Rf values and UV-spectra of the extracted spots found with the second system employed, that of HOTCHKISS[15], were also identical to those of the original, commercially available adenine, guanine, cytosine and thymine, respectively. Radioactivity could be found only in adenine, guanine and thymine in cells treated with C^{14}-MBH and C^{14}-formate, respectively. Furthermore, the spots of these bases were the only ones over the whole length of the paper chromatogram where a distinct radioactivity above background was found. Although the distribution of counts per min (cpm) per spot in the MBH experiment did not parallel those in the formate experiment, the patterns of distribution of radioactivity were similar in both experiments. These results indicate that in vivo the methyl group of MBH is metabolically labile, since it was oxidized and incorporated into adenine, guanine and the methyl group of thymine. Studies are under way to investigate whether methylation of the purine or pyrimidine bases also occurred[16].

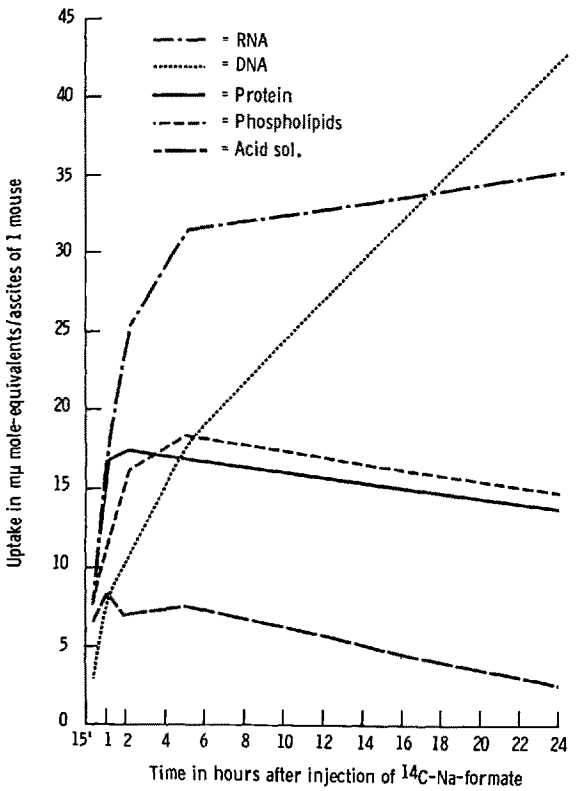

Fig. 2. Uptake of radioactivity into 5 fractions of P 815 leukemic cells after i.p. injection of ^{14}C-Na formate.

Comparison of deoxyribonucleic acids extracted from ascites of P815 leukemic cells treated with C^{14}-MBH and C^{14}-formate

Base	Treated with C^{14}-MBH		Treated with C^{14}-formate	
	cpm[a]	Molar ratio[b]	cpm[a]	Molar ratio[b]
Guanine	72	19.6	796	19.8
Adenine	108	29.5	776	28.9
Cytosine	0	21.0	10	21.0
Thymine	46	29.9	536	30.8

[a] cpm/spot (mean value of 3 to 4 determinations). [b] Mean value of 4 to 8 determinations.

Zusammenfassung. Die endständige N-Methyl-Gruppe von 1-Methyl-C^{14}-2-*p*-(isopropylcarbamoyl)benzylhydrazin-Hydrochlorid (MBH) (Ro 4-6467) erwies sich in In-vivo-Experimenten metabolisch labil, wird teilweise oxydiert und Bestandteil des Formiatpools.

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Effect of Na^+, Ca^{++} and Mg^{++} on the O_2 Consumption of the External Medulla of Dog Kidney

The effect of the external Na^+ concentration on the O_2 consumption of the external medulla of dog kidney slices was investigated by ULLRICH and PEHLING[1]. They demonstrated, by means of a particular technique, that a correlation exists between these two variables. No information is apparently available, however, on the influence exerted by the external depletion of Ca^{++} and Mg^{++} on the O_2 consumption associated with the Na^+ transport. This work was designed with the purpose of finding out this influence and incidentally establishing the functional relationship between Na^+ concentration and O_2 consumption.

The dogs were killed by a shot in the head, the kidneys were removed immediately, placed on iced glass and the external medulla was cut with a razor blade. The slices were incubated in Krebs-Ringer buffered with Tris containing also glucose and α-ketoglutarate.

[1] K. J. ULLRICH and G. PEHLING, Pflügers Archiv. ges. Physiol. *267*, 207 (1958).

In order to find out a Na^+ concentration suitable to test the influence of Ca^{++} and Mg^{++}, experiments were performed with Na^+ concentrations varying from 0 to 330 μeq/ml; choline chloride was added to attain 300 μosm/ml in the solutions ranging from 0 to 150 μeq/ml Na^+. In higher Na^+ concentrations no choline chloride was added because it was previously found that in this case the differences in osmolality did not affect the O_2 consumption.

The O_2 consumption was measured in a Warburg respirometer and expressed in μl of O_2 consumed per mg dry tissue/h and transformed as fractions of basal consumption (consumption in a medium without Na^+). These values minus one are the suprabasal O_2 consumption. An increase in O_2 consumption was obtained with the increase in Na^+ concentration. Plotting the suprabasal O_2 consumption against Na^+ concentration (in doses ranging from 0 to 330 μeq/ml) a curve was obtained which could be transformed into a straight line using the log of suprabasal O_2 consumption and the reciprocal of Na^+ concentration. This function is similar to that obtained for cortex O_2 consumption in rat kidney slices[2].

Converting the straight line into the original units a good adjustment ($p < 0.001$) to the following exponential curve was obtained:

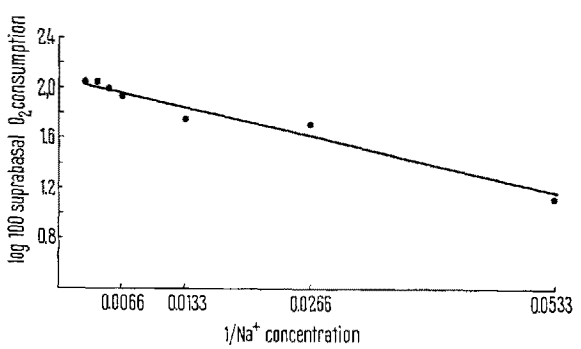
$$y = 1.21 \times 10^{-17.348/x}$$

where y = suprabasal O_2 consumption and x = Na^+ concentration (n = 62).

The data of the O_2 consumption under the effect of varying doses of Na^+ fit also a Michaelis-Menten function, except for the first dose (18.8 μeq/ml Na^+) in which the departure is due to a great variance. This could actually be predicted because of the nature of the data, that is, the reciprocal of values less than 1.

Several trials revealed that 300 μeq/ml of Na^+ was an adequate proportion of this ion to test the influence of the external depletion of either Ca^{++} or Mg^{++} and of both Ca^{++} and Mg^{++} on the O_2 consumption of the dog kidney external medulla. For this purpose, slices of this zone were incubated in Krebs-Ringer with 300 μeq/ml of Na^+ and containing: (1) Ca^{++} and Mg^{++}, (2) Mg^{++} but no Ca^{++}, (3) Ca^{++} but no Mg^{++}, (4) neither Ca^{++} not Mg^{++}, (5) neither Ca^{++} nor Mg^{++} but containing also 2 m*M* of EDTA (EDTA was added in order to chelate the Mg^{++} and Ca^{++} eventually released from the tissue).

The results were expressed as fractions of the basal O_2 consumption obtained by measuring the O_2 consumption of a solution with Ca^{++} and Mg^{++} but without Na^+ and adding choline chloride in the concentration necessary to attain 600 μosm/ml.



Regression of log 100 suprabasal O_2 consumption against $1/Na^+$ concentration. Each point represents the mean of a variable number of determinations for each dose (n = 62).

The results can be seen in the Table. (1) Comparing the O_2 consumption of the slices suspended in Krebs-Ringer with Mg^{++} and 300 μeq/ml of Na^+ with and without Ca^{++}, a significant lowering in O_2 consumption was obtained when the tissue was incubated in a Ringer without Ca^{++}. (2) The absence of Mg^{++} in the presence of Ca^{++} did not induce any modification in O_2 consumption. (3) The absence of both Ca^{++} and Mg^{++} apparently lowered the O_2 consumption when compared with a medium with Mg^{++}. (4) The presence of EDTA in a medium without Ca^{++} and Mg^{++} did not induce any further decrease in O_2 consumption.

In muscle it has been found that the external depletion of Ca^{++} accelerates the Na^+ efflux and increases the O_2 consumption[3]. From these experiments it can be concluded that the kidney external medulla apparently reacts differently, since the depletion of Ca^{++} or of both Ca^{++} and Mg^{++} lowers the O_2 consumption. On the other hand, the depletion of only Mg^{++} does not influence the O_2 consumption. Besides, the Ca^{++} and Mg^{++} which may leak from the tissue do not change the O_2 consumption.

A Ca^{++} (and Mg^{++}?) dependent enzymatic complex located in the cellular membrane might perhaps explain the lowering of O_2 consumption induced by the extracellular Ca^{++} depletion[4].

O_2 consumption of dog kidney slices (fractions of basal consumption) as affected by Ca^{++}, Mg^{++} and EDTA, in the presence of 300 μeq/ml of Na^+

EDTA	Ca^{++}	Mg^{++}	O_2	n	p
–	+	+	2.14	12	< 0.001
–	–	+	1.54		
–	+	+	1.97	10	> 0.10
–	+	–	2.05		
–	–	+	1.53	12	< 0.02
–	–	–	1.32		
–	–	–	1.45	10	> 0.10
+	–	–	1.295		

Résumé. Dans la moelle externe du rein du chien, la consommation d'oxygène (y) augmente avec la concentration du Na^+ (x), d'après la fonction

$$y = 1.21 \times 10^{-17.348/x}$$

L'absence du Ca^{++} et Mg^{++} diminue le QO_2. L'existence d'un complexe enzymatique dépendant du Ca^{++} et Mg^{++} pourrait expliquer ces résultats.

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[3] S. E. SIMON, M. MULLER, and G. D. SATCHELL, Biochim. biophys. Acta *60*, 126 (1962).
[4] Acknowledgment: The author is indebted to Dr. S. L. RABASA and F. BERGMANN from Instituto de Investigaciones Médicas, Rosario, for valuable advice and to Dr. E. BUENO, from Instituto de Fisiología, Facultad de Ciencias Médicas, Rosario, for helpful discussion.