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Instituto de Fisiología de Barcelona
(Prof. Dr. J. Jiménez-Vargas)

Action of *hydrocyanic acid and* its two tautomeric forms on *phospho-monoesterases* (*)

By J. MONCHE, J. JIMÉNEZ-VARGAS and A. SOLS

It being admitted that potassium cyanide behaves as an inhibitor of the activity of the phosphomonoesterases, we undertake to solve in this work the problem of the mechanism of its inhibiting action.

Potassium cyanide hydrolizes in a watery solution, as is well known, with formation of non-dissociated molecules of hydrocyanic acid, given the extraordinary weakness of the said acid. The hydrolisis indicated is particularly intense in the conditions in which it is employed as a phosphatasic inhibitor (HARMAN and WORLEY (1)).

The characteristics of hydrocyanic acid place it between the nitriles (cyanides) and the carbylamines (isocyanides). It is thus considered as a mixture in equilibrium of the two tautomeric forms, which will be discussed later on:

$$\underset{\text{(I)}}{H-C\equiv N} \rightleftharpoons \underset{\text{(II)}}{H-N=C}$$

According to structure (I) and comparing its physical properties with those of the nitriles, it is held to be the nitrile of formic acid or formonitrile (methylcyanide). Hydrolitically it behaves in the same way as the nitriles, but on the other hand, its combustion heat, its explosive properties and its organic reactions bring it nearer the carbylamines.

According to the aforesaid the watery solutions of potassium cyanide will contain the two tautomeric forms in equilibrium referred to, corresponding to the non-dissociated molecules of hydrocyanic acid formed by hydrolisis.

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As a working hypothesis we suppose that the inhibiting action of the phosphatasic activity exercised by potassium cyanide, should be intimately relationed with the presence of one of these two tautomeric forms. In order to come to sure conclusions, we should have employed each of the two forms separately; however, given the fundamental impossibility of isolating them, we have tried to solve the problem in an indirect manner. We therefore studied comparatively potassium cyanide, acetonitrile (methylcyanide), methylcarbylamine (methylisocyanide) and tested the inhibiting action of these compounds as to their phosphatasic activity, operating in identical experimental conditions.

We selected methylcarbylamine and acetonitrile in order to compare their activity with that of potassium cyanide, because the former are two isomers which correspond entirely to either of the two tautomeric forms of hydrocyanic acid. They differ from the latter only by the presence of the methyl group, which substitutes the respective atom of hydrogen. The influence of this group has therefore to be completely analogous.

The comparative study of these compounds is of special interest on account of the particularity of their chemical structure; but this interest lies also in their biological properties having studied very little, for in the literature we find no other references than those communicated by GAUTIER (2), CALMELS (3) and FALK (4).

EXPERIMENTAL PART

Obtention of phosphatases. — Following up ALBERS' technique (5), we have obtained preparations of liver, kidney, bone, spleen, brains and small intestine, utilizing adult dogs.

The yields of the final product, precipitated from the initial material, are the following:

Material	Initial Quantity	Precipitated Product	Percentage	Aspect
Liver	900 gr	3,3560 grs.	0,37 %	Earth coloured powder
Kidney	170 »	0,5391 »	0,31 %	Yellowish powder
Bones	470 »	0,9160 »	0,19 %	Id. id.
Spleen	115 »	0,2406 »	0,20 %	Id. id.
Brains	168 »	0,3126 »	0,10 %	White powder
Small Intestine	1.000 »	0,9578 »	0,095 %	Id. id.

We studied the activity of these preparations, obtained in pulverulent form, after dispersion thereof in physiological serum. For the quantitative estimation of the Phosphomonoesterases we followed the method of one of us (SOLS (6)).

The results obtained, corresponding to alkaline phosphatase (pH = 9,4) and acid phosphatase (pH = 5,0) — A_1 and A_2 of Folley and Kay —, expressed in miligrammes liberated per gramme and operating in the presence of Mg++0,01 M., are as follows :

Material	A_1 mgr. P/gr.	A_2 mgr. P/gr.	A_1/A_2
Liver	64,8	33,5	1,9
Kidney	82,2	20,0	4,1
Bone	87,0	7,6	11,4
Spleen	42,0	32,0	1,3
Intestine	146,0	19,1	7,6
Brains	139,0	38,4	3,6

In the preparation proceeding from intestine we observed a certain activity in an acid medium, although considerably inferior to that appreciated in an alkaline medium. In view thereof we tested its behaviour in the presence of sodium taurocholate M/100, with which we attained the experimental confirmation of its origin on observing a slight inhibition in an alkaline medium (of 16 %) and no activation, or only a very slight one, in an acid medium (López-Navarro (7)).

Methylcyanide and Methylisocyanide. — We have employed methylcyanide Merck which we carefully rectified by means of distillation (b. p. = 80-81,5° C.). We obtained methylisocyanide by the method of Gautier (8) starting from silver cyanide and methyl iodide. This method gives a practical quantitative yield (Bruylants (9)). Before using same, we rectified it by careful distillation (b. p. = 58-59° C.) by means of a fractioning column. We utilized in every treatment the least possible quantity of product so as to dimminish the risk of an explosive decomposition thereof (Lemoult (10)).

Methylisocyanide, as well as methylcyanide are soluble in water ; their employ is therefore easy for experimental quantitative study. The stability of the carbylamines (isocyanides) is perfect in a watery or an alkaline medium ; en the other hand, in an acid medium it is easy that a hydrolitic decomposition of the methylisocyanide be produced at room temperature, and the corresponding formiamidine be formed (Gautier (11)).

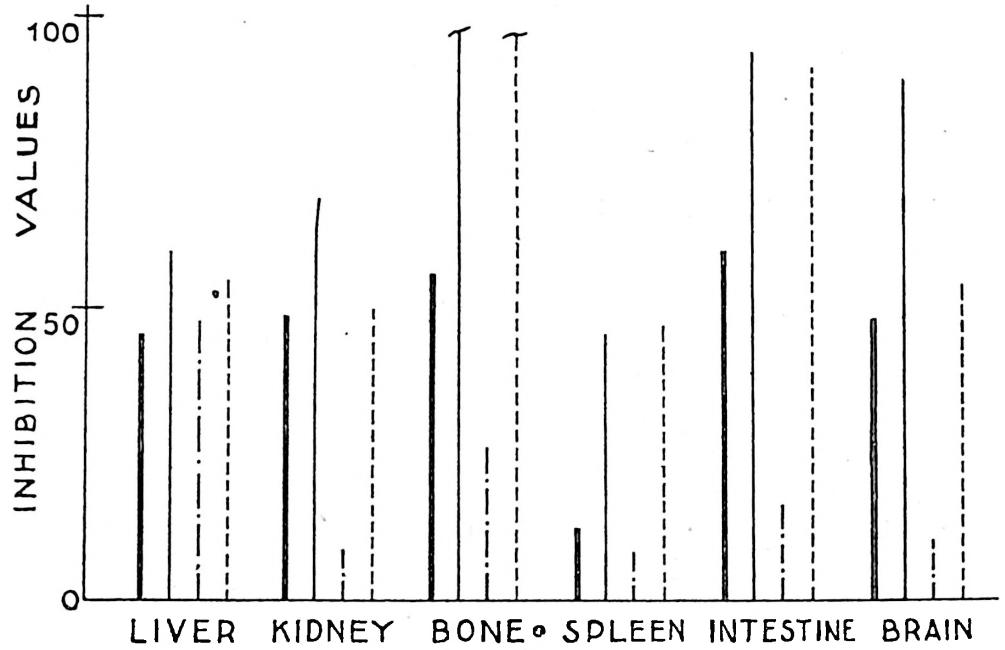
We have obtained the results which we are representing in the graph.

DISCUSSION

Our results show an evident difference between the actions exercised by potassium cyanide, methylisocyanide and methylcya-

Inhibition of purified phosphatases from several organes of the dog by the potassium cyanide, methyl cyanide, and methyl isocyanide, at concentrations M/100 in the presence of Mg^{++} M/100.

Methyl cyanide has no influence in any case

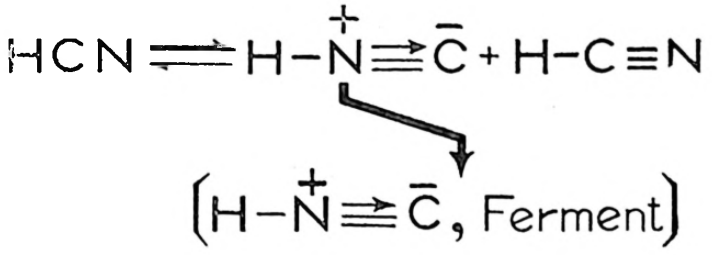


pH = 9,4 { KCN ; $CH_3-N=C$ } pH = 5,0

nide. Methylcyanide has no inhibiting action; on the other hand, methylisocyanide inhibits strongly and its action is more energic than that of potassium cyanide. All this agrees completely with the hypothesis most generally accepted at the actual moment, on the constitution of hydrocyanic acid: it is considered as a mixture in equilibrium of the two isomers mentioned at the beginning of this paper; but with a predominance of the isocyanide form (H — N = C), according to MICHAEL and W. HIBBER (12); J. F. THORPE (13); H. C. HETHERINGTON (14) and KURT MEYER and HOPFF (15). This hypothesis was posteriorily confirmed by means of the Raman spectrography (DADIEU (16) and LINDEMANN (17)), and also by HAMMICK, SIDGWICK and SUTTON (18). In agreement with the works of the last mentioned authors, as a consequence of measures of di-polar electric moments, the following structure was assigned to the functional isocyanide group (carbylamine): $-\overset{+}{N}\rightarrow\overline{C}$ (III). The said structure, characterized by the double semipolar bond, has afterwards been confirmed by the experimental way through determinations of parachors of its derivatives. On the other hand, the structures: — N = C (II), with a divalent carbon atom and also — N ≡ C (I), with a pentavalent nitrogen atom, accepted only by Dadieu and Kohbrausch (19), are more discussible. Structure (III) represents the existence of a process of intramolecular ionisation of the isocyanide group (carbylamine) with the corresponding electric charges in atoms C and N. In accordance therewith and bearing in mind the colloidal nature of the phosphomonoesterases, physico-chemical entities can be formed with said ferments, in the way of isocyanide-phosphomonoesterase complexes. Such complexes, by their marked stability, would be the determining factor of the inhibiting effect exercised by potassium cyanide and methylisocyanide. The complex would be more or less eliminated from the hydrolitic enzymatic system.

The greater part of the hydrocyanic acid formed hydrolitically, on being constituted by non-dissociated molecules, will determine the existence of the two tautomeric forms referred to. This is not applicable to the ions corresponding to both seeing that they are identical ($N \equiv C)^-$.

In accordance with the foregoing, we formulate the following hypothesis of the mechanism of inhibition of phosphomonoesterases by hydrocyanic acid:



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