency of a group of sympathomimetic amines in the frog heart has been found to be isoproterenol > epinephrine > norepinephrine (NICKERSON and NOMAGUCHI[1]; LAND and HOWARD[2]). ERLIJ et al.[3] reported that isoproterenol is 10,000 times more potent than epinephrine. The results of a study (SANCHEZ-GARCIA et al.) on isolated ventricle of the frog indicated that isoproterenol was approximately 10 times more potent than epinephrine. The discrepancy in these results might be attributed to changes on the uptake system of catechol-

Table I. Uptake and retention of H^3NE by isolated ventricle of frog at 25 °C, 35 °C and control (room temperature)

N [a]	Temperature (°C)	H^3NE present during incubation (ng/ml, 5 min)	H^3NE in tissue 45 min after washout (dpm/g) [b]
7	25	5	176.617 ± 38.037
6	35	5	164.522 ± 19.020
6	control [c]	5	226.692 ± 84.123

[a] Number of experiments. [b] Mean ± standard deviation of mean. [c] Room temperature (15 °C, approx.).

amines by adrenergic nerves, produced by the different temperatures at which preparations were exposed (SANCHEZ-GARCIA et al.[4].) On the other hand, if uptake mechanism is changed by temperature, or seasons (winter-summer) this fact could explain the variations in concentrations of catecholamines reported by several investigators in amphibia (DONOSO and SEGURA[5]; IZQUIERDO et al.[6] and others).

The purpose of this work was to study the influence of temperature, and seasons (winter-summer) on H^3-norepinephrine (H^3NE) uptake by strips of isolated frog ventricle.

Methods. Ventricles of frog (*Rana pipiens*) were prepared and mounted as previously described by FURCHGOTT et al.[7]. Ventricles of frog were perfused with regular ringer solutions containing 10 g/ml of ethylenediaminetetraacetic acid (EDTA). A mixture of 95% O_2 and 5% CO_2 was bubbled through the bathing solution. All preparations were electrically driven at a frequency of 30 beat min. Ventricles were attached to force-displacement transducer (Grass model FT 03), and mechanical activity was recorded by means of a Grass polygraph. Under their respective conditions, halves were then incubated with 5 ng/ml of D, L H^3NE for 5 min, and then thoroughly washed. Four additional washes were given over the subsequent 40 min period, at the end of which the halves were removed for analysis of radioactivity. The catecholamines analysis was performed according to the method of ANTON and SAYRE[8] and radioactivity was counted in a Nuclear Chicago liquid scintillation spectrometer model 725. All samples were corrected for quenching with an automatic external reference standard.

The present method did not suggest that radioactivity obtained in the analysis was due to deaminated metabolites (WAKADE and FURCHGOTT[9]). H^3NE uptake is expressed in terms of disintegrations per min (dpm)/g of tissue. When we refer to H^3NE uptake, we mean H^3NE uptake and retention by isolated ventricle of frog, 45 min after washout. Statistical significance of the difference between was determined by the *t*-test for non-paired data.

Drugs used. – D, 1-norepinephrine-7-H hydrochloride, specific activity, 16,7 C/mmol (New England Nuclear Corp.)

Regular ringer solution in m*M*/l: NaCl, 103,4; KCl, 1,013; $CaCl_2$, 0,9009; CO_3HNa, 1,851.

Results. In 7 experiments all halves were kept under standard conditions (25 °C) for 30 min and then were incubated with 5 ng/ml of H^3NE for 5 min. Wash solution also was at 25 °C. In each experiment one half of ventricle served as a control (room temperature, 15 °C approximately). Table I shows the H^3NE uptake by ventricle isolated of frog at 25 °C.

In another series of 6 experiments all halves were kept under standard conditions (35 °C) for 30 min and then were incubated with 5 ng/ml of H^3NE for 5 min. Wash solution also was at 35 °C. In each experiment one half of the ventricle served as a control (room temperature, 15 °C approximately). Table I shows the H^3NE uptake by ventricle isolated from frog at 35 °C.

The 4th column of the table shows that there is no statistically significant difference between H^3NE uptake in preparations at 25 °C ($P > 0.8$). The same table shows that there is no statistically significant difference between the H^3NE uptake in preparations at 25 °C, 35 °C, and those subjected at room temperature (control) ($P > 0.5$ for 25 °C and $P > 0.6$ for 35 °C).

The Table II shows the H^3NE uptake by strips of isolated frog ventricle during different seasons (winter-summer). In 24 experiments carried out in winter,

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Table II. Uptake and retention of H^3NE by isolated ventricle of frog at different seasons (winter-summer)

N [a]	Seasons [c]	H^3NE present during incubation (ng/ml, 5 min)	H^3NE in tissue 45 min after washout (dpm/g) [b]
24	Winter	5	221.293 ± 26.951
15	Summer	5	242.312 ± 69.310

[a] Number of experiments. [b] Mean ± standard deviation of mean. [c] Winter (15 °C approx.). Summer (20 °C approx.).

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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