used by MACHOVA. However, in a number of preparations this correlation was not observed (Figure).

These data imply that either glycoside = sensitive Na pumps are of different structure in cardiac muscle of cats and frog skin or the interaction of some glycosides with Na, K-ATPase in the whole organism differs from that in isolated biological membranes of frog skin.

ВЫВОДЫ. Сердечные гликозиды с $C\overset{O}{\underset{H}{<}}$ В 19-ОМ положении молекулы оказались наиболее эффективными ингибиторами натриевого насоса клеток кожи лягушки. Наличие ацетильного радикала увеличивало ингибирующую способность гликозида; агликон - оуабагенин оказался самым слабым инибитором.

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Effect of Pyruvate on the Acute Cyanide Poisoning in Mice

It is well known that, besides other collateral effects, e.g. block of the –SH groups, the acute toxicity of cyanide is due to its capacity to bind the terminal oxidase of the mitochondrial respiratory system[1].

At present, the most used antidotes to cyanide poisoning are: a) compounds able to produce methaemoglobin, such as sodium or amyl nitrite[2,3], *p*-aminopropiophenone and methylene blue[3] or b) thiosulfate[2,3], which is substrate for the enzyme rodhanase. The reason for employing the first groups of compounds is based on the fact that the competition between methaemoglobin and cytochrome oxidase for cyanide makes possible the formation of the non-toxic cyanmethaemoglobin. However, since the cytochrome oxidase-cyanide complex is much less dissociable than cyanmethaemoglobin[4], a high level of methaemoglobinemia is required. When thiosulfate is used, rodhanase catalyzes the following reaction: $CN^- + -S$ compounds $\rightleftharpoons CNS^-$[3]. The resulting thiocyanate is not toxic. The use of such antidote has also some limitations due to the presence of rodhanase almost exclusively in the tissues, so that its activity is restricted to the free cellular cyanide[3]. In addition, the rodhanase activity is counteracted by the activity of thiocyanate oxidase[3]. For a more detailed description of the present treatment of the acute cyanide intoxication, see DONE[3].

In a recent paper we presented evidence for the activity of pyruvate in removing quickly the inhibition of respiration induced by cyanide, leaving unaffected the integrity of oxidative phosphorylation of Ehrlich ascites cells in vitro[5]. The effect of this compound was attributed to its reaction with cyanide which leads to the formation of the non-toxic pyruvic-cyanhydrin. On this experimental basis and because of all the difficulties in the present therapy of acute cyanide intoxication, we thought it worth testing the effectiveness of sodium pyruvate on the cyanide poisoning in mice. The results presented here show that pyruvate is able to remove, even in vivo, the binding of cyanide to cytochrome oxidase. Moreover pyruvate, since is not toxic at the doses used and lowers significantly the lethality by cyanide, appears to be an antidote relatively more suitable than the others described above.

Materials and methods. Male albino mice of the Swiss strain weighing 23–25 g were used in all experiments. The animals were housed at random in stock cages, in groups of 12, and fed with a standard balanced diet and tap water ad libitum. The drugs were supplied to animals fasted 6 hours. NaCN, dissolved in bidistilled water, was given i.p.; sodium pyruvate, dissolved in sterile 0,9% NaCl, was injected i.v. 30 sec after NaCN. Groups of

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Effect of sodium pyruvate, injected i.v., on the mortality induced by cyanide in mice[c]

Treatment	% Mortality (cumulative values) $n = 12$[a] NaCN mg/Kg i.p.													LD_{50}	Limits (95% confidence)	PR[b]
	3.50	4.50	4.75	5.00	5.25	5.50	6.00	7.00	7.25	7.50	8.00	8.50	9.00			
Controls	25	41.7	67.7	91.7	100	100	100	100	100	—	—	—	—	4.785	(4.650–4.923)	
Sodium pyruvate 250 mg/kg i.v.	—	—	—	0	0	0	8.3	66.7	83.3	100	100	—	—	6.705	(6.487–6.931)	0.71
Sodium pyruvate 500 mg/kg i.v.	—	—	—	—	0	0	0	0	0	25	58.3	66.7	100	7.914	(7.565–8.280)	0.60

[a] Number of animals tested at each dose level. [b] LD_{50} NaCN/LD_{50} NaCN + sodium pyruvate. The slopes of all lines compared did not deviate significantly. [c] For the experimental conditions see materials and methods.

12 mice were used for each dose level. The doses used are reported in the Table. Groups of animals were kept in separate cages for 72 h. Results were subjected to probit analysis. Medium lethal dose (LD_{50}) with the fiducial limits and potency ratios with the 95% confidence limits were obtained. Acceptable tests for parallelism and heterogeneity were always obtained before the potency ratios were determined.

Results. The NaCN induced deaths of the control mice and of the mice treated i.v. with sodium pyruvate, which occurred within less than 10 min, are shown in the Table. There was no evidence of late toxicity.

Sodium pyruvate treatment resulted in a marked increase in the amount of NaCN required to produce death. The values of LD_{50}, reported in the same Table, deviate significantly ($p<0.05$).

From these data it is evident that pyruvate treatment has an antagonist effect against acute cyanide lethality in mice.

The prompt regression of the symptomatology of cyanide intoxication, together with the markedly decreased incidence of lethality, indicate that there is a strict correlation between these effects of pyruvate in vivo and the release of the cyanide-blocked respiration already observed in isolated cells[5]. Moreover, even though additional pharmacological experimentation is required, the present data suggest the possibility of using pyruvate as an antidote to acute cyanide poisoning.

Riassunto. Sono stati studiati gli effetti del piruvato di sodio nella intossicazione acuta da cianuro nel topo. I risultati, statisticamente validi, dimostrano che il piruvato iniettato per via endovenosa riduce notevolmente l'effetto letale del cianuro di sodio somministrato per via endoperitoneale[6].

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Increased Bilirubin Conjugation in the Liver and Intestinal Mucosa of Phenobarbital Treated Rats

It has been shown that the intestinal mucosa is able to conjugate several substrates with glucuronic acid and that this capacity is parallel to that of liver[1]. This ability has also been proved in vitro for bilirubin[2,3]. In a previous work[4] we demonstrated the presence of conjugated bilirubin in the intestinal mucosa of normal and hepatectomized rats infused intravenously with unconjugated bilirubin.

Phenobarbital administered to patients with jaundice, due probably to bilirubin UDP-glucuronyl transferase deficiency, led to a reduction of plasma bilirubin levels[5]. Since phenobarbital increased the maximum biliary excretion of bilirubin (Tm) in heterozygous Gunn rats with a partial defect of bilirubin conjugation, enzyme induction by drug administration had to be admitted[6]. On the other hand, the level reduction of plasma unconjugated bilirubin in jaundice by phenobarbital treatment was considered strong evidence of bilirubin conjugation deficiency[7].

In this investigation we found that phenobarbital increased the content of conjugated bilirubin in the intestinal mucosa of Wistar and heterozygous Gunn rats infused with unconjugated bilirubin intravenously. This effect was also observed after total hepatectomy.

Methods. 24 Wistar and 20 heterozygous Gunn rats of both sexes weighing from 260 to 400 g were used. 12 Wistar and 9 heterozygous Gunn rats were used as untreated controls. The remainders were injected daily with phenobarbital (100 mg/kg dissolved in 1 ml of 0.9% NaCl) i.p. during 3 days. Unconjugated bilirubin was infused i.v. as described[4] and the bile collected for Tm calculation[8]. Blood, liver, as well as the mucosa and content of the small intestine, were obtained as previously reported[4]. Total bilirubin was determined in serum and bile samples[9] and in liver, mucosa and intestinal content homogenates[4,10]. Diazotized samples were concentrated to a small volume[11] and chromatographed on paper[12]. Conjugated bilirubin was calculated from total bilirubin concentration and the proportion of azopigment B on the chromatograms determined by densitometry (Densicord 542 A, Photovolt, USA).

In another set of experiments, 4 Wistar and 5 heterozygous Gunn rats (2 rats of each group received phenobarbital) were subjected to total hepatectomy[4] and then injected i.v. with unconjugated-^{14}C bilirubin (200,000 to 350,000 dpm, specific activity 2,400 to 4,200 dpm/μg) mixed with unradioactive pigment (2 mg/100 g body wt.). The animals were sacrificed 30 min after the injection. Crystalline radioactive bilirubin was obtained from the bile of rats[13] that were injected with ^{14}C-ALA (δ-aminolevulinic acid-4-^{14}C hydrochloride, CEA, France)[14]. Mucosa and intestinal content homogenates were diazotized and chromatographed as described. Azopigments B were eluted from chromatograms, pooled separately, concentrated to a small volume and submitted to two-dimensional chromatography. After the first development, the sheet was removed, dried and azopigments B were hydrolized on the paper[2] by using bacterial β-glucuronidase (Sigma Che.Co.) (1% solution in distilled water alcalinized with 0.1 *N* NaOH at pH 6.2). After incubation at 37 °C the

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