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 However, when preparations were pretreated with IAA (unpublished results), the H^3NE uptake and retention decreased to 8%, in a similar manner to that previously described.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

days as a 10% suspension in a 1% aqueous suspension of gum tragacanth. Administration was performed during a slight ether anesthesia. Blood was obtained from ether anesthetized animals by puncture of the orbital vein plexus or of the heart. Sulfhydryl groups (SH groups) were determined with 0.1 ml of serum according to BUTLER et al.[2]. Copper determination was performed by Sächsisches Serumwerk Dresden following the DAB 7-DDR[3,4]. Diphenylamine reaction was performed according to AYALA et al.[5]. Inflammation units were determined according to GLENN et al.[6] and lysozyme according to PILIERO and COLOMBO[7] modified by GIESSLER[8]. The activity of leucine aminopeptidase (LAP, with leucine hydrazide as substrate) was determined according to SCHMOLLACK[9]. The activities of lactic dehydrogenase (LDH) and of glutamic-oxalacetic transaminase (GOT) were determined with the commercial sets of VEB AWD Dresden (UV-tests, see[10]).

Results and discussion. Arthritis appeared after daily administration of 125 or 250 mg/kg of 6-SAI for 8 consecutive days at the earliest at day 2. It developed in 93% of the rats by day 9 (Figure). With 250 mg/kg of 6-SAI, arthritis developed somewhat earlier than with 125 mg/kg. Forepaws were not involved. SIGG et al.[11] observed, after administration of 125 mg/kg of 6-SAI for 12 days, an arthritis in all animals.

The Table summarizes the results of blood determinations. At day 8–10, SH groups are significantly decreased and copper and DPA reaction are significantly increased. SIGG et al.[11] found plasma fibrinogen in 6-SAI arthritic rats significantly increased. The changes of these parameters are thus the same as in the adjuvant arthritis[2,12–14]. Inflammation units[6] and lysozyme activity[7,8] are also increased in adjuvant arthritis. In the 6-SAI arthritis they are surprisingly not significantly influenced. LDH and GOT activities, which are also increased in adjuvant arthritis[6,15], are likewise not altered in 6-SAI arthritis. While erythrocyte sedimentation rate and total white cell count are commonly increased in acute inflammation, MILLER et al.[16] found no change in 6-SAI arthritis; SIGG et al.[11], however, reported an increase of these parameters in 6-SAI arthritis. The specific pattern of the altered biochemical parameters in 6-SAI arthritis might include aspects of interest regarding the reaction of further biochemical parameters, the involvement of mediators of inflammation as well as the usefulness of 6-SAI arthritis for testing anti-inflammatory substances.

Zusammenfassung. Bei oraler Gabe von täglich 125 bzw. 250 mg/kg 6-Sulfanilamidoindazol zeigten am 9. Tag 93% der Ratten Arthritis der hinteren Extremitäten. Im Serum waren Sulfhydrylgruppen um 40% abgesunken, Kupfer um 82% und Diphenylaminreaktion um 55% angestiegen. Die Fermente Lysozym, LDH, LAP und GOT sowie die Entzündungseinheiten blieben unbeeinflusst.

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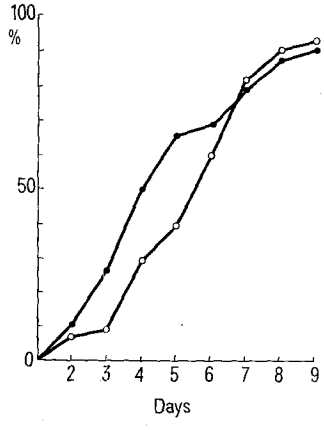
Biochemical parameters of the serum on day 8–10 after administration of 125 mg/kg of 6-SAI for 7–9 days

Parameter	Control value ($\bar{x} \pm s$)	N	Change (%) ($\bar{x} \pm s$)	N
SH groups	405 $\pm$ 50 μM	20	-40 ± 20 [a]	23
Copper	1.85 $\pm$ 0.22 ppm	15	$+82 \pm 27$ [a]	10
DPA	309 $\pm$ 60 [b]	14	$+55 \pm 36$ [a]	23
Lysozyme	7.4 $\pm$ 0.9 μg/ml	10	+11	8
LDH	164 $\pm$ 42 U/l [c]	6	+ 8	7
LAP	490 $\pm$ 147 IU/l	12	0	6
GOT	27 $\pm$ 11 IU/l	9	0	6
Inflamm. units	12.6 $\pm$ 4.7	25	+10	11

[a] $p < 0.001$. [b] DPA value: absorbance $\times 1000$. [c] Incubation temperature 20 °C.



Cumulative incidence of arthritis following daily administration for 8 consecutive days of 125 (○; $N = 44$) or 250 mg/kg (●; $N = 28$) of 6-SAI.

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