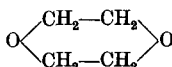


NOTE ON THE TOXICITY TO ANIMALS OF SOME OXIDATION PRODUCTS OF 1:4 DIOXAN .

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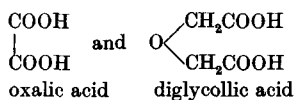
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THE toxicity of 1 : 4 dioxan



to animals by inhalation, intravenous injection, feeding and absorption through the skin, with the resulting lesions in the kidneys and in the liver, have been described in a previous paper (Fairley, Linton and Ford-Moore, 1935).

In vitro



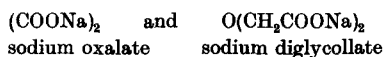
are produced as oxidation products of 1 : 4 dioxan.

The fact that nephritis may be produced by oxalic acid or by oxalates is stated in most text-books. But it was thought that, if renal and hepatic lesions, similar in type to those resulting from 1 : 4 dioxan, could be produced by oxalic acid and diglycollic acid or by their salts, some light might be thrown upon the reason for the toxicity of 1 : 4 dioxan, and upon the way in which it produces its effects in the tissues. The present study was undertaken with this object.

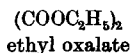
Unfortunately, owing to the pressure of work of more immediate urgency, it has not been possible to amplify the investigation on animals.

A few experiments are described, however, which are suggestive; no claim is made that they are conclusive.

The two oxidation products, under consideration, are acidic compounds and did not appear to be suitable, as such, for direct use on animals; therefore, the sodium salts



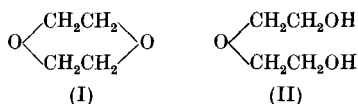
were used for the intravenous injections and the presumably lipoid-soluble ethyl ester



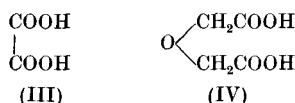
was selected for application to the skin.

CHEMICAL CONSIDERATIONS

When either 1 : 4 dioxan (I) or 2·2'-dihydroxyethyl ether (diethylene glycol) (II)



are oxidized with nitric acid, two acids are produced. The first acid was readily identified as oxalic acid (III). The second acid, which is considerably more soluble in water than oxalic acid, has been identified as diglycollic acid (IV):



When 88 g. (1 mol.) of dioxan are oxidized with fuming nitric acid, the yield of diglycollic acid, based on the calcium salt, varies between 29·5 g. (0·22 mol.) and 41·2 g. (0·31 mol.). At the same time, 10·2 g. oxalic acid (0·11 mol.) is formed.

When 106 g. (1 mol.) of diethylene glycol was oxidized by heating with concentrated nitric acid (2 parts) and water (1 part), 70 g. diglycollic acid (0·52 mol.) and 39·0 g. oxalic acid (0·43 mol.) were formed. No oxalic acid is produced when diglycollic acid is boiled with fuming nitric acid for 2 hours. Oxalic acid is formed, however, when diglycollic acid is boiled in neutral solution with potassium permanganate.

Since the oxidation of diethylene glycol proceeds with greater ease and gives a higher yield, this substance was used as the source of the diglycollic acid required for these experiments. The oxalic and diglycollic acids were separated by treating their hot aqueous solution with precipitated calcium carbonate till neutral, filtering from the calcium oxalate and excess of carbonate and allowing the calcium diglycollate to crystallize from the filtrate. Alternatively, the dried mixed acids were boiled with alcoholic hydrochloric acid, the ethyl oxalate and ethyl diglycollate being then separated by fractional distillation under reduced pressure. The first method is the more satisfactory when dealing with small quantities of material. The calcium diglycollate obtained in the first method is converted into the free acid by decomposition with the exact amount of oxalic acid. The diglycollic acid thus obtained was purified by converting into its ethyl ester which was distilled under reduced pressure. The pure ethyl diglycollate boiled at 120·5°/8 mm., froze at 19·4° and had $D_{15}^{19.5}$ 1·111. The acid obtained by the hydrolysis of the pure ester, after crystallising from ethyl acetate, melted at 148°.

Analysis: Calculated for $\text{C}_4\text{H}_6\text{O}_5$: C, 35·8; H, 4·5. Found: C, 36·02, 36·01; H, 4·42, 4·48.

As a further check, a small quantity of the acid was converted into its methyl ester which boiled at $109^{\circ}/7$ mm. and froze at 37.6° .

Sodium diglycollate was prepared from the pure acid by dissolving it in water and treating with the exact amount of pure anhydrous sodium carbonate (134 g. acid require 106 g. sodium carbonate). The salt was isolated by evaporation to dryness on the water-bath, grinding in a glass mortar with alcohol and drying at 110° .

The ethyl oxalate used in these experiments was obtained by redistilling a purchased sample till it boiled at $73.5^{\circ}/8$ mm. and had D_{15}^{20} 1.096.

The sodium oxalate used was an "Analytical Reagent" sample purchased from Messrs British Drug Houses, Ltd., and was used without further purification.

ANIMAL EXPERIMENTS

Sodium oxalate—intravenous

Rabbit 1.

This animal received twelve injections each of 5 c.c. 1 per cent. sodium oxalate in normal saline into the marginal vein of the ear during 22 days. It died in convulsions 5 min. after the last injection.

During the experiment the animal's weight decreased from 2.95 to 2.15 kg.

Post-mortem. Macroscopical: nothing abnormal. Microscopical: kidney—patchy cloudy swelling of the tubule cells in the cortex, and many hyaline casts in the medullary tubules; liver—normal.

Rabbit 2

Received twenty intravenous injections of sodium oxalate in normal saline as follows:

No.	Quantity c.c.	Strength %	
1	5	2	} during 47 days
2	4	2	
1	3	2	
13	5	1	
2	4	1	
1	3	1	

The animal's weight decreased from 3.18 to 2.72 kg. It was killed for examination on the 47th day.

Post-mortem. Macroscopical: kidneys—enlarged. Microscopical: kidney—cloudy swelling of tubule cells of cortex, casts in the tubules of the medulla; urine—deposit of oxalate crystals on centrifuging; liver—normal.

Ethyl oxalate—skin absorption

This compound was selected for the skin experiments, as it was a clear liquid convenient to apply. Being immiscible with water and readily miscible in all proportions with toluene, it was regarded as likely to have a high degree of lipid solubility and to be absorbed readily through the skin.

The technique employed was the same as that described in the investi-

gation of 1 : 4 dioxan, to which reference has been made. Eleven rabbits and six guinea-pigs were used. A patch was closely shorn on the back of each animal and each animal was placed in a box so designed that removal of the liquid from the skin by licking or scratching was impossible. Ten drops of the compound, in the case of each rabbit, and five drops in the case of each guinea-pig, were delivered on to the shorn patch at each application. Applications were made twice daily for 5 days, once on the 6th, with a free day on the 7th, *i.e.* eleven applications in each week.

The compound was readily absorbed through the skin. It had, however, a drying effect, and the skin tended to crust and crack slightly. The areas of application were varied as far as possible, but it is not claimed that absorption necessarily occurred through areas of skin which were entirely intact, and in which there was no solution of continuity.

The results are set out in Table I.

Table I

Animal	Duration of experiment days	Weight at start kg.	Weight at finish kg.	Remarks
Rabbit 1	106	2.50	2.27	Died 106th day
„ 2	92	2.72	2.05	Acutely ill 2 days, killed
„ 3	96	2.00	1.13	Rapid onset of illness 91st day; killed
„ 4	91	3.20	2.44	Died 91st day
„ 5	92	2.52	1.82	Acutely ill 2 days, killed
„ 6	30	2.31	1.95	} All animals out of condition and thin, killed for examination on days stated
„ 7	22	3.00	2.27	
„ 8	23	2.72	2.22	
„ 9	22	1.88	1.36	
„ 10	22	2.38	1.75	
„ 11	26	3.00	2.15	
Guinea-pig 1	117	0.800	0.800	} Animals remained in apparently normal health; killed for examination on the days stated
„ 2	135	0.800	0.800	
„ 3	135	0.800	0.805	
„ 4	142	0.720	0.790	
„ 5	142	0.680	0.700	
„ 6	149	0.800	0.800	

Post-mortem findings.

(a) *Macroscopic.* In rabbit 4 and in all six guinea-pigs nothing abnormal was noted in the post-mortem appearances.

In the remaining ten rabbits, the kidneys were larger and paler than normal.

(b) *Microscopic.* (i) The skin at the site of application (examined in rabbits 6-11 and the six guinea-pigs).

In rabbits 6, 8 and all six guinea-pigs sections of the skin showed no abnormal appearances.

In rabbits 7, 9, 10 and 11 the sections showed marked proliferation of the squamous layers of epithelium and a tendency to keratinization, especially in the hair follicles.

(ii) *The kidneys.* In all other cases, both rabbits and guinea-pigs, a patchy degeneration of the cells of the cortical tubules, varying in degree from well-

marked degeneration to a gross destruction occurred. In addition all cases showed a varying degree of vascular engorgement.

In rabbits 6, 8 and 11 the medulla of the kidney appeared to be normal, but in the remaining rabbits and in all six guinea-pigs, many tubules were blocked with casts.

Haemorrhages—mainly cortical—were seen in all the guinea-pigs. This was not noted in the rabbit sections.

(iii) *The liver.* In all animals there was well-marked vascular engorgement in the liver, and these were the only changes noted in rabbit 6 and guinea-pig 3.

Sections from all the remaining animals showed a well-marked cell degeneration which typically started at the periphery of the lobules. In some cases the peripheral origin of the degeneration was less marked.

Rabbits 7, 8, 9 and 10 showed a well-defined new formation of portal systems.

The blood-urea content after skin absorption of ethyl oxalate

Fairley, Linton and Ford-Moore (1935) examined the blood urea content (Maclean's method) in fifty healthy rabbits. The highest reading obtained was 46 mg. and the lowest 26 mg. per 100 c.c. of blood.

Observations were carried out on rabbit 1 as follows:

Day of experiment	Blood urea mg. in 100 c.c. blood
94	39
99	40
102	121
103	168
104	240
105	310
106	471

Rabbit 3 was found to have a blood urea content of 282 mg. in 100 c.c. blood shortly before its death.

In view of these findings a special investigation of the blood urea content was carried out on rabbits 6–11 with the results recorded in Table II.

Table II. *Blood urea content: mg. per 100 c.c. blood*

Rabbit No. ...	6	7	8	9	10	11
11th day	54	166	81	86	116	88
12th day	—	161	—	91	—	—
15th day	80	146	94	101	116	101
18th day	76	96	166	206	226	156
20th day	—	—	—	188	—	—
22nd day	74	212	—	181	386	—
23rd day	—	—	353	—	—	273
26th day	101	—	—	—	—	378
30th day	288	—	—	—	—	—

Sodium diglycollate—intravenous

(a) 1 per cent. in normal saline.

Rabbit 1 received fifteen 5 c.c. injections in 23 days. It remained in normal health, gaining in weight from 1.90 to 2.27 kg. The blood urea content was

26 mg. per 100 c.c. on the 8th day, and 31 mg. on the 23rd day, when it was killed for examination.

The post-mortem appearances were normal, and a slight degree of vascular engorgement in the kidney was the only microscopical change noted, the liver cells being quite normal.

Rabbit 2 received thirty 5 c.c. injections in 44 days. It appeared to remain in normal health, gaining in weight from 1.90 to 2.24 kg. The blood urea content was 28 mg. per 100 c.c. on the 8th day, and 26 mg. on the 44th day, when it was killed for examination.

In this animal also the post-mortem appearances were normal, and a few casts in the medullary tubules of the kidney were the only microscopical abnormality. Again, no changes in the liver were found.

(b) 2 per cent. in normal saline.

Rabbit 3 received thirty 5 c.c. injections in 44 days. It remained in normal health, increasing slightly in weight from 2.24 to 2.30 kg. Its blood urea content was 24 mg. on the 8th day and 32 mg. on the 44th day, when it was killed for examination.

No abnormality, either macroscopic or microscopic, was detected post-mortem.

Rabbit 4 received five 5 c.c. injections in 8 days. It rapidly deteriorated in condition, became sluggish, refused to eat, and its weight fell from 2.27 to 1.77 kg.

Its blood urea on the 8th day was 136 mg. per 100 c.c. and it was killed as not being likely to survive another 24 hours.

Post-mortem examination showed a pair of large pale kidneys.

On microscopical section, the kidney showed vascular engorgement with a glomerular nephritis and with casts in the tubules of the medulla. There was no evidence of cell degeneration in the cortical tubules. In the liver the only change noted was an increase in the lymphoid tissue around the portal systems.

(c) 4 per cent. in normal saline.

Rabbits 5 and 6 received one 5 c.c. injection each. The animals went into immediate convulsions and collapsed. They recovered rapidly, but rabbit 5 died on the 5th day, and rabbit 6 being obviously ill was killed at the same time.

The post-mortem appearances were normal, but sections of the kidneys of both animals showed advanced patchy cell degeneration of the same types that is typical after the administration of 1 : 4 dioxan or sodium oxalate. The liver in both was normal.

Rabbit 7 received two 5 c.c. injections. Convulsions occurred, immediately followed by collapse and recovery after each. On the 3rd day the animal was obviously ill and was killed.

The cortex of the kidney showed the same patches as rabbits 5 and 6, and sections of the liver were of normal appearance.

Rabbit 8 also received two 5 c.c. injections. The clinical course was exactly similar to that of rabbit 7, and it was killed on the 3rd day.

Sections of the kidney and liver, however, were quite normal, except for some degree of vascular engorgement.

SUMMARY

1. It has been shown that, *in vitro*, the final oxidation product of 1 : 4 dioxan is oxalic acid, with diglycollic acid either as an intermediate stage or as a by-product.

2. Renal changes have been produced in rabbits by the intravenous administration of sodium oxalate, and renal and hepatic changes in rabbits and guinea-pigs have followed the application of ethyl oxalate to the skin. These lesions were similar in type to those produced by 1 : 4 dioxan.

3. The rabbits, in which ethyl oxalate was applied to the skin, showed a well-marked rise in the blood-urea content.

4. Renal changes of a similar type have occurred in rabbits after the intravenous administration of sodium diglycollate. These changes were less constantly observed, and were less in degree than those following intravenous sodium oxalate. Weight for weight sodium diglycollate appeared to be less toxic than sodium oxalate, when given intravenously to rabbits.

5. A possible method by which 1 : 4 dioxan may produce its effects is suggested, namely that 1 : 4 dioxan is oxidised in the tissues to diglycollic and oxalic acids, which are neutralized to diglycollates and oxalates as soon as formed by the alkaline tissue fluids. It is possible that the relative amounts in which these oxidation products occur in the tissues, after the administration of 1 : 4 dioxan, may vary, and that the severity of the resulting lesions may depend upon this variation.

REFERENCE

- FAIRLEY, A., LINTON, E. C. and FORD-MOORE, A. H. (1935). *J. Hygiene*, **34**, 486.

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