some substances, which are released during shock, may be responsible for the activity of HLL hydrolase in plasma. NAKAHARA recently isolated a low molecular weight inhibitor in lysosomes of the skeletal muscle[3]. The present paper was designed to investigate the effects of epinephrine, norepinephrine, histamine, serotonin and corticosteroids, which might be secreted as part of the metabolic response to ischemia, on plasma HLL hydrolase activity.

Mongrel dogs were subjected to tourniquet shock by application of tourniquets to both thighs following the method of the previous paper[4]. Heparinized plasma was obtained before and after release of the tourniquets. 1 ml of plasma cleaves at the rate of 0.95 μM/min[3]. The activity of HLL hydrolase decreased after release of the tourniquets, and reached a minimum 30 min after release. Aliquots of each sample were ultrafiltered using an Amicon Ultrafiltration Chamber with UM-10 membrane. When each ultrafiltrate was added to normal plasma, HLL hydrolase activities decreased suggesting that HLL inhibitors appear in plasma after release of the tourniquets (Table I).

Increase in blood glucose and reduction in the tolerance for glucose after clamp release have been shown in shocked rats[5-7]. The glycogen level in uninjured muscles and the liver continuously decreases after limb ischemia, but a medullectomy can prevent hyperglycemia and loss of glycogen from uninjured muscles[5]. Plasma concentration of epinephrine and norepinephrine in hemorrhagic and anaphylactic shock increases[8,9]. In the present experiment, adrenaline and noradrenaline did not affect HLL hydrolysis of the plasma over a concentration range of 10^{-3} to $10^{-8}M$. Glucose also had no effect on its activity up to 500 mg/100 ml. However, insulin enhanced HLL hydrolysis of plasma; 4×10^{-3} insulin-units showed 42% enhancement of its activity compared with the control. The high initial glycogenolytic rate is generally attributed, in part at least, to secretion of epinephrine and norepinephrine[10].

Relatively large amounts of histamine were released in the peritoneal cavity of rats after release of the tourniquets, but only small amounts of serotonin were present[11]. Serotonin reduced HLL hydrolysis of the plasma; HLL hydrolase was significantly inhibited at a concentration of $10^{-4}M$, whereas histamine had no effect on its activity (Table II).

Hydrocortisone was effective in lowering the level of the lysosomal enzyme after tourniquet release, presumably by stabilizing lysosomal membranes[12]. An increased secretion of adrenal cortical steroids follows operative or traumatic injury[13,14]. It is also known that the adrenal corticosteroids of the 17-hydrocorticoid-type contribute to continued hyperlgycemia[10]. Corticosteroids used in this experiment effectively reduced the levels of the HLL hydrolase with increasing concentrations up to $10^{-4}M$ (Table II). It is thought from the above findings that corticosteroids and serotonin may be responsbile for the reduction of plasma HLL hydrolase levels after ischemia. But, there may be no relationship between the reduction of plasma HLL hydrolase activity by corticosteroids and survival rate following administration of corticosteroids in tourniquet shock. Hydrocortisones were ineffective for increasing the survival rate in tourniquet shock[7,12]. The significance of the reduction of this enzyme level in plasma is under investigation.

Résumé. L'activité de l'hydrolase de la hippuryl-L-lysine dans le plasma du chien est diminuée par l'épreuve du tourniquet. L'hydrocortisone, la prédnisolone, la dexaméthasone, la β-méthasone et la sérotonine réduisent l'activité de cet enzyme in vitro, tandis que l'histamine, l'adrénaline et la noradrénaline sont sans effet.

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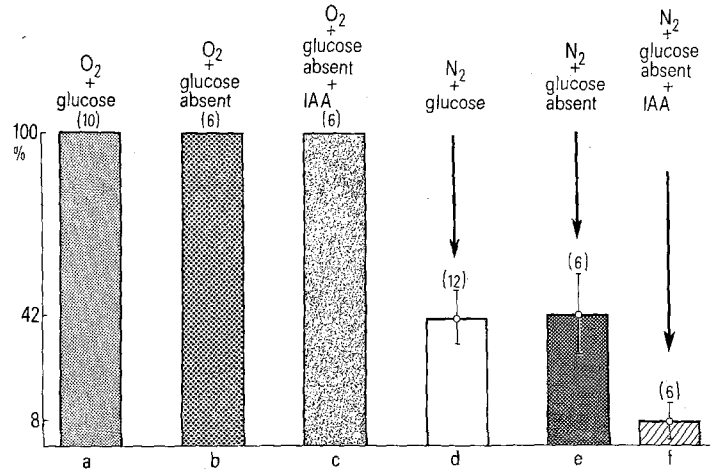
Sources of Metabolic Energy used by Isolated Strips Ventricle of Frog Heart for the Uptake and Retention of H^3-Norepinephrine

The uptake and retention of H^3-norepinephrine (H^3NE) by isolated strips ventricle of frog heart is an active process requiring metabolic energy. In a recent paper[1] we obtained evidence that under anoxia and glucose deprivation, the uptake and retention of H^3NE by isolated strips ventricle of frog heart was 42% compared with controls under standard conditions (O_2 plus glucose absent in the incubation medium) and that the energy for this uptake and retention was produced by the glycolysis of endogenous carbohydrates since the pretreatment with iodoacetate (IAA) reduced the uptake and retention to 8%. These results differ greatly from those obtained on isolated atrium of the guinea-pig[2].

However, further experiments carried out in our laboratory under similar circumstances to those described above (isolated strips ventricle of the frog heart, under anoxia plus glucose deprivation) showed significant differences in the H^3NE uptake and retention compared with the results previously described[1].

The present paper reports some of the results lately obtained, and an attempt to connect these results with those previously published. A theory is also proposed, elaborated with the data obtained in this work and in several previous publications[1,3,4], that would explain the sources of metabolic energy utilized for the uptake and retention of H^3NE by isolated strips ventricle of frog heart.

Methods. Ventricles of frog (*Rana pipiens*) were prepared and mounted as previously described by FURCHGOTT et al.[5], for isolated atrium of guinea-pig, Ventricles were suspended in an organ bath containing 20 ml of regular Ringer solution[6], containing 10^{-5} g/ml of ethylene diamine tetraacetic acid (EDTA). A mixture of 95% N_2



Comparison of the H^3-norepinephrine uptake and retention by isolated strips of frog ventricle under different circumstances. a) Controls (100% uptake and retention) were kept in glucose medium and 95% O_2 and 5% CO_2 for 30 min. H^3NE (5 ng/ml) was added for 5 min and then the ventricle was washed for 40 min with glucose-Ringer under 95% O_2 and 5% CO_2. b) Strips were incubated in a similar manner to a), but kept in glucose-free medium (wash solution also was glucose-free). c) Strips were incubated in a similar manner to b) for 30 min. Halves were then treated with IAA (10^{-4} g/ml) for 20 min and then incubated with H^3NE (wash solution was similar to b). d) Strips were incubated in a similar manner to a), but kept under a mixture of 95% N_2 and 5% CO_2 (wash solution also was under anoxia). e) Strips were incubated in a similar manner to d), but kept in glucose-free medium (wash solution also was glucose-free). f) All halves were kept under a combination of anoxia and glucose deprivation for 30 min. Halves were then treated with IAA (10^{-4} g/ml) for 20 min and then incubated with 5 ng/ml of H^3NE for 5 min. Wash solution also was under anoxia and glucose deprivation. The results (means ± SEM) are expressed as percent of controls for the numbers of experiments given in parentheses. Results previously reported by MARTINEZ-SIERRA[3,4] and MARTINEZ-SIERRA and LORENZO-VELAZQUEZ[1].

and 5% CO_2 or 95% O_2 and 5% CO_2 was bubbled through the bathing solution (see results). All preparations were electrically driven at a frequency of 30 beats/min. Ventricles were attached to force-displacement transducer (Grass model FT 03), and mechanical activity was recorded by means of a Grass polygraph. Each ventricle was subjected to a resting tension of 1 g. Under their respective conditions, halves were then incubated with 5 ng/ml of D, L-H^3NE for 5 min, and then throughly washed; 4 additional washes were given over the subsequent 40 min period, at the end of which the halves were removed for analysis of radioactivity. All preparations were performed at room temperature.

The catecholamines extraction was performed according to the method of ANTON and SAYRE[7] 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. Under our working conditions, the radioactivity present in the alumina eluates cannot be ascribed to metabolites of H^3NE but to H^3NE itself[2]. H^3NE is expressed in terms of disintegrations per min per g of tissue (dpm/g). Statistical significance of the difference between means was determined by the *t*-test for paired data.

Drug used: D, L-norepinephrine-7-H^3hydrochloride, specific activity, 16,7 C/mmol (New England Nuclear Corp).

Results. In 6 paired experiments, all halves were kept under anoxia and glucose deprivation for 30 min and then were incubated with 5 ng/ml of H^3NE for 5 min. Washing solution also was under anoxia and glucose deprivation. In each experiment one half of ventricle served as a control (O_2 and glucose deprivation). Table I shows a statistically significant decrease ($P < 0.001$) between the H^3NE uptake and retention in preparations under anoxia and their controls.

Discussion. The results obtained (Table I) show that the uptake and retention of H^3NE by strips ventricle of the frog heart under anoxia and a free-glucose medium was 21% of the controls under O_2. These data present a

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Table I. Uptake and retention of H^3 NE by isolated strips ventricle of frog under anoxia

N[a]	Gas mixture	H^3NE present during incubation	H^3NE in tissue 45 min after washout[b] (dpm/g)	Difference (%)
6	95% N_2 and 5% CO_2	5 ng/ml (5 min)	49,440 ± 9,802	79
	95% O_2 and 5% CO_2	5 ng/ml (5 min)	232,832 ± 35,552	

[a] Number of paired experiments. [b] Mean ± S.E.M.

Table II. Theory proposed to explain the results showed in Table I and in the Figure

Energy produced by	Columns of the Figure					
	a	b	c	d	e	f
Oxidative metabolism	4×	4×	4×.	Blocked by anoxia	Blocked by anoxia	Blocked by anoxia
Glycolysis endogenous substrates[a]	1×	1×	Blocked by IAA	1×	1×	Blocked by IAA
Exogenous glucose	0×	Absent	Absent	0×	Absent	Absent
Total energy produced	5×	5×	4×	1×	1×	—
Energy necessary	2×	2×	2×	2×	2×	2×
Uptake and retention (%)	100	100	100	50	50	0

[a] In the results reported in table I energy produced by this pathway would be 0.42×, whereas in the results reported in the figure would be 0.84×. Consequently in columns d) and e) the uptake and retention was 21% and 42%. See text for details.

great discrepancy compared with those previously published by us[1] in which the H^3NE taken up and retained under similar circumstances was 42% of the controls. However, when preparations were pretreated with IAA (unpublished results), the H^3NE uptake and retention decreased to 8%, in a similar manner to that previously described. This fact prompted us to think that the uptake and retention observed (21%) was obtained from the energy produced by endogenous carbohydrates.

This paradoxical finding that under the same experimental circumstances (anoxia plus glucose deprivation) the frog ventricle presents such differences in the H^3NE taken up and retained, and that a previous treatment with IAA produces a similar blockade, can be explained as follows: In the results previously published (42% of uptake and retention), the experiments were carried out with animals killed 1, 2, 3 days after being transported from their natural habitat, whereas in the experiments described in the present paper the animals were killed some weeks after their maintenance in our laboratory without food, with only water (some of them died). This fact induces us to think that their carbohydrates reserves were strongly depleted and the energy produced by a glycolytic pathway would be consequently smaller.

These results and some of previous publications suggest the following theory that would explain, under our experimental conditions, the diverse sources of metabolic energy utilized by isolated strips ventricle of frog heart for the uptake and retention of H^3NE: 1. We assign the hypothetical value of 2× to the metabolic energy necessary to obtain a 100% of H^3NE uptake and retention. 2. We assign the hypothetical value of 4× to the energy produced by an aerobic way. 3. According to the endogenous carbohydrate reserves in the animals, the energy produced by a glycolytic pathway would be 1×, 0.75×, 0.50× ... 4. Exogenous glucose is not utilized as a source of energy in this process.

In the Figure are shown the results previously reported by us in several publications[1,3,4], in which the metabolic requirements for the H^3NE uptake and retention by isolated strips ventricle of frog heart were studied. Table II would accounts for these results according to the theory above exposed.

Conclusions. Under our experimental circumstances, the uptake and retention of H^3NE by isolated ventricle of frog: 1. Is not affected by the presence or absence of glucose in the incubation medium (compare column a) and b) of the Figure and Table II). 2. IAA does not produce any effect per se on this mechanism (compare column c) and a) of the Figure and Table II). 3. The noncarbohydrate endogenous substrates oxidation provides all the energy necessary for this process (column c) of Table II and the Figure). 4. Anoxia produces some energy for this process (column d) and e) of Table II and Figure). 5. Under anoxia, exogenous glucose is not utilized as a source of energy (compare column d) and e)). 6. Under anoxia, energy is produced by endogenous carbohydrates (glycolysis) (compare column e) and f)). 7. Energy produced by the glycolysis of endogenous substrates, depends upon the reserves of the experimental animals. Compare Table I (with fasting animals), with column of Table II and the Figure (with animals well nourished). 8. The small residual uptake and retention of H^3NE in preparations under anoxia and IAA (column f) could be due to energy produced by oxidation of endogenous substrates by traces of O_2 in the gas mixture.

Resumen. La oxidación de substratos endogenos no carbohidratos produce la energia necesaria para obtener un 100% de incorporación y retención de H^3NE por el ventriculo aislado de rana. La glicolisis de substratos endogenos produce la necesaria para obtener de un 21–42%. La glucosa exogena no es fuente de energia utilizada para este proceso.

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Sulfhydryl Groups, Copper, Diphenylamine Reaction and some Enzymes in the Serum of Rats with 6-Sulfanilamidoindazole Arthritis

In 1964 MIELENS and ROZITIS[1] described an arthritis which appeared after oral administration of high doses of 6-sulfanilamidoindazole (6-SAI) in old rats, and which almost exclusively affected the hindlimbs. There is hitherto little knowledge about typical humoral changes in 6-SAI arthritis. Therefore we determined some biochemical parameters of the blood during this arthritis.

Material and methods. Male Wistar rats with a mean body weight of 410 g (range 300–570 g) received 125 or 250 mg/kg of 6-SAI orally once daily for 7–9 consecutive