LABORATORY AND CLINICAL STUDIES WITH NYSTATIN IN POST-ANTIBIOTIC MYCOTIC INFECTIONS

BY

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There are varied reports concerning the incidence of post-antibiotic mycosis. The comparative infrequency of reports about it in Great Britain suggests that the incidence at present is much lower than in the U.S.A., but the risk undoubtedly increases with the availability and prolonged use of oral antibiotic therapy (Sharp, 1954). The most common form of mycosis is probably that due to *Candida albicans*, which, in some areas at least, is by no means negligible in primary infections of the respiratory tract (Brown, 1947) as well as in the mouth and vagina. Devitalized epithelium favours the colonization and spread of pathogenic forms of candida, a circumstance which is aggravated in the pharynx and bronchi by the suppressive action of antibiotics on the salivary bacterial flora. During the past 18 months throat swabs and sputa from a large number of patients receiving antibiotic treatment in the Croydon and Carshalton groups of hospitals have been examined for species of candida, by noting the presence of yeast spores in direct films or in sweeps made from blood-agar cultures, and setting up primary or sub-cultures on "oxoid" Czapek-Dox selective medium. By this means it has been found that colonization by monilia occurred in 10% or more of patients who had received oral antibiotics, notably of the tetracycline series. Such superinfection was more prevalent, and tended to be more troublesome, in children and in elderly people (Tables I, II, and IV).

"Nystatin"* ("mycostatin"), an antibiotic prepared from *Streptomyces noursei* (Hazen and Brown, 1950), has shown promise in recent experimental and clinical studies in the U.S.A. (Sternberg *et al.*, 1953; Sloane, 1955). A sample of a crude batch of this substance was obtained in 1953 for laboratory studies; the results were encouraging. Further results obtained with various larger batches of purified, lyophilized, and other preparations having potencies of 2,000-3,500 units per mg. are recorded below.

Activity of Nystatin in Vitro

Nystatin is described by the manufacturers as an amphoteric crystalline polyene with the probable empirical formula $C_{46}H_{77}NO_{19}$. It is insoluble in water but soluble in various alcohols and glycols. The preparations used in these experiments were yellow powders, the most highly purified sample being a pale-yellow lyophilized powder which was fairly rapidly inactivated by heat, light, and oxygen. Tablets containing 500,000 units of the substance were also obtained for clinical use.

In peptone and other liquid media, nystatin inhibited typical strains of *C. albicans* at concentrations of 5-20 units per ml. In solid media higher concentrations were required. A strain inhibited by 5 units in liquid medium would grow to the edge of a hole in agar containing 10 units per ml. but showed a clear zone of inhibition at 20-40 units. Blotting-paper disks (9 mm. diameter) showed activity when

*The name "nystatin" is derived from the discovery of the antibiotic in the New York State Department of Health.

TABLE I.—Incidence of *C. albicans* in Pharynges of Children after Various Antibiotics

No. examined after antibiotic therapy ..	175
No. showing superinfection with <i>C. albicans</i> :	
After tetracycline series ..	9
.. multiple antibiotics ..	8
.. chloramphenicol ..	2
.. penicillin ..	2
.. erythromycin ..	2
	23 (13%)

TABLE II.—Details of 102 Successive Isolates of *Candida*

Source	Lesion	Primary Infection		Post-antibiotic	
		Adults	Children	Adults	Children
Mouth ..	Stomatitis	5 (3)	3 (1)	5 (3)	3 (1)
Throat ..	None	—	3	—	15
Sputum ..	Uncertain	21 (2)	1	26 (16)	5
Vagina ..	Thrush	7	—	—	—
Skin ..	Wound infection, eczema	—	1	1	—
Ear ..	Otitis externa	—	—	—	1
Stomach washings	Uncertain	—	—	—	1
Intestine (p.m.)	Generalized moniliasis	—	—	—	1 (fatal)

All the above strains were sensitive to nystatin. Only a small number were treated, and these are indicated in parentheses (see Tables III and IV).

TABLE III.—Therapeutic Effect of Nystatin in Mycotic Infection (1-2 million units daily for 7-10 days)

Diagnosis	No. of Cases	Results
Post-antibiotic moniliasis (pharyngeal or bronchial)	12 (adults)	9 cleared of infection; 2 cleared but recurred on withdrawal of drug; 1 not cleared
Stomatitis ..	6 (adults)	5 cleared; 1 unaffected by treatment
	2 (children)	Both cleared (0.5 million units daily)
Ringworm (topical application)	2 (children)	Lesions subsided during application of drug but recurred on withdrawal

TABLE IV.—Incidence of Mycotic Superinfection in 32 Elderly Adults Receiving Antibacterial Therapy with and without Nystatin (1-½ million units daily)

Candida	Antibacterial Drugs Only	Antibacterial with Nystatin	Totals
Absent throughout ..	12	7	19
Present throughout ..	—	2	2
Absent before present after ..	1*	4*	5
Present before absent after ..	1	5	6
Totals ..	14	18	32

* Cleared of infection after a subsequent course of nystatin.

impregnated with a solution of 250 units of nystatin per ml. in methanol and then dried.

To date, over a hundred strains of candida, isolated from various lesions or sources, have been tested (Table II) and all have proved sensitive to concentrations equivalent in liquid media to 5-20 units per ml. This inhibitory action was maintained in the presence of human serum and plasma, though only at higher concentrations. In medium containing 50% serum twice the concentration of nystatin was required.

The action of nystatin upon all the strains of candida tested was essentially directed at suppression of cell division. In experiments with counted inocula this effect was usually absolute at 5-20 units per ml., and no further reductions in count, or consistent alterations in the appearance of the organisms, were obtained by raising the concentrations to levels of 100 units or more per ml.

Much of the assay work upon nystatin has been performed with *Saccharomyces cerevisiae*, and this organism was therefore used as a known standard for studying metabolic aspects of the action of the compound. The addition of carbohydrates to plain peptone media fortified growth and diminished the inhibitory effect, glucose being more antagonistic in this respect than maltose, and maltose more than lactose (Fig. 1). At a concentration of 100 mg. per 100 ml.

in peptone, broth, or serum broth, glucose halved the fungistatic power of nystatin (10 units per ml.), as tested by cell counts after 18 hours' incubation, and caused further loss after 24 hours' incubation (Fig. 1). This antagonistic effect was maximal, against *S. cerevisiae* and two strains of *C. albicans* also tested, at nystatin concentrations of 6–10 units per ml.—which concentrations, in plain broth, prevented any increase in cell count. At nystatin concentrations of 40–50 units per ml. the antagonistic effect of glucose was lost. Similar but less intensive antagonistic effects were observed with maltose, lactose, and saccharose.

Since the action of nystatin in organic and probably in physiological solution was influenced by the participation of other organic substances in the growth and metabolism of yeasts, this question was further studied by incorporating various fatty alcohols and glycols in the media in equimolecular concentrations. Alcohols inhibited growth in proportion to their fatty-chain length, the *n*-forms of propanol and butanol being more active than the isomeric forms. These alcohols caused absolute arrest of cell division, and definite morphological abnormalities, but little or no lysis of the yeast cells, which remained largely viable on subculture. Amyl and octyl alcohol caused further suppression, and death or lysis of the inoculum. Ethylene and propylene glycol also possessed inhibitory properties, but to a much smaller extent than their homologous alcohols. Combinations of these substances with threshold concentrations of nystatin showed some additive inhibitory power, but no synergy (Fig. 2). A lytic effect was obtained by combining nystatin at 6 units per ml. with 0.4 molar *n*-butanol. Mycelial growth and cell-division were inhibited

by combinations of nystatin (2–6 units) with *n*-propanol (0.4 M) or with iso- or *n*-butanol (0.2 M). At higher concentrations (1 M and above), methanol and ethanol began to exhibit comparable effects in combination with nystatin. The growth-promoting effect of glucose and maltose was antagonized by the alcohols in proportion to chain length and molar concentration.

Aqueous suspensions of nystatin or solutions in organic solvents were unstable. This resulted in a sharp fall in its antifungal activity against liquid cultures after 18–24 hours' incubation, and may have accounted for the relatively poor results obtained with slowly growing cultures tested on solid media. Full potency was retained for seven days when the lyophilized powder—completely stable when dry—was suspended in water, dissolved in ethanol or methanol, or dispersed in ethylene or propylene glycol. An aqueous suspension kept at -20°C . for two weeks retained full potency, but losses of up to 50% of potency occurred in less than seven days in solutions and suspensions kept in the dark at room temperature and at 37°C .

Passage experiments were conducted by exposing inocula of 10^8 cells of *S. cerevisiae* and *C. albicans* to nystatin in concentrations of 6 and 50 units per ml. for periods of two and seven days, and then retitrating the inocula. In other experiments these strains were successively passed through 2, 6, and 50 units for 48-hour periods. The inocula maintained in 50 units were, of course, completely prevented from dividing, but no lysis was evident (see above), and retitration of the freed cells against the originally effective range of nystatin (2–12 units per ml.) showed no change in the endpoint.

Nystatin was tested in liquid and solid media against various strains of streptococci, staphylococci, and coliform bacteria, but showed no effect in concentrations up to 250 units per ml. Its action against *Candida* spp. and *S. cerevisiae* was, however, fully maintained in the presence of these bacteria. Conversely, the presence of nystatin in solid media did not antagonize the action of penicillin, the tetracycline series, chloramphenicol, and streptomycin against bacteria sensitive to these antibiotics, nor did the antibiotics interfere with the action of nystatin against *Candida*.

Clinical Results

When nystatin was first obtained, in relatively crude form, it was applied topically to pharyngeal lesions in two adults with monilial stomatitis. These lesions subsided in a few days, and swabs were found to be negative. A paste was then prepared from assayed powder, and given by mouth to patients with pharyngeal infections in doses of 1–2 million units daily, causing apparent clearance of infection. Finally, a series of twelve patients (Table III) with fungal hyphae demonstrable in direct examination of films made from sputum, and cultures positive for *C. albicans*, were treated with three to four daily oral doses of 500,000 units for seven days. In each of these (bronchitic) patients, moniliasis had developed as a sequel to antibacterial therapy with various antibiotics, and was characterized by copious greyish sputum containing epithelial fragments colonized by *C. albicans*. Apart from this, no symptoms attributable solely to the mycotic infection were present. The results, expressed in terms of eradication of the organism, showed that nine out of twelve were cleared. An effect was also apparent in seven out of eight cases of stomatitis, and a temporary effect in two cases of ringworm (Table III).

On the assumption that colonization of the pharynx and bronchi could proceed more freely during antibacterial therapy, alternate patients—mainly elderly people—with bronchitis were given nystatin in addition to whichever antibacterial antibiotic had been prescribed. The results (Table IV) showed that colonization was haphazard, and that nystatin given concurrently with an antibacterial drug had no definite prophylactic value.

Strains of *Candida* were re-isolated from twelve patients during or after courses of nystatin (Tables III and IV). Each

FIG. 1

FIG. 2

of these strains was retested but showed no change in degree of sensitivity to the drug *in vitro*. Six recurring infections were treated with second courses, and five cleared.

One of these patients, receiving 1½ million units a day, developed nausea and vomiting on the sixth and seventh days of her course. Several others complained of slight nausea at about the same stage, and three of the earlier patients, who were receiving a suspension of the powdered drug, complained of its offensive taste, but this difficulty did not arise when compressed chocolate-covered tablets were given. No other toxic symptoms or signs were noted. In concentrations of 30 units per ml., nystatin did not interfere with the phagocytic properties of human leucocytes when incubated at 37° C. with suspensions of cells prepared from buffy coat or cerebrospinal fluid.

Discussion

These results show that nystatin inhibits cell-division and mycelial growth of candida and saccharomyces, including pathogenic strains isolated from a variety of human lesions. This effect, detectable at unit strength against an assay organism, is fairly complete against *C. albicans* at concentrations of 5–20 units per ml.

Any assessment of the efficacy of nystatin against pharyngo-respiratory mycotic infections must be based upon a critical realization of the superficial nature and tendency to spontaneous amelioration of many monilial lesions. Establishment and colonization, in the first place, probably depend upon devitalization of the epithelium and alterations in the pharyngeal flora, and can be provoked by antibacterial therapy. Withdrawal of the responsible antibacterial drug often allows such mycotic superinfection to disappear. When colonization occurs—as evidenced by fungal growth around desquamated epithelial plaques in film preparations—it may persist in the presence of a mixed bacterial flora. A number of other factors—including dentures, atrophic mucosae in older people, poor nutritional states, uraemic and other toxic conditions, and the prevalence of pathogenic candida in the environment—all contribute to the initiation and intensity of the infection. These factors not infrequently combine to render moniliasis an awkward therapeutic problem. From the results now available it can be claimed that nystatin is an agent of very definite promise in this field. Its specificity of action against fungi, its lack of interference with antibacterial agents, and its apparent lack of toxicity would appear to be unique properties.

A clue to the mechanism of action may be present in the antagonisms and additive effects obtainable with various carbohydrate substances. The presence of a chain of CH₂ groups, as in the alcohols, favours activity, while CHOH and CHO groups, as in various sugars, are antagonistic. Glycols, with combination of both, show intermediate properties. These results may also explain in part some variations observed in prophylactic and therapeutic trials, in that the circulating level of blood glucose, and possibly substances present in exudates, could theoretically interfere with the action of low concentrations of nystatin. At its best this action is fungistatic, so that, in the absence of local tissue immunity or re-establishment of a competitive flora, recrudescence of candida infection could be expected to follow cessation of treatment.

The experiments with higher alcohols described above suggest that a more complete effect might be obtainable by adding these to topical forms of nystatin.

Exposure of candida and saccharomyces to subinhibitory and fully inhibitory concentrations of nystatin *in vitro* disclosed no tendency on the part of the surviving organisms to acquire resistance. Likewise, re-assay of strains re-isolated after treatment revealed no change in sensitivity. Since the mechanisms by which micro-organisms acquire resistance are many and varied, these limited results are far from conclusive, but they suggest that rapidly developing acquired resistance, of the type found with streptomycin, does not readily affect organisms exposed to nystatin.

Its action upon the growth and division of candida, together with therapeutic results obtained to date, suggests that nystatin is worthy of extended trial in patients with established mycotic infections. There is, however, no justification at present for its use prophylactically.

Summary

“Nystatin” (“mycostatin”), an antibiotic substance derived from *Streptomyces noursei*, inhibits the growth and cell division of species of candida and saccharomyces.

In its mode of action this substance is complex but highly specific. Activity is favoured by CH₂ chains and antagonized by CHOH. Bacteria and human leucocytes are not affected by concentrations which are highly toxic to fungal cells.

A therapeutic trial in 22 patients with simple mycotic infections, mainly with *C. albicans*, showed rapid and complete clearance of infection in 16 and temporary clearance in 4. Apart from transient nausea, no toxic effects were observed.

Nystatin and antibacterial agents showed no mutual interference *in vitro*, but when given prophylactically nystatin was not wholly successful in preventing mycotic superinfection in patients receiving antibacterial therapy. Resistance to nystatin was not found in strains after passage *in vitro* or on re-isolation during and after treatment.

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EFFECT OF NYSTATIN ON GROWTH OF CANDIDA ALBICANS DURING ANTIBIOTIC THERAPY

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The disturbing effect of the broad-spectrum antibiotics on the normal microflora of the host receiving them is one of the most important and potentially dangerous aspects of their use. Several observers have drawn attention to the increased frequency with which *Candida albicans* colonizes such regions as the mouth and bowel (Sharp, 1954; Cannon, 1955; Loh and Baker, 1955; Carpenter, 1955). The present investigation was carried out to see if the simultaneous administration of an anti-mycotic drug “nystatin” (“mycostatin”) to patients who were receiving tetracycline would have any effect upon the overgrowth of *C. albicans*. Nystatin is derived from cultures of *Streptomyces noursei*, and is described as a pale, almost colourless, crystalline substance (Hazen and Brown, 1950). *In vitro* it is reported to be most