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PROTEUS VULGARIS URINARY TRACT INFECTIONS IN RATS; TREATMENT WITH NITROFURAN DERIVATIVES

BY

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Ascending urinary tract infections with stone formation have been produced experimentally in rats, using a modification of the method of Vermuelen & Goetz (1954a, b). A zinc disc infected with a culture of *Proteus vulgaris* was inserted into the bladder by suprapubic cystotomy under ether anaesthesia. The *p*H of the urine rises from 6.9 to 8 or 9 and calculi develop in the bladder within a few days of infection. The bladder and ureters become swollen, distended and inflamed, and renal abscesses develop. Death from renal failure generally occurs within 10 days of infection. Oral treatment with nitrofurantoin was commenced three days after infection and continued for one month. This arrested the initial rise in urine alkalinity and stone formation, and few, if any, macroscopic lesions were found at post-mortem examination. Of nine nitrofuran derivatives examined for activity against this infection several showed slight activity, but only one, N-(5-Nitrofurfurylidene)-γ-butyric acid, was as active as nitrofurantoin when given at four times the dose, but it was also one-third as toxic. It is concluded that this technique is suitable for the examination of potential urinary antiseptics.

Vermuelen & Goetz (1954b) produced experimental ascending urinary tract infections in rats by inserting zinc discs infected with *Proteus morganii* into the bladder by means of suprapubic cystotomy, and demonstrated the efficacy of nitrofurantoin as a urinary antiseptic in these infections. The following work arose from the need for a method of examining compounds against urinary tract infections *in vivo*. The method of Vermuelen & Goetz appeared to be suitable for this purpose. These authors reached their conclusions regarding the suitability of the method as a result of a series of experiments with nitrofurantoin, and it was considered that an appraisal of this method could best be achieved if a series of compounds closely analogous to nitrofurantoin was examined in comparison with nitrofurantoin. Accordingly such compounds were devised and synthesized by my colleagues, Drs Holland and Rogers, in these laboratories.

Experiments with these compounds form the basis of this study, but, since Vermuelen & Goetz described their procedure for infection in general terms only, the technique adopted for our experiments is described here in some detail.

METHODS

Male albino rats of the Wistar strain, weighing from 200 to 250 g, were used. They received no special treatment before infection. The rats were anaesthetized with ether, their abdomens were shaved and the skin was liberally swabbed with cetrimide. A transverse skin

incision (1 to 2 cm) was made across the abdomen, to the right of the midline, to expose the body wall. The body wall and the peritoneum were then opened by a transverse cut which left the rectus abdominis muscle intact. The prostate gland, which was then revealed, was moved aside so that the bladder could be pulled through the incision. The apex of the bladder was cut with fine scissors and a zinc disc, 3 mm × 1 mm, previously immersed in an 18-hr broth culture of the test organism, was inserted. *Proteus vulgaris* N.C.T.C. No. 3156 was used, grown in a synthetic liquid medium (Wilson, 1954) at 37° C, and stored on agar slopes of the same medium at 4° C. A single silk suture was used to close the bladder and the peritoneum and body wall were then closed with one or two silk sutures and the skin wound closed with Michel clips. The procedures were carried out as cleanly as possible, for example, the instruments were kept in an antiseptic solution or 70% alcohol, but full aseptic precautions were not necessary.

The operative mortality in all the experiments, comprising over 180 rats, was less than 1.0%. A small number of infected animals died from urinary extravasation into the peritoneal cavity within 2 to 3 days of infection, but this never occurred when sterile discs were inserted.

The infected rats were randomized into groups of six and treatment was started on the third day of infection by administering tragacanth suspensions of the compounds by stomach-tube at a dose approximating to one-sixth of the LD50 as determined in mice. This dose was then given daily for one month by incorporating the compound into a powdered diet (M.R.C. Diet No. 41B, Bruce & Parkes, 1956), which was made wet and then dried again in the oven. The amount of compound mixed into the diet was calculated on the basis that the rats ate 20 to 25 g per day. During treatment the consumption of diet by the rats was checked periodically.

Several of the rats which died of the infection, and all those which survived to the end of the experimental period, were examined *post mortem* for the presence of urinary calculi and gross macroscopic lesions of the urinary tract, and the *p*H of the urine was determined.

Cultures were made from the urine and diagnostic tests with any resultant growth were made on Kligler Iron medium (Oxoid).

The compounds tested were:

M&B 5132; N-(5-Nitrofurfurylidene)-γ-hydroxybutyric acid hydrazide.
M&B 5507; N-(5-Nitrofurfurylidene)-4-hydroxyvaleric acid hydrazide.
M&B 5793; N-(5-Nitrofurfurylidene)-2-ethyl-4-hydroxybutyric acid hydrazide.
M&B 6041; N-(5-Nitrofurfurylidene)-crotonic acid hydrazide.
M&B 6042; N-(5-Nitrofurfurylidene)-senecioic acid hydrazide.
M&B 6093; N-(5-Nitrofurfurylidene)-glycollic acid hydrazide.
M&B 6294; N-(5-Nitrofurfurylidene)-L-lactic acid hydrazide.
M&B 6432; N-(5-Nitrofurfurylidene)-2-nonenoic acid hydrazide.
M&B 6480; N-(5-Nitrofurfurylidene)-pyruvic acid hydrazide ethylene glycol ketal.
Nitrofurantoin is N-(5-Nitrofurfurylidene)-aminohydantoin.

RESULTS

Control experiments. Sterile zinc discs were inserted into the bladders of rats. These animals remained uniformly healthy for periods of up to one month, and when the discs were recovered they were found to be clean with no trace of stone formation. The urinary *p*H was within the normal range and no macroscopic pathological signs were seen.

In further control experiments, a sample of *Proteus* was injected into the bladder in the absence of a disc. No signs of ill-health could be detected in these rats.

Treatment with nitrofurantoin. Of 48 rats treated with nitrofurantoin, 60 mg/kg/day, 42 survived (87.5%), and of 48 untreated animals 7 survived (14.6%). These results are very similar to those obtained by Vermuelen & Goetz (1954b) with 90

mg/kg/day of nitrofurantoin ; their figures were 22 out of 31 (66.7%) and 5 out of 31 (16.1%), respectively.

The *p*H of the urine in the untreated animals, whether they survived the experimental period or not, was 8.0 to 8.8 (normal *p*H 6.9 to 7), and there were massive calculi in the bladders and smaller stones and gravel in the ureters and kidneys. Inflammation, swelling and distension of the bladders and ureters were noted and the kidneys were distended, had gross abscesses and were frequently necrotic (see Fig. 1).

In nearly all the nitrofurantoin-treated rats the final reaction of the urine was about *p*H 7.1 to 7.4, that is, slightly higher than normal, although in one group

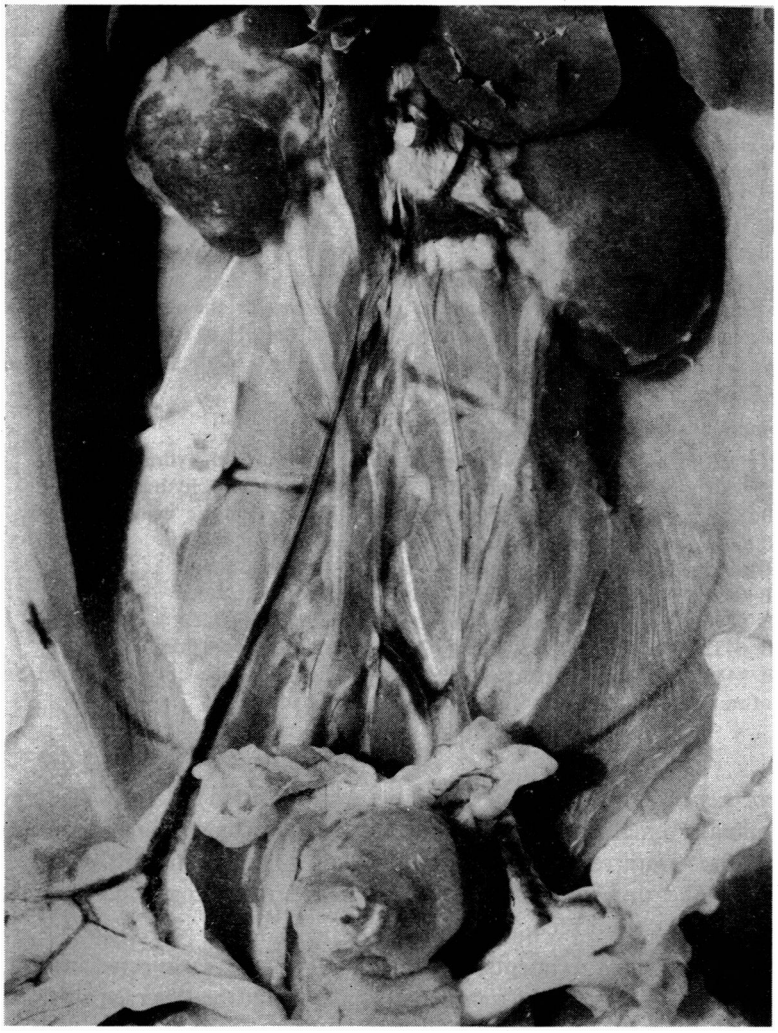

Fig. 1. Male rat, 200 g, infected with *Proteus vulgaris* on a 3 mm zinc disc inserted into the bladder 4 weeks previously. Note the abscesses in the right kidney, the distended ureters and enlarged bladder which contained numerous calculi and was highly inflamed.

TABLE 1

THE EFFECT OF NITROFURANTOIN AND OTHER NITROFURAN COMPOUNDS AGAINST *PROTEUS* URINARY TRACT INFECTIONS IN RATS

Expt. no.	Compound	LD50 (mouse) mg/g	Dose mg/g day	Surviving animals: Treated animals	Post-mortem examination of survivors
1	Nitrofurantoin	0·4	0·05	5/6	1 infected (urine *p*H 8·2; stone formation). Remainder healthy
	M&B 5132	1·3	0·2	5/6	All healthy, but one with small renal abscess. Small calculi
			0·05	2/6	Urine *p*H normal, but massive calculi and renal abscesses present
	Controls	—	—	0/6	—
2	Nitrofurantoin	0·4	0·06	6/6	Not examined
	M&B 5507	0·5	0·08	1/6	Not examined
	Controls	—	—	0/6	Not examined
3	Nitrofurantoin	0·4	0·06	4/6	All perfectly healthy. No stones
	M&B 5793	0·8	0·12	1/6	Urine *p*H 8·6. Inflamed bladder. Small stone
	Controls	—	—	2/6	Urine *p*H 8·4. Stones in bladder and kidneys. Abscesses and necrosis of kidneys
4	Nitrofurantoin	0·4	0·06	6/6	Urine *p*H high at 7·4 to 8·4. One with large calculi, one with renal abscesses
	M&B 6041	1·3	0·2	1/6	Urine *p*H 8·4. Large calculi
	M&B 6042	3·2	0·5	0/6	—
	Controls	—	—	1/6	Urine *p*H 7·7. Large calculi. Abscesses
5	Nitrofurantoin	0·4	0·06	5/6	All healthy, normal
	M&B 6093	0·6	0·1	2/6	Alkaline urine, *p*H 8·0. Calculi. Bladder inflamed, swollen
	Controls	—	—	1/6	Alkaline urine, *p*H 8·0. Massive calculi in bladder and kidneys. Renal abscesses and necrosis
6	Nitrofurantoin	0·4	0·06	4/6	All normal, healthy
	M&B 6294	0·68	0·11	3/6	Grossly infected, alkaline urine *p*H 8·4. Calculi. Renal abscesses
	Controls	—	—	0/6	—
7	Nitrofurantoin	0·4	0·06	6/6	2 grossly infected, 4 healthy
	M&B 6480	0·9	0·5	4/6	2 grossly infected, 2 healthy
	Controls	—	—	1/6	Grossly infected

TABLE 1—*continued*

Expt. no.	Compound	LD50 (mouse) mg/g	Dose mg/g day	Surviving animals: Treated animals	Post-mortem examination of survivors
8	Nitrofurantoin	0·4	0·06	6/6	Normal, healthy. 3 alkaline urine *p*H, small stones
	M&B 6432	>4·6	0·8	2/6	Grossly infected. Urine *p*H 8·4 to 8·7. Massive calculi. Abscesses, inflammation, necrosis
	M&B 6480	0·9	0·15	2/6	Grossly infected. Urine *p*H 8·4 to 8·7. Massive calculi. Abscesses, inflammation, necrosis
	Controls	—	—	2/6	Grossly infected. Urine *p*H 8·4 to 8·7. Massive calculi. Abscesses, inflammation, necrosis

(Table 1, experiment no. 4) the range was from *p*H 7.4 to 8.4. In the animals treated with nitrofurantoin gross macroscopic lesions of the urinary tract were absent although small urinary calculi were sometimes found, indicating that an infection had become established for a short time at least.

Cultures taken from the urine failed to give consistent results. Of the animals treated with nitrofurantoin only 15% had sterile urine. *Proteus* was isolated from about 48% and a variety of organisms (*A. aerogenes, E. coli* and *Salmonellae* of undetermined species) were found in the other 37%. The source of these organisms, whether introduced at infection or by a faulty sampling, is not known. Cultures taken from the surviving infected controls and from some which died during the experimental period all yielded *Proteus*. On several occasions *Proteus* and other Gram-negative organisms were isolated from the urine of healthy animals. Chemical analysis of the calculi showed them to be composed of magnesium ammonium phosphate.

Treatment with nitrofuran derivatives. Table 1 shows the results of tests with nine nitrofuran derivatives. Brief comments are given on the post-mortem examination in each group. Six of the compounds were inactive against the infection. Although 3 out of 6 animals survived on M&B 6294 the animals had alkaline urine, massive urinary calculi, swollen inflamed bladders and large renal abscesses, and it was consequently less effective than nitrofurantoin. Similarly for M&B 6480, for, although in two experiments 6 out of 12 rats survived, it was less effective than nitrofurantoin, with which all 12 rats survived, and the post-mortem findings also showed more infection in the animals treated with M&B 6480.

The only experimental compound active against the infection was M&B 5132, which was administered at two doses (Table 1, experiment no. 1). It was one-third as toxic as nitrofurantoin, and when given at four times the dose used for nitrofurantoin it was just as effective.

DISCUSSION

Treatment of urinary tract infections usually consists of sulphonamide or antibiotic therapy, but unfortunately many strains of *Proteus* and *Pseudomonas* are

refractory, and it is in these cases that nitrofurantoin finds some use. Urinary tract infections in animals have been produced experimentally by methods which have generally involved the injection of bacteria into the blood stream after renal trauma, for example, ureteral ligation (Lepper, 1921), renal massage (Braude, Shapiro & Siemienski, 1955) and electrocauterization (Rocha, Guze, Freedman & Beeson, 1958). Kidney infections in mice have also been produced in the absence of antecedent damage by the injection of suitable strains of *Staphylococcus aureus* by Heptinstall & Gorrill (1955), Smith & Dubos (1956) and Gorrill (1958).

However, as many urinary tract infections arise after surgery or catheterization and spread via the ascending rather than the haematogenous route (Vivaldi, Cotran, Zangwill & Kass, 1959) the method of Vermuelen & Goetz (1954b) was chosen for study. The rationale of this method is outlined by these authors as follows. When bacteria are simply injected into the bladder they are frequently eliminated spontaneously without causing a disease, but when a foreign body is simultaneously inserted this apparently prevents a spontaneous cure. If the organism is a strain of *Proteus* producing the enzyme urease, urea in the urine is broken down to ammonia and the *p*H of the urine is increased, thus favouring the precipitation of relatively insoluble crystalloids which with the foreign body as a nucleus eventually form massive urinary calculi. (The role of bacterial urease in pyelonephritis has been studied experimentally by Braude & Siemienski, 1960.) Presumably pressure of urine due to the obstruction in the bladder spreads the infection to the kidneys via the ureters, leading to renal failure and death.

A number of the experimental conditions described here differ from those described by Vermuelen & Goetz (1954b). The infecting organism they used was a strain of *Proteus morganii* isolated from a rat, whereas here a stock laboratory strain of *Proteus vulgaris* was used. Preliminary experiments were carried out with two strains of *Proteus vulgaris* isolated from clinical urinary tract infections, but both were unsuitable, since one was too virulent, causing death within 3 to 4 days, and the other failed to cause a fatal infection. All, however, were powerful urea splitters.

Although the rats were obtained from two different sources (Wistar and Harlan strains), they all produced calculi consisting of magnesium ammonium phosphate. Vermuelen & Goetz (1954a) found that another strain of rat (Holtzman) produced stones of calcium salts.

On the whole there was a very good agreement between Vermuelen & Goetz's results and our own, although we obtained a somewhat higher survival rate with a lower dose of nitrofurantoin without any increase in the survival of untreated animals. This may be partly the result of giving the initial dose directly by stomach-tube, and partly the result of differences in the strain of organism. An attempt was made to reduce the period of treatment from four weeks to one week, but this was found unsatisfactory.

It is concluded that the method as described is suitable for the examination of potential urinary antiseptics.

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