Fate of Halogenated Phenols in the Organism

By B. ZONDEK AND B. SHAPIRO, *Gynaecological-Obstetrical Department, Rothschild Hadassah-University Hospital and the Hormone Research Laboratory, Hebrew University, Jerusalem, Palestine*

(Received 19 August 1943)

Halogenated phenols, long employed for external disinfection, are also powerful internal disinfectants [Zondek, 1942*a*]. As the result of parenteral or percutaneous application in man, halogenated phenols are excreted in the urine, and prevent its bacterial decomposition on storage. In the clinical treatment of pyelitis and cystitis, their application may effect sterilization of the urine [Zondek, 1942*b*]. Blood from treated patients is bacteriostatic and inhibits growth of inoculated staphylococci. Successful treatment of septic infections in man is thus made possible.

The present paper is concerned with the fate in the body of *p*-chloro-*m*-xylenol (4-chloro-3:5-dimethylphenol, referred to briefly as *CX*), *p*-chloro-*m*-cresol (4-chloro-3-methylphenol, referred to as *CC*), and with the following derivatives of *p*-chloro-xylenol: acetate, benzoate, sulphate, methyl and ethyl ethers, and the *o*-iso-propyl derivative.

METHODS

The method of determining *CX* and *CC* in the presence of phenol, described by Zondek, Shapiro & Hestrin [1943], was used. *CX* and *CC* react with Millon reagent at room temperature, yielding coloured substances which can be extracted with ether and quantitatively determined therein. Phenol in the quantities present in body fluids gives no colour with Millon reagent under the conditions of these assays.

Glucuronic acid in urine was estimated according to Mozolowski [1940]. The quantity of sulphuric esters in urine was calculated by subtracting the free sulphate from the total sulphate (sulphate after acid hydrolysis).

RESULTS

Excretion of CX (p-chloro-m-xyleneol) and CC (p-chloro-m-cresol) in the urine

In rabbits. Rabbits (approx. 1 kg.) were injected subcutaneously with 1 g. of *CX* dissolved in 10 ml. of olive oil. The urine was collected daily and its content of free *CX* determined. The pH was usually 7.5–8.2. About 15% of the *CX* given was excreted during 4–5 days (Table 1). In an analogous experiment with *CC*, 15–20% was recovered in the urine.

In man. *CX* administered intramuscularly to man is not usually excreted in the free form if the pH of the urine is acid. The difference between

Table 1. *Excretion of p-chloro-m-xyleneol in urine of rabbits*

Days after injection	Excretion of free substance by rabbit no.			
	1 (mg.)	2 (mg.)	3 (mg.)	4 (mg.)
1	43	40	10	30
2	46	60	50	28
3	50	30	44	32
4	25	10	34	30
5	12	—	6	8
6	—	—	—	—
Total excreted	176	140	144	128

Average excretion = 150 mg. = 15% of quantity injected.

human beings and rabbits in respect of *CX* excretion was associated with the more acid reaction of human urine (pH 5.5–6.7). A *CX* content in urine as high as 50 mg./100 ml. and recoveries up to 8.3% were obtained in man when the urine was made alkaline by administration of NaHCO_3 . Concentrations of *CX* of 30 mg./100 ml. convert urine into a bactericidal fluid. If the urine is again rendered acid by administration of NH_4Cl , *CX* excretion ceases (see Table 2). But in spite of the fact that

Table 2. *Effect of urinary pH on the excretion of p-chloro-m-xyleneol in man*

Patient D.H.				
Date	Material injected (g.)	Additional treatment	pH of urine	Material excreted (mg.)
17–18 Jan.	7	None	—	—
19–21	12	NaHCO_3	7.6	1320
22	0	"	7.2	980
23	0	"	7.1	300
24–26	0	NH_4Cl	5.2–5.8	60
27–28	8	"	5.4	—
29	6	NaHCO_3	5.4	—
30	6	"	7.2	240
31	0	"	7.8	330
2 Feb.	3	"	7.8	170
3	0	None	6.4	60
4	0	None	6.2	—
5	0	None	6.3	—
Total given	42	Total excreted	= 3460 mg. = 8.3%	

no *CX* could be demonstrated chemically, some bactericidal substance seems to have been excreted, since with most treated patients no ammoniacal

decomposition of the stored urine occurred. In other experiments decomposition of urine was found to be prevented by concentrations of *CX* exceeding 2 mg./100 ml.

When *CC* was administered to several human patients intramuscularly in oil, it was in no case recovered in the urine in appreciable quantity. It was extremely difficult to make the urine of these patients alkaline, an effect which was apparently due to the administration of *CC*. Where alkalinization was effected a negligible excretion of *CC* was observed.

Combined forms of CX in urine

Since phenol appears in urine combined with glucuronic acid and with sulphuric acid as well as in the free state, it was to be expected that *CX* too would be found partly combined in these forms. The combined *CX* was determined by estimating glucuronic acid and bound sulphuric acid before and after *CX* treatment. In humans injected with 5 g. *CX*, approximately 700 mg./100 ml. were excreted combined with glucuronic acid, and 840 mg./100 ml. as a sulphuric ester; 14% of the quantity injected is thus found in the form of glucuronide, and 17% as sulphuric ester. The total recovery of *CX* in combined form is thus 31%.

Dissociation of combined CX by urine

Some urine samples, which give no chemical tests for free *CX* when fresh, may develop considerable quantities on standing. This finding was corroborated by bacteriological tests. Urine which contained *Escherichia coli* when fresh, became sterile after standing for some days, and in some cases then also destroyed added bacteria.

Urine specimens in which no free *CX* could be demonstrated were stored for 1 month. Control specimens were boiled before storing. Free *CX* was then determined, and 9–17 times as much was found in the unboiled urine as in the boiled specimens. These results suggest that the dissociating agent is destroyed by heat and is of an enzymic nature, presumably a glucuronidase or sulphatase. This point is under investigation.

Excretion of CX in urine after injection of CX esters and ethers

Ester derivatives of hormones have been of great value in hormone therapy (e.g. oestradiol benzoate, testosterone propionate, desoxycorticosterone acetate, etc.). The high activity of such esters is attributable to their slow absorption on subcutaneous administration and the consequent sustained liberation of active substance in the body. It seemed possible that in phenol-chemotherapy, too, the use of ester derivatives would be advantageous. The acetic acid and benzoic acid esters of *CX* were less

toxic in animals and more soluble in oil than was *CX* itself (solubilities—20 and 25% respectively for the esters, as against 10% for *CX*). These ester derivatives could therefore be safely administered in larger single doses. It was found, also, that *CX* dissolves in oil to a higher concentration in the presence of *CX*-esters. In oil containing a total of 30% of *CX*-acetate, for instance, 20% of *CX* could be dissolved as against only 10% in pure oil. The sulphuric acid ester presented a particular advantage, its sodium salt being soluble in water up to 5%. *CX*-methyl- and ethyl-ethers were liquids at 30° and could be injected without dilution.

It was thus of interest to ascertain whether esters and ethers of *CX* are decomposed in the body with regeneration of active *CX*. The excretion of free *CX* in rabbit urine after injections of the ester serves as an indication of such regeneration. The *CX*-esters and ethers do not give a positive colour reaction with Millon reagent.

CX-acetate and benzoate are readily absorbed and decomposed to *CX* in the animal body (Fig. 1).

Fig. 1. Total amount of *p*-chloro-*m*-xyleneol (*CX*) excretion in rabbits, as % of amount injected.

The total amount of free *CX* excreted in urine in such cases is quite as high as when *CX* itself is injected, though the rate of excretion is somewhat slower.

CX-methyl- and ethyl-ethers are also decomposed to give free *CX*, the extent of the excretion of *CX* being equal to that obtained when *CX* itself was injected. This is of particular interest, since it is not generally accepted that the animal organism can perform such dealkylations. Excretion of free *CX*, after the ethers had been given, continued for 11–17 days, as against a period of excretion of 4–6 days when free *CX* or *CC*, and esters of *CX*, were tested. Repetition of the experiment with various modifications consistently confirmed this finding. The slow absorption or decomposition of the test substance should be of clinical importance.

After injection of *CX*-sulphate no free *CX*, or only small amounts, appeared in the urine. *CX*-sulphate, which is a detoxication product of *CX*, is

presumably rapidly excreted, largely in an unchanged condition. After *o*-iso-propyl-CX had been given to human beings or to rabbits it was not found in the urine. Its water solubility is low and it is also practically insoluble in dilute alkali.

Concentration of CX in blood

Concentrations in blood of 3–10 mg./100 ml. are observed only when large amounts of CX have been given, e.g. 50 g. to man or 1–3 g. to a rabbit. These large doses can be administered by inunction of a 30% CX ointment. Concentrations in blood of 1 mg./100 ml. were obtained when 1 g. of CX was given to man by intramuscular injection. The maximal CX content was observed 1–5 hr. after treatment, but in most cases no CX was chemically demonstrable in the blood. Nevertheless, we have sufficient evidence from the urinary data of the completeness of the absorption of CX. Evidence was also provided by the CX content of cantharidin blisters in CX-treated patients. Despite our inability to detect CX in the blood by chemical means, we have found that the blood of the treated patients showed considerable bacteriostatic power towards inoculated cocci. Whereas chemically detectable quantities of CX disappear from the blood several hours after treatment, bacteriostasis sets in only after more than 24 hr. The cause of this disparity will be dealt with elsewhere.

CX in cerebrospinal fluid

In no instance were chemically detectable quantities of CX present in cerebrospinal fluid taken from rabbits or from men at various intervals 4–48 hr. after CX treatment.

Fate of CX injected in the body

As excretion in the urine of free and combined CX accounted for only part of the CX injected, it was of importance to establish what proportions of the amount administered are excreted in the faeces and retained in the body. Five rats (70–80 g.) were given 100–200 mg. of CX by injection, and the urine and faeces collected for 48 hr. The animals were then killed, minced and extracted first with cold and then with warm acetone, and CX determined in the extracts. About 15% of the CX injected was recovered in the faeces. Half an hour after injection, 85–90% of the CX was still present in an unchanged form in the whole body. After 48 hr. only 10% could be recovered from the minced carcass. The total quantities of CX found in urine (combined + free = approx. 30%), faeces (15%), and in the body (10%) after 48 hr. thus accounted for only approx. 55% of the quantity injected. It must be assumed that some part of CX injected is changed

by the body to a substance which cannot be detected by our procedure for the estimation of CX in tissues.

Absorption of injected CX and its distribution in the body

200 mg. of CX in 2 ml. olive oil were injected subcutaneously into the left forelegs of five rats weighing 100–120 g. After 24–48 hr. the animals were killed. The posterior and anterior parts of each were minced and analyzed separately for CX. The bulk of the recovered CX was found in all the animals in the anterior part of the body where the injection had been given. In one typical experiment, 24 hr. after the administration, 60% of the injected quantity was found in the anterior part and only 5% in the posterior. After 48 hr. the anterior part contained 6% and the posterior 1% of the quantity injected. Absorption of CX is therefore a comparatively slow process.

SUMMARY

1. When *p*-chloro-*m*-xylenol (CX) is injected into rabbits, approximately 15% of the amount administered is excreted in urine. In human urine of acid reaction only very small quantities (about 2 mg./100 ml.) are excreted. These quantities are usually sufficient to inhibit ammoniacal fermentation of the urine. When human urine is rendered alkaline by administration of NaHCO_3 , CX concentrations up to 50 mg./100 ml. are obtained, and up to 8% of the administered CX is excreted. Concentrations of CX above 30 mg./100 ml. render the urine bactericidal.
2. After administration to human beings or to rabbits *o*-iso-propyl-CX is not excreted in the urine.
3. *p*-Chloro-*m*-cresol injected into rabbits is rapidly excreted in the urine. In man no excretion was observed.
4. The acetic acid and benzoic acid esters and the methyl- and ethyl-ethers of CX decompose in the body with regeneration of CX, which is excreted in the free form in the urine. The absorption of the esters is somewhat slower than that of CX itself. The time required for complete absorption and excretion of CX-ethers is three times that for CX itself.
5. CX is partly (30%) excreted in the urine as glucuronide and sulphate ester (in equal amounts) after administration to man.
6. Even when large doses are administered the concentration of CX in blood seldom exceeds 1 mg./100 ml. Concentrations of 3–10 mg. are encountered occasionally, but only when very large amounts are given. The maximum quantities in blood are found 1–5 hr. after treatment.

7. No CX was detected in cerebrospinal fluid after administration of this substance.

8. The aggregate amount of CX found in the urine and faeces and recovered from the animal body after 48 hr. accounted for only approximately 55 % of the amount of CX given.

9. The absorption of injected CX is a slow process and is incomplete even 48 hr. after the injection.

We are indebted to the Teva Middle East Pharmaceutical and Chemical Works Co., Ltd., Jerusalem, for a generous supply of *p*-chloroxylenol.

REFERENCES

Mozolowski, W. [1940]. *Biochem. J.* **34**, 822.
Zondek, B. [1942a]. *Nature, Lond.*, **149**, 334.

Zondek, B. [1942b]. *J. Urol.* **48**, 563.
— Shapiro, B. & Hestrin, S. [1943]. *Biochem. J.* **37**, 589.

The Replenishment of Depleted Skeletal Reserves of Magnesium

By J. DUCKWORTH AND W. GODDEN, *The Rowett Institute, Aberdeen*

(Received 15 September 1943)

It has been shown that the Mg of the skeleton is mobilized in response to the threatened shortage of the element in the soft tissues that results from dietary insufficiency of Mg [Duckworth, Godden & Warnock, 1940]. In the face of the persistent demand for Mg in Mg deficiency it was found that the rate of skeletal growth determined the amount that could be liberated on account of the demand of the skeleton itself for Mg [Duckworth & Godden, 1941]. The most striking feature of the mobilization of Mg was the high speed with which large quantities were released.

The question that immediately suggests itself is whether the skeletal stores can be replenished as easily as they are depleted. The problem is of practical interest in the nutrition of farm animals and man, in which the dietary intake of Mg is liable to vary. This is especially true in the case of man for whom vegetables, the supply of which fluctuates throughout the year, are the most important single source of Mg [Duckworth & Warnock, 1942-3]. The result is that varying degrees of inadequacy or alternating periods of adequacy and inadequacy are more common than constant minor degrees of insufficiency. This state may be further accentuated by losses in sweat, which may become abnormal owing to seasonal change in temperature or conditions and intensity of work [Duckworth & Warnock, 1942-3].

The present study was undertaken to examine this problem, and because of the profound influence of the rate of bone growth on liberation of skeletal Mg, a combination of Mg deficiency with different degrees of Ca deficiency was used. This approach also allowed the examination of certain points of interest concerning the interrelation of Ca and Mg in the skeleton.

METHODS

Diets used. The preparation of the diet, the methods of analysis and the management of the animals have been reported [Duckworth *et al.* 1940; Duckworth & Godden, 1941] and need not be repeated here. The technique for preparing a diet low in Mg simultaneously removes the Ca with about the same degree of efficiency, so that no additional procedure is required. The diets contained less than 6 parts per million (p.p.m.) of Mg and about 1-2 p.p.m. of Ca.

Four diets were prepared, each with a P content of 0.35 %. By additions of CaCO₃, with slight compensatory adjustments in the starch content, these diets were arranged to contain 0.90, 0.30, 0.10 and 0.00 % of Ca, and are referred to as diets A, B, C and D respectively.

Animal management. One litter of eight rats (4 ♂ and 4 ♀) at weaning was allotted to each diet. Of each litter six rats were placed on the appropriate deficient diet while two remaining rats of the litter were given the same diet supplemented with sufficient MgSO₄ to raise the Mg content to 0.07 %. The six animals undergoing skeletal Mg depletion were observed closely, and upon the death of one member of the set another, judged clinically to be close to death, was dosed with several drops of a solution of MgSO₄ and then transferred to the same diet supplemented with Mg. Thus of each litter three animals (hereafter referred to as 'depletion rats') died of Mg deficiency and three others (subsequently referred to as 'curative rats') were sacrificed after each had received a curative period of 10 days on the appropriate Mg-supplemented diet. The two remaining animals were treated as controls, one being killed at what was estimated to be the average