Both steroids will regularize the cycles if an adequate dose has been given, and in the majority of cycles treated bleeding started within four days of finishing a course of treatment.

The endometrium of proved cases of metropathia was shown to have some secretory transformation even with 20 mg. of norethisterone acetate in 10 days, and subsequent bleeding was normal after biopsy in the majority, although occasionally a heavy menstrual loss followed a proved secretory transformation. A cyclical dose of 40–60 mg. of norethisterone acetate in 10 days or 90–120 mg. of norethisterone in the last phase of the cycle is generally satisfactory in producing regular normal bleeding, and is preferable, particularly in women who desire pregnancy. It also requires less steroid than if given from the 5th to the 25th day, and with the latter regime sometimes a breakthrough bleeding occurs before the patient has finished the 20 days' treatment, thus altering the cyclical rhythm.

A number of these patients treated for menorrhagia with cyclical progestogens at the end of the cycle complained of dysmenorrhoea, but, as Bishop and de Almeida (1960) found in their series, this was not severe enough to make them unwilling to continue treatment if the loss was successfully controlled.

Swyer's (1960) method of giving treatment for 20 days from the 5th to the 25th day of the cycle is more expensive, but has the advantage of inhibiting ovulation and so preventing dysmenorrhoea and acting as an oral contraceptive.

The number of cycles in this series when norethisterone acetate was given deliberately to attempt to postpone menstruation is small, partly because the quantity of this steroid available was not unlimited; but doses of up to 16 mg. daily for 20 to 30 days cannot be relied upon for this purpose. In this respect Swyer, Sebok, and Barns (1960) have shown that norethisterone acetate is less potent than norethisterone, of which 20–30 mg. daily will delay a normal menstrual period and induce a pseudo-pregnancy of as much as seven months (Greenblatt and Jungck, 1958).

Summary

During the last two and a half years norethisterone and more recently norethisterone acetate have been used in a total series of 90 women. Some were given either the one or the other, and some were treated with both steroids at different times. The conditions treated were primary and secondary amenorrhoea, oligomenorrhoea, some dysmenorrhoea, and a larger series of cases of menometrorrhagia.

For secondary amenorrhoea both steroids were equally effective when used with oestrogens to induce menstruation.

An assessment of the secretory transitional dose of norethisterone acetate in oestrogen-primed married women with secondary amenorrhoea was made, and the effect of similar doses on the endometrium of women suffering from metropathia was investigated.

The control of prolonged excessive regular or irregular menstruation was about equally effective with norethisterone and norethisterone acetate (83:75%), but, weight for weight, the latter was about twice as potent.

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TREATMENT OF URINARY INFECTIONS WITH CYCLOSERINE

BY

R. W. FAIRBROTHER, T.D., M.D., D.Sc.
F.R.C.P.
Director

AND

G. GARRETT, M.D., Dip.Path.*
Senior Registrar

Department of Clinical Pathology, Manchester Royal Infirmary

Infections of the urinary tract constitute one of the most difficult groups of infection encountered in a general hospital at the present time. Many are complicated by some underlying anatomical abnormality or neurological condition, and while infections with sensitive organisms, such as the coliforms, tend to respond readily to routine therapy, an increasing number are being caused by organisms resistant to the usual chemotherapeutic agents. The causative organism is often a *Proteus*, *Ps. pyocyanea*, or paracolon which tend to be resistant to the sulphonamides, nitrofurantoin, chloramphenicol, and tetracycline. These cases have consequently become a major problem, and the introduction of a new effective antibiotic would be an important development.

Cycloserine, an antibiotic prepared from *Str. orchidaceus*, was originally used for the treatment of tuberculosis. It has, however, a wide antibacterial spectrum, and, though it has a relatively low grade of *in vitro* activity, it has been found to produce concentrations in the urine which may be effective against some resistant organisms responsible for urinary infections (Welch *et al.*, 1955). Although toxic manifestations were a serious problem when cycloserine was used for the treatment of tuberculosis, only minimal side-effects were observed with the smaller doses required for urinary infections. Moreover, encouraging results were given in some patients with chronic resistant infections (Herrold *et al.*, 1955).

*Now Consultant Pathologist, Oldham.

It was therefore decided to carry out a trial of cycloserine in the treatment of refractory urinary infections—that is, those caused by organisms found to be resistant to, and/or had not responded to treatment with, the usual agents.

Methods

The sensitivity of the organisms, freshly isolated from the urinary infections, and the levels of cycloserine in urine were determined by the serial dilution technique. A stock solution in sterile distilled water was prepared with a special salt of cycloserine to give a final concentration of 10,000 $\mu\text{g./ml.}$ Suitable doubling dilutions were made with special assay broth containing glucose and an indicator; 0.5 ml. was the volume used. In all cases the bacterial inoculum was 0.02 ml. of an undiluted overnight broth culture. Readings were taken after overnight incubation (usually 18 hours) at 37° C.; visible inhibition of growth was the criterion on which the results were based.

The urine was passed through a Seitz filter, after the bacteriological examination, for the determination of the cycloserine levels; dilutions were made with the special broth. Only the first and last specimens from female patients were collected by catheter; clean or mid-stream specimens, collected daily, were used for the other investigations. In each series a control set of dilutions of the standard cycloserine solution was prepared and all tubes received the standard inoculum (0.02 ml.) of the test organism, which was a relatively sensitive strain of *Staph. aureus*.

The blood levels were determined by a modification of the chemical technique described by Jones (1956).

Results

Various observations were made during the investigation, and the results are presented in four parts: (1) sensitivity *in vitro* to cycloserine of urinary pathogens, (2) blood and urine levels obtained after various schemes of dosage, (3) clinical results, and (4) development of resistance by the organisms *in vivo* and *in vitro*.

In Vitro Sensitivity Tests

A wide range of organisms isolated from simple and complicated urinary infections were subjected to routine testing (Table I).

TABLE I.—*In Vitro* Activity of Cycloserine. Minimal Inhibitory Concentration on Urinary Pathogens ($\mu\text{g./ml.}$)

Organism	No. Tested	$\mu\text{g./ml.}$					
		1,280	640	320	160	80	40
<i>E. coli</i>	107	2	8	45	50	2	—
<i>Proteus</i>	71	15	48	8	—	—	—
<i>Str. faecalis</i> ..	34	—	7	24	3	—	—
<i>Ps. pyocyanea</i> ..	30	3	17	10	—	—	—
<i>Staph. albus</i> ..	30	—	—	5	2	7	16
<i>Staph. aureus</i> ..	20	—	—	3	2	7	8
Paracolon strains ..	25	—	4	9	11	1	—
<i>Aerogenes</i>	17	—	6	7	4	—	—
Total ..	334	20	90	111	72	17	24

Cycloserine did not prove to be a very active antibiotic. Only 113 out of 334 organisms were inhibited by concentrations of 160 or less $\mu\text{g./ml.}$; these organisms were principally staphylococci and coliforms. Out of 334 organisms 110 were inhibited only by 640 or more $\mu\text{g./ml.}$; these were mainly strains of *Proteus* or *Pseudomonas* which are frequently responsible for obstinate urinary infections.

Blood and Urine Levels

Cycloserine was given orally to the first four patients in the following dosage: 250 mg. (one pulvule) statim, with 250 mg. after three hours and then 250 mg. at six-hourly intervals. In an attempt to obtain higher levels more quickly, the remainder received the 250-mg. dose at once, after intervals of three, six, and nine hours, and then six-hourly throughout the course of 5–10 days (average 7 days). The maximum total dosage was 10 g. Blood and urine estimations were usually made on samples collected during the morning, four hours after the previous dose of cycloserine, and in the afternoon two hours after the dose.

The blood levels were low; a range of 13–15 $\mu\text{g./ml.}$ was soon reached, and levels of 19–27 $\mu\text{g./ml.}$ were maintained.

Cycloserine is greatly concentrated in the urine, and much higher levels were obtained than in the blood; these tended to be consistent and also to remain steady throughout the course (see Chart). Levels of 960 $\mu\text{g./l.}$

Urine levels of cycloserine in 22 patients under treatment. Shaded area is range of results, and the line is the average result. (One case was treated for 2½ days, one for 4 days, ten for 5 days, two for 6 days, four for 7 days, two for 8 days, and two for 10 days.)

ml. were attained in 10 out of 21 cases, while in all cases a level of at least 400 $\mu\text{g./ml.}$ was reached, being maintained throughout the course.

Clinical Results

A total of 22 cases (14 males) were treated with cycloserine, and of these 17 were complicated by neurological or anatomical disorders. Of the uncomplicated cases, four were infected with *E. coli* and one with *Ps. pyocyanea*. Two of five uncomplicated but only 3 of 17 complicated cases had not received any previous treatment for the infection; 20 of the 22 patients received treatment for at least five days, four for seven days, and two for 10 days.

The drug was well tolerated by most patients. One patient, however, became incoherent and incontinent after 55 hours' therapy but rapidly recovered on withdrawal of the drug. Three others also showed definite side-effects, after 4 g. in two cases and 5 g. in the other; these were mainly undue drowsiness and dizziness, and were intensive enough to warrant cessation of the drug. Recovery was rapid and complete.

The clinical response varied greatly and the results have been divided into four separate grades: (1) com-

plete clinical and bacteriological cure; (2) temporary cure—relapse within 48 hours after cessation of treatment; (3) some clinical improvement but the urine never freed of organisms; and (4) no obvious effect—clinical or bacteriological.

The results have been so graded in relation to the nature of the infecting organism or organisms. Only 21 cases have been included, and four of them had a mixed infection (Table II).

TABLE II.—*Urinary Infections. Results of Treatment with Cycloserine (21 Cases with 26 Organisms)*

Organism	No. Isolated	Pre-treatment: M.I.C. $\mu\text{g./ml.}$	Grade of Response			
			1	2	3	4
<i>E. coli</i> ..	5	320	1*	2		1
	4	160	1	2		
<i>Proteus</i> ..	7	640		2	2	3
	2	320				2
<i>Ps. pyocyanea</i>	1	1,280			1	
	3	640				3
<i>Str. faecalis</i>	1	320			1	
	2	640	1*			
	1	320	1*			

* Mixed infection with bacteriological clearance only.
M.I.C. = Minimum inhibitory concentration.

Only three complete cures were obtained, and in each case the causative organism was a coliform with a relatively wide range of sensitivity to the antibiotics. Two of these cases were uncomplicated; the other was a case of tabes dorsalis with tidal drainage. Two other uncomplicated cases, infected with *E. coli*, showed only temporary improvement, and the fifth case was infected with a resistant strain of *Ps. pyocyanea*. In two cases of mixed infection the sensitive organisms were eliminated but the resistant organisms persisted.

In 6 out of 21 cases there was temporary improvement, with relapse at the end of treatment; four of these were caused by coliforms and two by *Proteus* strains. In 12 out of 21 cases there was little or no response. The 16 complicated cases included four in an advanced stage of disseminated sclerosis, two with tumours of the spinal cord and paralysis, while two had had operations for carcinoma of the bladder.

These results, though disappointing, were not unexpected, as we were deliberately dealing with unpromising material. The *in vitro* tests had, moreover, indicated quite clearly that in the majority of cases the urinary levels of cycloserine were unlikely to have any appreciable effect, particularly on the difficult *Pseudomonas* and *Proteus* strains.

Development of Increased Resistance to Cycloserine

The capacity of urinary pathogens to habituate themselves to cycloserine was examined under *in vitro* and *in vivo* conditions.

For the *in vitro* tests, various organisms were subcultured in broth containing increasing subinhibitory concentrations of the antibiotic, and were subjected to sensitivity tests. Some increase in resistance was found in all the organisms examined. While strains of *E. coli* did not readily develop increased resistance, *Aerogenes* strains showed an eightfold increase after four or five subcultures. An eightfold increase in resistance was produced by three out of three strains of *Ps. pyocyanea* in four subcultures and by three out of three strains of *Proteus* in five subcultures.

Resistance did not readily develop during routine treatment. Nevertheless, some increase was given by all

strains examined, although it was usually only twofold. This was, however, sufficient in some cases to render such borderline organisms as *Proteus* and *Ps. pyocyanea* resistant to the antibiotic concentrations found in the urine.

Discussion

Disappointing results were obtained from the use of cycloserine in the treatment of stubborn urinary infections, most of which were selected for trial because they had failed to respond to the usual chemotherapeutic agents and were presenting a serious problem. Toxic symptoms, such as vertigo and drowsiness, were observed in 4 out of 22 patients (18%), and these were severe enough to warrant the withdrawal of the antibiotic. Recovery from these side-effects was, however, rapid and complete.

Clinical and bacteriological recovery from the urinary infection was produced in only 3 out of the 21 patients in the series. Two of these infections were uncomplicated and followed catheterization. *E. coli* was the causative organism of these three infections, and in each case it was sensitive to most of the usual chemotherapeutic agents, which would probably have proved equally effective. Although cycloserine is undoubtedly effective in coliform infections (Murdoch *et al.*, 1959), it cannot be recommended as a first choice in view of the risk of side-effects and the number of other possible active agents, including the sulphonamides.

The other 18 cases showed only temporary or no improvement; most of these were difficult and complicated cases, caused by resistant strains of *Proteus* and *Pseudomonas*.

The results, although disappointing, were not surprising. *In vitro* tests had shown that many strains of *Proteus* and *Ps. pyocyanea* are inhibited only by at least 640 $\mu\text{g./ml.}$, while the maximum level demonstrated in the urine during therapy was 960 $\mu\text{g./ml.}$ It is, however, possible that slightly higher levels might have been produced for short periods during the course. Nevertheless, under such conditions, cycloserine was unlikely to be effective.

There was no marked development of resistance by the causative organisms during the treatment, but any slight increase was sufficient to take some of the doubtful organisms out of the therapeutic range. When such a change in resistance was observed treatment was stopped before the full course was completed. It was considered that under such conditions continued therapy would almost certainly be ineffective, and would, moreover, expose the patient to the risk of side-effects.

These results indicate clearly that if cycloserine is to be used for the treatment of urinary infections it is desirable, if not essential, to have adequate laboratory control, particularly the isolation and sensitivity-testing of the causative organisms and the determination of urinary levels during treatment. With such control, the cases likely to respond to treatment can be selected and any unnecessary risk of toxic reactions avoided.

Summary

Twenty-two cases of urinary infection were treated with cycloserine. Side-effects, warranting cessation of the drug, were experienced by four patients.

Three infections with *E. coli*, which proved to be a relatively sensitive organism, were eradicated.

The response of the other infections, usually complicated and due to such resistant organisms as *Proteus* and *Ps. pyocyanea*, was disappointing. Temporary improvement was experienced by 6 out of 21 patients, but 12 out of 21 failed to respond.

Strict laboratory control is essential when cycloserine is used for treatment of urinary infections.

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THE NEUTRALIZATION OF HEPARIN AFTER PERFUSION

A COMPARISON OF PROTAMINE AND "POLYBRENE"

BY

N. G. ROTHNIE, F.R.C.S.

AND

J. B. KINMONTH, M.S., F.R.C.S.

Surgical Professorial Unit, St. Thomas's Hospital Medical School, London

The complete neutralization of heparin is essential after perfusion for open heart surgery because residual heparin can cause or aggravate a bleeding state. The proper correction of heparin by a neutralizer can be ensured by the use of a simple test, the thrombin clotting-time of plasma, which can be made very sensitive to traces of heparin. Its method of performance and use in practice have been previously described (Rothnie and Kinmonth, 1960).

The rapid reversal of the effect of heparin and the restoration of haemostasis after operation requires an efficient heparin neutralizer. The variation and uncertainty of adequate heparin neutralization which we have found with protamine led us to an investigation of the use of the new antiheparin agent "polybrene" (hexadimethrine bromide). We report in this paper the results with this antidote in a series of experimental perfusions in the dog and in patients undergoing perfusion for the correction of varying cardiac abnormalities. These results are compared with those obtained when protamine was used as the antidote. From this investigation we have determined the most satisfactory amount of polybrene needed for complete heparin neutralization after perfusion.

Methods

In all the perfusions the Melrose rotating disk oxygenator was used. The blood samples were fixed with 3.8% (w/v) of trisodium citrate in the ratio of 9 volumes to 1. They were obtained through a small cannula in the inferior vena cava inserted through the saphenous vein in the groin (Rothnie *et al.*, 1960).

Citrated platelet-poor plasma was obtained by centrifuging the blood at 3,000 r.p.m. for 5–10 minutes.

The thrombin clotting-time was obtained by adding a thrombin-calcium solution to citrated plasma and timing the clot formation. If the thrombin clotting-time was prolonged beyond the control time of 10–12 seconds, the test was repeated after adding toluidine blue to the plasma sample. If the result was more normal this was taken to indicate that the original prolongation had been due to heparin (Rothnie and Kinmonth, 1960).

The control pre-perfusion thrombin clotting-time was obtained in each case from a sample of plasma taken before heparinization, and subsequent clotting-times after perfusion were compared with this.

Heparin was given intravenously to each case immediately before caval and femoral cannulation and its antidote by the same route after removal of these cannulae, about 10–15 minutes after stopping perfusion.

Results with Protamine

In a series of 19 perfused dogs, which were given heparin 2.5 mg./kg. (donor blood 25 mg./500 ml.) and protamine as the antidote, 11 had troublesome bleeding afterwards due to residual heparin. Of these 11 bleeders, five died of continuous oozing after operation and had received protamine 2.5 or 3.75 mg./kg. (ratio of protamine to heparinizing dose of 1:1 or 1.5:1). Haemothoraces occurred in the other six bleeders and contributed to the death of two of them. These six cases were given varying doses of protamine from 2.5 to 7.5 mg./kg. (1:1 to 3:1). The remaining eight dogs in the series were non-bleeders, and most of them received protamine 7.5 or 10 mg./kg. (3:1 or 4:1).

Thus as a result of increasing the dose of protamine and ensuring that there was no residual heparin by means of the thrombin clotting-test, we were able to reduce the incidence of oozing after perfusion and eliminate the immediate mortality from this cause. There was, however, a variation in the amount of protamine necessary for the complete neutralization of heparin. In some dogs a ratio of 2:1 was sufficient and in others a ratio of 4:1 was required.

A similar variation in protamine requirements was found in six patients (average weight 30 kg.) who underwent perfusion for the correction of varying cardiac abnormalities. They received heparin 2.5 mg./kg. and doses of protamine varying from 3.75 mg./kg. (1.5:1) to 7.5 mg./kg. (3:1). After perfusion two of them had troublesome bleeding due to residual heparin and had received a total dose of protamine of 5 mg./kg. (2:1) and 7.5 mg./kg. (3:1). Details of the thrombin clotting-times in these two patients were previously reported by us in stressing the importance of the proper correction of unneutralized heparin (Rothnie and Kinmonth, 1960). One other patient, who had a trace of heparin after the administration of protamine 5 mg./kg. (2:1), had an increased post-operative drainage compared with the three non-bleeders. None of these six patients, who were perfused for an average time of 32 minutes, had any evidence of a defect of a major clotting factor. The average post-operative drainage in 24 hours was 40 ml./kg. (lowest 15 ml./kg.; highest 80 ml./kg.).

The results in these experimental and clinical cases showed that for the adequate neutralization of heparin there was a variation in the requirements of protamine from 1.5:1 to 4:1 (see Table).