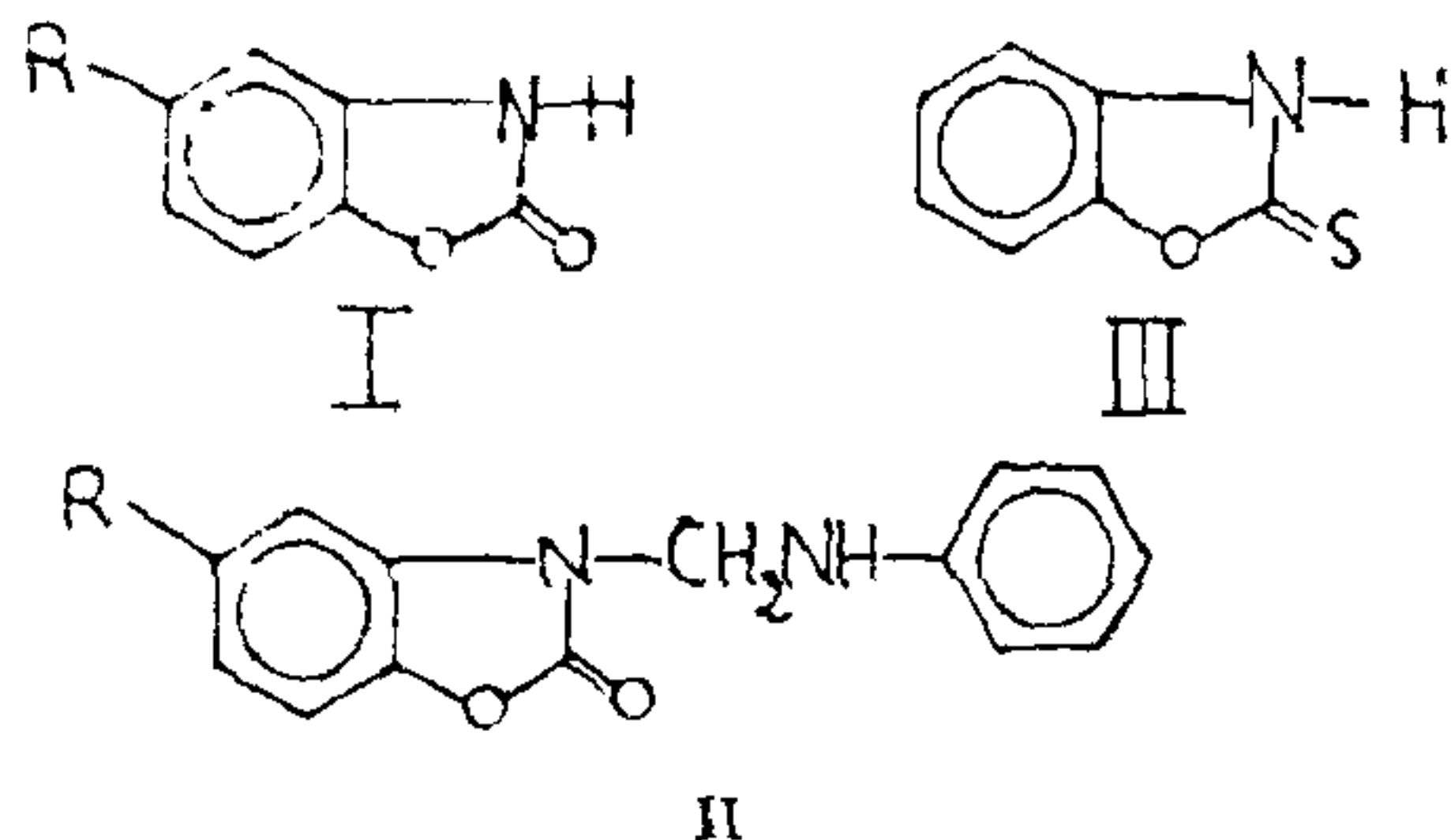


The second method (B) involved the desulfurisation of benzoxazolin-2-thiane⁸ (III) in DMSO. The latter (0.01 mole) in DMSO containing 5 drops of conc. H_2SO_4 was kept at room temperature for 2 weeks.



Benzoxazolin-2-one (I, $R = CO_2Me$) was prepared similarly by methods A and B. In another method 5-carboxy-benzoxazolin-2-one (1.79 gm, 0.01 mole), dry methanol (15 ml) and thionyl chloride (1.5 ml) were refluxed for 12 hrs. The solvent was removed and the residue neutralised with 5% sodium bicarbonate. The product was recrystallised (water), m.p. 197° (Lit.⁹, m.p. 196.5°), yield 1.46 gm (76%). The product obtained was identical with that obtained by methods A and B (m.p., m.m.p., I.R. and T.L.C.); I.R. (KBr): $\nu_{max} = 3210$ (NH), 1780 ($C=O$, ring), 1690 cm^{-1} ($C=O$, ester). Calcd. for $C_9H_7NO_4$, N = 7.25, Found N = 7.51%.

5-Carbomethoxy-3-phenylaminomethylbenzoxazolin-2-one (II, $R = CO_2Me$)

Aniline (0.93 gm) and formalin (1 ml) were added to a boiling ethanolic suspension of I (1.93 gm, $R = CO_2Me$) with shaking. The reaction mixture was stirred for 10 min. and the product was recrystallised from ethanol, m.p. 188–189° yield 2.0 gm (70%); I.R. (KBr): $\nu_{max} = 3400$ (NH), 1760 ($C=O$, ring) 1700 cm^{-1} ($C=O$, ester); N.M.R. ($CDCl_3$): $\sigma = 3.90$ (Me), 4.74 (CH_2), 5.30 (NH), 6.70–7.90 (Ar-H). Calcd. for $C_{16}H_{14}N_2O_4$, N = 9.39. Found N = 9.39%.

II ($R = CO_2Me$) prepared from I ($R = CO_2Me$) obtained by all the three methods was found identical (m.p., m.m.p., I.R. and T.L.C.).

5-Carboxybenzoxazolin-2-one (I, $R = CO_2H$)

Method A: 3-Amino-4-hydroxybenzoic acid (15.3 gm, 0.1 mole) and urea (6.60 gm, 0.11 mole) were refluxed in 30 ml of dry pyridine for 14 hrs. The product thus obtained was recrystallised from ethanol, m.p. > 297°; (Lit.¹⁰, m.p. 336–338°), yield 14.6 gm (82%).

Method B: 3-Amino-4-hydroxybenzoic acid (15.3 gm) and urea (8.0 gm) were fused at 150° and kept at this temperature for 4 hrs. Working up the reaction mixture gave the expected product, m.p. > 297°, yield 10.7 gm (60%).

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THERAPEUTIC EVALUATION OF ANTIMYCOTIC DRUGS IN REPEAT BREEDING BOVINES DUE TO MYCOTIC INFECTIONS

THE role of mesophilic and thermophilic fungi in causing repeat breeding, metritis, abortion in females and seminal vesiculitis and orchitis in males has recently been recognised by Zeverva and Repko¹, Saxena and Pathak² and Ainsworth and Austwick³.

In the present investigation an attempt has been made to evaluate a broad spectrum antifungal drug that could be effective against fungi found in repeat breeders.

Fungi were isolated to the extent of 83% from mucopurulent discharges of 92 repeat breeders on Sabouraud's dextrose agar (Saxena⁴ and Lachenicht and Potel⁵). They included *Aspergillus* 21% (*Aspergillus fumigatus*, *A. niger*, *A. terreus*, *A. cheveleri*, *A. flavus*), *Candida albicans* and other *Candida* species 6%, *Cladosporium* 10%, *Penicillium* 6%, *Alternaria* 3%, *Mucor* 9%, *Rhizopus* 6%, *Geotrichum* 2%, *Cryptococcus* 1%, *Curvularia* 8%. Other typed fungi were 11% (*Phialophora*, *Sporotrichum*, *Scopulriopsis*, *Botrytis*, *Allescheria* and *Fusidium*) closely matching the

isolates of Indian soil. No fungi could be isolated from 17% cases of repeat breeders.

In vitro drug sensitivity was tried against *A. cheveleri*, *A. terreus*, *A. fumigatus*, *Fusidium*, *Candida* and *Curvularia* species of fungi with 18 drugs (Table I) and the zone of growth inhibition recorded (as slight and marked) after the technique of Grigorin and Grigorin⁶. Only three drugs, useful in *in vitro* drug sensitivity test, were tamponed as 1% solution in the vagina and cervix of repeat breeders, at four days intervals in three groups and the result of therapeutic efficacy compared by the conception of animals (Table II). These three groups had all the fungal isolates as noted above from their cervicouterine discharges.

Out of the 18 compounds tested in *in vitro* (Table I) 1% solution of copper sulphate and mercurochrome had the same fungicidal activity as possessed by the expensive drugs Otamadyl (Dibromopropamide isoethionate 15% and diamidinodiphenylamine dihydrochloride 5%) and Talsutin ovulets (Amphotracin B 50 mg and Oxytetracycline 100 mg). Mycostatin (Nystatin) was slightly inhibitory to the growth of *Fusidium* and *Candida albicans* species. Crystal violet, potassium permanganate lotion and Ampicillin had growth inhibitory effect against *Candida* only while Lugol's iodine the most widely used vaginal antiseptic in veterinary practice had very slight effect against *Aspergillus* species.

Apart from the antifungal activity shown against limited species of fungi in the *in vitro* study, copper sulphate and mercurochrome appeared clinico-therapeutically efficacious to the extent of 78% and 56% respectively in repeat breeders against other isolates also (*Penicillium*, *Alternaria*, *Mucor*, *Rhizopus*, *Allescheria boydii*, *Scopulriopsis* and *Phialaphora*) present in the uterus and not tested in the *in vitro* test. One repeat breeder cow with *Cryptococcus neoformans* isolation responded well to 1% crystal violet intravaginal tamponing (Table II).

The intravaginal application of these cheap antiseptics may find wide use in overcoming repeat breeding problem in animals. Earlier workers (Ciszowski⁷, and Saxena⁸), have also commented over the superiority of copper compounds in fungicidal activity. Talsutin infusion in 15 ml distilled water after the technique of Sachii *et al.*⁹ has also given encouraging results in reducing bacterial as well as fungal infection of the repeat breeders.

Copper sulphate and mercurochrome both as 1% aqueous solutions and Otamadyl (M and B) intra-uterine tamponing, were found to possess antimycotic therapeutic efficacy to the extent of 78%, 56% and 50% respectively in preventing mycotic repeat breeding. Their antimycotic spectrum appeared against *Aspergillus cheveleri*, *A. fumigatus*, *A. terreus*, *Candida*

TABLE I
In vitro drug sensitivity against fungi

Drugs	Fungi isolated from repeat breeders					
	<i>A. cheveleri</i>	<i>A. terreus</i>	<i>A. fumigatus</i>	<i>Fusidium</i>	<i>Candida albicans</i>	<i>Curvularia</i>
Otamadyl (M and B)	M	M	M	..	M	..
Talsutin (Squibb)	M	M	M	S	S	..
Copper sulphate 1%	M	M	M	S	M	..
Mercurochrome 1%	M	M	M	S	M	..
Sodium chloride 1%	S	M	S	S	S	S
Potassium permanganate lotion 1:1000	S	..
Lugol's iodine 1%	S	S	S
Mycostatin 20 mg/ml	S	S	..
Crystal violet 1%	S	..
Ampicillin 20 mg/ml	S	..
Lactic acid 1% potassium metabisulphite 1% sodium metabisulphite 1% sodium thiosulphate 1-4%
benzyl benzoate 1% Nebasulph. (Pfizer) Grisofulvin 20 mg/ml Bifuran (SKF)

M = Marked, S = Slight ; .. = Not sensitive.

albicans, *Fusidium* in *in vitro* drug sensitivity trials and, in addition, against infections of *Penicillium*, *Alternaria*, *Mucor*, *Rhizopus*, *Allescheria boydii*, *Phialaphora* and *Scopulriopsis* species of fungi in repeat

TABLE II
Clinical trials against fungal repeat breeding cases

Number of repeat breeding cases		Fungal isolates	Drug tried	Results
Cows	Buffaloes			
2 (Brand Nos. 702 and 351)	7 (Brand Nos. 321, 43, 394, 37, 393, 145, 81)	<i>C. albicans</i> , <i>Penicillium</i> , <i>Alternaria</i> , <i>Mucor</i> , <i>Aspergillus</i> , <i>A. terreus</i> , <i>Rhizopus</i> , <i>Phialophora</i> , <i>Curvularia</i>	Copper sulphate 1% tamponing	Pregnancy restored and calved
1 (155)	2 (376, 146)	<i>Aspergillus</i> , <i>Alternaria</i> , <i>Rhizopus</i> , <i>Scopulriopsis</i>	—do—	Chronic cases of cervicitis; did not respond; non-pregnant
2 (125, 141)	2 (146, 74)	3 <i>Aspergillus</i> species, <i>Geotrichum</i> , <i>Candida</i> , <i>Penicillium</i>	Mercurochrome 1%	Cases of cervicitis; non-pregnant
...	4 (410, 93, 94, 425)	<i>Aspergillus</i> , <i>Allescheria boydii</i> , <i>Scopulriopsis</i> , <i>A. terreus</i>	—do—	Pregnant and calved
1 (884)	..	<i>Aspergillus</i> and <i>Penicillium</i>	Otamadyl	Cervicitis, non-pregnant
1 (136)	..	<i>Aspergillus</i> , <i>Mucor</i>	—do—	Pregnant and calved
..	2 (403, 385)	<i>A. terreus</i> , <i>Penicillium</i>	Control	Remained repeat breeder
..	2 (144, 83)	<i>Mucor</i> and <i>Curvularia</i>	—do—	Pregnant and calved
1 (Private)	..	<i>Cryptococcus neoformans</i>	Crystal violet	Pregnant and calved

breeding bovines. A single case of Cryptococcal repeat breeding cow responded well to 1% crystal violet intrauterine tamponing.

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BLOOD COPPER CONTENT AND MINIMUM MOLECULAR WEIGHT OF HAEMOCYANIN OF *CRYPTOZONA LIGULATA* (FERRUSAC)

THERE is extensive literature on the chemistry and biology of the haemocyanins¹⁻⁴. The arthropod and molluscan haemocyanins have been shown to be distinct in their copper content and molecular weights¹. In the present note an attempt has been made to determine the copper and haemocyanin content and the minimal molecular weight of the haemocyanin in the blood of active, aestivated and naturally revived snail, *Cryptozona ligulata*.

The snails were collected and maintained in the laboratory as described earlier⁵. Haemocyanin was

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