EXPERIMENTAL TUBULAR NECROSIS OF THE KIDNEYS ACCOMPANIED BY LIVER CHANGES DUE TO DIOXAN POISONING

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(With 3 Graphs)

Barber (1934) reported the death of five factory workers, who were employed in processes involving the use of dioxan. The principal lesions found at autopsy were a central zonal necrosis of the liver and a symmetrical necrosis of the cortex of the kidneys. The similarity of the renal condition to that found in the cortical necrosis of pregnancy first prompted the present investigation.

Previous work

Yant, Schrenk, Waite and Patty (1930) investigated similar cases in U.S.A. where dioxan is used commercially as an industrial solvent, especially for paints, varnishes and lacquers. They exposed guinea-pigs to atmosphere containing 3 per cent. dioxan vapour with resulting irritation of nose and eyes, retching movements suggesting gastric irritation, and apparent narcosis. They concluded, therefore, that due warning would be given to workers before toxic or lethal effects could be produced.

Van Oettingen and Jirouch (1931) investigated the pharmacology of ethylene glycol and its derivatives, of which dioxan is one. They produced acute nephrosis in rats with doses of 2·5–5·0 c.c. per kg. of a 50 per cent. solution of ethylene glycol. On the basis of experiments on isolated muscle they concluded that all the ethylene glycol compounds depressed muscular and nervous tissues, but that dioxan was the least toxic of the group.

Fairley, Linton and Ford-Moore (1934) investigated the toxicity to guinea-pigs, rats and rabbits by inhalation, feeding and inoculation experiments. They described a peripheral zonal necrosis of the liver and necrosis of the tubular epithelium of the kidney in fatal cases.

It was hoped that the experimental production of cortical necrosis by administration of dioxan would elucidate the mechanism of the renal necrosis of pregnancy. At the same time, in view of the widespread use of dioxan commercially and its recent introduction to histology as a dehydrating and fixing agent, the limits of its toxicity require definition. Furthermore, the present experiments were undertaken to investigate more fully lethal dosage, the

question of tolerance and the mechanism of the disturbed renal function which follows tubular necrosis.

METHODS

Rabbits and guinea-pigs on basic diets of greens 200 gm., oats 60 gm. and bread 60 gm. for rabbits, and greens 80 gm., oats 30 gm. and bread 20 gm. for guinea-pigs, were placed in metabolism cages. After a few preliminary experiments it was found that similar lesions were produced in the two species, so it was decided to employ rabbits only. Dioxan was purchased from Messrs British Drug Houses and Messrs Hopkins and Williams, and both samples were found to have a boiling point of 101–2° C. The product was suitably diluted and given by stomach tube or intravenously in doses calculated in c.c. of dioxan per kg. of body weight. In order to include a species of carnivor, Prof. Wright very kindly performed similar feeding experiments on cats.

Renal function was estimated by examinations of the blood urea and total urine excreted. Sections of liver and kidney were stained with haemotoxylin and eosin, Best's carmine stain for glycogen, Sharlach R for fat and McGregor's modification of Mallory-Heidenhain's stains for the glomerular basement membrane.

FINDINGS

Immediate symptoms of non-lethal dosage

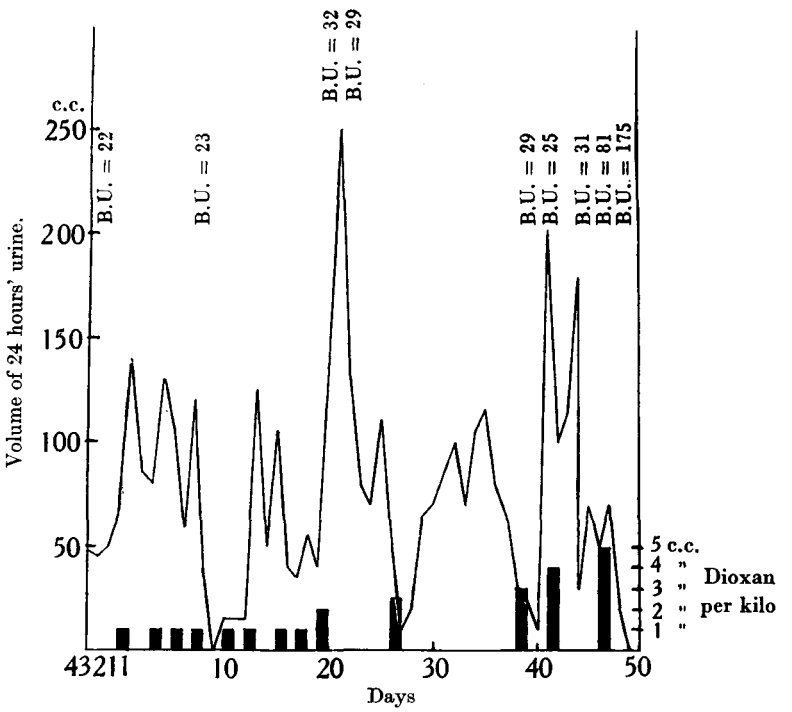
Dioxan in doses of 0·2 c.c. per kg. of body weight dissolved in 10 c.c. of water, given intravenously, were without visible effect either during the injection, which was given slowly, or after. Doses of 1·0 c.c. per kg. in 5 c.c. of water were well tolerated during the injection, but a few minutes later the rabbit appeared limp and dazed; on moving, the animal's gait was slow and ataxic with a tendency to roll from side to side. When it found support, such as a wall, it would lean against it and slowly slide to the ground with all the appearances of drunkenness. The reflexes were still present, though diminished in force and sluggish. These symptoms usually lasted from 2 to 4 hours with gradual improvement to normal. Cats were similarly affected. Similar doses *given by stomach tube gave rise to the same effects which, however, were* later in onset.

Lethal dosage

Five rabbits were given a single dose of 1·5 c.c. of dioxan per kg. in 5 c.c. of water intravenously, death resulting in 2–6 days. A similar number were given 2·0 c.c. in 20 c.c. of water by stomach tube without previous fasting with the same result. Doses of 1·0 c.c., apart from giving the above symptoms, were not lethal. Doses of 0·2 c.c. repeated at weekly intervals did not appear to affect the animals at all, one rabbit receiving as many as fifteen weekly doses of 0·2 c.c. without effect. The lack of any cumulative action with repeated sublethal doses was remarkably constant. The minimum lethal dose was therefore found to be 1·5 c.c. per kg. when given intravenously and 2·0 c.c. when introduced into the stomach.

Tolerance

It was soon found that animals which received sublethal doses either intravenously or by stomach tube required increasing amounts to produce symptoms or changes in renal function. This tolerance was independent of the mode of administration and was constantly demonstrated. One rabbit (No. 14) received the following amounts by stomach tube at 2–3 day and weekly intervals: seven doses of 1·0 c.c. and one dose each of 2·0, 2·5, 3·0 and 4·0 c.c., which last dose produced symptoms of drunkenness but with no disturbance of



Graph I. Rabbit No. 14. Effects of repeated doses of dioxan on renal function.
B.U. = Blood urea in mg. %

renal function beyond polyuria with complete recovery. This animal required 5·0 c.c. per kg. to produce a terminal uraemia. Another rabbit (No. 8) received eight doses of 0·5 and five "lethal" doses of 1·5 c.c. intravenously at weekly intervals without disturbance of renal function except for transient polyuria, the blood urea remaining within normal limits of 20–45 mg. per cent. This animal was killed, and at autopsy both liver and kidneys appeared normal.

Graph I, representing rabbit No. 14, illustrates clearly the great increase in tolerance with succeeding doses of dioxan as well as the complete lack of any cumulative effect with chronic poisoning. A number of similar animals with acquired tolerance is shown in Table I.

Effects of a lethal dose

The symptoms of drunkenness abate and the animal returns to normal a few hours after injection. The first change observed in renal function was a polyuria. The amount of urine excreted by a normal rabbit on the above diet during the 24 hours varied from 25 to 50 c.c. with a specific gravity above 1035 and a *p*H slightly on the alkaline side. The amount of urine passed after the injection of dioxan was increased up to treble the normal amount for the

Table I

Rabbit no.	No. of doses in c.c. per kg.	End	Kidneys	Liver	Renal function		
1	2 × 1·5	Died	Necrosis + + +	Normal	Polyuria	Anuria	B.U. = 357
2	15 × 0·2	—	—	—	,,	—	,, = 41
	3 × 1·5	—	—	—	,,	—	,, = 129
	3 × 2·0	—	—	—	,,	—	,, = 31
	2 × 3·0	—	—	—	,,	—	—
	1 × 4·0	Died	Necrosis + + +	Normal	—	Anuria	B.U. = 170
3	1 × 1·5	,,	,, + + +	Glycogen + +	Polyuria	,,	,, = 333
4	1 × 1·5	Killed	,, + +	Normal	—	—	,, = 87
5	2 × 2·0	Died	,, + + +	Glycogen + + +	Polyuria	Anuria	,, = 294
6	1 × 1·5	,,	,, + + +	,, + +	,,	,,	,, = 300
7	1 × 2·0	,,	,, + + +	,, + + +	,,	,,	,, = 364
8	8 × 0·5	—	—	—	—	—	—
	5 × 1·5	Killed	Normal	Normal	Polyuria	—	B.U. = 29
9	3 × 1·0	,,	,,	,,	,,	—	,, = 44
10	9 × 0·5	—	—	—	—	—	—
	1 × 2·0	Died	Necrosis + + +	Glycogen +	Polyuria	—	—
11	1 × 1·0	Killed	,, +	,, +	,,	—	B.U. = 26
12	13 × 0·5	,,	Normal	Normal	,,	—	,, = 37
13	4 × 1·0	—	—	—	—	—	—
	1 × 1·5	,,	Necrosis +	Glycogen + + +	Polyuria	—	B.U. = 43
14	7 × 1·0	—	—	—	,,	—	,, = 23
	1 × 2·0	—	—	—	,,	—	,, = 29
	1 × 2·5	—	—	—	,,	—	,, = 25
	1 × 3·0	—	—	—	,,	—	,, = 31
	1 × 4·0	—	—	—	,,	—	,, = 81
	1 × 5·0	Died	Necrosis + + +	Normal	—	Anuria	,, = 175

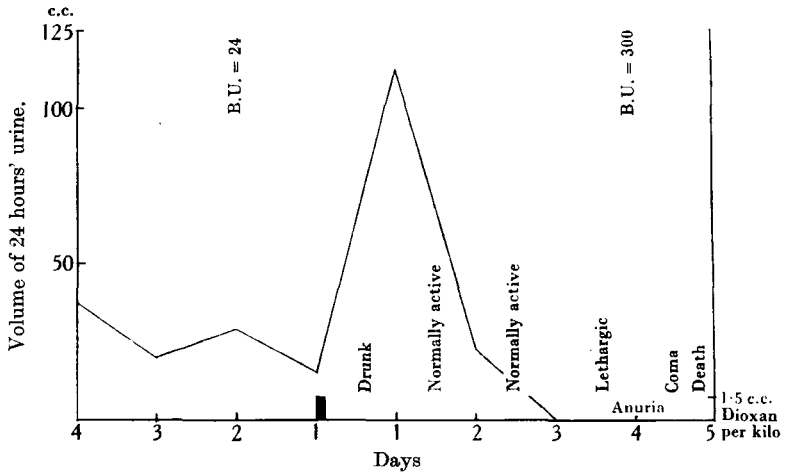
B.U. = Blood urea in mg. per cent.

next 24–48 hours with corresponding diminution in the specific gravity, unaccompanied by any constant change in the *p*H. This polyuria was then followed by anuria which was usually complete after 48 hours. During the period of anuria, the blood urea rose rapidly, reaching 300 mg. per cent. or over, 4 or 5 days after the injection. Abnormal constituents such as pus, red cells or casts were never found in the urine. The animal loses weight in the meantime, becomes progressively lethargic, and eventually dies in coma after 3 or 4 days complete anuria. Occasionally paralysis of the hindlimbs occurs before death. Graphs II and III illustrate the sequence of changes in typical cases following the injection of a single lethal dose.

Morbid anatomy

The animal appeared dehydrated and wasted. No abnormalities were seen in any of the viscera with the exception of the liver and kidneys. The liver was of normal size and showed an accentuation of its lobular anatomy, which was

due to a diffuse mottling of pale areas surrounded by congested zones, the reverse of what is seen in a nutmeg liver. The consistency was soft and friable but not fatty. Jaundice was absent. The kidneys were enlarged, weighing 9·5 and 9 g. respectively (normal 6–7 g.), pale and oedematous with stretched capsule and bulging parenchyma on section. The cortex was increased in thickness and extremely pale with loss of vascular markings. Its consistency was very soft, as though œdematous. The boundary zone was well defined,



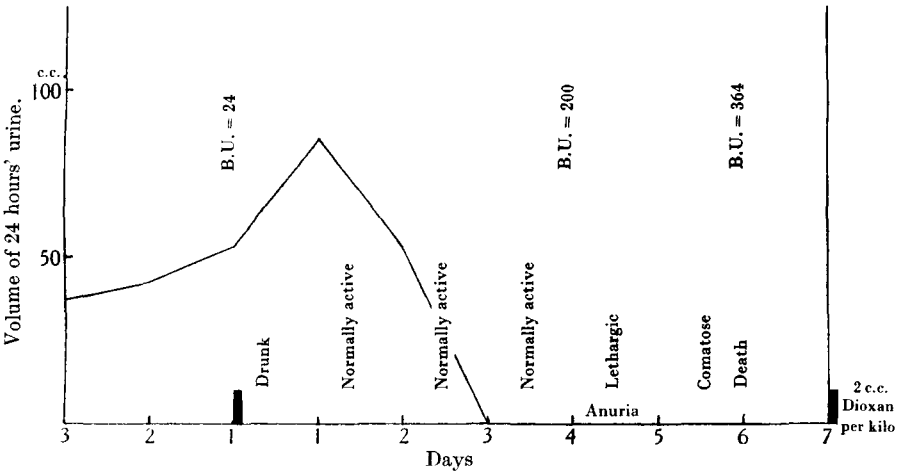
Graph II. Rabbit No. 6. Effects of a single lethal dose of dioxan on renal function.
B.U. = Blood urea in mg. %

while the medulla, pelves and ureters appeared normal. The bladder was empty. The above renal changes were constantly present in every animal which died as a result of dioxan poisoning. The degree of liver changes was less constant and varied irrespective of dosage or mode of administration.

Histology

The heart, lungs, spleen, suprarenals, stomach and intestines were devoid of lesions. The kidneys showed a characteristic and constant change, differing only in degree. The glomerular tuft appeared contracted and empty of blood, but no changes in the character or number of cells or in the basement membrane were seen. The capsular space, however, was markedly dilated to four or five times the normal size at its widest part. The spaces were partially filled with eosinophile albuminous clear material in which were entangled the remains of necrotic cells with an occasional red cell. The epithelium of the capsular membrane was destroyed, leaving the basement membrane denuded. The most marked change was seen in the secretory tubules of the cortex. Their lumina were completely occluded by outlines of swollen cells, from which all trace of nucleus and cytoplasmic stain had disappeared leaving clear intracellular spaces. These faintly outlined cell membranes were in turn surrounded by the tubular basement membrane. These intracellular spaces were devoid of fat or

glycogen and their ballooned appearance suggested a fluid content which remained unstained. This dissolution of cytoplasm stopped short at the boundary zones, the epithelium of the collecting tubules being normal, though albuminous fluid with cellular debris was present in the lumen. The pelvis was normal. In view of the well-defined vascular lesions seen in human cases of dioxan poisoning, it was surprising that no evidence of vascular damage was found in any of the animals under experiment.



Graph III. Rabbit No. 7. Effects of a single lethal dose of dioxan on renal function
B.U. = Blood urea in mg. %

The liver, when stained with haemotoxylin and eosin, showed areas, usually confined to the central zone of the lobule and in parts spreading to include several lobules, where the lobular arrangement had become distorted by marked swelling of the liver cells, which had lost their usual normal cytoplasmic structure. The cytoplasm appeared vacuolated and replaced by brownish granules of irregular shape but roughly uniform size. The nuclei varied greatly in shape, intensity of staining and in number. Many cells showed twin nuclei, while in others the nucleus was absent. This was not due to the sections being cut too thin and therefore missing the nucleus of the large liver cell, as nuclear counts, in the same measured area in sections cut 15 μ in thickness, as compared with 6 μ, showed no significant difference. The appearance of the liver was typical of what is described as "hydropic" degeneration. When stained with Best's carmine, however, these vacuolated cells were seen to be packed with bright red granules of glycogen, which showed a maximum concentration around the central vein and diminished in intensity towards the periphery. This change always appeared to begin in the central zone and to spread peripherally, becoming continuous with similar changes in adjacent lobules in many cases, though in others the changes remained strictly zonal. This glycogen accounts for the spurious degeneration which is described in sections stained with

haemotoxylin and eosin only. No fatty change was evident in sections stained with Sharlach R.

Table I is a protocol of the rabbits employed and demonstrates the constancy of the renal lesions and the variability of the liver changes.

Discussion

Renal changes

The symmetrical cortical necrosis of the kidneys described by Barber in human cases of dioxan poisoning was shown by De Navasquez (1935) to be histologically identical with cortical necrosis of pregnancy. Both conditions were due to primary necrosis of the walls of the intralobular arteries and their efferent glomerular branches, which resulted in ischaemia of the parenchyma. No such lesions were present in this investigation. In the laboratory animals examined, dioxan has a selective action on the secretory epithelium of the nephron without affecting the vessels in any way. The convoluted tubules undergo a rapid form of degeneration associated with loss of cytoplasmic structure and nuclei and "ballooning" of the cells with fluid, which exhibits no characteristic staining properties. This is considered to be an acute form of "hydropic" degeneration. The tubules are blocked as a result of this swelling, causing anuria and uraemia to develop rapidly. The transient polyuria which precedes the anuria is probably an expression of early damage which diminishes the concentrating power of the tubule. Animals which received sublethal doses of dioxan, were killed in the polyuric state, and mild degrees of hydropic degeneration were present. At this stage, the damage is reparable, as animals ordinarily return to normal after sublethal dosage and, if killed later, exhibit no lesions at autopsy. Fairley, Linton and Ford-Moore described haemorrhages in the renal cortex, but none were found in the present series.

Liver changes

The histology in both the human and animal livers is similar. In the rabbits which showed liver changes, it is doubtful whether these can be considered pathological. Turnbull (1920) warned investigators that the histological changes seen in rabbits' livers when infiltrated with glycogen resemble closely those of severe "dropsical" degeneration. He produced such changes in rabbit's liver with suitable carbohydrate feeding and regards the absence of cytoplasmic structure and the nuclear changes as transitory manifestations in the course of physiological glycogen metabolism. It is not improbable that the "central necrosis" seen in human cases is of a similar nature. The zonal distribution of glycogen shows a maximum intensity around the central vein. The change is an inconstant one and bears little relationship to the degree of poisoning, as some animals which received lethal doses of dioxan exhibited normal livers. Furthermore, this condition leaves no trace of damage, as of rabbits which received sublethal doses of dioxan, some showed marked glycogen infiltration, while others killed later had a liver normal in appearance. No fibrosis was observed

after repeated doses. It is difficult to exclude a similar change in the human cases, as it is seldom possible to cut sections of liver sufficiently soon after death to demonstrate glycogen, which rapidly disappears; nor is alcohol, which is an essential fixative for glycogen, often used.

Toxicity

Yant, Schrenk, Waite and Patty exposed five persons for 1 min. to air containing 0·55 per cent. by volume of dioxan. The symptoms noticed were irritation of the eyes and a burning sensation in the nose and throat accompanied by a transitory vertigo. The atmosphere was not intolerable but noticeable. No permanent damage appeared to have been suffered. Their conclusion was that the vapour could only have a very low toxicity. The present series of experiments confirms the low toxicity of dioxan. Lethal effects were only produced in guinea pigs, rabbits and cats in comparatively large doses of 1–2 c.c. per kg. of body weight, when introduced intravenously or by stomach tube, so that an intense concentration of vapour would be necessary to raise the dioxan in the blood to a level to produce renal failure. Though lethal dosage in laboratory animals may not be directly applicable to man, the fact that several species were similarly affected does offer some indication of the probable toxicity to workers in industry. In view of the easily acquired tolerance demonstrated in these animals, some degree of tolerance would also be expected in man, and these experiments confirm Barber's opinion that the five deaths which he records were referable to a few intense exposures and not to chronic poisoning. On the evidence of Yant and his colleagues, however, exposure to atmosphere containing small doses of dioxan causes irritation which renders it very noticeable. It is therefore surprising that work should have continued in atmospheres containing dioxan in sufficiently high concentration to cause death from renal failure. Fairley, Linton and Ford-Moore exposed human observers for 3 min. to atmosphere containing nominal concentrations of 1/500 and 1/1000 and only very mild symptoms, which rapidly diminished, were produced. These observers exposed four rabbits, four guinea-pigs, six rats and five mice to similar concentration and only one rabbit died after exposures totalling 69 hours. Although this animal showed renal lesions at autopsy, these may well have been transitory, as the highest blood urea recorded in the other rabbits which remained alive and even maintained their weight after 99 hours exposure was 32 mg. per cent., which excludes the possibility of renal failure. It is therefore improbable that sufficient dioxan vapour concentrations to cause toxic or lethal effects would escape the notice of workers employed in such processes.

SUMMARY

1. Dioxan when given intravenously and by stomach tube to guinea-pigs, rabbits and cats produces a state of drunken intoxication.

2. The minimum lethal dose for rabbits and cats is 1·5 c.c. per kg. intravenously and 2·0 c.c. per kg. by stomach tube.

3. Tolerance is rapidly acquired and recovery is complete after repeated sublethal doses.

4. In human beings lethal doses of dioxan produce primarily a necrosis of the intralobular arteries of the kidney with resulting necrosis of the parenchyma; whereas in laboratory animals, it has a selective action on the convoluted tubules, which undergo an acute hydropic degeneration, causing intrarenal obstruction and anuria with subsequent death from uraemia.

5. The so-called "necrosis" of the liver produced in rabbits with dioxan is a transitory phenomenon due to the presence of glycogen. This may be equally so in the "central zonal necrosis" seen in dioxan poisoning in human beings.

6. It is suggested that the toxicity of dioxan to human beings is relatively low and that large doses are required to produce lethal effects.

It is a pleasure to express my thanks to Prof. G. Payling Wright for many helpful suggestions and much critical advice.

REFERENCES

BARBER, H. (1934). *Guy's Hosp. Rep.* **84**, 267.

DE NAVASQUEZ, S. (1935). *J. Path. Bact.* **41**, 386.

FAIRLEY, A., LINTON, E. C. and FORD-MOORE, A. H. (1934). *J. Hygiene*, **34**, 486.

TURNBULL, H. M. (1920). *Brit. J. Exp. Path.* **1**, 310.

VAN OETTINGEN, W. F. and JIROUCH, E. A. (1931). *J. Pharmacol. and Exp. Therap.* **42**, 355.

YANT, W. P., SCHRENK, H. H., WAITE, C. P. and PATTY, F. A. (1930). *U.S. Pub. Health Rep.* **45**, 35, 2023.

(*MS. received for publication* 5. x. 1935.—Ed.)