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SOME OBSERVATIONS ON THE ECOLOGY OF *CANDIDA ALBICANS*, A POTENTIAL MAMMALIAN PATHOGEN

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INTRODUCTION

Candida albicans is a yeast-like organism, belonging to the Family Cryptococcaceae. Its normal habitat is found in the alimentary tract of warm-blooded vertebrates, where it lives as a budding yeast on the mucosal surface and in the mucosal secretions. The organism is, however, an opportunist pathogen. When alterations occur in its environment, through changes in the host tissues or in the microbial community exploiting them, *Candida albicans* becomes invasive and penetrates the living layers of the substrate. This invasiveness is associated with a change in morphology, so that branching filamentous hyphae to a great extent replace the budding yeast-like form.

Although *C. albicans* must be constantly inoculated onto the skin and its appendages it only rarely establishes itself in this habitat and, when it does so, usually behaves like a pathogen. In the following paragraphs only the colonisation of the skin is considered. The results of invasion of the intestinal wall and the deeper organs are much more serious for the host, but the factors involved in the pathogenesis of systematic candidiasis are too complex to be summarised in this paper.

HEADQUARTERS AND RANGE

Candida albicans is found as a member of the normal flora of a large number of mammals and possibly of birds, and it seems reasonable to designate the alimentary tract of mammals as the headquarters of this yeast. Van Uden (1960) has divided the intestinal yeasts into 3 groups:

(1) obligate saprophytes
(2) facultative saprophytes
(3) passers-by

He includes *C. albicans* in the first group, defined as organisms which have a natural habitat exclusively in the mammalian gut. Table 1 shows the distribution of *C. albicans* in the intestinal tract of some New Zealand mammals, other than man. It is clear that a number of mammals can be carriers of this yeast. It may not however be present in the alimentary tracts of all species, for Parle (1957) failed to recover it in samples taken from cows, guinea pigs, mice or rats.

TABLE 1. *Carriage of* Candida albicans *by various mammalian species in New Zealand.*

Animal	No. examined	% carriage	Reference
Hedgehog *(Erinaceus europaeus)*	10	90	Parle (1957)
Hedgehog	78	87	Smith (1964)
Pig	20	5	Parle (1957)
Monkey	10	30	
Dog	10	20	
Sheep	15	54	
"Possum" *(Trichosurus vulpecula)*	5	60	
"Possum"	35	0.0	Marples (1961)
Cat	36	14	

It is difficult to reconcile the findings of Parle (1957) with those of Marples (1961) concerning the carriage of *C. albicans* by "possums" *(Trichosurus vulpecula)*. In the later survey both oral and faecal swabs were examined but no yeasts were isolated from 35 trapped opossums. Parle (1957) examining only the intestine, recovered *C. albicans* from 3 out of 5 animals sampled. Since much of his work was carried out in the Zoo, the animals may have been caged ones, and therefore living in a different environment from those examined in 1961.

It is interesting that so high a proportion of New Zealand hedgehogs *(Erinaceus europaeus)* should be carriers of *C. albicans*, for Van Uden (1960) has indicated that yeasts are not prominent members of the intestinal flora of animals feeding mainly on arthropods. Brockie (1958) found that arthropods, and land molluscs formed the major items in the diet of hedgehogs in New Zealand, so that diet does not appear to account for this high incidence.

Candida albicans IN MAN

The distribution of *Candida albicans* in human hosts has been studied in many parts of the world. Tables 2, 3, and 4 show the carriage of the yeast in the mouth and in various other sites by subjects sampled in Dunedin. In general, a rather higher proportion of persons living in New Zealand carry *C. albicans* than has been recorded elsewhere. This may, however, reflect different methods of sampling rather than a true difference in incidence.

TABLE 2. *Distribution of* Candida albicans *in mouths of subjects in different age groups in New Zealand.*

Age Group	No. examined	% carriage	Reference
—Gums—			
Infants 4-7 days	104	11.5	Somerville (1964)
Children 2-13 years	278	16.3	Marples and di Menna (1952)
Students 18-30 years	103	15.5	
Students 17-22 years	72	18.0	Marples and Somerville (unpublished)
Geriatrics 60+ years	63	47.6	

TABLE 3. *Distribution of* Candida albicans *on the skin of subjects in different age groups in New Zealand.*

Age Group	No. examined	% carriage	Reference
—Hands—			
Children 2-13 years	278	0.0	Marples and di Menna (1952)
Students 18-30 years	175	0.0	Marples and di Menna (1952)
Geriatrics 60+ years	63	4.8	Marples and Somerville (unpublished)
—Interdigital spaces—			
Children 11-13 years	387	0.3	Marples and Chapman (1959)
Students 17-22 years	72	1.4	Marples and Somerville (unpublished)
Geriatics 60+ years	63	1.6	
—Other skin sites—			
Students 17-22 years	72	0.0	
Geriatrics 60+ years	63	19.0	
—Total skin carriage—			
Students 17-22 years	72	1.4	
Geriatrics 60+ years	63	27.0	

The yeast is a dominant member of the oral flora of many subjects. If mouth washings are used as the sample 40 to 50% of adults can be shown to be carrying *C. albicans*. This technique is, however, unsuitable for the examinations of infants or of geriatric subjects, so that the results of swabbing the lower gums is shown in Table 2. A significantly higher incidence of the yeast in the mouths of those over 60 years as compared with younger subjects is recorded. This probably represents the presence of very dense populations, since the yeast could be isolated from so many individuals even when only a small saliva sample was available. The yeast can also be recovered from a proportion of faecal samples (Table 4), and there is evidence that it is actively multiplying in the large intestine, and that its presence in faeces is not the result of the passage of undigested oral cells (Marples & di Menna 1952).

C. albicans is recovered from the vagina in only a small proportion of non-pregnant adult females. Its increased incidence during pregnancy is thought to be due to increased concentrations of glycogen in the vaginal mucosa. This habitat appears to return to an unfavourable one scon after parturition, as is shown in Table 4 and can be regarded as forming the edge of the yeast's range.

Candida albicans OUTSIDE THE MAMMALIAN BODY

Although *C. albicans* is so frequently found in the mammalian alimentary tract, it is only rarely isolated from the non-living environment. In New Zealand di Menna (1955) isolated only two strains from 100 soils, and Marples (1961) recovered 5 strains from 167 samples. In both surveys the soils which

TABLE 4. *Distribution of* Candida albicans *in faeces and vagina in New Zealand.*

Group	No. examined	% carriage	Reference
—Faeces—			
Infants 4-7 days	104	13.3	Somerville (1964)
Children 2-13 years	188	30.8	Marples and di Menna (1952)
—Vagina—			
Non-pregnant females	50	4.0	Marples (unpublished)
Pregnant females	127	30.4	Somerville (1964)
Post-partum females	104	8.7	Somerville (1964)

yielded *C. albicans* were moist and subject to heavy animal contamination.

It is possible that the yeast survives longer on the surface of leaves. Di Menna (1958) isolated 3 strains from one out of 24 samples of freshly collected leaves. She regarded the presence of the yeast as a result of animal contamination, and did not consider that it was a member of the Phyllosphere flora. It is possible that the factors which play a part in the rapid disappearance of the yeast from soil are similar to those which make the human skin an unfavourable habitat for this organism.

Candida albicans AS AN INHABITANT OF THE HUMAN SKIN

Table 3 shows the carriage of *C. albicans* on different skin sites, in New Zealand subjects. It has been stated (Drouhet 1957) that the healthy intact skin does not provide a suitable habitat and this appears to be true of the younger age groups. The yeast has not been isolated from the general skin surface of children or young adults, during numerous surveys, not all of which are included in Table 3. The skin of elderly hosts does, however, appear to provide a more favourable environment. *C. albicans* was isolated from one or more areas in 27% of 63 geriatric subjects. Some, but not all, were oral carriers. This difference in incidence of the yeast on the skin of the two age groups is statistically significant and suggests that the cutaneous layers of the elderly differ in some way from those of young people.

DISAPPEARANCE FROM THE SKIN

Since *C. albicans* is so commonly present in the human mouth it must constantly be transferred from this habitat to different sites on the skin, yet in most hosts it is unable to establish itself as a member of the cutaneous community. The self-sterilising power of the human intact skin contaminated by potentially pathogenic bacteria has been extensively studied, and the results of many investigations have been summarised by Gleeson-White (1960). Two mechanisms appear to operate varying in importance with the potential invader. The most important factor leading to the disappearance of the gram positive cocci is probably chemical in nature. Sebaceous secretion contains a number of unsaturated fatty acids, and these can be shown *in vitro* to inhibit the growth of pathogenic staphylococci and streptococci. In sufficient concentration they may be bactericidal. The gram negative bacilli are less susceptible to the antagonistic effects of sebum components, and their eradication appears to result from physical characters of the skin. Exposed cutaneous areas are too dry for the successful colonisation by coliforms, but Rebell and his colleagues have shown (1950) that if a cutaneous area is kept moist by suitable means *Escherichia coli* can become a member of the flora of undamaged skin.

The factors leading to the disappearance of *Candida albicans* from healthy skin have been little studied, but the investigations described below suggest that desiccation is of major importance in the self-sterilising process. Heavy suspensions of the yeast were painted onto the skin of the forearm of two adults and approximately equal strips of the area were sampled by swabbing at regular time intervals. No yeasts could be recovered 3½ hours after the inoculum was applied (Marples, unpublished). This could be attributed to loss of viable cells from the skin surface, rather than to the death of cells still attached to the epidermis. It is however possible to show that desiccation leads to a true reduction in viability of the cells of *C. ablicans*.

EFFECTS OF DESICCATION

The following experiments to show the effects of desiccation on *C. albicans* were undertaken using a strain recently isolated from the mouth of a healthy adult.

Twenty-four hour cultures of this stock yeast in a Dextrose Yeast Extract (D.Y.E.) broth were prepared for each experiment, the number of viable mycelial units present being determined by the Miles, Misra & Irwin (1938) technique. The equipment for each experiment consisted basically of 2 trays, each holding 2 12-welled haematology tiles. One tray was left open to the air, the other, the control, was enclosed by a smaller inverted tray bedded on to layers of absorbent cotton wool kept saturated with distilled water.

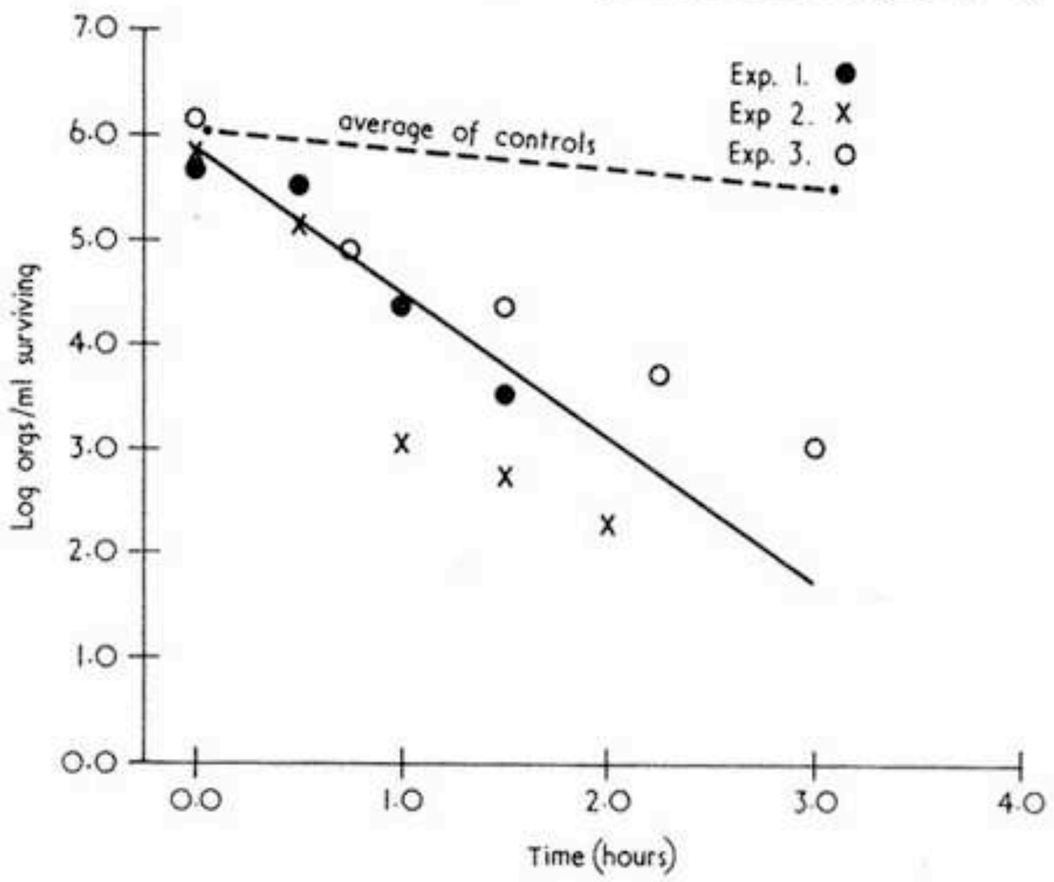
Throughout the experiments a pipette calibrated to give 50 drops per ml. was used. Five drops of the culture were placed in each well of the test and control tiles. These were all held open to the air at 37°C. for 1 hour so that the suspending fluid could evaporate. The tiles were then placed in the apparatus described and maintained at 27°C. or 37°C. At specified intervals the organisms were removed from 4 control and 4 test wells by

adding 1 ml (50 drops) of D.Y.E. broth and pipetting up the resulting suspension. Serial ten-fold dilutions were made, and drop plates on Sabouraud medium adjusted to pH 4 with IN. HCl were prepared. The plates were incubated for 24-48 hours and the number of colonies counted, each being regarded as arising from a single viable mycelial unit. The experiment was repeated 3 times at 37°C. and 4 times at 27°C.

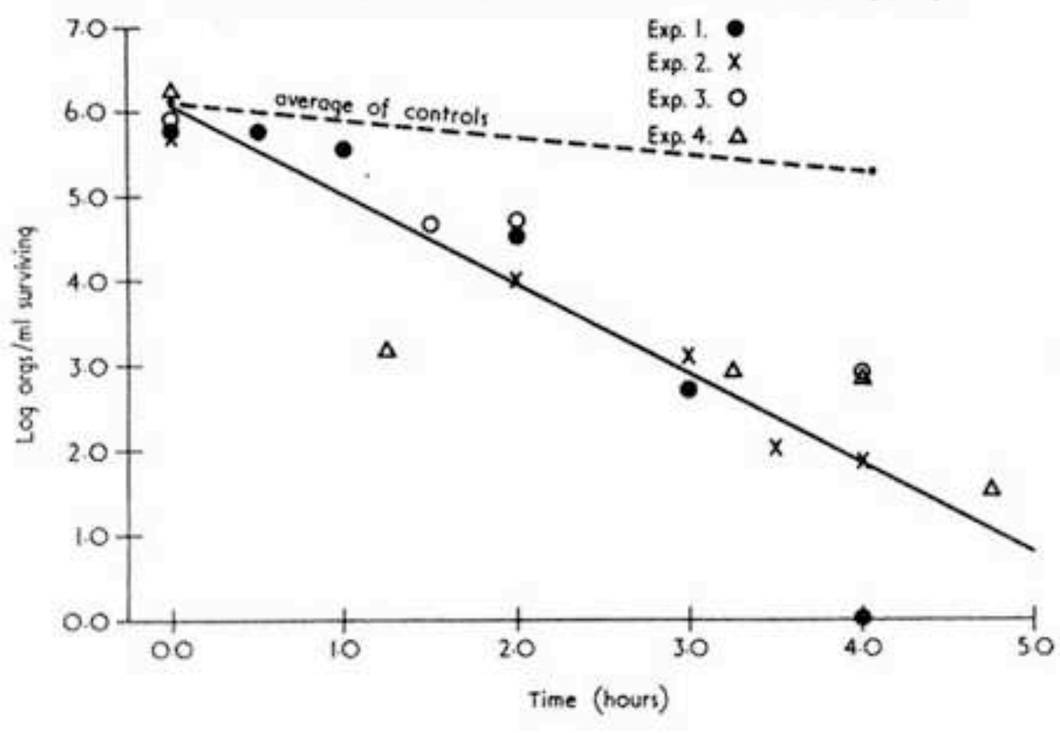
RESULTS

Figures 1 and 2 are graphs illustrating the results obtained at 37°C. and 27°C. respectively. The average number of viable cells exposed to desiccation fell from 10^5 to 10^2 in 3 hours regardless of the environment temperature.

GRAPH 1.
EFFECT OF DESICCATION ON CANDIDA ALBICANS (37°C)



GRAPH 2.
EFFECT OF DESICCATION ON CANDIDA ALBICANS (27°C)



The graphs also show an average of the final values obtained in the controls maintained in a saturated atmosphere. These show a slight overall fall in viable cells but the reduction is not nearly as great. The difference between control and test numbers is significant in both series ($p < 0.001$ at both temperatures). Comparable experiments, using a strain of *Rhodotorula* isolated from the soil, showed only a slight loss of viable cells during 3 hours exposure to desiccation.

Further evidence of the importance of a high humidity in the establishment of *C. albicans* as a cutaneous inhabitant is provided by a study of the sites in which spontaneous cutaneous candidiasis develops. Lesions are extremely rare on the general skin surface, but are found in such areas as the external auditory meatus, the submammary folds in women, the perineal area and the nail-folds, paronychia occurring most frequently in individuals whose occupation leads to the constant wetting of the hands. Cutaneous infection is particularly prevalent in young infants and the yeast causes approximately one third of all napkin rashes. The importance of *C. albicans* in the causation of these cutaneous lesions appears to involve not only the permanently moist condition of the skin of the infants' buttocks but also a special susceptibility in this age group.

Discussion

The survival of *C. albicans* on the skin of elderly human hosts is not readily explained and requires further investigation. It is possible that changes in skin texture and composition might lead to an increased moisture content of the epidermis, which would make it a more favourable habitat. Changes in the quantity of sebaceous secretion do not appear to be related to the ability of the yeast to exploit this habitat. Although it has been shown that the quantity of sebum secreted per unit area of skin is reduced in elderly females, no such diminution of secretion has been demonstrated in males of the same age group (Kirk 1948). In the survey of the normal flora of geriatric subjects carried out in Dunedin *C. albicans* was isolated from the skin of approximately the same proportion of males and females.

Little is known of the coactions of *C. albicans* with other cutaneous micro-organisms. Isenberg and his colleagues (1960) have separated intestinal bacteria into two groups: those which inhibit and those which stimulate the growth

of *C. albicans*. Similar studies have not been carried out on mixed cultures of the yeast and the staphylococci and diphtheroids which are found as regular inhabitants of the skin. These members of the normal flora may play a part in preventing the establishment of the yeast in the cutaneous habitat. Further studies may not only elucidate these coactions but may furnish information on the homeostatic mechanisms which preserve the integrity of natural populations.

SUMMARY

(1) *Candida albicans* has been shown to be an inhabitant of the alimentary tract of man and 7 other mammals in New Zealand.

(2) Although the yeast was present in the mouths of up to 40% human subjects, it was not isolated from the general skin surface of 278 children aged 2 to 13 years.

(3) Skin carriage of *Candida albicans* was demonstrated in only 1.4% of 72 students aged 17 to 22 years while the yeast was recovered from 27.0% of geriatic subjects aged more than 60 years. The difference in incidence is statistically significant.

(4) The possible factors leading to the disappearance of *C. albicans* from young healthy skin are discussed and it is suggested that desiccation is of major importance in the self-sterilising process. Experiments on the effect of desiccation on the viability of *C. albicans in vitro* were carried out. The average number of viable cells of the yeast exposed to desiccation fell from 10^5 to 10^2/ml in 3 hours, regardless of whether the environment temperature was 37°C. or 27°C. The number of viable cells in control preparations, maintained in a saturated environment, remained more or less constant during the same period. The difference in number of viable cells after 3 hours in the presence or absence of desiccation was statistically significant ($p < .001$). Desiccation failed to produce an equal loss of viability in cells of a *Rhodotorula* species isolated from the soil.

(5) The distribution of spontaneously acquired cutaneous lesions of candidiasis in man is noted. Since these occur in areas of skin which are normally maintained in conditions of high humidity, this distribution supports the hypothesis that *Candida albicans* requires moist conditions for its survival.

(6) The reasons for the survival of *C. albicans* on the skin of geriatric subjects are unknown and require further investigation. It is unlikely that diminution of sebaceous secretion accounts for the ability of the yeast to establish itself as a member of the normal cutaneous flora of elderly subjects.

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