compound, respectively, in infected medium to the cells, incubating, and subsequently pouring away the medium and replacing with fresh medium, containing the appropriate compound, every 24 h. The effect of replacing the medium was to delay onset of CPE in all 3 sets of tubes from day 2 onwards. However, this effect was greater for IUdR-containing tubes than for tubes containing the HBB-derivative. With daily replacement of active compounds, there was little overall difference in protective activity between 5-bromo-1-propyl-HBB (50 μM) and IUdR (50 μM). However, the toxicity of IUdR for cells is much less than that of the benzimidazole drivative, although, of course, its long-term effect on cells in general might be suspect.

The observed cell protective effects of the 1-butyl-, 1-phenyl-, 1-benzyl-, and 5-bromo-1-propyl-derivatives of HBB suggest that further investigation of the activities of lipophilic benzimidazole derivatives against DNA viruses is desirable, particularly in relation to the dependence of activity on the specific cell culture employed.

Zusammenfassung. 5-Bromo-1-propyl-, 1-Benzyl-, 1-Phenyl- und 1-Butyl-2-(α-oxy-benzyl-benzimidazol hemmen die cytopathische Wirkung (HeLa Zelle) von Vaccinia-Virus.

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Oxidation and Excretion of D-Lactic Acid by Rats

The role of L(+)lactic acid as the 'physiological isomer' in animal lactate metabolism is since long established. As the classical experiments of CORI and CORI[1] with rats have shown, D(−)lactic acid is poorly utilized and 30 to 40% of the dose ingested is excreted in the urine. These results were fully confirmed by more recent investigations[2] using ^{14}C-D-lactate. However, the wide-spread occurrence of D-2-hydroxyacid dehydrogenase (EC1.1.99.6) in liver, kidney, heart, brain and spleen of the rat and other animals, as well as reversible activation of this enzyme in vitro[3], indicated the need for a re-examination of D-lactate metabolism.

When rats of 250–300 g body weight (BW) were fed a purified diet containing DL-lactate, only 1–2% of the D-lactate ingested was recovered in urine (Table I). Contrary to earlier experiments, a specific enzymatic assay (Boehringer, Mannheim) was used for D-lactate determinations. The excretion of such low levels on the first days of lactate feeding excluded long-term adaptation. In further experiments, oxidation and excretion of D-lactate were measured by $^{14}CO_2$-exhalation radiometry (Exhalameter Berthold & Frieseke, Karlsruhe) in 24 h-fasted rats injected with ^{14}C(u)-D-lactate (Amersham) in appropriate dilution with inactive D-lactate (Serva, Heidelberg). As indicated by the results (Table II), D-lactate was readily oxidized. The somewhat higher excretion in urine after i.p. injection as compared to feeding, can be attributed to faster absorption and to increased diuresis due to sodium surplus. The fraction of metabolites of D-lactate in urine was calculated from radioactivity and enzymatically determined D-lactate. Intermediary isomerization to L-lactate could not be excluded since the metabolites were not isolated. Enzymatic determination of L-lactate in urine indicated, however, that this isomer accounted for only 0.9 and 1.1% of the D-lactate injected into animals on control diet and lactate diet, respectively. Thus, isomerization could not have occurred to a large extent. Surprisingly, lactate feeding did not enhance D-lactate oxidation but tended to increase the excretion of D-lactate and its metabolites in urine. The mechanisms envolved may deserve further investigation.

Table I. Urinary excretion of D-lactate in rats fed a purified diet with 5% DL-Ca-Na-lactate

Measurements	Days on DL-lactate diet	
	1	2
Number of animals	5	5
D-lactate consumed (mg/kg BW)	490 ± 135	629 ± 143
D-lactate in urine (mg/kg BW) [a]	7.4 ± 1.9	7.1 ± 0.4
Dosis (%)	1.5	1.1

[a] 24 h collections.

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Table II. Oxidation and excretion of D-lactate after i.p. injection in rats fed lactate and control diets

Measurements [a]	Lactate-free diet	5% DL-lactate diet
Number of animals	12	12
D-lactate injected (mg/kg BW) [b]	247.0 ± 20.2	247.0 ± 8.4
% of dosis recovered within 6 h as:		
CO_2 in exspiration	84.4 ± 1.3	81.9 ± 2.3
D-lactate in urine	2.9 ± 1.5	4.7 ± 2.7
Metabolites in urine	3.0 ± 2.2	5.5 ± 3.2
Total	90.3 ± 2.2	92.1 ± 5.3

[a] Groups of 3 animals measured simultaneously, $\bar{x} \pm s$ based on $n = 4$. [b] 22 nCi ^{14}C(u)D(−)lactic acid/mg, neutralized with NaOH i.p.

The present results differ markedly from those reported in the literature[1,2]. This discrepancy is not easily explained. Unpublished evidence suggests that lactoyllactic acid and higher polymers in the preparation would escape intermediary oxidation but not recovery in urine by non-enzymatic method. The physiological significance of the metabolism of D-lactate may be largely based on the intestinal production of D,L-lactate by many kinds of bacteria[4,5]. It may also gain importance for the utilization of D-lactate by man and animals consuming fermented vegetable or milk diets.

Zusammenfassung. Mittels spezifischer Methoden wurde der Stoffwechsel von D-Lactat bei Ratten untersucht. Nach peroraler Gabe erschienen (entgegen den bisherigen Befunden) nur 1–2% im Harn. Nach i.p. Gabe wurde D-Lactat rasch oxydiert und binnen 6 h als CO_2 exspiriert (83.2%) sowie in Form von D-Lactat (3.8%) und Metaboliten (4.2%) im Harn ausgeschieden.

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Effect of Low Calcium Concentration on the Oxidation of NAD-Linked Substrates in Rat Liver and Tumor Mitochondria

The uptake of Ca^{2+} and its effect on mitochondrial structures and functions has been extensively studied[1]. Recently Vinogradov et al.[2] have reported an inhibitory effect of high Ca^{2+} concentrations on respiration of rat liver mitochondria in the presence of NAD-linked substrates.

In this paper we describe the effect of low Ca^{2+} concentrations on rat liver and tumor mitochondria under different metabolic conditions. The results obtained indicate that Ca^{2+} can play, in rat liver mitochondria, a significant role on the oxidation of NAD-linked substrates, depending on the energetic state of mitochondria upon addition of the cation. Moreover Ca^{2+} has no effect on tumor mitochondria regardless of their energetic state.

Materials and methods. Rat liver mitochondria were prepared according to Chance and Hagihara[3]. Ehrlich ascites tumor cells mitochondria (Lettré-hyperdiploid strain) were isolated by the method of Kobayashi

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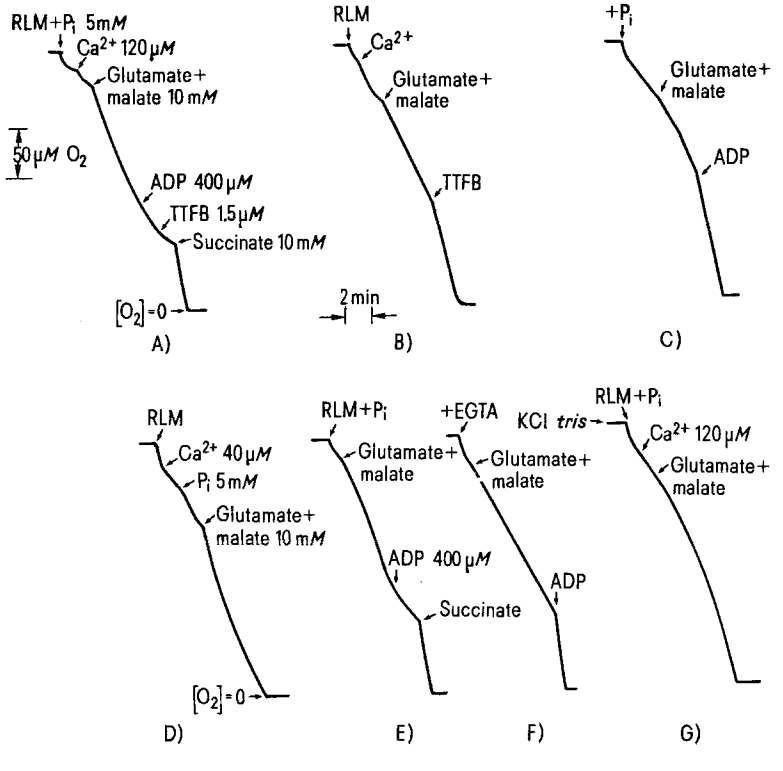

Fig. 1. Polarographic recordings of O_2 uptake in rat liver mitochondria showing the effect of Ca^{2+}, in the presence of P_i, on NAD-linked substrates respiration. 0.1 ml of mitochondrial suspension, containing 5–6 mg protein (A, B, C, D and G) or 2.5–3 mg protein (E and F), were added to 2.4 ml of an isotonic medium composed by: 0.225 *M* mannitol, 0.075 *M* sucrose and 0.01 *M* MOPS (morpholino propane sulfonic acid) pH 7.4, except in G, where the medium used was 0.08 *M* KCl and 0.02 *M Tris* pH 7.4. All the additions are reported on the figure. EGTA concentration: 1.0 m*M*.