THE DISTRIBUTION OF CANDIDA SPECIES AND THEIR ANTIBODIES IN PREGNANT WOMEN AND THE NEWBORN

(A CLINICAL AND EXPERIMENTAL STUDY)

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ABSTRACT

Mycoses caused by Candida albicans are amongst the commonest infections of pregnant women and their offspring. The fungus is often isolated from the healthy vagina or the mouth. Therefore, the diagnosis of vaginal or oral thrush cannot be established solely from the cultural findings. The diagnosis is made on clinical grounds, and the mycological findings are corroborative.

There have been many attempts to devise serological tests which will have diagnostic significance, and most promising amongst these are tests based on the precipitin reaction. Some experimentalists have suggested that precipitins to antigens of C. albicans and to related species of Candida are diagnostic of deep-seated infection with the thrush fungus; others have suggested that the presence of such precipitins relates to the weight of infection, and does not necessarily reflect its site.

A review of surveys of thrush in pregnant women and the newborn, together with an account of the incidence of oral and vaginal thrush based on an examination of past records at Queen Charlotte's Maternity Hospital, London, is given. Factors relevant to the declining incidence of oral thrush are discussed. This is followed by a section dealing with the various serological tests used or advocated for use in the diagnosis of superficial or deep-seated candidosis.

An ecological survey made prospectively on 1,085 pregnant women and their newborn was undertaken to determine the percentage of the maternal and newborn population harbouring the fungus. 18.6 per cent of pregnant women harboured yeasts in the vagina; half of these

patients were symptomless. C. albicans was isolated from 82.8 per cent of patients with symptoms and from 80.4 per cent without symptoms.

A yeast which appears to be a new species of Candida was isolated from the vagina of a pregnant woman and was studied in detail.

Sera of 306 pregnant women were examined for precipitins to antigens of C. albicans prepared by:-

(i) Fehling extraction (mannan antigen);
(ii) Mickle disintegration (cytoplasmic antigen); and
(iii) Culture filtrate antigen.

Some of the offspring were examined both culturally and serologically and evidence is adduced that precipitating antibody to C. albicans crosses the placenta and is thus passively acquired by the foetus.

The relationship between detectable precipitating antibodies to candida carriage and clinical infection is analysed. Precipitating antibodies to mannan antigen are found in 14.2 per cent of sera from pregnant women, and their presence is related to candida vulvovaginitis.

Antibodies to the protein antigen of culture filtrate of C. albicans occur in pregnant women without evidence of deep-seated candidosis but they are not related to candida vulvovaginitis.

Antibody to the protein antigen of cytoplasmic extracts was not found in unselected pregnant women. It is suggested that the precipitin test could be used in the diagnosis of clinical vaginal thrush if the sensitivity of the test could be improved and if a standardized mannan antigen were available.

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INTRODUCTION

The term "yeast" was used in the nineteenth century for the agent of alcoholic fermentation, but today it denotes a "heterogeneous, ill-defined group of organisms" (Lodder, 1970). The yeasts have been classified, according to Lodder (1970) into four groups. The first group includes the ascomycetous yeasts which can form asci with ascospores. The second group consists of yeasts in the two genera Leucosporidium and Rhodosporidium, which have been included in the order Ustilaginales of the Basidiomycetes. The third group comprises the yeast-like organisms forming ballistospores and these are classified in the family Sporobolomycetaceae. The fourth group consists of yeasts in which no sexual reproduction has been observed and which do not form ballistospores. This group is included in the Fungi Imperfecti and consists of twelve genera:-

1. Brettanomyces
2. Candida
3. Cryptococcus
4. Kloekera
5. Oosporidium
6. Pityrosporum
7. Rhodotorula
8. Schizoblastosporion
9. Sterigmatomyces
10. Torulopsis
11. Trichosporon
12. Trigonopsis

There are 81 species and 7 varieties in the genus Candida (van Uden and Buckley, 1970). Type species of the genus Candida is Candida vulgaris Berkhout (= Candida tropicalis (Castellani) Berkhout).

Origin of the generic name Candida

The term "thrush" has been used to describe the white patches and ulcers which appear in the course of severe diseases. In 1839 Langenbeck demonstrated the causative organism of thrush in smears taken from oral lesions of a patient suffering from typhoid fever. However, he regarded the organism to be causally related to typhoid and not to the oral lesions of thrush. Since then the causative organism of thrush has been placed in a number of different genera, including Oidium, Monilia and Candida. In 1847, the thrush fungus was placed in the genus Oidium by Robin and six years later it was given the specific name of albicans. The parasite of thrush in man had been incorrectly named by Zopf in 1890 as Monilia albicans. In the 18th century, the name Monilia had been given by botanists to fungi isolated from vegetable sources and bearing no resemblance to the causative organism of thrush (Benham, 1931). From 1890 to 1923, the name Monilia was applied by botanists for fungi isolated from vegetable sources and by medical mycologists for the thrush fungus. It was Berkhout in 1923 who proposed the generic name Candida for the medical monilias. In September 1939, at a meeting of medical mycologists, it was agreed to substitute the generic name Candida for Monilia and the Eighth Botanical Congress at Paris in 1954 adopted Candida as a nomen conservandum (Winner and Hurley, 1964). This designation of the genus was legalized by the Ninth Inter-

national Botanical Congress at Montreal in 1959 (van Uden and Buckley, 1970).

Morphology of C. albicans

All members of the genus Candida produce budding cells or blastospores and mycelia. The principal pathogenic member of the genus, and the causative agent of most cases of thrush is C. albicans. Van Uden and Buckley (1970) studied 22 strains of C. albicans isolated from various sources and described the organism in detail.

C. albicans exists in three morphological forms; yeast cells or blastospores, pseudomycelium and/or mycelium and chlamydospores. Cultures of C. albicans on suitable media show a rich development of pseudomycelium. Pseudomycelia are septate, branched, filaments in which the long cells are formed one from another by budding. The development of ball like clusters of blastospores on the pseudomycelium is very characteristic of the species. Very characteristic, also are the large, round chlamydospores which are usually terminal but may be intercalary, lying laterally or, rarely, in clusters (Fig. 1). The thin walled basal cells on which chlamydospores arise are round, elongated and somewhat bottle-shaped to pear-shaped. Yeast cells vary in size from 3.5 - 6μ x 6 - 10μ and grow well in aerated nutrient media containing a high level of sulphydryl groups and glucose (Fig. 2). A low oxygen tension favours mycelium and chlamydospore production. Chlamydospores range in diameter from 6.9 - 17.2μ.

Methods of identification of the genus Candida

(i) Culture

The identification of different species of yeasts proved difficult, and Benham (1931) stated that definite and rigid

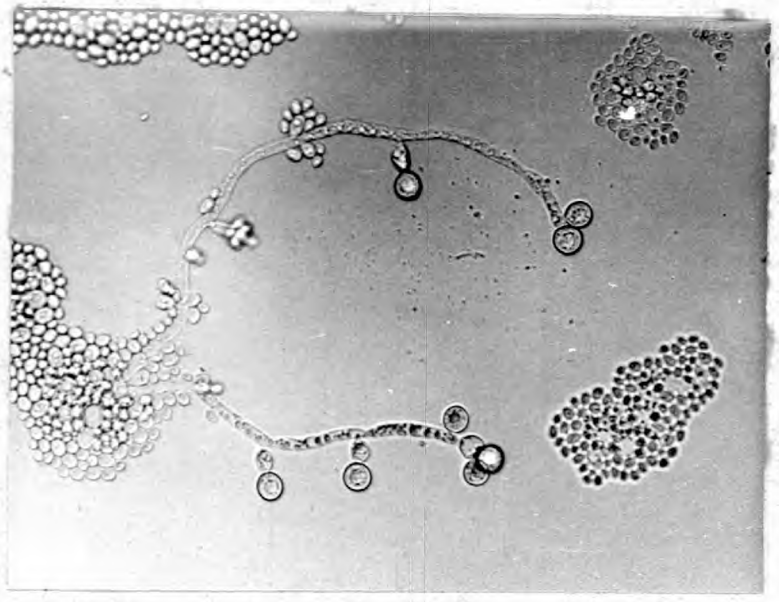

Fig. 1. Chlamydospores and hyphae of C. albicans from 24 hour culture on cornmeal agar with Tween 80. Unstained x 475.

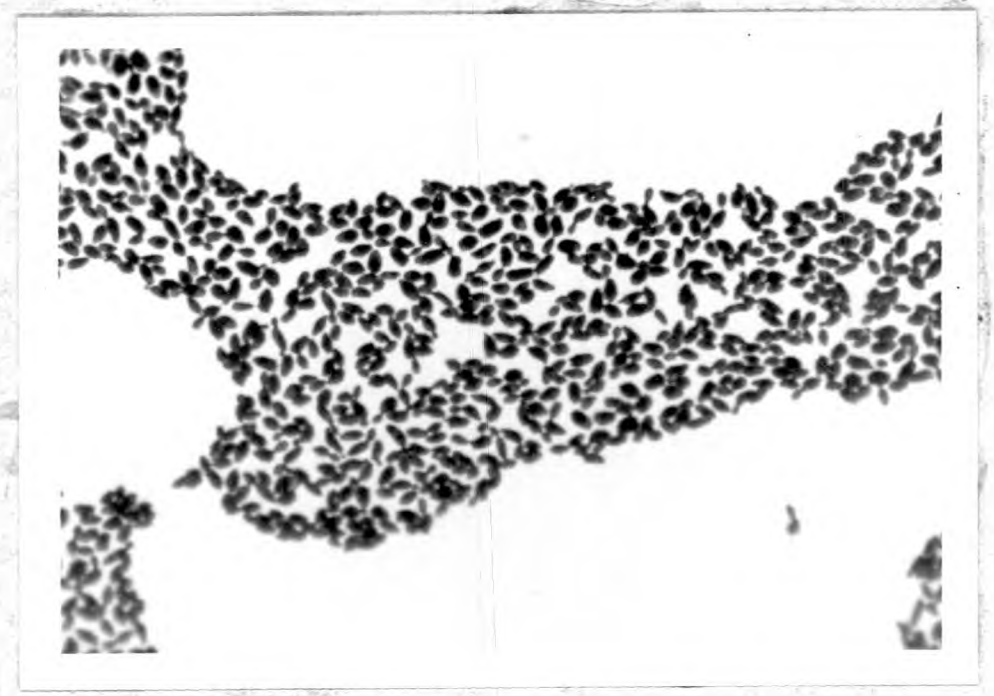

Fig. 2. Budding cells (blastospores) of C. albicans from 24 hour culture on Sabouraud's glucose peptone agar. Gram stained x 360

criteria for the identification of yeast species were needed. She found that Monilia albicans, later called Candida albicans, but not the other Monilia species produced chlamydospores when grown on cornmeal agar, due to its low nutritive properties. Various workers have attempted to modify the growth medium to facilitate the formation of chlamydospores. This was successfully achieved by Taschdjian (1957) using cream of rice infusion with Tween 80 and Dawson (1962) using Czapek Dox synthetic medium plus magnesium glycerophosphate and Tween 80. The method of inoculation recommended by Bakerspigel (1954) was to scratch the yeast on and into the plates while Kligman (1950) suggested a surface streak inoculation covered by a coverslip. This produced areas of relative anaerobiosis and also facilitated scrutiny microscopically.

(ii) Germ tubes

Taschdjian et al. (1960) showed that C. albicans could be detected within a few hours by the production of filamentous structures called "germ tubes" when the yeast phase was inoculated into serum and incubated at 37°C. (Fig. 3).

(iii) Fermentation

Biochemical methods for the identification of yeasts were used by Castellani (1927) who found that species of Candida and other yeasts differed in their ability to ferment sugars, with the production of acid and carbon dioxide. This has been used to provide fermentation patterns for the genus Candida (Lamb and Lamb, 1935; Martin and Jones, 1940). More recently, Barnet (1960) has used a photoelectric cell to

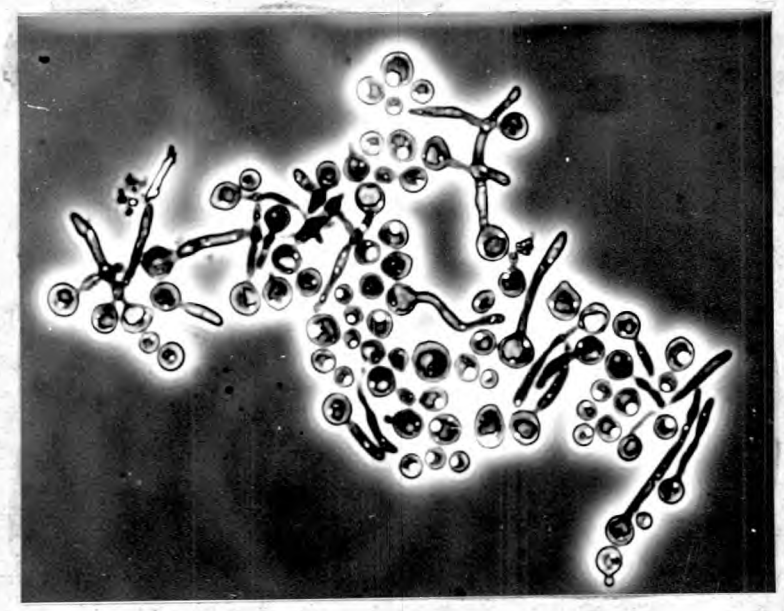

Fig. 3. Germ tubes of C. albicans after incubating in serum for two hours at 37°C. Phase contrast x 800.

measure the growth rates of a large number of the yeasts on varying sources of carbon and nitrogen. Barnet (1960) and Biguet et al. (1961) studied the exogenous material excreted into the media by different Candida species.

(iv) Auxanograms

Lodder and Kreger-van Rij (1952) used the auxanographic test for assimilation patterns in yeasts. This test has been used to establish sugar assimilation patterns for the genus Candida.

Pathogenicity of C. albicans in man

Species of the genus Candida are found in numerous sites in human beings, both as commensals and as pathogens. Pathogenicity describes the ability of a microorganism to produce disease, either natural or experimental, in a given host species. It is a general attribute of a species or genus of microorganisms. Virulence is a term used to indicate the degree of pathogenicity of an organism as observed in a particular host species, under defined conditions. The term infection is applied to invasion of the body by pathogenic microorganisms and the reaction of the tissues, in their presence (Dorland's Medical Dictionary, 1965). Others refer to infection as the presence of microorganisms in the tissues, whether or not it results in detectable pathological effects (Dubos, 1958). In this thesis, the term infection is used as defined in Dorland's Dictionary. C. albicans is isolated from various sites of the human body more frequently than any of the other species of Candida (Mackenzie, 1961) and is considered to be the principal pathogenic member of the genus. C. albicans causes a mild superficial infection, involving the mouth, skin, vagina and nails, or a fulminating, deep seated infection. It

may also cause an acute disseminated, septicaemic disease or a disease mainly localized in the heart or other organs.

Superficial candidosis

Though not a usual commensal on the skin, C. albicans may cause chronic skin disease in susceptible subjects. This may spread over a large proportion of the body surface and may persist for years. Such widespread infection is often associated with lesions elsewhere, e.g. oral and vaginal mucous membranes. Diabetes mellitus favours infection. In the newborn cutaneous candidosis occurs in the 'diaper' region. C. albicans is often present in the human mucosa as a commensal, exciting neither reaction nor symptoms. On the other hand it causes "thrush" of the mucous membranes of healthy persons and in physiologically abnormal states such as pregnancy and in the newborn period. In this thesis, diseases caused by organisms belonging to the genus Candida are called candidoses, the term thrush being used for the oral and vaginal manifestations of the disease.

C. albicans as a pathogen in vaginitis

J.S. Wilkinson (1849) was the first to suggest that vaginitis could be caused by a fungus. He reported the presence of yeast-like organisms in the vaginal discharge of a woman of 77. Castellani (1916) showed that yeasts could be present in the vagina without giving rise to symptoms. The typical symptoms and signs of vaginal candidosis are pruritus, a thin irritant discharge, reddening of the mucosa of the labia minora and lower third of the vagina and lard-like patches or flakes (Plass et al., 1931). C. albicans the principal pathogenic member of the genus Candida is the most important of the specific agents causing vaginitis. Table 1 (taken from Winner &

Hurley, 1964) shows the incidence of C. albicans in the vagina. All the studies indicate the increased incidence during pregnancy.

An account of the numerous surveys of thrush in pregnant women follows. Plass et al. (1931) examined vaginal smears and cultures from 64 pregnant women. They found Candida species in 12 out of 18 (66.6%) pregnant women with vaginal or vulval irritation. Among 46 patients without irritative symptoms, Candida was found in 15 (32.6%). The identification of yeast species isolated in that study was, according to Castellani's classification, based on fermentation reactions.

Woodruff and Hesseltine (1938) cultured vaginal swabs taken routinely from 402 pregnant women in the third trimester, to determine the incidence of vaginal thrush. The patients were questioned about the presence of genital symptoms and observations were made on the amount of vaginal discharge and clinical appearance. Among 100 black pregnant women of poorer social class, 41 per cent had evidence of vaginal mycoses in the third trimester, when compared with 33.3 per cent of 150 white patients of the same social class. Among 152 patients from a better social class, 14.4 per cent had evidence of vaginal mycoses. The overall incidence of vaginal mycoses was 28 per cent in their series of patients.

Bret and Coupe (1958) investigated 300 pregnant women at term, a third of them at entry into hospital and two-thirds at delivery. Specimens were taken from the vaginal wall and cul de sac. 20 per cent were shown to carry C. albicans in the vagina.

Harris et al. (1958) studied vaginal cultures taken from

TABLE 1

Incidence of C. albicans in the Vagina

* no species differentiation

Copied from Winner and Hurley, 1964, p.135

Authors	Place	No. and types of patients studied	No. with *C. albicans*	Percent. with *C. albicans* or yeasts
Plass *et al.* (1931) . .	U.S.A.	39 non-pregnant		15*
		46 pregnant		35*
Negroni (1935b) . .	Argentine	100 non-pregnant		8
		Pregnant		33
Fisher and Arnold (1936)	Illinois	73 antenatals		15
		195 in gynæcology clinic		6
Woodruff and Hesseltine, (1938)	U.S.A.	300 all classes in third trimester of pregnancy		28*
Carter *et al.* (1940) .	U.S.A.	200 pregnancies		43*
Vaccaro and Ferrada Urzua (1952)	Uruguay	550 vaginal secretions		2
Dawkins *et al.* (1953) .	G.B.	500 family planning clinic patients	38	7·6
Grasset *et al.* (1954) .	France	424 leucorrhœas		1
Yo bwan Hie (1954) .		300 pregnancies		19
Garnier and Vieu (1955)	France	200		15
Halde and Aragon (1956)	Philippines	171 pregnancies		25
Kostic (1957) . .	Belgrade	2,440	197	8·1
Niño (1957) . . .	Argentine	456 samples of vaginal fluid	67	20·4
Bret and Coupe (1958) .	France	300 mothers at term	59	20
Giunchi (1958) . .	Italy	Normal		13·7
		In childbirth		35·9
Harris *et al.* (1958) .	Canada	1,442 expectant mothers	254	17·6
Rieth (1958) . . .				22·7
Stough and Blank (1958)	Florida	334 gynæcological	60	17
		156 obstetrical	46	29·5
Clark and Solomons (1959)	U.S.A.	739 pregnant	202	27·3
		277 non-pregnant gynæcological	45	16·2
		78 postmenopausal	10	12·8
		199 premenopausal non-pregnant	35	17·6
Gillespie *et al.* (1960) .	U.S.A.	259 at delivery	40	15
		116 to 6 weeks post-partum	6	5
Mackenzie (1961) . .	Edinburgh	150 hospital patients	13 yeasts 7 *C. alb.*	8·7 yeasts 4·6 *C. alb.*
Miguens (1961) . .	Spain	100 gynæcological and obstetric	19	17
Mizuno (1961) . .	Japan	11,781 without symptoms of candidosis	1,893	16·1
		(4,254 pregnant	882	20·7)
		(7,527 non-pregnant	1,011	13·4)

Acknowledgement:

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1,442 pregnant women in early labour. 254 (17.6%) had C. albicans in the vagina. 53 per cent of all pregnant women in their series gave a history of vaginal discharge while 69 per cent of patients with positive cultures for C. albicans complained of vaginal discharge. These findings suggest that 31 per cent with positive cultures do not complain of any discharge.

Dawkins (1958) found C. albicans in the vagina in 20 per cent of pregnant women at the 16th, 28th or 36th week of pregnancy, but in only 8.3 per cent was C. albicans isolated on all three occasions. Stough and Blank (1958) noted that C. albicans was isolated from 29.5 per cent of pregnant women.

Clarke and Solomons (1959) studied the general incidence of candida in 739 consecutive pregnant women at the Maimonides Hospital, New York. Using Nickerson's medium (Nickerson, 1953) they isolated Candida species from 27.3 per cent of vaginal swabs collected from the lateral vaginal fornices in these patients.

Taubert and Smith (1960) using Taschdjian's medium (Taschdjian, 1957) for isolation of yeasts noted that 53.7 per cent of pregnant women harboured C. albicans in the vagina. However, 37.5 per cent of patients with clinical evidence of candida vaginitis showed no evidence of fungal infection. The incidence of candida vaginitis in pregnant women in the third trimester is given by Daftary et al. (1963) as 38 per cent.

Jennison (1966) made a retrospective study of 7,222 deliveries over the period 1962 to 1963 at St. Mary's Hospital, Manchester, to study the true incidence of candida vaginitis in pregnancy. He found

that 16 to 17 per cent of patients attending the antenatal clinics had symptoms or signs of vaginitis, severe enough to warrant sending a specimen for diagnosis. C. albicans was cultured in one-third of them. The overall incidence of C. albicans was 4 to 5 per cent in his series.

40 per cent of pregnant women examined antenatally by Somerville (1964) carried C. albicans in the vagina and 10 per cent postnatally.

Hurley and Morris (1964) noted an incidence of candida vaginitis of 10 per cent in maternity patients. They analysed culture reports of specimens sent from patients with signs and symptoms of vaginitis and showed that yeasts were isolated from between a third to a quarter of them. The yeasts isolated from cases of vaginal thrush were C. albicans, C. tropicalis, C. krusei and C. stellatoidea.

Shrand (1961) made a retrospective study of thrush in pregnant women attending the antenatal department of Queen Charlotte's Maternity Hospital, London, over the period 1955-1959. There were 14,873 live births during this period, and 232 mothers had vulvovaginitis due to C. albicans, giving the incidence of candida vaginitis as 1.5 per 100 live births.

My analysis of case records over the period May 1965 to May 1967 showed that among 6,402 antenatal patients, 470 had thrush vaginitis giving an incidence of 7.3 per cent. Analysis of laboratory records at Queen Charlotte's Hospital over a five year period (1966-1970) showed that there were 1,538 yeast positive specimens amongst those sent for diagnosis of vaginitis in pregnant women. There were 18,137 deliveries during this period giving the incidence of candida

vaginitis as 7.9 per cent. C. albicans was isolated from 94 per cent of specimens containing yeasts (Leask, 1971).

Just as pregnancy predisposes to the multiplication and establishment of yeasts in the vagina and increases the susceptibility of pregnant women to candida vulvo-vaginitis, so the newborn are particularly vulnerable to thrush of the mucous membranes, especially of the mouth. In the newborn period there may be symptomless carriage of Candida species in the mouth (Bret and Coupe, 1958). Others regard the appearance of C. albicans in the mouth of the newborn infant as a prodromal carrier state or "latent thrush" (Taschdjian and Kozinn, 1957). Table 2 gives the incidence of C. albicans in the newborn as found by the above authors.

C. albicans as a pathogen in the newborn

The commonest manifestation of disease caused by Candida species in the neonatal period is oral thrush. Other manifestations are skin thrush, especially of the 'diaper' region, and systemic candidosis. The first case of in utero infection of the amniotic sac was reported by Benirschke and Raphael (1958). Lopez and Aterman (1968) reviewed the literature on intrauterine infection by Candida and Aterman (1967) described the pathology of candida infections of the umbilical cord.

Oral thrush was described briefly in a textbook of Paediatrics by Rosen von Rosenstein in 1771, but the etiological agent was unknown at this time. According to Bennet (1844) the credit for discovery of the etiological agent of oral thrush belongs to Eschricht of Copenhagen who described vegetations in the disease called aphthae. However, it was Berg in 1846 who made a scientific study of thrush in infants and

TABLE 2

INCIDENCE OF C. ALBICANS IN THE NEWBORN

	Number	Percentage with C. albicans
Bret and Coupe (1958)	300	16.3 (oral & nasal swabs)
Kozinn et al. (1958b)	2,175	4.6 (oral swabs & stools)

demonstrated the yeast and mycelial forms from the lesions. The typical signs and symptoms of oral thrush are vesicles on the mucous membranes, followed by white patches on the lining of the gums, sides of the tongue and buccal mucosa. White patches of thrush can be removed only with difficulty by swabbing and leaves a raw surface. In more serious infection, there is erosion and ulceration of the mucosa. According to Lehner (1966a) candida infections of the mouth could be classified into acute and chronic varieties as shown:-

Acute -	Acute pseudomembranous candidosis (thrush)
	Acute atrophic candidosis
Chronic -	Chronic hyperplastic candidosis
	Chronic atrophic candidosis (denture sore mouth)

The pseudomembranous and hyperplastic types appear as white patches and the atrophic types show diffuse erythematous lesions. In acute pseudomembranous candidosis (thrush) the pseudomembrane consists of "desquamated epithelium, keratin, fibrin, necrotic tissue, food debris, leucocytes and bacteria; all this is matted together and anchored down to the epithelium by fungal hyphae".

Table 3 (taken from Winner and Hurley, 1964) shows the reported incidence of oral thrush in the newborn, and Table 4 shows the incidence of oral thrush in selected groups of infants (according to Kozinn et al., 1958). Epstein (1924) studied 1,000 healthy babies aged 1 to 10 days and found an incidence of oral thrush of 2.4 per cent. In a study on 1,000 children aged between 6 days to 1 year, the incidence was 24.6 per cent. Manifest thrush was highest (54%) between the 2nd to 6th week of life. The incidence of "latent"

TABLE 3

REPORTED INCIDENCE OF ORAL THRUSH IN THE NEWBORN
(Winner and Hurley, 1964)

Author or Institution	Newborns in series	Oral thrush	
		Number	Per cent
Epstein (1924)	2,500	150	6.0
Ludlam and Henderson (1942)	2,540	163	6.4
Anderson et al. (1944)	107	20	18.8
*Beth El Hospital (1955)	3,500	5	0.14
*Maimonides Hospital (1955)	3,600	36	1.0
*Mount Sinai Hospital (1955)	4,000	20	0.5
*Maimonides Hospital (1956)	2,175	69	3.1
Queen Charlotte's Hospital (1955-1959) (Shrand, 1961)	14,875	95	0.66
Birmingham Maternity Hospital (1960-1961) (Dunn, 1962)	3,700	40	1.0
M.G.M. Med. Coll., Indore (Kaul et al., 1960)	713	29	4.0

*Quoted by Kozinn, et al., 1958

TABLE 4

INCIDENCE OF ORAL THRUSH IN SELECTED GROUPS OF INFANTS
(after Kozim et al., 1958)

Group	Number of infants in series	Oral thrush	
		Number	Per cent
Premature	87	3	3.5
Full term	2,175	69	3.1
Breast fed	158	7	4.4
Bottle fed	2,017	62	3.4
Antibiotic therapy	370	10	2.7

thrush was also most frequent in a similar age group, 6 days to 6 weeks. Thrush did not occur in the first four days of life. The incidence of thrush fungus in the stools of 270 healthy infants, 1 to 8 days old is given as 23 per cent while of 20 babies with manifest oral thrush, all had the fungus in the stools. Woodruff and Hesseltine (1938) give the low figure of 1 per cent as the incidence of oral thrush in the infant population of the Chicago Lying-in-Hospital. They state that the chance of oral thrush occurring in a baby born of a mother infected with thrush fungi is 35 times greater than that of one born to an uninfected mother. Ludlam and Henderson (1942) investigated thrush in the newborn at the Royal Infirmary at Edinburgh. In 1939 the incidence was 7.2 per cent and in 1940 it was 6.4 per cent in 2,540 infants surviving over 48 hours. In infants aged 2 to 10 days, the incidence was 4.1 per cent. In a study on 60 unselected infants, 18.3 per cent had C. albicans in oral swabs taken during the first ten days. The incidence of thrush in this special group was 23.3 per cent. The discrepancy in the incidence of thrush during the short space of three years is attributed to the intensity of the special study. According to them, oral thrush rarely manifests itself before the seventh day of life (Fig. 4).

Anderson et al (1944) investigating the frequency with which 107 normal newborns had Candida in the oral cavity found an incidence of 18.7 per cent. Clinical oral thrush developed in 10.8 per cent of cases with positive oral swabs.

Shardt and Roy (1957) give the incidence of thrush in the newborn as 4.7 per cent.

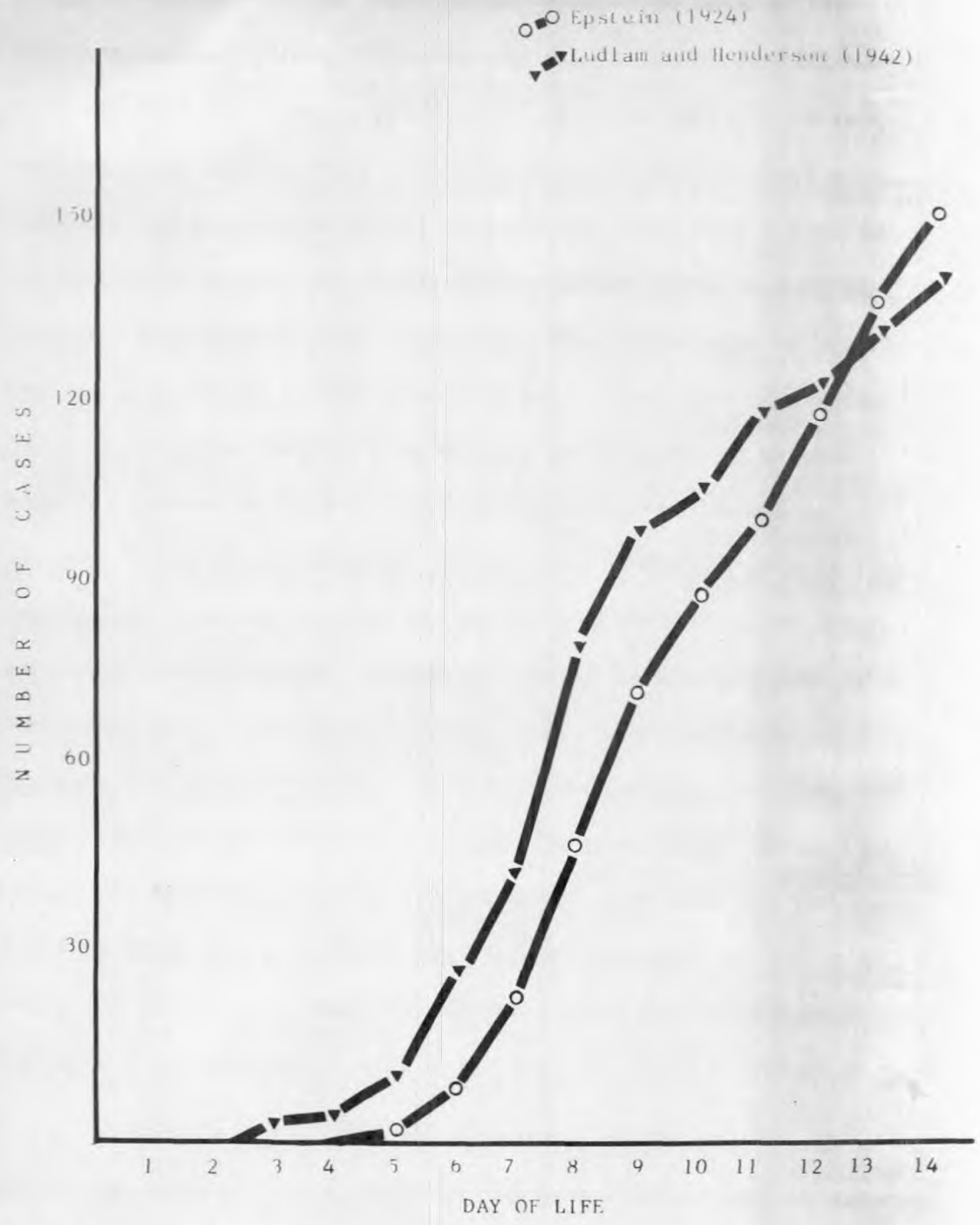


Fig. 4. Cumulative incidence of oral thrush during the first 2 weeks of life according to Epstein (1924) and Ludlam and Henderson (1942). (after Kozinn et al., 1958).

Kozinn et al. (1958) examined oral swabs from 2,175 consecutive newborns at the Maimonides Hospital, New York. They found C. albicans in the oral cavity in 90 (4.1%). Examination of 520 stools from newborns showed an incidence of C. albicans in 11 (2%). The overall incidence of C. albicans was 4.6 per cent. In all these cases clinical thrush developed 2 to 5 days after C. albicans was isolated. The incidence of oral thrush in these 2,175 infants was 3.1 per cent, cutaneous thrush occurred in 6 per cent and "latent thrush" in 0.9 per cent. According to Taschdjian and Kozinn (1957), 55 per cent of infants born to infected mothers develop oral thrush showing that the primary source of neonatal infection is maternal vaginal infection.

Harris et al. (1958) showed that 20 per cent of infants born to women with positive vaginal cultures harboured C. albicans in the mouth and 11.4 per cent developed thrush. In babies born to mothers with negative cultures, only 1 per cent had C. albicans in the mouth, and 0.45 per cent developed thrush.

Bret and Coupe (1958) examined oral and nasal swabs from 300 babies at birth and at different intervals after birth. They found that 16.3 per cent carried C. albicans in the mouth, at birth. A similar incidence (16%) was found in 200 day old infants. By the 4th day the incidence of C. albicans in the mouth had fallen to 4 per cent. In another series of 100 the incidence was 17 per cent at 24 hours and 7 per cent on the 4th day, and 10th day. 4 per cent developed thrush by 2 weeks. The incidence of C. albicans in the stools was 9 per cent at 24 hours.

Shrand (1961) studied thrush in the newborn over a period of five years. 14,873 newborn infants were examined daily for evidence of oral thrush. Thrush developed in 95 babies giving an incidence of 0.6 per cent. The incidence of thrush among babies born to mothers with untreated monilial vulvovaginitis was 77 per cent, while in adequately treated cases, the incidence was 2 per cent.

The incidence of Candids species, among Indian newborn infants swabbed on three occasions during the first week of life, was 15.8 per cent and 4.1 per cent developed clinical thrush (Kaul et al., 1960). The predominant species isolated were C. tropicalis and C. pseudotropicalis.

Dunn (1962) states that the incidence of oral thrush in newborn infants in a Birmingham Maternity Hospital was 10 per cent.

According to Jennison (1966) clinical thrush occurred in 1.7 per cent and 2.6 per cent of live births at St. Mary's Hospital, Manchester in 1962 and 1963 respectively.

At Queen Charlotte's Maternity Hospital, London, the incidence of oral thrush in newborns was 0.66 per cent during the period 1955 to 1959 (Shrand, 1961). During a two year period, May 1965 to May 1967, there were 6,354 live births at this hospital, of which 47 developed oral thrush, giving an incidence of 0.73 per cent (White-Franklin, 1968). My study of records at Queen Charlotte's Maternity Hospital over the period 1965 to 1969 shows an apparently declining incidence of thrush in the newborn (Table 5).

TABLE 5

INCIDENCE OF ORAL THRUSH IN THE NEWBORN AT QUEEN CHARLOTTE'S MATERNITY HOSPITAL, (1965-1969)*

Year	Number of live births	Number with thrush	Percentage incidence
1965	3,526	12	0.34
1966	3,406	13	0.38
1967	3,425	20	0.57
1968	3,328	21	0.63
1969	3,421	11	0.32

*Taken from hospital records

SEROLOGICAL STUDIES OF YEAST LIKE ORGANISMS

Serological tests for the detection of candida antibodies have been made (i) to elucidate the antigenic structure of the organisms, and (ii) to aid in the diagnosis of mycoses, particularly in cases of obscure or deep-seated infection of doubtful origin. A brief account of the serological methods that have been used in the detection of antibodies to C. albicans in man follows.

Agglutination

Todd (1937) made a survey of 1,150 sera from healthy human beings for agglutinins to C. albicans. He showed that 22.5 per cent of sera agglutinated C. albicans and that 3 per cent gave titres of 1:150 or higher. Drake (1945) using slide agglutination, examined sera from 114 students for agglutinins, using antigens derived from different yeasts; 95 per cent reacted with at least one organism and 45 per cent with C. albicans. He concluded that agglutinins are probably natural antibodies which arise as a result of exposure to an organism, or to related organisms containing common antigens, or that they are normal constituents of human serum. The spatial configuration of these natural antibodies in serum enables them to function as antibodies and the reaction is therefore fortuitous, and not specific. Norris and Rawson (1947) showed that 64 per cent of a hospital population had agglutinins to C. albicans, concluding that measurement of candida agglutinins was unreliable in the serological diagnosis of candida infections. They found a high frequency of positive agglutinins in healthy persons and a lack of correlation between agglutinin titres and active disease. Winner (1955) screened 2,017 sera received for Wasserman tests by a slide

agglutination technique, finding that 31.6 per cent were positive. Females had agglutinins more often than males. Estimation of agglutinin titres in infected and non infected cases did not reveal any association between high agglutinin titres (over 1:16) and infection, and there was no evidence that a rise in titre occurred during the course of infection. High agglutinin titres occurred in the absence of overt infection and low titres in the presence of infection. He concluded that agglutinins are of little or no diagnostic significance. Maibach and Kligman (1962) repeatedly injected killed candida cells, subcutaneously in five human volunteers. No agglutinin response was detected. They concluded that serological tests were not of diagnostic value in cutaneous candidosis. Comaish et al. (1963) studied agglutinin titres in patients with clinical candida infection of the skin and with other dermatoses and in controls with no evidence of skin or gastrointestinal infection. They suggested that a titre of 1:8 or higher indicates the presence of C. albicans somewhere while a titre of 1:16 or higher is likely to signify clinical disease. However, they, too found that several patients with undoubted candida infections had titres of less than 1:8, so that a low titre does not exclude candida infections. Murray et al. (1969) measured agglutinin titres in the sera of 68 patients undergoing open heart surgery. They found agglutinin titres greater than 1 in 16 in 60 of them. Rosner et al. (1971) measured agglutinin titres in the sera of 8 patients with acute leukemia, who had concomitant systemic candidosis. They found markedly elevated serum agglutinin titres (1 in 5120) against C. albicans, in six of them. There was a good correlation between serum agglutinin titres and recovery of

the organism from body fluids in these patients receiving anti-leukemic therapy. They suggested that periodic serological studies be performed on these high risk patients to facilitate early diagnosis of systemic candidosis. The high incidence of agglutinins in all groups of subjects studied is shown in Table 6.

Complement fixation test

Koerth et al. (1941) thought that a positive complement fixation reaction indicated the etiological role of a particular fungus in bronchomoniliasis. Peck et al. (1955) examined sera of 793 patients with skin infections by a complement fixation test. 13.5 per cent of the total gave positive reactions, while 75 per cent with clinical candidosis gave positive results. These results did not suggest that the complement fixation test, as performed, could be of much value in diagnosis, as such antibodies were present in many people who had no overt clinical infection.

Immunofluorescence

Lehner (1965 and 1966b) used fluorescent techniques for the detection of candida antibodies in 244 human sera, 76 of which were from cases of candidosis. He used whole antihuman globulin conjugated with fluorescein isothyocyanate and an indirect fluorescent antibody test to determine serum fluorescent antibody titres. He showed that a titre greater than 16 was evidence of candidosis, while controls had a titre of 8 or less. Esterly (1968) studied 207 sera by a similar indirect fluorescent antibody test and confirmed these findings. Lehner (1970) used monospecific conjugates of the immunoglobulin classes, Ig G, Ig M and Ig A, to

TABLE 6

INCIDENCE OF AGGLUTININS IN GROUPS OF PATIENTS

Investigator	Number tested	Group of patients tested	Titre taken as positive	Percentage positive
Todd (1937)	1,150	Healthy subjects	1:10	22.5
Drake (1943, 1945)	114	Students	1:10	45
Norris and Rawson (1947)	469	Hospital patients	1:5	64
Winner (1955)	2,017	Hospital patients	1:6	31.6
Murray et al. (1969)	68	Patients undergoing open heart surgery	1:16	88
		(6 patients with endocarditis)		(100)
Rosner et al. (1971)	9	Acute leukemic patients with systemic candidosis	1:160	75

determine serum fluorescent antibody titres in 65 cases. He showed that the antibodies in candidosis were detected in all three classes. The rise in antibody titre was most frequently of the Ig G type, to a less extent Ig M and least commonly of the Ig A class. Estimation of the concentration of a particular immunoglobulin class showed no correlation between concentration and fluorescent antibody titres.

Immune adherence

Brody and Finch (1960) showed lack of correlation between serum antibody titres and human candidosis as measured by an immune adherence technique.

Immuno-osmophoresis

This is an agar gel precipitin test using osmophoresis and was used by Culliford (1964) for detection of soluble antigens in forensic specimens. When an electric current is passed through agar gel, which is negatively charged, endosmosis occurs, i.e. water molecules move from anode to cathode. Endosmosis slows the migration of negatively charged bodies and may cause slow moving ones to reverse their direction. Electrophoresis of gamma globulins in agar gel at pH between 7.2 and 9 causes them to migrate to the cathode. Antigens which migrate from the cathode to anode under similar conditions, may be made to converge on the globulins. If antibodies to these antigens are present in the gamma globulins, precipitation will occur. Jameson (1968) devised an immunoosmophoresis test for the diagnosis of Farmer's lung, and found it to be more sensitive than conventional immuno-diffusion tests for detection of precipitins. Murray (1969) used immunoosmophoresis in the detection of candida precipitins.

Immunoelectrophoresis

In this serological method developed by Grabar and Williams (1953) electrophoretic separation of a macromolecular mixture is combined with double diffusion in agar. A micromethod of immunoelectrophoresis described by Scheidegger (1955) has the advantage of economy of material and time. Taschdjian et al. (1964a) used immunoelectrophoresis to demonstrate that serum from a patient with Candida albicans septicaemia and serum from a patient with Candida parapsilosis endocarditis, reacted with different antigenic fractions of a cytoplasmic extract of C. albicans. Taschdjian et al. (1964a) electrophoresed concentrated precipitin positive serum and allowed it to diffuse against antihuman serum on the one hand and somatic antigen of C. albicans on the other. The precipitation arc evoked by the antigen was located in the beta macroglobulin (IgM) region. Pepys et al. (1968) tested precipitin positive sera by immunoelectrophoresis and showed that reactions to mannan antigen of C. albicans occurred in the region of the immunoglobulins of the Ig G type.

The precipitin test which is based on the precipitin reaction has been used in the serological investigation of human sera for the presence of antibodies to the antigens of C. albicans.

The precipitin reaction

The formation of a precipitate when an antigen in solution and its corresponding antibody are mixed, was first recognised by Kraus in 1897. He observed that cell free filtrates of broth cultures of plague bacillus mixed with antiplague serum formed a precipitate. Precipitating antibodies called precipitins can be

produced against proteins and some polysaccharides.

The mechanism of the precipitin reaction is the formation of a specific precipitate, as a result of a firm but reversible combination of antigen and antibody molecules, followed by separation from solution of the antigen - antibody complex. The combination occurs in two stages: a complexing stage, when specific rapid but invisible combination of antigen with antibody molecules occurs, with the formation of small soluble complexes, and an aggregation stage, when a growing network or lattice of these complexes is formed. The lattice of complexes becomes so large that it becomes insoluble and visible in the form of a precipitate. The first stage is aided by electrolytes and can occur at temperatures ranging from 15 - 40°C, in the case of antisera from warm blooded animals. The second stage of visible precipitation is aided by electrolytes and occurs at an optimal pH and temperature range. A pH of less than 6.5 causes non-specific precipitation of serum proteins and a pH above 8.2 causes dissociation of complexes. Higher temperatures favour rapid diffusion of reactants and accelerate precipitation but resolution of the band of precipitate is decreased.

Antigen antibody combination occurs in different ratios but strongest precipitation occurs most rapidly when antigen and antibody are in optimal proportion. An excess of one reactant may prevent visible precipitation.

The characteristics of the antigen and the antibody determine the reversibility of the antigen-antibody combination - for example, horse antibodies against proteins, form readily reversible

combinations with the antigen, and an excess of antigen or antibody will prevent visible precipitation. Such antibodies are called 'H' or flocculating antibody. Horse antibodies to polysaccharides and rabbit antibodies to proteins and polysaccharides form more tenacious combination with the antigens. However, an excess of antigen may prevent visible precipitation or dissolve formed precipitates. These more tenacious antibodies, formed typically by rabbits are called 'R' or precipitating antibody. Both types occur in human sera (Roitt et al. 1958).

The precipitin reaction could be made quantitative (Kabat and Meyer, 1961). Complete precipitation of the antibody by addition of excess antigen, and subsequent analysis of the washed precipitate enables the antibody nitrogen to be estimated.

An up-to-date account of the theory and methodology in relationship to precipitin tests is given by Williams and Chase (1971).

The precipitin test

The precipitin test used for the detection of antibodies to C. albicans antigens is an immuno-diffusion test. According to Crowle (1961), the first description of an immuno-diffusion test was by H. Bechhold in 1905. He described an experiment in which rabbit antiserum to a goat serum was incorporated in 1 per cent gelatin, and allowed to set in test tubes. This was overlaid with goat serum, when two, heavy precipitation bands appeared. The immuno-diffusion test was put to practical use by Petri (1932) who used it in the identification of microorganisms. He incor-

porated an antiserum specific for a microorganism, in an agar plate and then cultured the organism on it. The production of a halo of precipitate, round the colony due to a reaction between antigen from the organism and the antiserum on the plate, indicated the identity of the organism. Its application to modern immunochemistry started in 1946 when Jacques Oudin devised a single diffusion, tube test where one reactant diffused actively into the medium containing the other. He layered an antigen over an agar gelled antiserum contained in a tube. A precipitin band formed due to diffusion of the antigen into the antibody column.

Immuno-diffusion techniques can be classified into two groups (Fig. 5).

I. Single diffusion systems where only one of the reactants, antigen or antibody, diffuses. There are two types of this system:-

   (a) Diffusion in one dimension, e.g. Oudin tube technique;

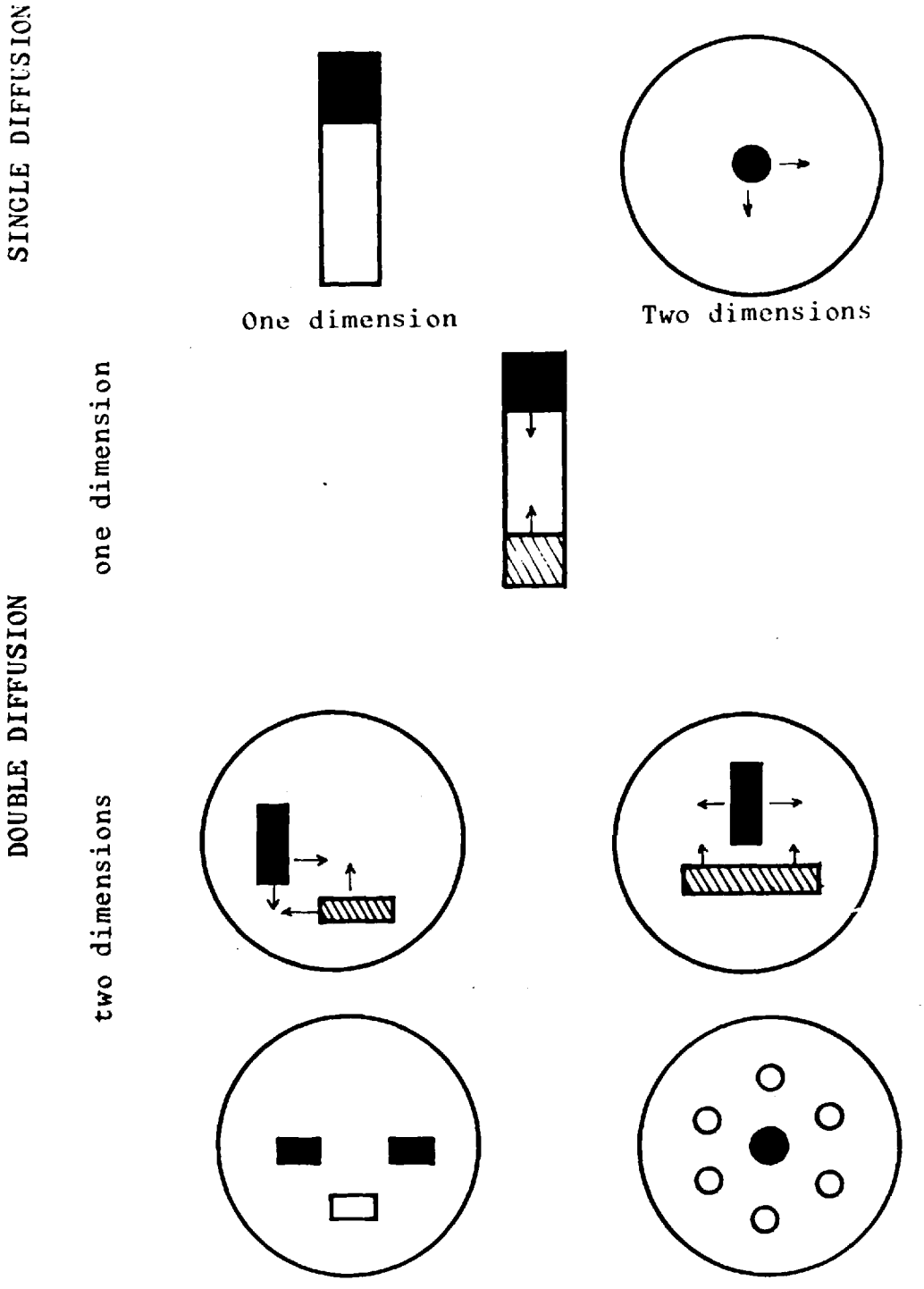
   (b) Diffusion in two dimensions, e.g. Halo reaction of Petri.

II. Double diffusion systems where both reactants are diffusing. There are two types of this system:-

   (a) Diffusion in one dimension, e.g. tube technique of Oakley and Fullthorpe (1953);

   (b) Diffusion in two dimensions, e.g. two cup or multiple cup, plate technique of Ouchterlony (1948) and technique of Elek (1948).

Fig.5 IMMUNODIFFUSION SYSTEMS: Antigen - black
Antibody- shaded



The term "single system" is applied to a diffusion arrangement comprised of only two reactants - antigen and antibody. When several sources of diffusion are employed, so that interaction between separate systems is possible, the term "combined system" is applied.

Hydrophilic substances such as agar or gelatin, called gels, are used in immuno-diffusion tests. Filter paper and strips of cellulose acetate have also been used as media for immuno-diffusion. The concentration of agar can be varied from 0.3 per cent as in Oudin tubes and 0.7-1.5 per cent as in Ouchterlony plates. The pH may vary between 6 and 9 but usually pH 7-7.4 is used. A preservative such as merthiolate 1:10,000 or sodium azide can be used. Immuno-diffusion should be carried out at a suitable constant temperature, in a humid atmosphere, to prevent formation of artifacts. At the low concentrations of agar used, diffusion of antibodies and most antigens is not impeded and the laws of free diffusion apply.

In a gel diffusion test, the antigen and antibody reaction occurs in the semisolid medium, with the formation of visible bands of precipitate. When a precipitate forms there is an increased flow of reactants into the zone of precipitation. The number of bands indicates the minimal number of antigen-antibody systems present, provided that artifacts due to temperature fluctuations, excessive antigen concentration, and nonspecific reactions are excluded.

The gel diffusion test has a high degree of sensitivity and even a few micrograms of antigen and antibody are detected by it.

Its limitations are that it fails to resolve components in some complex mixtures and to detect non-precipitating antibody. Three gel diffusion procedures are widely used:-

1. Single diffusion tube test of Oudin. This involves one dimensional diffusion of one reactant into a gel containing the other.

2. Double diffusion in one dimension. This method was first described by Oakley et al. (1953). Antiserum is placed at the bottom of a tube, an agar layer poured over it and the antigen layered on top of the agar. Antigen and antibody diffuse towards each other and bands of precipitate develop in the middle layer of agar which contained neither reactant. A miniature form of this double diffusion tube test was described by Preer (1956) and was used by Chew and Theus (1967) for the detection of precipitins to C. albicans.

3. Double diffusion in two dimensions. This technique was developed independently in Sweden, by Ouchterlony (1948) and in England by Elek (1948), for tests on the toxigenicity of Corynebacterium diphtheriae.

In the Ouchterlony method, antigen and antibody are present as fluids in separate wells in an agar plate. In its simplest form, a solution of 1 per cent clarified agar in saline, containing a preservative is poured into a petri dish to give a layer 3 to 6 mm. thick. A central well and three circumferential wells are cut at

equal distances apart, with a cork borer and the agar removed by suction. The size of the wells and the distance between the central and circumferential wells may be varied. A template or a gel cutter may be used to cut a pattern of holes, or penicillin assay cups may be used. Instead of round wells, square, triangular or rectangular wells may be used, in various arrangements (Fig. 6). Mansi (1958) designed a micromodification of the Ouchterlony method using a layer of agar on a slide. Crowle (1961) modified this further using a layer of perspex with conical cups so that bands of precipitate form in the body of the agar.

According to the design of the experiment antiserum may be placed in the central well and antigens in the peripheral wells, or vice versa. Plates are incubated at a constant temperature with moistened filter paper to prevent drying. They are observed frequently.

The reactants diffuse towards each other in two dimensions, and are precipitated in the medium that originally contained neither. Bands of precipitate occur where there are optimal concentrations of antigen and antibody, beginning at the point at which the two wells are closest and extending in length. The precipitation line formed is a straight line if the antigen and antibody have about the same molecular weight (Korngold et al., 1957). The line curves slightly towards the antibody reservoir, if the molecular weight of the antibody is greater and slightly away from the antibody reservoir if the molecular weight of the antigen is greater.

When an antiserum contains antibodies to several antigens and a mixture of antigens is used, the number of precipitin bands

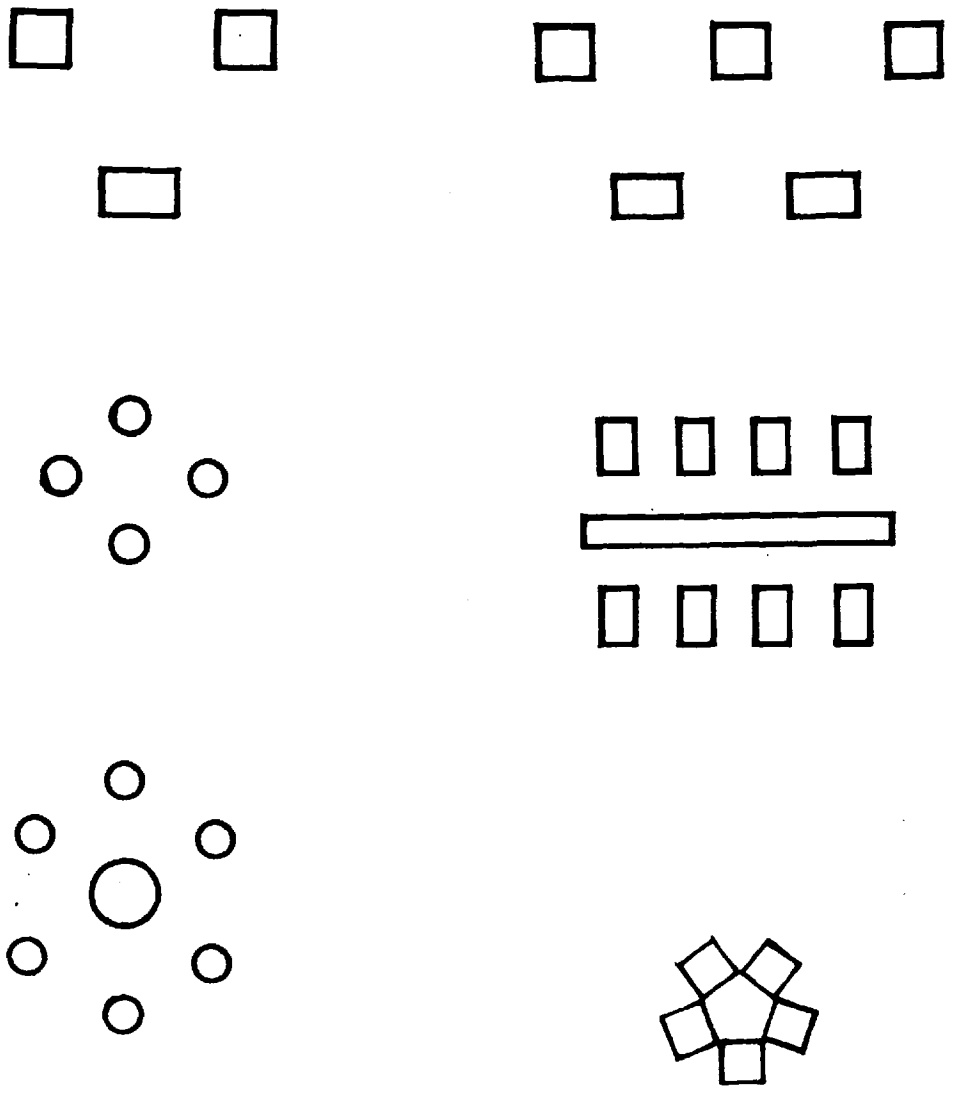


Fig.6.Some arrangements of sources of diffusion in plates

developing can be interpreted as representing the minimum number of distinct precipitating systems present. In such a system if one of the antigenic components is added to an adjacent circumferential well, it will form a band which will fuse with a band due to a similar antigen in the mixture, forming a line of identity. It will, however, intersect any bands due to unrelated antigen antibody systems. If two cross reacting antigens are placed in adjacent wells, there will be partial fusion of the bands but the homologous antigen will form a 'spur' beyond the point of fusion (Fig. 7).

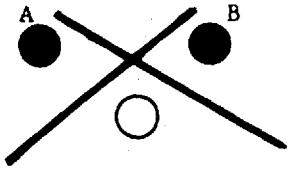
A rough titration of antigen or antibody may be made by placing serial dilutions of antigen in the peripheral wells and various sera may be compared this way (Gell, 1955).

Precipitation bands formed in gel diffusion may be stained with various dyes (Bjorklund, 1954), after washing in several changes of saline. The agar can be dried down to a thin layer on filter paper, so that the agar layer can be kept as a permanent record (Gell, 1955). A method of washing, drying and preserving agar gel from a petri dish was described by Rondle et al. (1956).

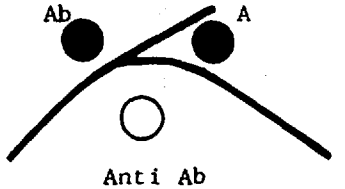
The Precipitin Test in serodiagnosis of candidosis

Akiba et al. (1957) first used a precipitin test according to the ring test method, to assess the value of a polysaccharide antigen of C. albicans in the serological diagnosis of candidosis. The polysaccharide fraction was extracted from dried C. albicans cells, using phenol, and used at a concentration of 0.5 mgm. per ml. in the ring test. They found that 0.8 per cent of healthy individuals, 100 per cent of patients with bronchopulmonary candi-

Anti A
Reaction of Identity



Anti AB
Reaction of Non identity



Anti Ab
Reaction of Partial identity "Spur Formation"

● Antigen
○ Antiserum

Fig. 7 Patterns observed in double diffusion plates where two antigen solutions are compared using antiserum as the analytical agent.

dosis and 72.7 per cent of patients with superficial candidosis gave positive reactions.

Taschdjian et al. (1964a) first used an agar gel precipitin test in the serodiagnosis of mycoses caused by C. albicans. They demonstrated candida precipitins in human sera in two patients, one with meningeal candidosis and another with candida granuloma, using the Ouchterlony double diffusion technique. Two antigens were tested:-

(i) Antigen containing cytoplasmic constituents of C. albicans. This antigen was prepared by disruption of C. albicans cells in a high intensity sonicator. The supernatant after centrifugation contained soluble cell wall and cytoplasmic components. It was evaporated to dryness and reconstituted to such a density that a reading of two was obtained using a refractometer. This antigen was designated 'S' antigen to indicate its somatic nature as well as the method of extraction and it was similar to the antigen extracted from "ground up" C. albicans by Biguet et al. (1962).

(ii) Undiluted, commercial Oidiomycin prepared from culture filtrates containing metabolites and soluble cell wall components was used as a control antigen.

One precipitin band to 'S' antigen was demonstrable by agar gel double diffusion in the one year old boy with candida meningitis

and two precipitin bands in the man with monilial granuloma, while four patients with superficial candidosis did not have precipitins. They concluded that precipitating antibody was formed only in candida infections involving living tissues. They suggested that the test might prove valuable as a diagnostic aid in systemic candidosis. This possibility was explored further in a study of sera from 8 patients in whom some of the clinical and laboratory findings suggested a diagnosis of systemic candidosis and 10 patients without systemic candidosis (Taschdjian et al., 1964b). Precipitins to 'S' antigen were found in the sera of 62.5 per cent of patients with systemic candidosis and in one case of candida granuloma. They confirmed their previous observation that candida precipitins are formed during the course of chronic systemic infection and are not found in superficial candida infection. However, the reliability of the test remained to be evaluated.

Stallybrass (1964) examined sera from 6 immunized rabbits and 834 human sera (which included at least 215 sera from pregnant women), for precipitins to antigens of C. albicans. He used the Dreyer method and double diffusion in agar, with a pattern of wells each 6 mm. in diameter, for demonstration of precipitins. He tested three antigens:-

(i) Antigen containing cytoplasmic constituents of C. albicans and designated 'C' antigen. This was prepared by disintegration of cells in a Mickle disintegrator. The supernatant after centrifugation, an opalescent solution, was

used as the antigen.

(ii) Formamide extract of C. albicans cells. This was prepared by extraction of cells with hot formamide, and precipitation of soluble polysaccharides with acid alcohol and acetone. The precipitated polysaccharides were redissolved in saline and used as antigen.

(iii) Formamide extract of cell walls of C. albicans. This was prepared by formamide extraction of the deposit remaining after preparation of 'C' antigen.

He showed that there are multiple polysaccharide antigens in the cell wall and a protein antigen in the cytoplasm. Each antigen was tested at the dilution which gave maximal precipitation with a positive rabbit serum (1:10 dilution) when titrated by the constant serum method of Dean and Webb (1926). He showed that immunized rabbit serum reacts with a polysaccharide antigen from cell wall and a protein antigen from cytoplasm. Of the human sera examined only one from a case of candida septicaemia had a single diffuse precipitin band to the polysaccharide antigen of the cell wall, but not to the cytoplasmic protein antigen, when tested by double diffusion against 'C' antigen, and formamide extracts of cells and cell walls. The occasional weak precipitation by the Dreyer method was interpreted as a nonspecific reaction. He too suggested that candida precipitins to either polysaccharide or protein antigens do not occur in healthy persons or in patients with superficial candidosis; and that they may be expected in man only when there is systemic infection with C. albicans.

Murray and Buckley (1966) tested 160 sera obtained from a blood bank but could not demonstrate precipitins to Mickle disintegrated antigens of C. albicans in them.

In a study on 20 patients with clinical evidence of systemic candidosis, Taschdjian et al. (1967) showed that 85 per cent had precipitins to somatic antigen; in a group of 49 controls with no evidence of superficial or systemic candidosis none had precipitins. However, 15.7 per cent with superficial candidosis had precipitins to somatic antigen and to Oidiomycin. The precipitin positive sera in this group were from one case of candida granuloma and 7 cases of endocrinopathy associated chronic mucocutaneous candidosis. The positive reactions to commercial Oidiomycin, a culture filtrate, in 5 of 43 sera from miscellaneous superficial candidosis were considered false positive reactions as these were also seen with sera from rabbits infected superficially. They suggested that cytoplasmic sonicates of C. albicans showed greater specificity than culture filtrates for systemic candidal infection, and that the development of a standardized antigen for diagnosis was desirable.

Chew and Theus (1967) investigated the sera of healthy subjects for candida precipitins using concentrated and unconcentrated sera and concentrated globulin fractions, and of subjects with mucocutaneous involvement using unconcentrated sera. They used three antigens in their study:-

(i) Purified cell wall polysaccharide (mannan) prepared by the method of Peat et al. (1961).

(ii) Cytoplasmic extract of C. albicans prepared by ultrasonic disintegration, according to the

method of Taschdjian et al. (1964a), used at a concentration of 100 mg. per ml. dry weight.

(iii) Cytoplasmic extract of C. albicans prepared according to the method of Stallybrass (1964).

Double diffusion was carried out in Preer tubes and in Ouchterlony plates using a pattern of wells 8 mm. in diameter. They found that when unconcentrated sera from healthy subjects were tested against mannan antigen by the Preer tube method, 48 per cent were positive for precipitins but only 3 per cent were positive in Ouchterlony plates. However, fifteenfold concentration of the globulin fraction of these sera, gave 100 per cent positive reactions when tested in Preer tubes and 30 per cent positive reactions in Ouchterlony plates. In tests on unconcentrated sera, from cases of mucocutaneous candidosis, 69 per cent were positive when tested in Preer tubes but only 10 per cent were positive in Ouchterlony plates. These results contradict the reports by Taschdjian et al. (1964) and Stallybrass (1964) who suggested that precipitins were found only in systemic candida infections. Chew and Theus comparing the purified mannan used in their study with candida antigens used by Taschdjian et al. (1964a and b) and Stallybrass (1964), showed that some sera formed two precipitin bands against cytoplasmic extracts, but only one band against mannan. Further, the mannan precipitation band formed a line of identity with the broad band obtained with cytoplasmic extracts, showing that antigens used by Taschdjian et al. (1964a and b) and Stallybrass (1964) contained mannan. The additional precipitin band obtained when some sera were tested with cytoplasmic extracts suggested the presence of an additional antigen in such

extracts. Purification and characterization of this additional antigen was necessary before its distribution and function could be investigated. Recently Buckley et al. (1970) have described a method of isolation of a pure protein antigen from disintegrated C. albicans cells.

Pepys et al. (1968) investigated precipitin reactions to C. albicans in respiratory disease in man, using purified mannan extracted from cell walls by a modification of the method of Peat et al. (1961), formamide extract of cells, and a culture filtrate extract of C. albicans. Precipitin tests were made by double diffusion in agar with a pattern of wells, such that the central serum well was 12.5 mm. in diameter and peripheral wells 4 mm. in diameter. They demonstrated two types of precipitation arcs. Fuzzy precipitation arcs of the 'H' type associated with polysaccharide antigens were given by mannan antigen. The culture filtrate gave 'H' type arcs giving reactions of identity with mannan and well defined 'R' type arcs which were due to a protein antigen. The protein nature of the antigen giving 'R' type arcs was shown by gel electrophoresis of culture filtrate and subsequent staining with a protein dye which gave a protein staining area corresponding with the 'R' type reaction. They showed that 4.5 per cent of unconcentrated sera and 22.7 per cent of concentrated sera from healthy adults had precipitins to mannan while unconcentrated sera from 25 per cent of patients with asthma and 33.3 per cent with asthma and pulmonary eosinophilia had precipitins against the mannan antigen of C. albicans showing that there was some association between these precipitins and respiratory disease in man. The frequency

of precipitins in healthy subjects in their study, contrasts markedly with reports by Taschdjian et al. (1964a and b) and Stallybrass (1964) although the formamide extract of C. albicans used by Stallybrass was shown to contain mannan by Pepys et al. (1968).

Taschdjian et al. (1969) maintained the diagnostic validity of the precipitin test in systemic candidosis, and supported it with positive autopsy findings in 14 out of 17 patients.

Murray et al. (1969) examined sera of patients undergoing open heart surgery using antigens prepared by disintegration of C. albicans cells, in a Mickle shaker or a hydraulic press. The optimal concentration for precipitation was determined by testing the antigen against a serum from a hyperimmunized rabbit. This antigen was comparable with that used by Taschdjian et al. (1964a and b) and Stallybrass (1964). Precipitin tests were carried out in agar buffered to a pH 8.6 and a pattern of wells was cut such that the central and four peripheral wells had a diameter of 6 mm. while two peripheral wells had a diameter of 2 mm. C. albicans antigen was placed in one small and one large peripheral well to give a volume ratio of 1:10, as sera with low precipitin titres reacted better with a smaller volume of antigen and those with higher titres reacted better with a larger volume. By this method, none of the 47 sera obtained from patients before operation had precipitins. High levels of precipitins to Candida species were found in 33 of 68 patients after open heart surgery. Thirteen sera gave precipitin reactions to Mickle disintegrated antigens of one species of Candida and twenty-two had precipitins to several species of Candida. Six of the patients who had precipitins to several

species of Candida developed candida endocarditis. According to Murray et al. (1969) the high levels of precipitins in patients after open heart surgery suggested a diagnosis of candida endocarditis complicating the operation or inapparent candida infection. Rosner et al. (1971) measured serum precipitin titres in patients with acute leukemia in whom clinical signs suggested secondary systemic candidosis. They found that 75 per cent of these had positive precipitin titres (as high as 1 in 64). They suggested that periodic determination of precipitin and agglutinin titres could facilitate early diagnosis of systemic candidosis in such patients.

A striking feature of all the investigations on precipitins to C. albicans in healthy and diseased persons, is the lack of a standard procedure for precipitin tests and the use of antigenic extracts of different chemical composition and concentration as shown in Table 7.

In the investigation on precipitins to C. albicans in pregnant women, described in this thesis, three antigens were used:-

(i) A purified cell wall mannan prepared by the method of Peat et al. (1961) as modified by Faux (1968).

(ii) A cytoplasmic extract of C. albicans cells prepared by a modification of the method of Stallybrass (1964). This extract has been shown to be a mixture of polysaccharide and protein antigens by Stallybrass (1964). Chew and Theus (1967) showed that an antigenic extract prepared by the method of

TABLE 7

Precipitation reactions to antigens of C. albicans using agar gel diffusion methods

Investigator	Method of antigenic extraction	Group of patients tested	Number tested	Percentage positive
Stallybrass (1964)	"somatic" formamide	Candidosis (1 systemic)	16 (1)	6.2 (100)
		V.D. clinic and antenatal cases	403	-
Taschdjian et al. (1964)	"somatic" oidiomycin	Systemic candidosis	8	62.5
		No systemic candidosis (1 granuloma)	9 (1)	- (100)
(1967)	"somatic" oidiomycin	Systemic candidosis	20	85
		Superficial candidosis	51	15.7
		Controls (negative)	49	-
Murray et al. (1965)	"Mickle"	Blood donors	160	-
(1969)	"Mickle" or hydraulic press	Patients before open heart surgery	47	-
		Patients after open heart surgery	62	43
		Patients with candida endocarditis	6	100
Chew and Theus (1967)	Mannan	Healthy	31	3
		Mucocutaneous candidosis	62	10
Pepys et al. (1968)	Mannan	Healthy	1	4.5
		Asthma	10	25
		Asthma & pulmonary eosinophilia	12	33.3
		Miscellaneous lung diseases	1	6.2

Stallybrass (1964), contained mannan antigen as well as an additional antigen whose nature had not been characterized.

(iii) A culture filtrate extract prepared by the method of Pepys et al. (1968). This has been shown to contain a protein antigen in addition to a small amount of mannan.

Precipitin tests were carried out by the Ouchterlony double diffusion method, in agar plates, with a pattern of wells described under methods.

Sera for precipitin tests were collected at the first booking visit from unselected pregnant women. At the same time, a high vaginal swab was taken for culture for yeasts, and clinical findings recorded.

The purpose of this investigation on precipitins to C. albicans in pregnant women, with and without symptoms of candida vulvovaginitis, was to determine whether precipitins to the polysaccharide and protein antigens of C. albicans occur in pregnant women and if so, to assess the relationship between the demonstration of precipitins, the presence of the fungus, and clinical thrush. The distribution and significance of candida precipitins in newborn infants was also studied in a small number of cases.

MATERIALS AND METHODS

Pregnant Women

1,085 pregnant women attending the antenatal clinics of Queen Charlotte's Maternity Hospital were studied prospectively. Cyclostyled sheets were used for recording histories, clinical observations and laboratory findings (see Appendix I). Direct questions regarding pruritis vulvae and burning sensation were asked and recorded. Clinical signs of discharge and adherent white plaques were noted. With the patient in the lithotomy position a sterile speculum was inserted and a high vaginal swab (HVS) taken.

207 of these patients were followed up at 36 weeks of pregnancy and a second high vaginal swab taken.

A report is made of the distribution of yeast species in the vagina of these patients; the occurrence of yeast species is correlated with clinical signs and symptoms of vulvovaginitis. This distribution of vaginal yeasts is compared with the distribution occurring in a group of 1,031 pregnant women studied retrospectively, at the same hospital. A report is also made of the distribution of yeasts in high vaginal swabs sent for diagnosis of vulvovaginitis, from a retrospective study of laboratory records over a 5 year period (1966-1970).

Newborn Babies

648 babies born to the pregnant women studied prospectively, had an oral and an anal swab taken within the first three days of life. A dry cotton swab on an applicator was rolled gently over

the tongue and buccal mucosa of the cheeks, while the mouth of the infant was kept open by depressing the chin with one hand. A dry cotton swab on an applicator was introduced into the anal canal to collect faecal material.

605 of these babies had a second oral and anal swab taken at the age of one week (43 of the babies studied in the first three days had been discharged by the 7th day).

Microbiological methods

Direct Microscopy

Smears were made on glass slides from the swabs. Smears were dried, heat fixed, stained by Gram's method and examined microscopically for Gram-positive yeasts and hyphae (Fig. 8).

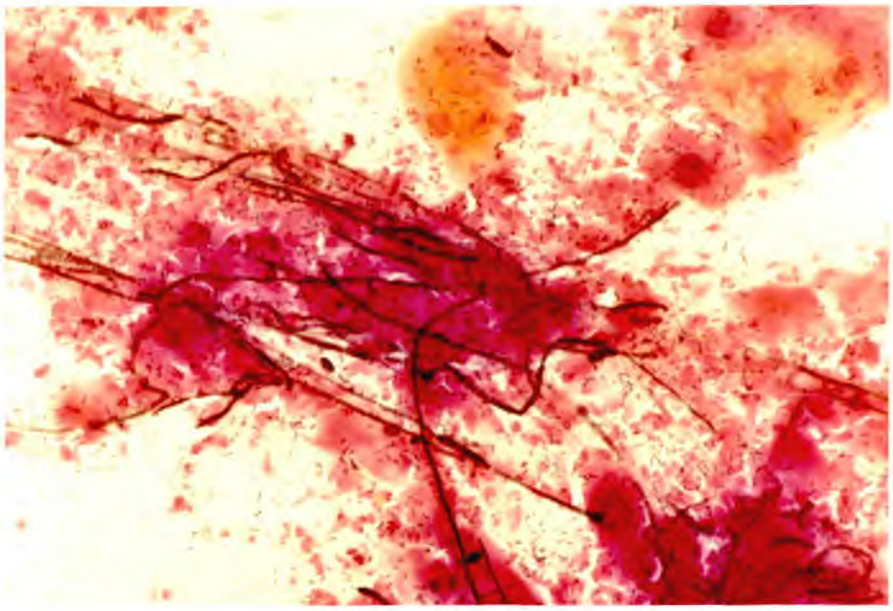
Culture

The swabs were inoculated on Sabouraud's glucose peptone agar medium (Appendix II) and incubated at 37°C overnight. A smear was made from the resulting growth and stained by Gram's method. Different colonial types of yeast-like fungi were picked off and purified on Sabouraud's medium. Incubation was carried out at 37°C for 24 hours. The primary culture plates were observed up to one week. The results of yeast growth were recorded as negative or positive.

Identification of yeasts (see Appendix II)

1. Formation of chlamydospores and hyphae

A single colony was picked up from the pure culture plate, using a sterile needle on a holder. This was inoculated in three lines on corn meal agar with added 10 per cent Tween 80,



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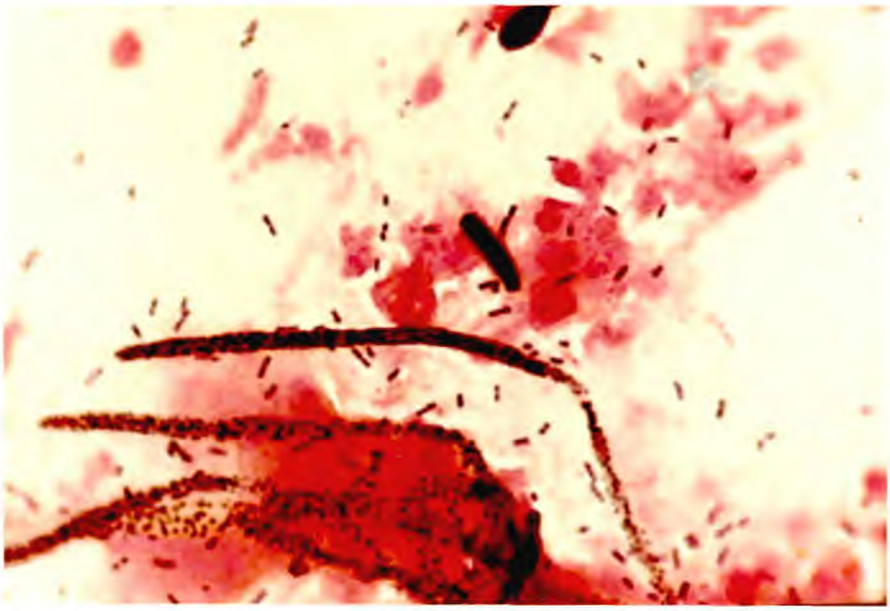


x 1,500

Fig. 8. Yeast cells and hyphae of C. albicans in vaginal smear of a pregnant woman with candida vaginitis. Gram stained.

covered with a sterile coverslip and incubated at 25°C. Daily examinations of the inoculated lines, under the low power of the microscope, for chlamydospores and hyphae, were made for at least one week.

2. Formation of germ tubes in serum

A single colony was inoculated into 0.5 ml. of horse serum in a bijou bottle, to give a faintly turbid suspension and incubated at 37°C for 2 hours. A drop was placed on a clean slide, covered with a coverslip and examined under the high power objective of the microscope for the production of germ tubes.

3. Auxanograms

Further identification was made by determining assimilation patterns of carbon and nitrogen compounds by the auxanogram method according to the system of Lodder and Kreger-van Rij.

(a) Carbon compounds

A synthetic basal medium devoid of a source of carbon was melted and cooled to 45°C and a drop of vitamin solution (as described by Lodder and Kreger-van Rij, 1952) added to it. An opalescent suspension of the yeast was made in normal saline and 2 ml. placed in a petri dish. The molten auxanogram medium was poured into the petri dish and the liquids were mixed well and allowed to set. Coloured filter paper discs which had

been impregnated beforehand, in a 5 per cent solution of glucose, maltose, sucrose, galactose and lactose respectively were placed on the surface of the agar. The plates were incubated at 37°C overnight and examined for zones of growth round each disc. A zone of growth round a disc indicated the ability of the yeast to assimilate the carbon compound on the disc.

(b) Nitrogen compounds

A basal medium not containing a source of nitrogen was used, and assimilation of 10 per cent potassium nitrate was tested for zones of growth as in 3 (a). A filter paper disc impregnated with 2 per cent asparagine was used as a control. (See Appendix II for assimilation patterns of Candida species isolated from clinical material and of T. glabrata and S. cerevisiae).

4. Fermentation reactions

These were used in the further identification of yeasts other than C. albicans and C. stellatoidea. Tubes containing 5 ml. amounts of glucose, maltose, sucrose, galactose, lactose (3%) and raffinose solutions (6%) in peptone water with phenol red indicator, were inoculated with 1 ml. of an opalescent suspension of the yeast. Incubation was carried out at 37°C for 10 days. Gas production during fermentation was collected in a Durham's tube. The production of acid and gas was considered a positive fermentation reaction. (See Appendix II for fermentation patterns of some Candida species, T. glabrata and S. cerevisiae).

5. Sporulation studies

Yeasts which did not form hyphae on cornmeal agar, were

inoculated on sodium acetate medium (Appendix II) and incubated at 37°C for up to 25 days, examining the culture for sporulation, daily for the first 5 days and at 5 day intervals thereafter.

Demonstration of ascospores

A smear of the yeast culture from sodium acetate medium was made on a slide and heat fixed. 2.5 per cent malachite green was poured on the smear and the slide heated to near boiling, for 2 minutes, washed in tap water and counter stained with 0.5 per cent safranin for 1 minute. The slide was washed in tap water, dried and examined under oil immersion for green staining spores (Fig. 9).

Serological methods

Sera

Blood was collected from the following groups of patients:-

(a) 3 ml. venous blood was collected from 306 pregnant women in the first trimester, at the same time as the high vaginal swab.

(b) 5 ml. of cord blood was collected from 34 babies born to these patients.

(c) 3 ml. venous blood was collected from 30 pregnant women with clinical and mycological evidence of vaginal candidosis.

(d) Six babies who had demonstrable candida precipitins in their cord blood, had 2 ml. venous blood taken after 3 months of age.

(e) Four babies with oral thrush and two babies with skin thrush had 2 ml. venous blood taken.

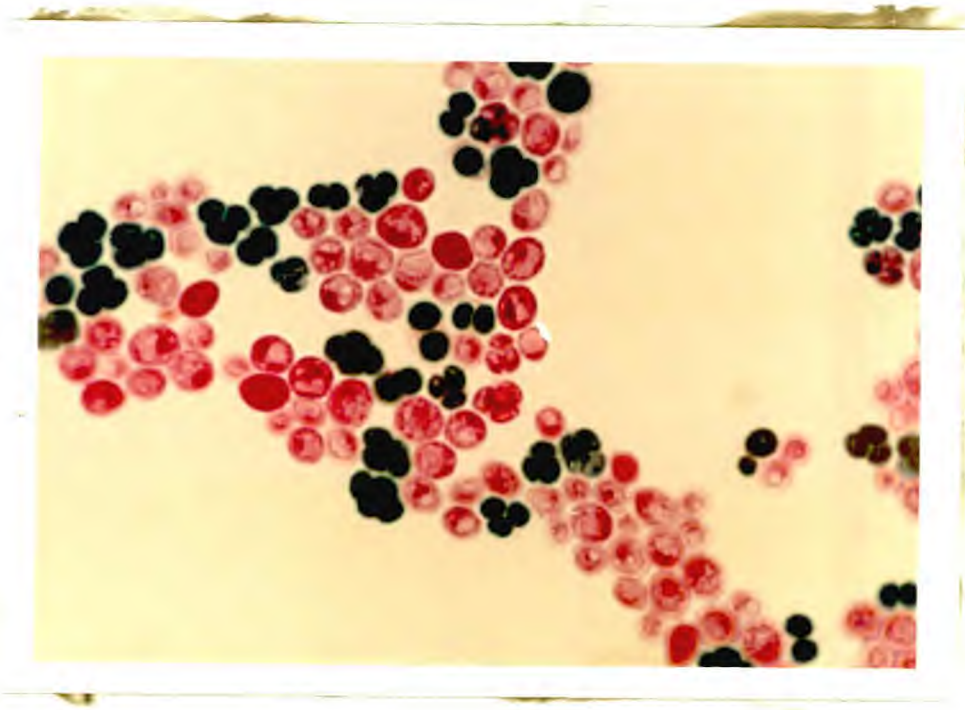


Fig. 9. Ascospores of S. cerevisiae from 3 day culture at 25°C on sodium acetate medium. Stained with malachite green x 1,500.

The blood was allowed to clot, serum separated, 0.1 per cent sodium azide added as preservative and stored at 4°C until tested.

Antigens

C. albicans group A, strain 3153 maintained at the Mycological Reference Laboratory was used throughout in the preparation of antigens and was kindly provided by the late Dr. I.G. Murray (London School of Hygiene and Tropical Medicine).

The antigens tested were:-

(a) Cell wall, mannan;

(b) Somatic antigen;

(c) Culture filtrate.

Method of culture of C. albicans cells

C. albicans was grown on a Sabouraud's dextrose agar plate for 24 hours. 1 ml. sterile saline was used to wash the surface growth and this was inoculated into 1 litre of modified Sabouraud's broth (2% glucose, 1% peptone, pH 6.8) in a 5 litre flask. The flask was incubated at 37°C in a water bath, with shaking for 24 hours. The culture was centrifuged at 3,000 r.p.m. to separate the yeast cells.

(a) Method of preparation and purification of cell wall mannan

Fehling extraction (Peat et al., 1961) as modified by Faux (1968).

C. albicans cells were washed twice in 10 ml. distilled water, by centrifugation. 20 gm. of yeast cells (wet weight) were autoclaved with 0.02 M citrate buffer, pH 7 (50 ml.) at 121°C for 2½ hours. The gelatinous precipitate was removed on centrifugation.

The supernatant was preserved. The precipitate was autoclaved again with distilled water (100 ml.) at 121°C for 2½ hours. The precipitate was removed on centrifugation. The supernatants were combined and concentrated under reduced pressure to 40 ml. The solution was made up to normal with glacial acetic acid (2.5 ml. acetic acid was added to 40 ml. solution). The gelatinous precipitate formed was removed by centrifugation and the cloudy supernatant preserved. The precipitate was washed with 10 ml. N acetic acid. The cloudy solution and washings were combined and neutralized with 6 N sodium hydroxide. The clear solution obtained was concentrated under reduced pressure to 25 ml. Ethanol (50 ml.) was added to this solution at 40°C and left overnight. The resultant precipitate was washed twice with 60 per cent aqueous ethanol. The precipitate was dissolved in distilled water (25 ml.) and made alkaline with 6 N sodium hydroxide. Freshly prepared Fehling solution (45 ml.) was added gradually. A greyish flocculent precipitate appeared on scraping the sides of the container. Excess Fehling solution causes the precipitate to dissolve. The precipitate was washed twice with warm distilled water and suspended in distilled water (5 ml.). Concentrated hydrochloric acid was added dropwise till the precipitate dissolved and the resultant solution was acid, when tested with litmus paper. This solution was filtered through a sintered glass funnel into 15 ml. ethanol at 4°C and left overnight at 4°C. The white precipitate obtained was removed by centrifugation and washed with 60 per cent ethanol. The precipitate was dissolved in distilled water (5 ml.) and reprecipitated with ethanol (15 ml.) at 4°C. The precipitate was removed on centrifugation, and washed with 60 per cent ethanol, dissolved in distilled water (5 ml.), dialysed overnight in distilled water, and freeze dried. The freeze dried material (crude mannan) was dissolved in distilled water (5 ml.)

and purified by passage through a Sephadex G 25 column (Pharmacia, Upsala, Sweden).

Sephadex G 25 gel filtration

Preparation of column

42 gm. Sephadex G 25 was washed in 200 ml. distilled water in a beaker, and allowed to settle at room temperature for 1 hour. The supernatant was decanted to remove the "fines". Distilled water was added to make up to the original volume, the contents stirred with a glass rod and allowed to settle. This procedure was repeated three times more and finally left overnight to settle. The supernatant was removed the next day, and distilled water added to make up to the original volume. The contents were mixed and packed into a vertical glass column (2.6 cm. x 38 cm.). The column was allowed to settle for 2 hours, removing any supernatant at the top of the column of Sephadex.

5 ml. of crude mannan solution was carefully applied to the top of the Sephadex column and allowed to diffuse in completely. Distilled water (5 ml.) was applied carefully to wash down the sample of crude mannan. More distilled water was added to fill the glass column and 10 ml. fractions of the effluent were collected at room temperature. A column of distilled water, above the Sephadex column was maintained constantly, as fractions were collected. The fractions giving a positive Molisch test for polysaccharide were pooled and freeze dried to give a white powder (mannan).

Molisch test for polysaccharide (Kabat and Meyer)

To 0.2 - 0.5 ml. of solution to be tested, 1 drop of 2 per cent α naphthol in ethanol was added. Concentrated sulphuric acid (0.5 - 1 ml.) was added slowly down the side of the tube, to form a lower layer. The formation of a violet ring at the inter face, indicates the presence of carbohydrate. Visual examination of the depth of colour obtained after the thorough mixing of the test reactants was used as a guide in the elution of polysaccharide.

Yield of Fehling Extraction

Wet weight of cells	Crude mannan	mannan after Sephadex G 25
20 gm.	32 mgm.	23 mgm.

(b) Method of preparation of Somatic antigen by Mickle disintegration (Stallybrass, 1964).

C. albicans cells were washed twice in 0.85 per cent saline. The cells were suspended in 0.85 per cent saline in polythene tubes, at a packed cell volume of 40 per cent, by haematocrit estimation. The cell suspension was subjected to Mickle disintegration with an equal volume of 1/16 inch glass beads, for 20 minutes, at maximum amplitude. The contents after Mickle disintegration were centrifuged at 3,000 r.p.m. to recover the liquid phase, which was stored at -20°C overnight. The contents were thawed and ultracentrifuged at 35G for 1 hour and the resultant supernatant was freeze dried as antigen 'C'. This antigen was reconstituted in N saline at 50 mgm./ml. The optimal concentration of the antigen for double diffusion tests was determined by testing different dilutions of the antigen against a serum from a hyperimmunized rabbit and a known positive

human serum. The optimal concentration of 'C' antigen most suitable for testing human serum was lower than the concentration suitable for testing positive rabbit serum.

(c) Method of preparation and purification of culture filtrate of C. albicans (Faux, 1968)

C. albicans (3153) was grown in 1 litre of Sabouraud's broth in a 5 litre flask, in a 37°C water bath for 7 days with aeration by an air pump. The culture was centrifuged, supernatant Seitz filtered and dialysed overnight in running tap water using 32/32 visking tubing. The retentate was freeze dried to give crude culture filtrate. 1 gm. of crude culture filtrate was dissolved in 20 ml. distilled water, centrifuged to remove undissolved particles and filtered through filter paper. 5 ml. of this solution was applied to a Sephadex G 25 column to remove low molecular weight particles which remained after dialysis. 10 ml. fractions were collected and the presence of polysaccharide in each fraction detected by the Molisch test, and protein detected by the Biuret test. Fractions giving a positive Molisch test and Biuret test were pooled together and freeze dried as culture filtrate antigen. The antigenic activity of the culture filtrate antigen was tested by double diffusion against known positive human sera and the optimum concentration for use determined.

Biuret test for protein

To 0.2 - 0.5 ml. of test solution, an equal volume of Biuret reagent ($CuSO_4$ $5H_2O$ 0.3%

Sodium potassium tartrate 0.9%

Potassium iodide 0.5% in 0.2M sodium hydroxide)

was added. A pink colour on visual examination indicates the presence of protein.

Immunological Procedures

1. Agar gel diffusion methods

(a) Double diffusion

3 gm. of Ionagar II (Oxoid) was dissolved in 100 ml. isotonic saline, by steaming for 45 minutes. An equal volume of warm, McIlvaine's citric acid phosphate buffer, pH 7, with 0.1 per cent sodium azide was added to the molten agar and mixed well. 46.5 ml. of buffered 1.5 per cent agar was poured into flat bottomed, glass petri dishes (diameter 14 cm.) to a depth of 3.5 mm. The agar was allowed to set overnight and a pattern of wells was cut with a Shandon gel cutter, such that the central serum well (diameter 12.5 mm.) was surrounded by 6 peripheral wells (diameter 4 mm.). The distance between the periphery of the central and peripheral wells was 6 mm. The central serum well was filled with the serum to be tested and the antigens placed in the peripheral wells. Five sera were tested on each plate, a solution of 1 per cent alcian blue being used to write particulars on the agar surface. The plates were incubated at 28°C for 7 days and observed daily for precipitin lines. The gel was removed from the plate and washed in normal saline with 0.05 per cent sodium azide, for 5 days, dried between sheets of Whatman 3 mm. filter paper at room temperature, on a flat surface, overnight. The dried agar layer was removed from the filter paper by soaking in tap water, and then stained with azocarmine B (Varley, 1962). The agar layer was stained with azocarmine B for 15 minutes, washed twice in 7 per cent acetic acid till the background was colourless, placed in 10 per cent glycerol in ethanol for 10 minutes and dried between filter

paper. Final results of precipitin lines were recorded from stained plates.

(b) Immunoelectrophoresis

An adaptation of the micro method of Scheidegger (1955) was used. Chemically clean glass slides (8 cm. x 8 cm.) were dried and coated with a thin film of 0.1 per cent aqueous agar. 1 gm. of Ionagar II (Oxoid) was dissolved in 25 ml. barbitone buffer (pH 8.2) and 75 ml. distilled water and 20 ml. amounts dispensed in universal containers. 7.5 ml. of 1 per cent agar in barbitone buffer was poured on the coated slides to form a layer 1 mm. thick. A central well 2 mm. diameter and lateral troughs 1 mm. x 50 mm. were cut in the agar. Precipitin positive serum was placed in the central well and electrophoresis allowed to proceed for 1½ hours with a potential drop of 4 volts/cm. across the slide. The potential drop was measured by a voltmeter. After electrophoresis, mannan antigen was placed in one lateral trough and goat-antihuman globulin serum (Burroughs-Wellcome) in the other trough. The precipitation reaction was allowed to proceed at 28°C for 24-36 hours, in a humid atmosphere. The slides were washed in saline with 0.05 per cent sodium azide, overnight, and then dried with a layer of filter paper (Whatman 3 mm.) pressed over the surface of the agar. The dried slides were stained with 0.1 per cent Naphthalene black (Amidoschwarz stain) in a solvent mixture of methanol: distilled water: glacial acetic acid 5:4:1, for 30 minutes. The slides were washed in successive changes of the solvent mixture until

the background was colourless and then blotted dry with filter paper. This test was employed for electrophoresis of precipitin positive test sera to show the production of precipitation reactions in the Ig G region against mannan antigen of C. albicans.

(c) Immunoosmophoresis (Jameson, 1968)

1 gm. Ionagar II (Oxoid) was dissolved in 40 ml. of barbitone buffer, pH 8.7, and 60 ml. deionised water. The agar was steamed for 1 hour and 20 ml. amounts dispensed in Universal containers. Chemically clean, dry microscope slides were coated with 1 per cent aqueous molten agar, allowed to set and dried in an incubator at 37°C. The slides were placed on a level surface, with coated surface uppermost. 2.25 ml. of molten buffered agar was poured on each side, spread evenly, and allowed to set. Two longitudinal slots 3/4 in. long and 1/8 in. wide were cut in the agar, and the agar removed from them. A strip of Whatman 3 mm. paper (3/4 in. x 3/8 in.) was immersed in a solution of mannan antigen (2 mgm./ml.), excess fluid was drained off and the antigen strip was laid transversely across the slide, at a distance of 1/2 in. from the end of the slots (Fig. 10).

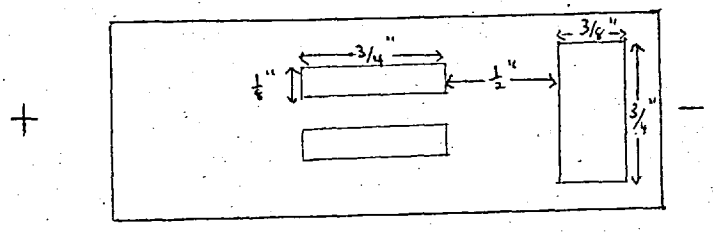


Fig. 10

The test sera were pipetted into the slots. The slide was placed across the bridges of a horizontal, electrophoresis tank (Shandon Scientific Company) such that the antigen strip was nearest the cathode. Barbitone buffer, pH 8.7 diluted with an equal volume of deionised water was used as tank buffer. Filter paper wicks were used to connect the agar on the slide and the buffer in the tank. Electrophoresis was allowed to proceed at a current of 1mA/slide, for 18 hours. On completion of osmophoresis the antigen strip was removed by rinsing in water. The slides were washed in isotonic saline with 0.05 per cent sodium azide overnight and dried. The slides were stained with 0.1 per cent naphthalene black as in immunoelectrophoresis. Immunoosmophoresis was employed to determine whether it was a more sensitive method for detecting precipitins to C. albicans, than Ouchterlony double diffusion in agar gel. Ten sera of patients with clinical and mycological evidence of vaginal candidosis, who had no detectable precipitins when tested by the Ouchterlony method, were also tested by immunoosmophoresis.

2. Agglutination

(a) Preparation of yeast suspension

C. albicans was grown on Sabouraud's agar plates for 24 hours at 37°C, harvested and washed in 0.9 percent saline. Cells were suspended in N. saline to give a packed cell volume of 2 per cent by haematocrit estimation. Sufficient 40 per cent formaldehyde was added to give a final concentration of 0.08 per cent and left for 24 hours. Sterility tests on the sus-

pensions were carried out. Cells were washed and resuspended in saline to give a concentration of 2 per cent P.C.V.

(b) Agglutination test (Murray and Buckley, 1966)

Perspex trays containing 80 cups were used. 0.4 ml. of double dilutions of serum was used in each cup and 0.02 ml. of 2 per cent yeast suspension added. The plates were incubated at 37°C for 2 hours and agglutination readings made. The plates were kept at 4°C overnight and a second reading taken. This test was employed to compare agglutinin titres with the presence of precipitins in pregnant women.

RESULTS

PROSPECTIVE STUDY ON PREGNANT WOMEN

Results of culture of high vaginal swabs

(a) In the first trimester of pregnancy

1,085 pregnant women had a high vaginal swab taken on one occasion during the first trimester of pregnancy and cultured for yeasts (Table 8).

202 (18.6%) of these pregnant women harboured yeasts in the vagina.

420 (39%) of the total number complained of one or more symptoms of vulvovaginitis while 665 (61%) did not complain of symptoms. Of the 420 with symptoms, only 105 (25%) carried yeasts in the vagina, while 75% had no yeasts. Of the 665 who did not complain of symptoms, 97 (14%) carried yeasts in the vagina.

Of the 202 women harbouring yeasts in the vagina, 105 (9.6%) complained of symptoms while 97 (9%) did not.

(b) At 36 weeks gestation

207 of the pregnant women were followed and had a second vaginal swab taken at 36 weeks of pregnancy. The results of culture are given in Table 9.

31 (14.9%) of them harboured yeasts in the vagina at 36 weeks.

Of these 12 (5.8%) had yeasts on the first occasion as well, while 19 (9.1%) did not.

TABLE 8

Carriage of yeasts in the vagina of 1,085 pregnant women in the first trimester

	Number	Percentage
Patients with symptoms of vulvovaginitis	420	39
Patients without any symptoms	665	61
Patients with yeasts + in HVS	202	18.6
Patients with yeasts and symptoms +	105	9.6
Patients with yeasts + no symptoms	97	9

TABLE 9

Incidence of yeasts in the vagina of 207 pregnant women at 36 weeks

	Number	Percentage
Patients with yeasts + at 36 weeks	31	14.9
Patients with yeasts + in 1st trimester as well	12	5.8
Patients with yeasts only at 36 weeks	19	9.1

Of the 176 who had no yeasts at 36 weeks, six had positive cultures in the first trimester and all six had been treated with nystatin pessaries.

(c) Species of yeasts isolated

(i) In the first trimester

202 out of 1,085 pregnant women in the first trimester had yeasts isolated from a high vaginal swab culture (Table 10). Of these 202 positive swabs, 165 (81.6%) were C. albicans. The second commonest yeast isolated was Torulopsis glabrata - in 25 (12.3%) of cases. The remaining 6.1 per cent isolates consisted of other Candida species, Saccharomyces cerevisiae and Rhodotorula mucilaginosa. The other Candida species isolated were C. tropicalis, 4 (1.9%) and C. stellatoidea, 2 (0.99%). Of the remaining 6 yeasts isolated, 4 (1.9%) were S. cerevisiae, 1 was R. mucilaginosa and 1 was not identifiable by me, nor by Miss B.G.S. Leask or by Dr. Helen Buckley (see Appendix IV).

(ii) At 36 weeks

31 out of the 207 pregnant women at 36 weeks had yeasts isolated from a high vaginal swab culture (Table 11). Of these positive swabs, 28 (90.3%) were C. albicans and 3 (9.7%) were T. glabrata.

(d) Incidence and correlation of symptoms with species of yeasts isolated in 105 pregnant women with yeast vulvovaginitis (Table 12).

Of the 202 patients harbouring yeasts in the vagina in

TABLE 10

The distribution of yeasts in the vagina of 1,085 pregnant women in the first trimester

		Total	Percentage
Yeasts cultured		202	18.6
Species isolated:	C. albicans	165	81.6
	C. tropicalis	4	1.9
	C. stellatoidea	2	0.99
	T. glabrata	25	12.3
	S. cerevisiae	4	1.9
	Rhodotorula mucilaginosa	1	0.49
	Unclassified spp.	1	0.49

TABLE 11

The distribution of yeasts in the vagina of 207 pregnant women at 36 weeks

		Total	Percentage
Yeasts cultured		31	14.9
Species isolated:	C. albicans	28	90.3
	T. glabrata	3	9.7

TABLE 12

Relationship of symptoms to species isolated in 105 pregnant women with yeast associated vulvovaginitis

Symptoms	No. of patients	Species of yeasts					
		C. albicans	C. tropicalis	C. stellatoidea	T. glabrata	R. mucilaginosa	S. cerevisiae
Pruritus	4	2	-	-	2	-	-
Discharge	60	45	2	1	8	1	3
Burning sensation	-	-	-	-	-	-	-
White patches	2	2	-	-	-	-	-
Pruritus & discharge	21	20	-	-	1	-	-
White patches & discharge	7	7	-	-	-	-	-
Discharge, pruritus, burning sensation	11	11	-	-	-	-	-
Total:-	105	87	2	1	11	1	3

the first trimester, only 105 complained of symptoms of vulvo-vaginitis. Vaginal discharge was the commonest, occurring in 99 (94%) either as the only symptom or accompanied by pruritis or white plaques. 36 (34%) patients complained of pruritus alone or together with other symptoms. White patches of vaginal thrush were noted in only 9 (8.5%) of patients.

Of the 105 patients complaining of symptoms, 87 (82.8%) had C. albicans in the vagina and 11 (10.4%) had T. glabrata.

(e) Correlation of vaginal discharge with different species of yeasts isolated (Table 13).

Of the 99 patients complaining of vaginal discharge, 83 harboured C. albicans and 9 harboured T. glabrata. 2 patients had C. tropicalis and 1 had C. stellatoidea. The remaining 3 patients with vaginal discharge had S. cerevisiae in the vagina.

(f) Correlation of pruritus with different species of yeasts isolated (Table 14).

36 patients complained of pruritus of whom 33 were found to have C. albicans and 3 had T. glabrata. The other species of Candida were not associated with pruritus.

(g) All 9 patients with white patches of vaginal thrush had C. albicans.

(h) Comparison of the distribution of yeasts in patients with and without symptoms of vulvovaginitis (Table 15).

C. albicans was isolated in 82.8 per cent of patients with vulvovaginitis and in 80.4 per cent without. T. glabrata was isolated in 10.4 per cent and 14.4 per cent respectively in the two groups.

TABLE 13

Correlation of vaginal discharge with different species of yeasts isolated

Symptoms	No. of patients	Species of yeasts					
		C. albicans	C. tropicalis	C. stellatoidea	T. glabrata	R. mucilaginosa	S. cerevisiae
Discharge only	60	45	2	1	8	1	3
Discharge & pruritus	21	20	-	-	1	-	-
Discharge & white patches	7	7	-	-	-	-	-
Discharge, pruritus & burning sensation	11	11	-	-	-	-	-
Total:-	99	83	2	1	9	1	3

TABLE 14

Correlation of pruritus with different species of yeasts isolated

Symptoms	No. of patients	Species of yeasts					
		C. albicans	C. tropicalis	C. stellatoidea	T. glabrata	R. mucilaginosa	S. cerevisiae
Pruritus only	4	2	-	-	2	-	-
Pruritus & discharge	21	20	-	-	1	-	-
Discharge, pruritus & burning sensation	11	11	-	-	-	-	-
Totals-	36	33	-	-	3	-	-

TABLE 15

Distribution of yeasts in patients with and without symptoms of vulvovaginitis

	With vaginitis	Symptomless
C. albicans	87 (82.8%)	78 (80.4%)
C. tropicalis	2	2
C. stellatoidea	1	1
T. glabrata	11 (10.4%)	14 (14.4%)
R. mucilaginosa	1	-
S. cerevisiae	3	1
Unclassified species	-	1
Total:-	105	97

RESULTS OF RETROSPECTIVE STUDY ON PREGNANT WOMEN AT QUEEN CHARLOTTE'S MATERNITY HOSPITAL

During 1967 and 1968, two groups of patients were studied by workers at Queen Charlotte's Maternity Hospital, London (Hurley et al., 1971). 511 unselected pregnant women booking at the antenatal clinics, between November and January, and 520 between March and May were studied. High vaginal swabs were taken at the first antenatal visit.

Table 16 shows the incidence of yeasts and Trichomonas vaginalis in 1,031 pregnant women.

The incidence of yeasts in the vagina in the first trimester of pregnancy was 17 per cent - the incidence in spring being higher (20%) than in winter (14%). Seventeen per cent of these women carried only yeasts, while 0.8 per cent had T. vaginalis as well.

Table 17 shows the percentage distribution of yeast species in the isolates examined.

139 (76%) of the isolates were C. albicans.

27 (15%) were T. glabrata.

TABLE 16

TABLE

Incidence of Trichomonas vaginalis

	No. of patients Nov 1967-Jan 1968	Number of patients Mar-May 1968
Yeasts only present	71	103
T. vaginalis only present	30	17
T. vaginalis + yeasts	6	2
Total examined:-	511	520

16

and yeasts in pregnant women

Total number of patients	Incidence		
	% winter	% spring	Total
174	14%	20%	17%
47	6%	3%	5%
8	1%	0.4%	0.8%
1,031			

TABLE 17

TABLE

Identification and percentage distribution

	November 1967 to January 1968 (511 patients)
Total number of vaginal swabs containing yeasts	77
C. albicans	57 (74%)
T. glabrata	14 (18%)
C. albicans and T. glabrata	-
S. cerevisiae	1
C. stellatoidea	2
C. tropicalis	1
C. parapsilosis	1
T. holmii	1
T. inconspicua	-

17

of yeast species from 1,031 pregnant women

March to May 1968 (520 patients)	Total (1,031 patients)	Percentage distribution of yeasts
105	182	
82 (78%)	139	76
13 (12%)	27	15
1	1	1
5	6	3
1	3	2
1	2	1
1	2	1
-	1	1
1	1	1

RESULTS OF RETROSPECTIVE STUDY OF RECORDS AT QUEEN CHARLOTTE'S MATERNITY HOSPITAL (1966-1970)

A study of records at Queen Charlotte's Maternity Hospital over the period 1966 to 1970 showed that 6,629 vaginal swabs were sent for diagnosis of vaginitis in pregnant women. Yeasts were isolated from 1,538 of them. There were 18,137 deliveries during this period, giving the incidence of mycotic vaginitis as 7.9 per cent.

Table 18 shows the distribution of yeasts in vaginal swabs sent as diagnostic specimens over the period 1966-1970. Yeasts were isolated from 1,538 of 6,629 vaginal swabs sent for diagnosis of vulvovaginitis. C. albicans was isolated in 93 - 95 per cent of specimens, T. glabrata in 3 - 5 per cent. Five other Candida species, two other species of Torulopsis, S. cerevisiae and Rhodotorula glutinis were isolated from the rest. The percentage distribution of the yeasts remained remarkably constant over the five year period.

Table 19a and b compare the distribution of yeasts isolated from consecutive pregnant women with that obtaining from specimens sent for diagnosis during the same period, in 1967, 1968 and 1969. While C. albicans was isolated from 90 - 97 per cent of high vaginal swabs sent for diagnosis, it was isolated from 74 - 78 per cent of specimens taken from unselected pregnant women. T. glabrata was isolated from 2 - 8 per cent of diagnostic specimens and 12 - 18 per cent of unselected patients.

TABLE 18

TABLE

Identification of yeasts

	1966	% of Total
Total number of vaginal swabs containing yeasts	270	
Total *C. albicans*	257	95%
C. albicans only	253	
C. albicans & *C. tropicalis*	-	
C. albicans & *C. pseudotropicalis*	-	
C. albicans & *C. krusei*	-	
C. albicans & *S. cerevisiae*	-	
C. albicans & *T. glabrata*	4	
C. stellatoidea	-	
C. tropicalis	-	
C. pseudotropicalis	-	
C. parapsilosis	-	
C. krusei	1	
T. glabrata	9	3%
T. inconspicua	1	
T. holmii	-	
S. cerevisiae	2	
Rhodotorula glutinis & *Cryptococcus diffluens*	-	

18

in vaginal swabs (1966-1970)

1967	% of Total	1968	% of Total	1969	% of Total	1970	% of Total
262		276		293		437	
245	94%	261	95%	272	93%	411	94%
239		255		262		399	
1		-		-		-	
-		-		-		1	
-		-		-		1	
-		1		-		1	
5		5		10		10	
1		5		3		2	
2		-		1		-	
-		-		-		1	
-		-		1		2	
-		-		-		-	
10	4%	9	3%	15	5%	16	4%
-		-		1		-	
1		-		-		-	
2		1		-		5	
1		-		-		-	

TABLE 19a

TABLE

Comparison of percentage distribution of diagnostic specimens and from consecutive

S P E C I E S	SPECIMENS (HVS) Mar-May 1967	
TOTAL NUMBER OF VAGINAL SWABS CONTAINING YEASTS	59	
C. albicans	56 (95%)	
C. albicans & T. glabrata	-	
Total specimens containing C. albicans	56 (95%)	
T. glabrata	1 (2%)	
C. stellatoidea	-	
C. tropicalis	1	
C. parapsilosis	-	
R. glutinis & Cr. diffluens	1	(3%)
T. inconspicua	-	
S. cerevisiae	-	

19a

Yeast species in pregnant women isolated from patients at booking (March - May)

SENT FOR DIAGNOSIS		PROSPECTIVE SURVEY (HVS) OF 520 PATIENTS
Mar-May 1968	Mar-May 1969	Mar-May 1968
54	63	105
49 (91%)	60 (95%)	82 (78%)
2	1	1
51 (95%)	61 (97%)	83 (79%)
2 (4%)	2 (3%)	13 (12%)
1	-	1
-	-	1
-	-	1
- (2%)	- (0%)	- (9%)
-	-	1
-	-	5

TABLE 19b

TABLE

Comparison of percentage distribution of
from diagnostic specimens and from consecutive

S P E C I E S	SPECIMENS (HVS) Nov 1966 to Jan 1967	
TOTAL NUMBER OF VAGINAL SWABS CONTAINING YEASTS	86	
Candida albicans	78	(91%)
C. albicans & T. glabrata	1	
Total specimens containing C. albicans	79	(92%)
T. glabrata	5	(6%)
C. stellatoidea	-	
C. tropicalis	-	
C. parapsilosis	-	
C. krusei	1	(2%)
T. holmii	1	
Saccharomyces cerevisiae	-	

19b

yeast species in pregnant women isolated patients at booking (November - January)

SENT FOR DIAGNOSIS		PROSPECTIVE SURVEY (HVS) OF 511 PATIENTS	
Nov 1967 to Jan 1968	Nov 1968 to Jan 1969	Nov 1967 - Jan 1968	
77	84	77	
69 (90%)	74 (88%)	57 (74%)	
-	3	-	
69 (90%)	77 (92%)	57 (74%)	
6 (8%)	6 (7%)	14 (18%)	
1	1	2	
-	-	1	
-	-	1	
- (3%)	- (1%)	- (8%)	
-	-	1	
1	-	1	

PROSPECTIVE STUDY ON THE NEWBORN

Results of culture of oral and anal swabs from the newborn

(a) Up to 3 days of life

648 babies born to the 1,085 pregnant women studied prospectively had an oral and an anal swab taken routinely within the first three days of birth, and cultured for yeasts. *Candida* species were recovered from the mouth in only 6 of 648 babies (i.e. 0.84%) and from the anal canal in 8 of 648 babies (i.e. 1.2%).

C. albicans was isolated from all 6 mouth swabs and from 7 of the 8 positive anal swabs. One anal swab grew *C. tropicalis*. Of the 6 babies with *C. albicans* in the mouth, 4 (66%) were born to mothers who harboured *C. albicans* in the vagina during pregnancy.

Of the 8 babies with positive anal swabs, 4 (50%) were born to mothers with *Candida* species in the vagina. The baby with *C. tropicalis* in the anal swab, was the offspring of a mother who harboured *C. tropicalis* in the vagina during pregnancy.

(b) At one week

605 babies had a second oral and anal swab taken at the age of one week and cultured for yeasts. *Candida* species were recovered from the mouth in 13 out of the 605 (i.e. 2.1%) and from the anal canal in 25 out of the 605 (i.e. 4.1%).

C. albicans was isolated from all the positive mouth

swabs and from 22 out of 25 anal swabs. Two out of the 25 anal swabs grew C. parapsilosis, and from the remaining one, T. glabrata was isolated.

Of the 13 babies with a positive mouth swab, 8 (61%) were born to mothers who had C. albicans in the vagina during pregnancy.

Of the 25 babies with a positive anal swab 18 (72%) were born to mothers who had Candida species in the vagina.

The overall incidence of C. albicans by the 10th day in the mouth of newborn infants at Queen Charlotte's Maternity Hospital was 1.5 per cent, and in the anal canal 2.3 per cent.

SEROLOGICAL STUDIES

I. Results of Precipitin Tests

1. Sera from pregnant women

Three groups were studied:-

Group A consisted of 190 sera collected from unselected pregnant women in the first trimester of pregnancy. Sera were collected on the same day that a high vaginal swab was taken for culture for yeasts. The unconcentrated sera were examined for precipitins using two antigens of C. albicans.

(i) purified cell wall, mannan;

(ii) somatic antigen containing cytoplasmic constituents and mannan.

Nature of precipitin bands

A broad, diffuse precipitin band of the 'H' type was obtained when some sera were tested against mannan antigen. A similar single broad, band was obtained with the somatic antigen placed in an adjacent peripheral well. The two broad bands showed a reaction of identity (Fig. 11).

Results

Of the 190 sera tested, 27 (14.2%) gave 'H' type precipitin reactions to the mannan antigen. All of these also gave a single broad band with the somatic antigen. None of the sera gave more than one band against the somatic antigen (Table 20).

Group B consisted of 116 sera collected from unselected pregnant women in the first trimester of pregnancy. Sera were collected at the same time as a high vaginal swab for culture for yeasts.

Fig. 11. The serum of a pregnant patient showing the 'H' type precipitin reactions when tested against somatic antigen and cell wall mannan.

(1) Cell wall mannan (1 mg./ml.)
(2) Somatic antigen (40 mg./ml.)

Note the common broad band ('H' type reaction) given with both antigens and no 'R' type reactions given to the somatic antigen (2).

TABLE 20

TABLE

Positive precipitin reactions

W O M E N

Group	No. tested	Precipitin test positive
		No.
A. Unselected pregnant women in 1st trimester	190	27
B. Unselected pregnant women in 1st trimester	116	22
C. Candida Vulvovaginitis	30	9

20

with unconcentrated sera using three antigens of C. albicans

TYPES OF SEROLOGICAL REACTIONS

Mannan antigen	Somatic antigen		Culture filtrate antigen	
'H'	'H'	'H' + R^1	'H' + R^2	R^2
No.	No.	No.	No.	No.
27 (14.2%)	27 (14.2%)	-	Not tested	
12 (10.3%)	Not tested		5 (4.3%)	5 (4.3%)
6 (20%)	6 (20%)	1 (3%)	3 (10%)	-

R^1 fine band to somatic antigen

R^2 fine band to culture filtrate antigen

'H' broad band to mannan antigen

Sera were tested using two antigens of C. albicans:-

(i) Purified cell wall, mannan;

(ii) Culture filtrate antigens.

Nature of precipitin bands

Two types of precipitin lines were recorded - fuzzy, broad 'H' type arcs associated with polysaccharide antigens and well defined 'R' type arcs associated with protein antigens.

Mannan antigen gave only 'H' type reactions while culture filtrate antigens gave both 'H' and 'R' type reactions with some sera.

Results

'H' and 'R' type precipitin reactions were detected in 22 of the 116 (18.9%) sera (Table 20).

12 out of the 22 (10.3%) gave broad band reactions to the mannan antigen only (Fig. 12).

5 out of the 22 (4.3%) gave only well defined, fine, precipitin reactions to the protein antigen of the culture filtrate (Fig. 13). The remaining 5 (4.3%) gave both broad precipitin lines to the mannan and fine lines to the protein antigen as well (Fig. 14).

Thus a total of 17 (14.6%) had precipitins to the mannan antigen, either alone or together with precipitins to the protein antigen, and a total of 10 (8.6%) had precipitins to the protein antigen of culture filtrate, either alone or together with precipitins to the mannan antigen.

Group C consisted of 30 sera collected from pregnant women with clinical and mycological evidence of vaginal candidosis due to

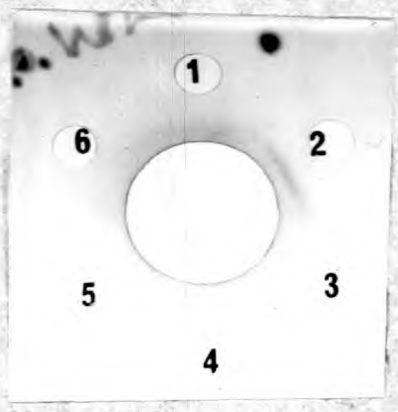


Fig. 12. Serum of a pregnant patient showing only an 'H' type precipitin reaction to the cell wall mannan antigen of C. albicans.

(1) Culture filtrate antigen (20 mg./ml.)

(2) Cell wall mannan (1 mg./ml.)

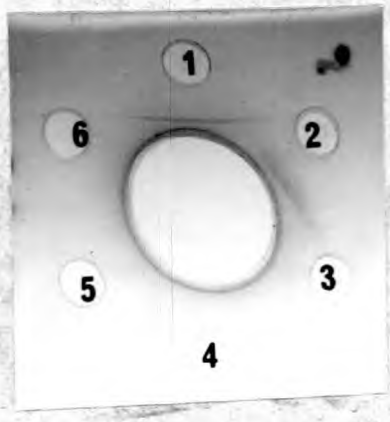


Fig. 13. Serum of a pregnant patient showing only a well defined 'R' type precipitin reaction to the culture filtrate antigen of C. albicans.

(1) Culture filtrate antigen (20 mg./ml.)
(2) Cell wall mannan (1 mg./ml.)

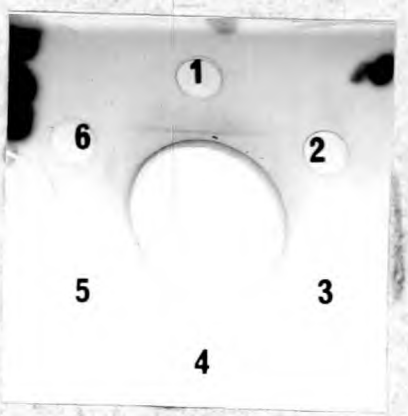


Fig. 14. Serum of a pregnant patient showing both 'H' and 'R'.types of precipitin reactions.

(1) Culture filtrate antigen (20 mg./ml.)
(2) Cell wall mannan (1 mg./ml.)

Note the broad diffuse band of the 'H' type to the mannan antigen and the well defined line of the 'R' type to the culture filtrate antigen. There is a reaction of non-identity between the 'H' and 'R' type bands.

C. albicans. Sera were examined for precipitins using three antigens:-

(i) purified cell wall mannan;

(ii) culture filtrate antigen;

(iii) somatic antigen.

Results

'H' and 'R' type precipitin reactions were detected in 9 of the 30 (30%) sera (Table 20).

6 (20%) gave broad precipitin bands to mannan antigen (Fig. 15).

3 out of the 9 (10%) gave fine precipitin bands to the protein antigen of the culture filtrate as well (Fig. 16).

1 out of the 9 gave a broad precipitin band to mannan antigen, a fine precipitin band (R^2) to protein antigen of the culture filtrate and a fine precipitin line (R^1) to a component (protein) in the somatic antigen. The fine precipitin band to the protein antigen of the culture filtrate did not show a reaction of identity with the fine band formed to the protein component contained in the somatic extract (Fig. 17).

2. Sera from newborn babies

Cord sera were collected from 34 babies born to mothers in Group B. Sera were tested with the two antigens used for testing the mothers' sera.

Six cord sera had precipitins to both polysaccharide and protein antigens (Fig. 18 a and b). All 6 babies were offspring born to women who had similar precipitins during pregnancy, suggesting that the precipitating antibodies against antigens of C. albicans were capable of crossing the placenta.

Fig. 15. Serum of a pregnant patient with candida vulvovaginitis showing only the 'H' type precipitin reaction when tested against the cell wall mannan antigen and somatic extract of C. albicans.

(1) Cell wall mannan (1 mg./ml.)
(2) Somatic extract (40 mg./ml.)
(3) Culture filtrate antigen (20 mg./ml.)

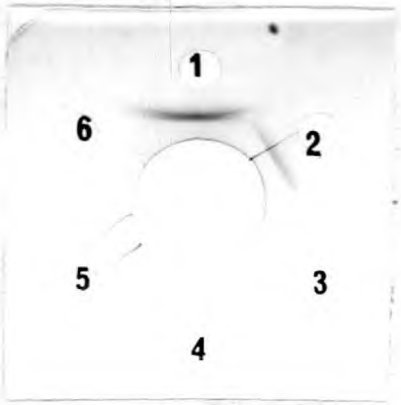


Fig. 16. Serum of a pregnant patient with candida vulvovaginitis tested against:-

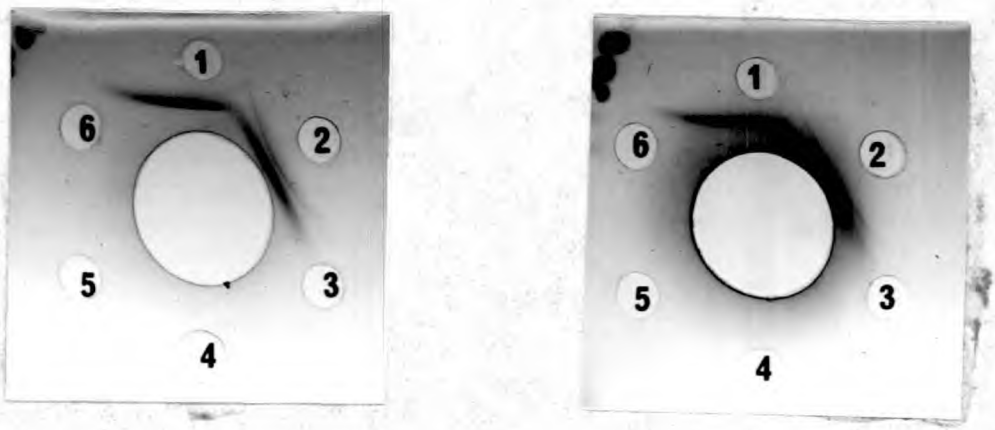
(1) Culture filtrate antigen (20 mg./ml.)
(2) Mannan antigen (1 mg./ml.)
(3) Somatic extract (40 mg./ml.)

Note a well defined 'R' type precipitin reaction to the culture filtrate. The second band given by the culture filtrate shows a reaction of identity with the broad 'H' type reaction of the mannan antigen.

Fig. 17. The serum of a patient with candida vulvovaginitis showing the 'H' type precipitin reaction given to the mannan antigen, and 'R' type reactions given to the culture filtrate and somatic antigen.

(1) Mannan antigen (1 mg./ml.)
(2) Culture filtrate antigen (20 mg./ml.)
(3) Somatic antigen (40 mg./ml.)

Note reaction of non-identity between 'R' type reactions to culture filtrate (2) and somatic antigen (3).



(a) (b)

Fig. 18. (a) The cord serum of a baby showing both 'H' and 'R' type reactions, and

(b) The serum of its mother showing the identical 'H' and 'R' type precipitin reactions when tested against:-

(1) Mannan antigen (1 mg./ml.)

(2) Culture filtrate antigen (20 mg./ml.)

28 negative cord sera were from babies born to mothers who had no precipitins in pregnancy.

3. Sera from 6 babies at 3 months of age

Six babies who had detectable precipitins in their cord blood were examined for precipitins to polysaccharide and protein antigens of C. albicans, after the age of 3 months. Precipitins were no longer detectable in their sera (Fig. 19 a and b).

4. Sera from babies with mucocutaneous candidosis

Four babies with clinical oral thrush and 2 babies with skin thrush in whom C. albicans had been isolated, were examined for precipitins. Four of them had precipitins to mannan antigen and culture filtrate antigen (Fig. 20a).

Sera from the mothers of these six babies were also tested for precipitins to C. albicans. The mothers of the four precipitin positive babies also had precipitins in their sera (Fig. 20b).

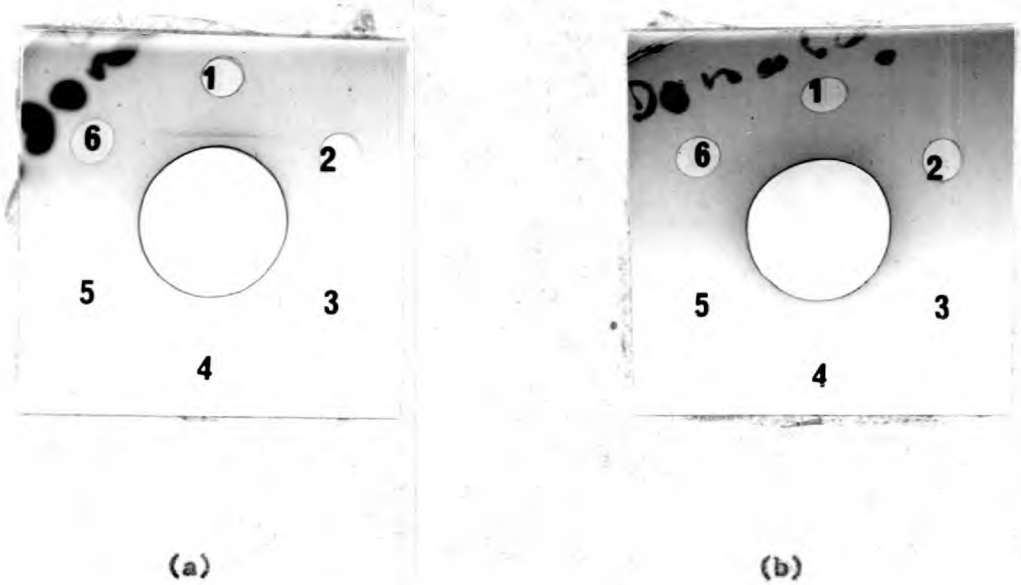
II. Results of Agglutination Tests

Agglutinin titres

(a) Sera collected from 116 unselected pregnant women in Group B were tested for agglutinins to C. albicans using C. albicans cells. Table 21 shows the agglutinin titres of the sera.

100 (86.2%) had agglutinin titres of $< 1:16$ or none at all;

16 (13.8%) had agglutinin titres of $> 1:16$.



(a) (b)

Fig. 19. (a) The cord serum of a baby showing a fine precipitin band to culture filtrate; and

(b) Serum of the same baby aged 3 months tested against:-

(1) Culture filtrate (20 mg./ml.)

(2) Mannan antigen (1 mg./ml.)

Note: disappearance of antibodies to culture filtrate antigen at age of 3 months

(a) (b)

Fig. 20 (a) The serum of a newborn baby with oral thrush showing both 'H' and 'R' type precipitin reactions, and

(b) The serum of the baby's mother showing similar 'H' and 'R' type precipitin reactions when tested against:-

(1) Mannan antigen (1 mg./ml.)
(2) Culture filtrate antigen (20 mg./ml.)
(3) Somatic antigen (40 mg./ml.)

TABLE 21

Agglutinin titres in the sera of 116 pregnant women

Agglutinin titre	No. of sera	
Negative	16	
1:2	16	
1:4	24	86.2%
1:8	28	
1:16	16	
1:32	8	
1:64	3	
1:128	3	13.8%
1:256	1	
1:512	1	

(b) Comparison of agglutinin titres with precipitins

The histogram (Fig. 21) shows the agglutinin titres and precipitin reactions of the sera from these 116 unselected pregnant women.

Of the 100 patients with agglutinin titres of less than 1:16, thirteen (13%) had precipitins to mannan in their serum. Of the 16 with agglutinin titres over 1:16, four (25%) had demonstrable precipitins to mannan.

One patient with an agglutinin titre of 1:512 had very strong precipitin reactions to mannan (Fig. 22). Two sera with a negative agglutinin titre gave positive precipitin reactions to mannan and three to protein antigen of culture filtrate.

III. Results of Immunoelectrophoresis

10 sera from pregnant women with precipitins to mannan antigen (by the double diffusion method) were also subjected to immunoelectrophoresis in agar gel and tested against mannan antigen and against goat-antihuman globulin serum.

In one serum it was possible to demonstrate that the precipitin reaction to the mannan antigen occurred in the region of IgG fraction of the immunoglobulins (Fig. 23).

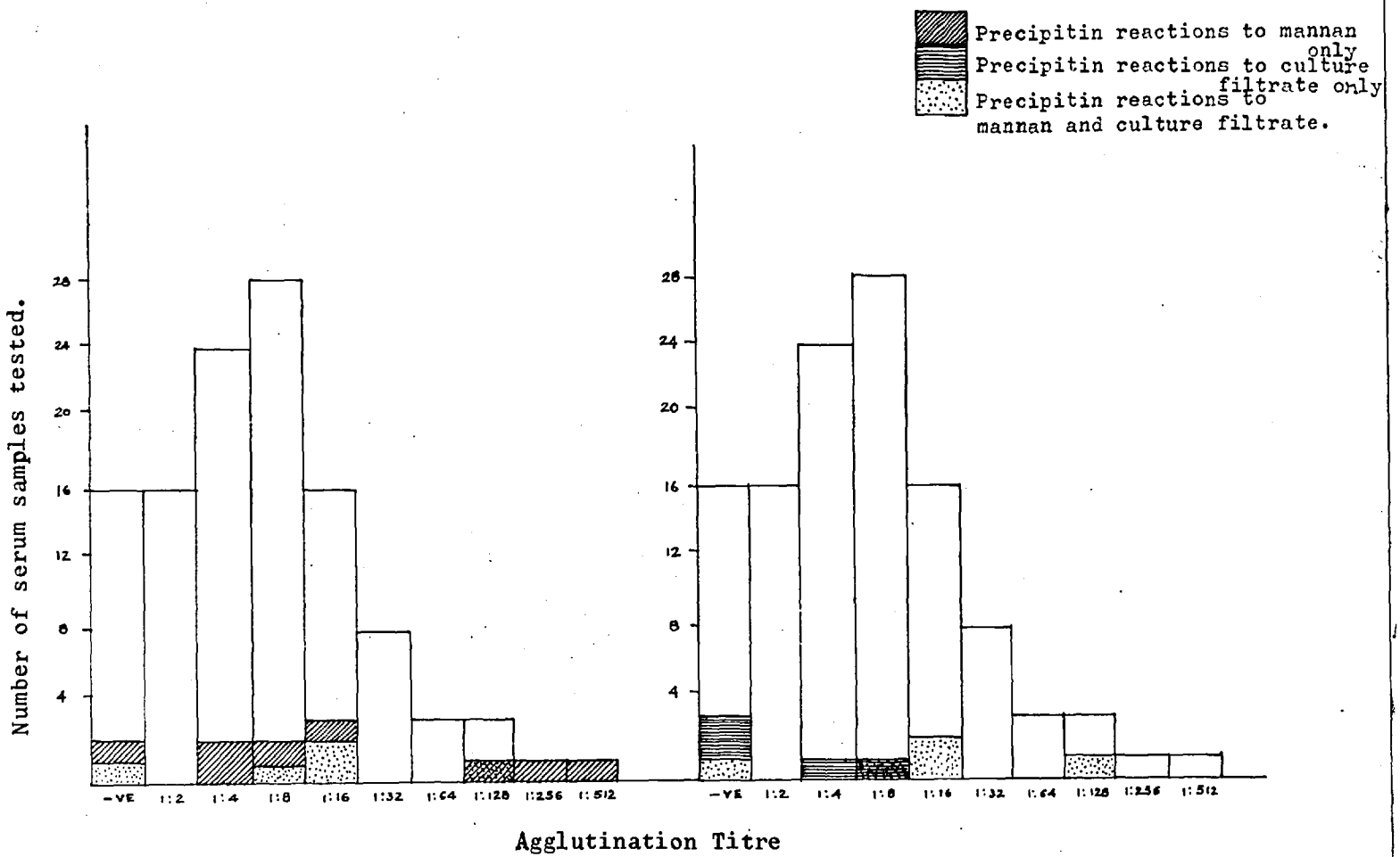


Fig. 21. A histogram showing the yeast cell agglutination titres and the precipitin reactions to mannan and culture filtrate antigens, of the serum samples from 116 pregnant women.

Fig. 22. Serum with an agglutinin titre of 1:512 showing strong 'H' type precipitin bands when tested against:-

(1) Mannan antigen (1 mg./ml.)
(2) Somatic antigen (40 mg./ml.)

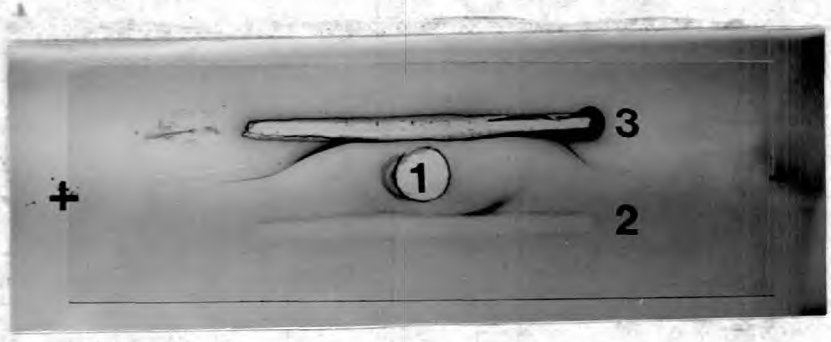


Fig. 23. Immunoelectrophoresis of a precipitin positive serum from a pregnant patient (1), tested against mannan antigen (2) and goat-antihuman globulin serum (3).

Note the precipitin arc given to the mannan antigen in the IgG region of the immunoglobulins (3).

IV. Results of Immunoosmophoresis

Ten sera from pregnant women with candida vulvovaginitis, which had no demonstrable precipitins to mannan antigen when tested by double diffusion, were also negative when tested by immunoosmophoresis. Sera which had precipitins when tested by double diffusion also gave positive reactions by immuno-osmophoresis (Fig. 24).

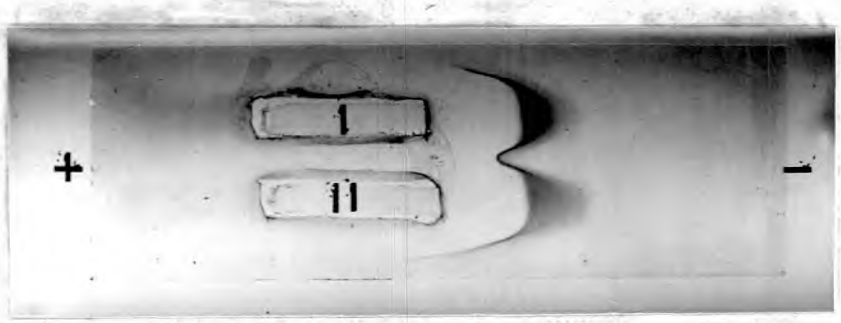


Fig. 24. Immunoosmophoresis test of two precipitin positive sera from pregnant patients, against mannan antigen. Slots I and II contain precipitin positive sera. Mannan antigen strip has been removed.

Note the reaction of identity between the two precipitin arcs obtained after osmophoresis.

ANALYSIS OF SEROLOGICAL RESULTS

CORRELATION OF SEROLOGICAL, MICROBIOLOGICAL AND CLINICAL STUDIES IN PREGNANT WOMEN

1. Relationship of precipitins to mannan antigen and candida carriage and candida vaginitis

Group A consisted of 190 and Group B of 116 unselected pregnant women all in the first trimester. Both groups were tested for precipitins to mannan antigen by double diffusion, and both groups were examined clinically for evidence of vulvovaginitis and culturally for the presence of yeasts in the vagina. High vaginal swabs were taken for culture at the same time as a specimen of blood for precipitins. The two groups differed only in the second antigen used for testing the sera for precipitins.

Thus a total of 306 sera from unselected pregnant women were tested for precipitins to mannan antigen. Table 22 shows that the distribution of precipitins to mannan antigen throughout the population studied is not uniform. There is a higher incidence of precipitins to mannan antigen in the sera of women with vaginal thrush where the diagnosis was confirmed by culture, as well as in women carrying C. albicans even though they did not have symptoms. This distribution is unlikely to have occurred by chance ($p < 0.05$).

Table 23 shows the relationship between the demonstration of precipitins to mannan antigen and the isolation of C. albicans

TABLE 22

TABLE

Distribution of precipitins to mannan antigen

	C. albicans isolated*	
	symptoms of vulvovaginitis +	no symptoms of vulvovaginitis
Precipitating antibodies positive	8	5
Precipitating antibodies negative	18	14
Total observed	26	19
% with precipitins	30	26

$\chi^2 = 10.02$

$n = 3$

$p < 0.05$

* HVS

22

of C. albicans in 306 unselected pregnant women

No C. albicans isolated*		
symptoms of vulvovaginitis +	no symptoms of vulvovaginitis	Total
12	19	44
69	161	262
81	180	306
14	10	14.6

TABLE 23

Relationship between demonstration of precipitins to mannan antigen and isolation of C. albicans in 306 unselected pregnant women

	Number of women		
	harbouring C. albicans in the vagina	not harbouring C. albicans in the vagina	Total
Precipitin positive	13	31	44
Precipitin negative	32	230	262
Total observed	45	261	306
% with precipitins	29	13	14

χ^2 (Yate's correction) = 7.693

n = 1

$p < 0.01$

from the vagina. Precipitins to mannan antigen occurred in 13 of 45 women harbouring C. albicans in the vagina and in 31 of the remainder (261) of the population studied. This distribution is significant ($P < 0.01$).

Table 24 shows the relationship between the demonstration of precipitins to mannan antigen and candida vulvovaginitis which had been confirmed by isolation of C. albicans.

Precipitins to mannan antigen occurred in the sera of 8 of 26 women with candida vulvovaginitis diagnosed on clinical and mycological evidence, and in 36 of the remainder (280) of the population studied. This distribution is significant ($P < 0.05$).

Table 25 shows the relationship between demonstration of precipitins to mannan antigen and vulvovaginitis diagnosed on clinical findings only.

It is not possible to show a significant relationship between precipitins to mannan antigen and vulvovaginitis, diagnosed on clinical criteria alone ($P > 0.2$).

2. Relation of precipitins to the protein antigen in culture filtrate of C. albicans and candida carriage and candida vaginitis.

116 sera from unselected pregnant women in the first trimester of pregnancy (Group B) were tested for precipitins to the culture filtrate antigens of C. albicans. They were examined clinically for evidence of candida vulvovaginitis and high vaginal swabs were cultured for yeasts.

TABLE 24

Relationship between demonstration of precipitins to mannan antigen and candida vulvovaginitis confirmed by isolation of C. albicans

	Number of women		
	with vulvovaginitis confirmed by isolation of C. albicans	without vulvovaginitis	Total
Precipitin positive	8	36	44
Precipitin negative	18	244	262
Total observed	26	280	306
% with precipitins	30	13	14

χ^2 (Yate's correction) = 4.83

n = 1

$p < 0.05$

TABLE 25

Relationship between demonstration of precipitins to mannan antigen and vulvovaginitis diagnosed on clinical findings

	Number of women		
	with symptoms of vulvovaginitis	without symptoms	Total
Precipitin positive	20	24	44
Precipitin negative	87	175	262
Total observed	107	199	306
% with precipitins	18	12	14

χ^2 (Yate's correction) = 1.569

n = 1

$p > 0.2$

Table 26 shows the distribution of precipitins to the protein antigen of culture filtrate in 116 pregnant women. This distribution is unlikely to have arisen by chance ($P < 0.01$).

Precipitins to the protein antigen of culture filtrate occurred in the sera of 4 of 15 women harbouring C. albicans in the vagina and in 6 of the remainder (101) of the population studied (Table 27). There is an association between the demonstration of precipitins to the protein antigen of culture filtrate and the isolation of C. albicans from the vagina. ($P < 0.05$).

It is not possible to show a relationship between the demonstration of precipitins to the protein antigen of culture filtrate and candida vulvovaginitis where the diagnosis was confirmed by the isolation of C. albicans (Table 28) $P > 0.8$.

TABLE 26

TABLE

Distribution of precipitins to the protein antigen of culture

	C. albicans isolated*	
	symptoms of vulvovaginitis+	no symptoms of vulvovaginitis
Precipitating antibodies positive	1	3
Precipitating antibodies negative	7	4
Total observed	8	7
% with precipitins	12.5	42.8

$\chi^2 = 12.96$

$n = 3$

$p < 0.01$

* HVS

26

filtrate of C. albicans in 116 unselected pregnant women

No C. albicans isolated*		
symptoms of vulvovaginitis +	no symptoms of vulvovaginitis	Total
1	5	10
25	70	106
26	75	116
3.8	6.6	8.6

TABLE 27

Relationship between demonstration of precipitins to protein antigen of culture filtrate and isolation of C. albicans in 116 unselected pregnant women

	Number of women		
	harbouring C. albicans in the vagina	not harbouring C. albicans in the vagina	Total
Precipitin positive	4	6	10
Precipitin negative	11	95	106
Total observed	15	101	116
% with precipitins	26	6	8.6

χ^2 (Yate's correction) = 4.732

n = 1

$p < 0.05$

TABLE 28

Relationship between demonstrations of precipitins to protein antigen of culture filtrate and candida vulvovaginitis confirmed by the isolation of C. albicans

	Number of women		
	with vulvovaginitis confirmed by isolation of C. albicans	without vulvovaginitis	Total
Precipitin positive	1	9	10
Precipitin negative	7	99	106
Total observed	8	108	116
% with precipitins	12	8	8.6

χ^2 (Yate's correction) = 0.0613

n = 1

$p > 0.8$

DISCUSSION

The importance of candida vulvovaginitis has led to many studies of the yeast flora of the female reproductive tract. In many of these studies there is no clear distinction between those who harbour the fungus as a commensal and those with vulvovaginitis. Some of the reports on the distribution of yeast species in pregnant women are merely lists of fungi isolated from material sent to the laboratory for diagnosis. There has been no effort to assess the true incidence and distribution of yeasts in pregnant women by prospective studies or to correlate the findings with clinical and immunological data.

C. albicans, the most important pathogenic member of the genus Candida is often found in the human vagina as a commensal. It is also one of the most important of the specific agents causing vulvovaginitis. The carriage of yeasts as well as the incidence of candida vulvovaginitis increases during pregnancy. The increased acidity of the vagina during pregnancy favours the establishment and multiplication of yeasts (Cruickshank and Sharman, 1934; Cruickshank, 1934; Davis and Pearl, 1938). The increased susceptibility of pregnant women to candida vulvovaginitis has been demonstrated by experimental inoculation (Bland et al., 1937).

The reported incidence of C. albicans in the vagina of non-pregnant women varies from 7.6 to 17 per cent (Dawkins et al., 1953; Stough and Blank, 1958; Clark and Solomons, 1959; Taubert and Smith, 1960; and Mackenzie, 1962). There is a wide variation in the reported incidence of yeasts in the vagina of pregnant women. The

incidence has been given as 35 per cent (Plass et al. 1931); 28 per cent (Woodruff and Hesseltine, 1938); and 43 per cent (Carter et al. 1940) respectively. Species differentiation was not undertaken by these authors.

The incidence of C. albicans in the vagina of pregnant women, according to Bret and Coupe (1958) is 20 per cent and according to Harris et al. (1958) it is 17.6 per cent. Stough and Blank (1958) and Clark and Solomons (1958) reporting from the United States of America showed that the incidence of C. albicans in the vagina of pregnant women was 29.5 per cent and 27.3 per cent respectively. These last two figures show a remarkable degree of agreement.

The incidence of yeasts in the vagina of pregnant women, irrespective of symptoms, was found to be 17 per cent at Queen Charlotte's Maternity Hospital, London (Hurley et al. 1971). The incidence was slightly higher (20 per cent) in spring and lower (15 per cent) in winter. The incidence of C. albicans in the vagina of pregnant women was 13.1 per cent.

In my study carried out prospectively in 1,085 pregnant women in the first trimester of pregnancy, the overall incidence of yeasts in the vagina was found to be 18.6 per cent. C. albicans was present in 15.9 per cent of them. The figures obtained by both groups of workers in the same hospital are in agreement. The carriage of yeasts in the vagina of pregnant women with symptoms of vulvovaginitis was 25 per cent and in those without symptoms it was 14 per cent.

The incidence of candida vulvovaginitis during pregnancy has been given as 4 to 5 per cent by Jennison (1966), although 16 to 17 per cent of the women studied had symptoms and signs severe enough to

warrant sending a specimen. These figures were obtained from a retrospective study of deliveries at St. Mary's Hospital, Manchester. Hurley and Morris (1964) state that the incidence of candida vulvovaginitis in maternity patients is 10 per cent. These figures were obtained from a retrospective study of diagnostic specimens sent from maternity patients and included specimens sent in the puerperium as well. Daftary et al. (1963) noted an incidence of candida vulvovaginitis in 38 per cent of women in the third trimester of pregnancy. In my study of 1,085 unselected pregnant women in the first trimester, the candida vaginitis rate was found to be 8 per cent, i.e. about half the carriage rate. This figure is again in agreement with that given by Hurley and Morris (1964). Unlike the earlier studies of Jennison (1966) and Hurley and Morris (1964) this study shows both the carriage rate as well as the vaginitis rate due to Candida.

In this study 48 per cent of patients with positive cultures for yeasts had no symptoms of vulvovaginitis. Harris et al. (1958) state that 31 per cent of patients with positive vaginal cultures were symptomless. Symptomless carriage is as common as vulvovaginitis associated with yeasts and there is nothing in these figures to suggest that yeasts were superficial pathogens.

Symptoms of vulvovaginitis were present in 52 per cent of pregnant women carrying yeasts. The commonest symptom was discharge, in 94 per cent, often accompanied by pruritus. Clinically typical thrush, i.e. white plaques and discharge or discharge, pruritus and burning sensation is invariably associated with C. albicans. The other yeasts isolated are associated with dis-

charge only, with the exception of T. glabrata which is sometimes associated with pruritus.

Vaginal morbidity including discharge, pruritus, burning sensation and white plaques was prevalent in 39 per cent of pregnant women studied. This high incidence of vaginal morbidity is not evident from retrospective surveys of laboratory specimens for in practice diagnostic specimens are sent only when a patient complains, or a clinical diagnosis needs confirmation.

Recently, Carroll et al. (1971) have reported a much higher incidence of minor vaginal morbidity, (89 per cent), among pregnant women attending the antenatal clinics at Queen Charlotte's Maternity Hospital. In that study, clinical data were collected by one observer, while in my study, it was done by different observers.

Although 39 per cent of the women examined had symptoms of vulvovaginitis, in 75 per cent of them no yeasts could be cultured from the vagina. Taubert and Smith (1960) noted that 37.5 per cent of patients with vaginitis had no yeasts on culture. According to Jennison (1966) two-thirds of patients with vulvovaginitis and according to Hurley and Morris (1964) two-thirds to three-quarters of patients with vulvovaginitis had no yeasts. The figure obtained in this study shows agreement with that of Hurley and Morris (1964). The aetiology of the remaining cases of vulvovaginitis needs to be established. Some of these may have other microbial aetiologies. According to Hurley et al. (1971) 3 per cent of pregnant women at Queen Charlotte's Maternity Hospital have trichomonadal vaginitis and 1/5,000 have gonococcal infection (Hurley, 1970).

About 10 per cent have fungal vaginitis. Therefore, in two-thirds of patients the aetiology of the vaginitis is unknown and needs investigation.

C. albicans, the principal pathogenic member of the genus is the commonest member isolated from the human vagina. Species of the genus Candida other than C. albicans are demonstrable pathogens (Winner and Hurley, 1964, and Hurley, 1970). They include C. tropicalis, C. krusei, C. pseudotropicalis, C. stellatoidea, C. parapsilosis and C. guilliermondii. Hurley and Morris (1964) isolated C. tropicalis, C. krusei and C. stellatoidea in addition to C. albicans from typical cases of vaginal thrush.

The percentage isolation rates of the different species of yeasts depend on the classification employed (Brodie and Henderson, 1961); and not all studies of the yeast flora of the vagina are comparable.

Using the criteria of Lodder and Kreger-van Rij (1952) for identification of yeasts, Hurley et al. (1971) have shown the following distribution of yeast species in positive specimens from unselected pregnant women: C. albicans 76 per cent, T. glabrata 15 per cent, and other Candida species, other Torulopsis species and S. cerevisiae 9 per cent. In my study on unselected patients, C. albicans was isolated from 81 per cent, and T. glabrata from 12.3 per cent of positive specimens. The rest consisted of two Candida species, S. cerevisiae and Rhodotorula mucilaginosa. In both series C. albicans predominated and T. glabrata was the yeast next most commonly isolated.

A comparison of the percentage of yeast species isolated from patients with and without symptoms of vulvovaginitis in this study (Table 15) showed that C. albicans was isolated from 82.9 per cent with symptoms and from 82.4 per cent without symptoms. T. glabrata was isolated in 10 per cent and 15.4 per cent respectively in the two groups.

A comparison of the percentage distribution of yeast species isolated from diagnostic specimens sent from pregnant women with vulvovaginitis and from unselected pregnant women attending the antenatal clinics was made by Hurley et al. (1971). C. albicans was isolated from 90 to 98 per cent of specimens sent for diagnosis but from only 74 to 78 per cent of unselected specimens. T. glabrata was the next most frequently isolated yeast from both groups. The percentage distribution differed in those sent for diagnostic culture and in those taken from the unselected series. C. albicans predominated in both series, but its percentage distribution was lower in unselected patients. In both series, T. glabrata was the species next most commonly isolated but it was more common in unselected patients. The percentage of species other than C. albicans and T. glabrata was higher in unselected patients. Thus it seems that species other than C. albicans are important in the pathogenesis of vulvovaginitis in pregnant women. An association between T. glabrata and vulvovaginitis has been noted by Wickerham (1957) and Kearns and Gray (1963), although Benham (1935) has shown that it is probably not pathogenic. Little experimental work has been done on these fungi but experiments by Stanley (1968) showed that S. cerevisiae exerts a minimal cytopathic effect on cultured cells of mammalian origin.

The possible role of these fungi in the pathogenesis of vaginitis needs further investigation.

Importance of uniform criteria for the identification of yeasts

Although C. albicans is the most important pathogen causing vaginitis in pregnancy, the correct identification of yeasts is important. Reliance on fermentation reactions and chlamydospore production only, gives an unduly high proportion of C. stellatoidea. This yeast does not ferment or assimilate sucrose. C. albicans according to Martin et al. (1937) ferments sucrose; it also assimilates sucrose. Lodder and Kreger-van Rij (1952) regard the fermentation of sucrose by C. albicans as variable, and do not rely on fermentation reactions to differentiate Candida species. Many yeasts reported in the past as C. stellatoidea would be regarded as C. albicans today. In this study, the identification of yeasts was made according to the system of Lodder and Kreger-van Rij (1952). C. stellatoidea was isolated from the vagina in less than 1 per cent of pregnant women.

The incidence of C. albicans in newborn infants has been given as 16.3 per cent (Bret and Coupe, 1958) and 4.6 per cent (Kozim et al. 1958).

In my study, 1.5 per cent of newborn infants in the first ten days were found to harbour only C. albicans in the mouth while 2.3 per cent harboured Candida species and T. glabrata in the anal canal. It is of interest to note that C. tropicalis was isolated from the anal canal of a baby whose mother harboured the same species in the vagina. The source of C. parapsilosis and T. glabrata isolated from the anal canal of two babies could not be traced to their mothers as they did not harbour any yeasts in the vagina. C. parapsilosis has

been found commonly in chronic paronychia (Reiersol, 1962).

Although Bret and Coupe (1958) found a fall in the incidence of C. albicans in newborns from 24 hours to 10 days, the incidence increased slightly in this study.

The reported incidence of oral thrush in the newborn at different institutions throughout the world varies from 0.14 per cent to 23 per cent. The incidence of oral thrush among newborn at Queen Charlotte's Maternity Hospital, was 0.66 per cent during the period 1955 to 1959 (Shrand, 1961). This incidence is similar to that reported from institutions of similar standing in the United States. Kozinn et al. (1958) attribute this very low incidence in recent surveys to the great improvement in oral hygiene of the newborn that has taken place within the past few years. Lincoln et al. (1965) think that the low incidence reported in some studies might in part be due to the fact that they were carried out in the newborn during their stay in hospital before the disease has manifested itself. Ludlam and Henderson (1942) state that oral thrush can rarely be detected clinically till after one week of birth. Kozinn et al. (1959) found that 2.9 per cent out of 786 newborn babies presented with clinical thrush only about the 8th or 9th day. The intensity of the diagnostic study of thrush in the newborn may account for the wide discrepancy in the incidence of oral thrush reported (Ludlam and Henderson, 1942).

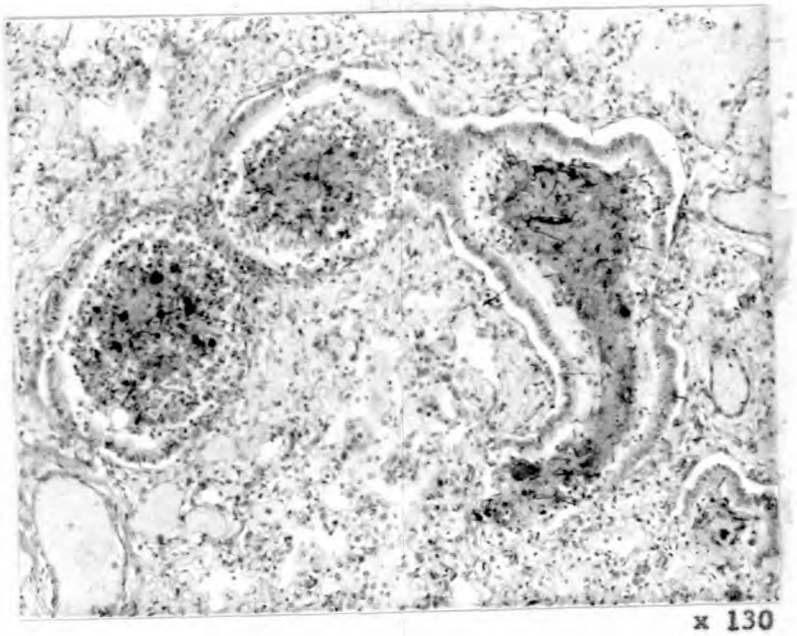
In this study of thrush in the newborn at Queen Charlotte's Maternity Hospital, a retrospective search of records showed that over the five year period 1965 to 1969, the incidence of thrush in the newborn was 0.45 per cent. During the period May 1965 to May 1967,

there were 6,402 deliveries and 47 developed oral thrush giving an incidence of 0.73 per cent (White-Franklin, 1968). In my special study of thrush in 648 newborn infants 1.5 per cent were found to harbour candida species in the mouth and 2.3 per cent in the anal canal, while 0.9 per cent developed oral thrush.

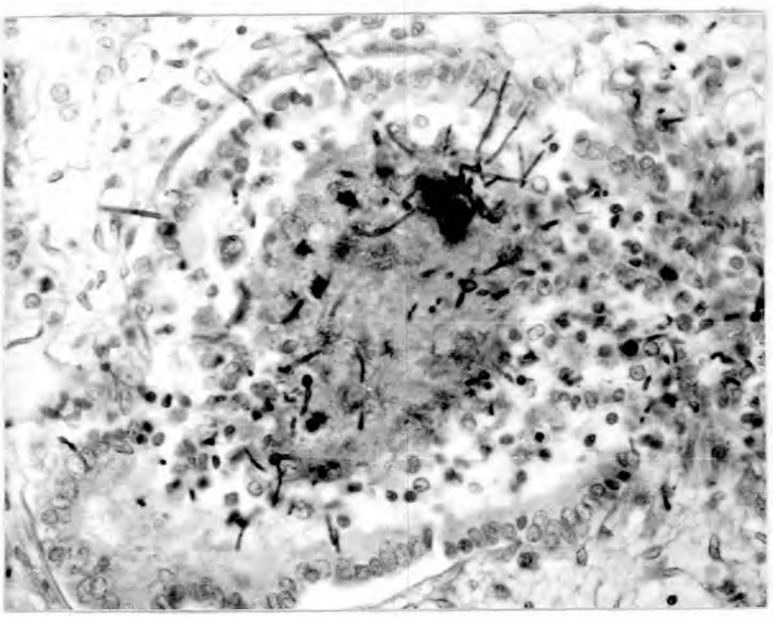
Although the incidence of oral thrush is low, it is not entirely a benign condition. Oral thrush can spread to the pharynx leading to inco-ordination of the swallowing mechanism and regurgitation, causing aspiration pneumonia and death (Fig. 25). Therefore, its complete eradication from the nursery is indicated.

The obvious source of infection of the newborn is the mother's vagina either directly or indirectly. Figure 26 summarises the mode of spread of C. albicans in a Maternity Unit. Woodruff and Hesseltine (1938) showed that a baby born of a mother harbouring thrush fungi in the vagina had a 35 times greater chance of developing thrush than one born of an uninfected mother. Ludlam and Henderson (1942) and Kaul et al. (1960) did not find any such relationship. Kozinn et al. (1958) and Shardt and Roy (1957) confirmed the importance of the vagina as a source of infection.

Adequate treatment of maternal vaginal thrush antenatally by insertion of nystatin pessaries into the vaginal vault has contributed to the declining incidence of oral thrush. The schedule of treatment advocated is:- 4 pessaries initially in the first 24 hours followed by one pessary every night and morning for 2 to 6 weeks or until delivery. Thrush in pregnancy is often self limiting, spontaneous cure being effected after delivery. The reduction in incidence of vaginal thrush after delivery has been attributed



x 130



x 640

Fig. 25. Section of lung from a 10 day old premature infant who died of aspiration pneumonia with terminal septicaemia. There are aggregates of macrophages and debris with yeast cells and hyphae predominantly within the bronchi, and show infiltration through the walls into the lung. P.A.S.H.

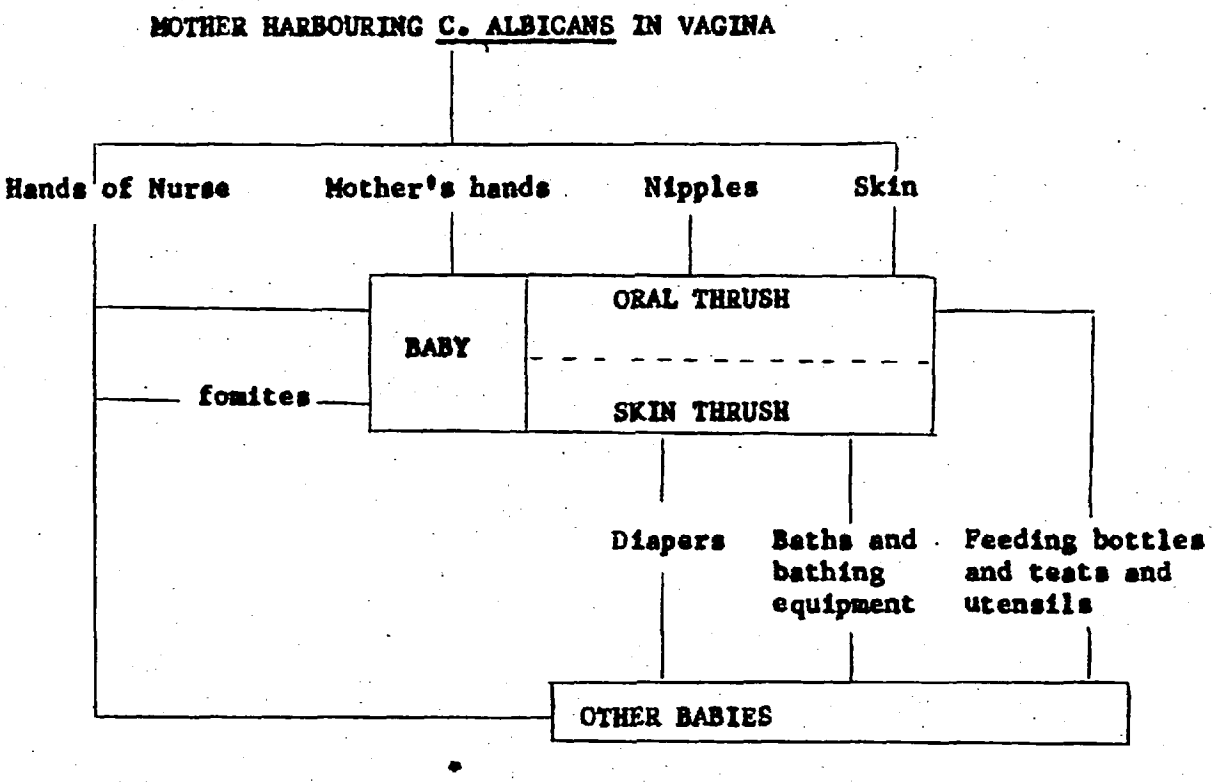


Fig. 26. Spread of C. albicans in a maternity unit

to the cleansing action of the lochia and a return of the vaginal environment to normal.

Prophylactic administration of nystatin to all infants born to mothers harbouring the thrush fungus has been suggested to eliminate thrush from the newborn nursery. Harris et al. (1958) have shown conclusively that the incidence of thrush in infants with a positive oral culture who were treated with prophylactic nystatin was lower (13 per cent) when compared with those who were not treated (74 per cent). The incidence of thrush in unselected newborn infants, all of whom were treated prophylactically with nystatin was 0.4 per cent. In a control group not treated with nystatin, the incidence of thrush was 4 per cent. These results were statistically significant.

A preliminary study of nystatin prophylaxis in the newborn was carried out over a 2 year period at Queen Charlotte's Maternity Hospital. Oral nystatin suspension (100,000 I.U. per ml.) was given before each feed for 5 days to babies born to mothers who harboured the thrush fungus. The incidence of thrush in infants born to mothers harbouring thrush fungi was 1.49 per cent while in those born to uninfected mothers was 0.67 per cent but there was no significant difference between those receiving nystatin and those not given nystatin, in the group at risk (White-Franklin, 1968).

Infected feeding bottles and teats have been incriminated as another source of infection (Ludlam and Henderson, 1942). Sterilization of bottles and teats and other utensils in preparing feeds has eliminated this source. Kozinn et al. (1958) and Shrand (1961) showed that there is no difference in the incidence of candidosis

between bottle fed and breast fed infants. Winner and Hurley (1964) state that chemical sterilization of bottles and teats is not satisfactory. Robson and Anderson (1964) state that boiling is preferable to Milton (sodium hypochlorite) although C. albicans is susceptible to its action. Autoclaving is the method of choice for sterilization. Ludlam and Henderson (1942) regard the nurse's skin as an important source of infection and the use of hibitane hand cream has contributed to elimination of this source. However, the thrush fungi can survive in hexachlorophane emulsions up to 8 days and in chlohexidine creams for 2 hours (France, 1968). Care must be taken to avoid contamination of these disinfectants.

In spite of preventive measures to eliminate possible sources of infection in the nursery there is still a small percentage of 0.5 to 1 per cent of newborn babies who develop thrush. This is a feature of all well run newborn nurseries both in the United Kingdom and the United States. Many investigators have suggested predisposing factors for the development of clinical disease and these seem related to the mode of transmission from the source.

Ludlam and Henderson (1942) and Harris et al. (1958) reported a higher incidence of thrush in premature than in full term infants but Kozinn et al. (1958) and Shrand (1961) found no such differences. Dunn (1962) reported a high incidence of thrush among infants nursed in incubators and considered contamination of the incubators with C. albicans and the increased temperature and humidity of the incubator as playing a part, but regular monitoring of incubators at Queen Charlotte's Maternity Hospital shows no C. albicans. Further the risks of cross infection to the premature infant are greater.

Although the incidence of candida infections in older people is increased with the use of broad spectrum antibiotics, this is not a factor in the newborn (Shrand, 1961; Kozim et al., 1958; Harris et al., 1958).

Trauma of the buccal mucosa caused during resuscitative procedures in the newborn has not been found to be a predisposing cause (Shrand, 1961; Kozinn et al., 1958). The factors which determine the development of infective thrush in the newborn merit further study. Wells (1970) suggests that in chronic oral candidosis one of the causes may be an abnormality which is genetically determined and inherited as an autosomal recessive trait.

Although mycoses caused by C. albicans are amongst the commonest infections of pregnant women and newborn babies, the fungus is often isolated from the healthy vagina and the mouth. The diagnosis of vaginal and oral thrush cannot therefore, be established solely from the cultural findings. The diagnosis is made on clinical grounds and the mycological findings are corroborative.

It is estimated that 10 per cent of maternity patients have clinical evidence of vaginal thrush which can be confirmed by culture of the thrush fungi (Hurley and Morris, 1964). It has been found in this study that many more have minor degrees of vaginal morbidity which may be causally associated with thrush fungi. Further, serious infections caused by C. albicans and other pathogenic species of Candida occur in obstetrics and gynaecological practice. Fox (1971) reported five fatal cases of systemic candidosis and reviewed eleven other cases. It is clear that a serological test would be of value in diagnosis of superficial as well as systemic candidosis.

There have been many attempts to devise serological tests which will have diagnostic significance. These include tests for agglutinins, complement fixing antibodies, fluorescent antibodies and precipitins. The presence of candida antibodies, particularly agglutinins, in a high proportion of the population has made serological tests difficult to assess. Most promising among these are tests based on the precipitin reaction.

Precipitins against C. albicans antigens have been found only in the serum of patients with deep seated infections by Akiba et al. (1957), Elinov and Zaikina (1959), Stallybrass (1964) and Taschdjian et al. (1964 a and b, and 1967). Precipitin reactions to the purified mannans of C. albicans group A have, however, been given with the concentrated serum of all subjects tested by Chew and Theus (1967) and in 22.5 per cent of healthy subjects tested by Pepys et al. (1967). Precipitin studies on limited numbers of patients with superficial thrush (Chew and Theus, 1967; and Taschdjian et al., 1967), and on patients undergoing open heart surgery (Murray et al., 1969) and in patients with respiratory diseases (Pepys et al., 1967) have been made. There is no documentation of precipitin studies on pregnant women and their offspring, using a purified, polysaccharide cell wall mannan, a culture filtrate extract and a somatic extract of C. albicans group A.

The antigens of C. albicans are diverse and methods of preparation are not standard. In this study antigenic extracts of C. albicans were isolated from the yeast cells (cell wall, mannan and somatic antigen) and from culture filtrates.

Structure of the yeast cell

The sturcture of the yeast cell as determined by Mundkur (1960) showed that the cell wall consists of two layers, an outer layer consisting of a mannan and an inner layer consisting of a glucan. The principal component of the cytoplasm is glycogen. Antigenic analysis of the various components (Kessler and Nickerson, 1959; Siegert et al., 1963; Muller and Hirsch, 1967) showed that the cell wall contained mainly a thermostable antigen and that the cytoplasm contained both a thermostable and thermolabile antigen. Bishop et al. (1960) isolated a glucan, mannan and chitin from the cell wall of C. albicans. Summers et al. (1964) using the Fehling method of Peat et al. (1961) found that the major component of the yeast cell wall and the major surface antigen was a mannan. Faux (1968) analysed mannan extracted by the method of Peat et al. (1961) and purified by Sephadex gel filtration, and found it to contain mannose, and traces of glucose.

Mannan antigen used in this study on precipitating antibodies in pregnant women was extracted by the method of Peat et al. (1961) as modified by Faux (1968). Double diffusion tests with mannan antigen against human sera gave diffuse, fuzzy precipitin arcs of the 'H' type (Pepys et al., 1967).

A somatic extract, containing both cytoplasmic and cell wall components was prepared by mechanical disintegration of yeast cells, in a Mickle disintegrator, by the method of Stallybrass (1964). Somatic extracts prepared in this manner have been shown to contain mannan antigen as well as an additional antigen whose nature has not been characterised (Chew and Theus, 1967).

A culture filtrate antigen, rich in antigenic components was prepared by growing C. albicans in an aerated Sabouraud's medium for 7 days, according to the method of Faux (1968). The culture filtrate has been shown to contain heat labile, protein antigenscapable of giving fine precipitin arcs of the 'R' type and heat stable polysaccharide antigens capable of giving diffuse precipitins arcs of the 'H' type (Pepys et al., 1967).

Analysis of the literature

Previous workers have used either the somatic or cell wall extracts of C. albicans for precipitin tests. These extracts are of dubious composition. Precipitins were found only in sera of patients with systemic candidosis when tests were performed with the various antigenic extracts, Akiba et al. (1957) using a phenol extract of C. albicans, Elinov and Zaikina (1959) using a β naphthol extract, Stallybrass (1964) using a formamide extract and Mickle disintegrated somatic extract, Taschdjian et al. (1964 a and b, 1967), using an ultrasonically disintegrated somatic extract, and Murray et al. (1969) using a Mickle disintegrated somatic extract. These extracts have been crude and it is not possible to calculate the actual concentration of the antigens used from the information provided. Akiba et al. (1957) using their crude extract at the concentration of 0.5 mgm./ml. found more positive reactions, than when the antigen was tested at 0.2 mgm./ml. They were not able to show the presence of mannose in their extract, although Summers et al. (1964) have shown that the major surface antigen is mannan. Stallybrass (1964) suggested that candida precipitins may be expected in man only when there is systemic infection with C. albicans; they would not be expected when there is superficial

candidosis. Taschdjian et al. (1964 a and b) suggested that precipitating antibody was formed during candida infection involving living tissue. The low number of precipitin positive sera among patients other than those with deep seated infections reported by Elinov and Zaikina (1959), Stallybrass (1964) and Taschdjian et al. (1964 a and b, and 1967) has been attributed to the use of antigens which were in too high concentration (Pepys et al., 1967). The use of too strong concentrations of mannan reduces the number of visible precipitation reactions either by causing the 'H' type precipitation reaction given by mannan, to dissolve in antigen excess, or by causing it to take place in the serum well. This would probably explain why positive reactions have been reported only in patients with deep seated infections as such patients would have large amounts of precipitins which would be capable of reacting with the high concentrations of the antigen used while the number of reactions of weak sera would be reduced. Taschdjian et al. (1967) demonstrated precipitins to somatic extracts and commercial culture filtrate (oidiomycin) of C. albicans in candida granuloma and endocrinopathy associated chronic mucocutaneous candidosis. Faux (1968) noted that only patients with endocarditis showed both diffuse and fine precipitin reactions with the somatic extract when it was used at a high concentration of 40 mgm./ml. whereas sera of patients without endocarditis gave diffuse precipitin reactions when the antigen was used at a lower concentration of 20 mgm./ml. Murray et al. (1969) using a somatic extract of C. albicans, in two different sized wells (volume ratio 1:10) showed that sera from patients before open heart surgery did not have precipitins, while six patients who

developed candida endocarditis after surgery had precipitins to several species of Candida. They also demonstrated precipitins in 27 other patients, about a fortnight after operation although none of them developed endocarditis or other evidence of deep-seated candidosis. They do not indicate the type of precipitin arcs observed although the antigen they used is stated to have contained little mannan. Murray et al. (1969) did not discount the possibility that precipitin positive patients without endocarditis had undetected candidosis. It is clear that precipitins to somatic antigens of C. albicans occur in forms of candidosis other than acute disseminated candidosis. Recently Chew and Theus (1967) using a purified mannan extract of C. albicans Group A found precipitins in the serum of all healthy subjects tested. They used two immunodiffusion methods, the Ouchterlony double diffusion and the Preer tube method (Preer, 1957) and found the latter to be more sensitive. Using the Ouchterlony method 3.2 per cent of the unconcentrated sera tested gave positive reactions and after concentration of the serum globulins 15 fold, 30 per cent of all healthy subjects gave positive precipitin reactions. Similar results were obtained by Pepys et al. (1967) using the Ouchterlony double diffusion method. 4.5 per cent of the unconcentrated sera gave positive reactions and after three fold concentration of the sera, 22.7 per cent of the healthy subjects tested gave positive reactions. However, using the Preer tube method Chew and Theus (1967) showed that 100 per cent of the 15 fold concentrated serum globulins gave positive reactions. They also showed that 10 per cent of unconcentrated sera from patients with mucocutaneous candi-

dosis gave positive reactions in Ouchterlony double diffusion plates. Pepys et al. (1967) found precipitins to mannan in concentrated sera in 55 per cent of patients with asthma and in 72.2 per cent of patients with asthma complicated by pulmonary eosinophilia.

Thus there is much controversy regarding the significance of precipitins to antigens of C. albicans, mainly due to lack of a standard procedure and standardized antigens for precipitin tests. However precipitins have been observed more frequently in those with candidosis be it deep seated or superficial. Further all the antigens used by previous workers have been shown to contain mannan, although the method of preparation differed. The choice of antigens used in this study on precipitins to C. albicans was based on the immunological studies of antigens of C. albicans made by Faux (1968). Three candida antigens - somatic extracts, purified cell wall mannan and culture filtrate antigen were used in this study.

Ouchterlony double diffusion method in Petri dishes with the size of wells described was selected for precipitin tests, as it provided good resolution of the bands of precipitate. It had the disadvantage that a period of fourteen days was necessary, before final results could be recorded, and at least 0.5 ml. of serum was required for each test. Immunoosmophoresis, though much quicker and more economical than the double diffusion test used in this study, did not detect precipitins to C. albicans in some sera that were negative in double diffusion.

Analysis of double diffusion tests

Precipitins to mannan antigen ('H' type reactions)

Precipitin tests by the Ouchterlony double diffusion method with unconcentrated sera from unselected pregnant women (Groups A and B) and a group of pregnant women with clinical and mycological evidence of candida vulvovaginitis (Group C) showed that the number of precipitin reactions to mannan antigen was higher in the group with candida vaginitis (30%) than in the group of unselected pregnant women (14.3%).

Tests with maternal and paired cord blood sera gave similar reactions to the mannan antigen of C. albicans indicating that the precipitating antibody reacting with this antigen was capable of crossing the placenta.

Of the total 306 sera from unselected pregnant women (i.e. Groups A and B) tested against mannan antigen 44 (14.3%) gave 'H' type precipitin reactions.

Precipitins to protein antigen of culture filtrate ('R' type reactions)

Tests by the Ouchterlony double diffusion method with unconcentrated sera from a group of 116 unselected pregnant women (Group B) and a group of 30 pregnant women with clinical and mycological evidence of candida vulvovaginitis (Group C) showed that the number of fine, 'R' type precipitin reactions to the protein antigens in culture filtrate was almost the same in the two groups - 8.6 per cent and 10 per cent respectively. This is in contrast to the percentage incidence of precipitins to mannan antigen, in the 2 groups.

Tests with maternal and paired cord blood sera gave similar reactions to the protein antigens of culture filtrate showing that

the precipitating antibody reacting with these antigens was capable of crossing the placenta.

Of the group of 116 sera from unselected pregnant women tested against culture filtrate antigen, 10 (8.6%) gave 'R' type precipitin reactions either alone or together with 'H' type reactions.

Precipitin reactions to somatic extracts

Precipitin reactions to somatic extracts prepared by Mickle disintegration of C. albicans cells occurred in 14.2 per cent of unselected pregnant women (Group A). These were 'H' type reactions. None of the 190 sera from unselected pregnant women gave fine precipitin reactions of the 'R' type when tested against somatic extracts. Only one serum from the selected group of 30 sera of pregnant patients with clinical and mycological evidence of candida vaginitis (Group C) gave 'R' type reactions to the somatic extract. This serum had in addition, a broad precipitin band to the mannan antigen and a fine precipitin band to the culture filtrate antigen. The fine precipitin band to the culture filtrate and somatic extract did not show a reaction of identity indicating that the two antigens in these two extracts which gave rise to the fine lines were not the same. It is of interest to note that 'R' type reactions to somatic extracts of C. albicans have been reported in the serum of rabbits inoculated intravenously with killed C. albicans cells (Stallybrass, 1964) and in sera from patients with candida endocarditis (Faux, 1968).

Precipitins to C. albicans antigens in babies

Sera of 6 babies who had candida precipitins in their cord blood, when tested after the age of 3 months, using the same method and antigens for precipitin tests on both occasions, showed no precip-

itins in any of them indicating that the precipitating antibody detected in the cord blood was of maternal origin and had disappeared. Of 6 sera from babies with clinical and mycological evidence of mucocutaneous candidosis, 4 had precipitins to mannan antigens. It seems that precipitating antibody derived from the mother does not confer immunity against oral or skin thrush in the newborn. The infectivity of Candida and most other fungi is thought to be suppressed predominantly by cellular immunity rather than circulating antibodies.

The frequency with which precipitins have been found in this study on pregnant women and in studies by Chew and Theus (1967) and by Pepys et al. (1968), contrasts strikingly with the reports by Stallybrass (1964), Taschdjian et al. (1964 a and b, and 1967) that precipitins were found only in patients with deep seated infections with C. albicans, in response to tests with a variety of extracts of C. albicans, all of which were shown by Pepys et al. (1968) and by Chew and Theus (1967) to contain mannan.

Agglutinin titres

There is an enormous difference of opinion about interpretation of serum agglutination titres, in response to candida antigens. Comaish et al. (1963) regard titres as low as 1:8 as an indication of the presence of Candida and a titre of 1:16 as an indication of clinical disease, while Seeliger (1968) is sceptical about titres as high as 1:160 although he considers it a positive response, in pulmonary infection due to Candida. Murray (1968) thinks that agglutination titres rising to the thousands is definitely significant. Analysis of sera from selective population groups would be

necessary before stating with certainty what titres are significant.

In agglutination tests on 116 sera from pregnant women, 86.2 per cent had titres of less than 1:16 while 13.8 per cent had titres over 1:16.

13 per cent of sera with agglutination titres of less than 1:16 and 25 per cent of sera with titres over 1:16, had demonstrable precipitins to mannan antigen. There does not seem to be a close correlation between agglutination titres over 1:16 and precipitins. However, one serum with an agglutinin titre of 1:512 gave a very strong precipitin reaction to mannan antigen. This is to be expected as mannan is a major surface antigen of yeast cells.

Summary of serological study in pregnant women and the newborn

Precipitins to C. albicans were found in 14.2 per cent of unselected pregnant women, when sera were tested against the two antigens, purified mannan and somatic antigen of C. albicans, by the Ouchterlony double diffusion technique. Only one type of precipitin reaction ('H' type) was observed. All the sera which gave reactions with purified mannan also gave reactions (sometimes weakly) with somatic antigen.

Precipitins to C. albicans were found in 18.9 per cent of unselected pregnant women, when sera were tested against the two antigens, purified mannan and culture filtrate of C. albicans, by the Ouchterlony double diffusion method. Both 'H' and 'R' types of precipitin reactions were observed. Some sera (10.3%) reacted only to the mannan antigen; 4.3 per cent reacted only to the culture filtrate, and 4.3 per cent reacted to both mannan and culture filtrate. A total

of 14.6 per cent sera reacted to mannan antigen and a total of 8.6 per cent reacted to culture filtrate.

On testing sera from pregnant women who had clinical evidence of candida vaginitis in whom the diagnosis was confirmed by the isolation of C. albicans from the vagina, precipitin reactions of the 'H' type, to mannan antigen were found in 30 per cent. Precipitin reactions of the 'R' type to culture filtrate were found in 10 per cent. It seems that precipitin reactions to mannan antigen increase in candida vaginitis. Emphasis has therefore been laid on the significance of precipitins to mannan, in relation to (a) isolation of C. albicans from the vagina and (b) the presence of candida vulvovaginitis.

It has been shown in this study that there is a significant relationship between the demonstration of precipitins to mannan antigen of C. albicans and the isolation of the fungus from the vagina. There is also a significant relationship between the demonstration of precipitins to mannan, and vulvovaginitis where the diagnosis has been confirmed by the isolation of C. albicans from a high vaginal swab. It has not been possible in this study, to show a significant relationship between the demonstration of precipitins to mannan antigen and mycotic vulvovaginitis, diagnosed on clinical criteria alone. This may be due to the fact that clinical findings were reported by different observers, all of whom were not equally interested to report in detail. Thus some patients with minor degrees of mycotic vulvovaginitis may not have been reported.

There is no close correlation between agglutinin titres of $>1:16$ and the demonstration of precipitins.

Precipitating antibody to C. albicans present in maternal sera is capable of crossing the placenta and is passively acquired by the foetus. Such passively acquired antibody has been shown to disappear from the circulation, after the age of three months. Further, passively acquired precipitating antibody to C. albicans does not appear to confer immunity against thrush in the newborn.

The candida precipitin test when first described and used was thought to be diagnostic of systemic candidosis or disseminated thrush with granuloma formation. It seems that this interpretation can no longer be maintained. Several workers have demonstrated the presence of precipitins to antigens of C. albicans in healthy persons, in patients with superficial thrush and in patients undergoing cardiac surgery who did not develop systemic candidosis. From this study evidence is presented that the demonstration of precipitins to mannan antigen in pregnant women, shows a statistically significant relationship to both candida carriage as well as thrush vaginitis which has been confirmed by the isolation of C. albicans from the vagina. It is possible that utilization of a uniform, standardized antigen as well as methodological adjustment of the precipitin test might align it with clinical thrush, with the presence of the fungus or with a sudden increase in the weight of infection. The precipitin test shows great promise as a diagnostic test in superficial candidosis.

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APPENDIX I

MOTHER

Name: Hospital No: Age:

Consultant: Parity:

L.R.M.P.: E.D.D.:

Symptoms: Pruritus

Discharge

White patches

Burning sensation

Diabetes

Steroid or Antibiotic therapy:

Asthma or Skin tests:

Treatment: Nystatin from: to:

Date of H.V. Swab (1) (2)

Trimester:

Blood for antibodies

Reports H.V.S. (1)

(2)

APPENDIX II

IDENTIFICATION OF YEASTS OF MEDICAL IMPORTANCE
TECHNICAL METHODS

1. Sabouraud's Glucose Agar

This medium is used for:-

(a) Isolation of yeasts from clinical specimens;

(b) Purification of yeasts in mixed culture with another yeast species or bacteria;

(c) Sometimes identification of yeast species, e.g. Candida krusei has typical rough colonies; Torulopsis glabrata has a whiter colony than C. albicans; Rhodotorula spp. have a red pigment.

(d) Sensitivity tests with discs of nystatin, or other antifungal antibiotic.

2. Serum Test (Germ tube test: practically specific for C. albicans)

The yeast under test is inoculated into c 0.5 ml. of horse serum in a bijoux bottle to give a faintly turbid suspension and incubated at 37°C for 1½ - 2 hours. Filamentous outgrowths called 'germ tubes' are formed by C. albicans and C. stellatoidea and can be seen in wet preparations under a microscope using the high power objective.

3. Corn Meal Agar

(a) Chlamydospore production

The test yeast is inoculated by means of deep cuts into

the agar using a sterilised needle or scalpel. The inoculum cut is then covered with a coverslip so that the cut extends beyond the edge of the coverslip. Three or four test yeasts may be cut into one plate. The plates are incubated at c 26°C for 1 - 3 days and examined under a microscope using the low power objective. Thickwalled structures known as chlamydospores are produced by C. albicans usually after 24 hr. incubation. In C. albicans these are borne terminally on hyphae; blastospores will also be seen. They are very often found first on the inoculum site at the edge of the coverslip. C. stellatoidea is the only other yeast which may produce a few chlamydospores.

(b) Pseudomycelium production

When chlamydospores are not formed by the test yeast, it should be noted whether or not pseudomycelium is produced. Candida spp. produce pseudomycelium and species identification can sometimes be aided by microscopic morphology on corn meal agar, e.g. giant cells are produced by C. parapsilosis. Cryptococcus spp., Torulopsis spp. and usually Saccharomyces spp. do not produce pseudomycelium.

When pseudomycelium is produced, but neither germ tubes nor chlamydospores, the yeast is presumed to be a Candida species other than albicans and should be inoculated into sugars and auxanogram plates for species identification. C. stellatoidea may pro-

duce both germ tubes and chlamydospores and can only be distinguished from C. albicans by its reactions on auxanograms. Auxanogram plates should therefore always be set up, on isolates of importance.

4. Auxanograms

Two agar media are used for this test. One contains a carbon source but no nitrogen; the other a nitrogen source but no carbon. The two media are melted and cooled to 45°C. A suspension of the test yeast is made in 5-6 ml. of sterile 0.85 per cent saline and half poured into each of two plates. The two molten media are poured into separate plates and allowed to set. Coloured discs impregnated with 10 per cent solutions of glucose, galactose, maltose, sucrose or lactose are placed on to the agar medium containing no carbon. Discs impregnated with a 10 per cent solution of potassium nitrate or a 2 per cent solution of asparagine (as control) are placed on agar medium containing no nitrogen. The plates are incubated at 30°C for 24 hours. If the test yeast can assimilate one of the sugars or nitrogen compounds, growth occurs round the disc. Some strains of yeasts require vitamins for growth and these can be added to the molten media either in the form of a vitamin solution (as described by Lodder (1970) or by adding 2 drops of a 1 per cent (w/v) sterile solution of yeast extract (e.g. Oxoid). The following results would be obtained for the Candida spp. occurring most commonly in the laboratory.

	Glucose	Galactose	Maltose	Sucrose	Lactose	Asparagine	Nitrate
C. albicans	+	+	+	+	-	+	-
C. stellatoidea	+	+	+	-	-	+	-
C. tropicalis	+	+	+	+	-	+	-
C. krusei	+	-	-	-	-	+	-
C. pseudotropicalis	+	+	-	+	+	+	-
C. parapsilosis	+	+	+	+	-	+	-
C. guilliermondii	+	+	+	+	-	+	-
T. glabrata	+	-	-	-	-	+	-
S. cerevisiae	+	+	+	+	-	+	-

+ = assimilation

5. Sugar Peptone Waters

Glucose, galactose, maltose, sucrose, lactose and raffinose are inoculated with the yeast and incubated at 37°C for 2 - 14 days. The following fermentation reactions would be obtained for the more commonly occurring Candida spp.

Fermentation reactions

	Glucose	Galactose	Maltose	Sucrose	Lactose	Raffinose
C. albicans	+	+ (often weak)	+	-	-	-
C. stellatoidea	+	-	+	-	-	-
C. tropicalis	+	+	+	+	-	-
C. krusei	+**	-	-	-	-	-
C. pseudotropicalis	+	+	-	+	+	+ or weak
C. parapsilosis	+	- or +	-	-	-	-
C. guilliermondii	+	+ or weak	-	+ or weak	-	+ or weak
T. glabrata	+	-	-	-	-	-
S. cerevesiae	+	+	+	+	-	+ (⅓)

+ = acid and gas production

- = neither acid nor gas

** = surface pellicle

SPORULATION OF YEASTS

With rare exceptions, yeasts of medical importance have no known sexual reproduction, and are classed amongst the Fungi Imperfecti. Perfect yeasts, such as Saccharomyces or Hansenula are occasionally encountered in clinical specimens. The spores of these ascomycetes are demonstrated by inoculation of sodium acetate medium with a young culture of the yeast under investigation. Cultures are examined daily for 5 days, then at 5 day intervals up to 25 days, for sporulation.

Demonstration of ascospores

Smear and heat fix yeast on glass microscope slide. Stain 2 minutes with near boiling malachite green 2.5 per cent. Rinse in tap water several times, and counterstain with safranin 0.5 per cent for 1 minute, again thoroughly washing in tap water. Examine, using an oil immersion lens. At least 2,000 cells should be examined before the yeast is regarded as asporogenous. Ascospores of the yeasts stain green.

M E D I A

Sabouraud's Glucose Agar

Peptone	10 g.
Glucose (commercial)	40 g.
Agar (New Zealand)	15 g.
Water	1,000 ml.

Heat to dissolve and bottle

Autoclave 10 lb./15 min.

Final pH 5.5

Corn Meal Agar

Corn Meal	62.5g.
Water	1,500 ml.

Place in water bath 60°C for 1 hr. Filter through paper and make volume up to 1,500 ml. with hot water.

Add Agar	23g.
and Tween 80	15 ml.

Autoclave 15 lb./15 min.

Auxanogram Media

1. No carbon source

Potassium dihydrogen phosphate	1 g.
Magnesium sulphate	0.5 g.
Agar (unwashed)	22 g.
Distilled water	1,000 ml.
Ammonium sulphate	5 g.

Heat to dissolve and bottle. Autoclave 15 lb./15 min.

2. No nitrogen source

Potassium dihydrogen phosphate	1 g.
Magnesium sulphate	0.5 g.
Agar (washed)	20 g.
Glucose	20 g.
Distilled water	1,000 ml.

Heat to dissolve and bottle. Autoclave 10 lb./15 min.

Sugar Peptone Waters (3% solutions)

Peptone water	1,000 ml.
Sugar	30 g.

Dispense 5 ml. amounts into test tubes with Durham tubes, plug with cotton wool or Oxoid polypropylene coloured caps and steam for 20 min. on three successive days.

Sporulation Medium

Sodium Acetate Agar

$CH_3\ COONa\ 3H_2O$	10 g.
Glucose	1 g.
Oxoid yeast extract powder	2.5 g.
Oxoid ionagar	20 g.
Distilled water	1,000 ml.

Adjust pH 6.9 - 7.1 before sterilizing

Autoclave 15 lb./15 min.

Disc Sensitivity Tests

Discs of nystatin (100 units) and amphotericin B (20 g.) are placed on Sabouraud's glucose agar plates previously covered by streaking with the test yeast. (Confirmatory test only).

A complete account of taxonomy and identifying criteria of all yeasts is found in:-

Lodder, J.W., Editor, The Yeasts, 1970.

North Holland Publishing Company.

APPENDIX III

THE PREPARATION OF THIS THESIS

1. The experimental work described in this thesis was carried out in the Bernhard Baron Memorial Research Laboratories of Queen Charlotte's Maternity Hospital, London, W.6., under the direction and supervision of Dr. Rosalinde Hurley, M.D.

2. The high vaginal swabs from pregnant women were collected by the Obstetric staff at the antenatal clinics of Queen Charlotte's Maternity Hospital.

3. The oral and anal swabs from newborn babies were collected by me.

4. Culture and identification of all yeasts isolated was done by me. The identification of some isolates was confirmed by Miss Barbara Leask. Occasional isolates were referred to Dr. Helen Buckley.

5. Candida antigens, for the preliminary serological studies were provided by Dr. J.A. Faux of the Institute of Chest Diseases, Brompton Hospital. The preparation and purification of mannan antigen, somatic antigen, and culture filtrate antigens for precipitin tests on the rest of the sera was done by me.

6. All the agar gel diffusion tests, immunoelectrophoresis and immunoosmophoresis were done by me.

7. The media used in this investigation were prepared by Mr. C.A. Cole.

8. The photographs were taken by Mr. J.P. Arthur and Miss B.G.S. Leask.

9. The histological section in Figure 25 was provided by Dr. J. Pryse-Davies.

10. All records of the prospective study on candida vaginitis were kept by me.

11. During the preparation of this thesis, I was Honorary Senior Registrar at the Institute of Obstetrics and Gynaecology, London, W.6.

12. I started preparation of this thesis in 1968 and finished it in 1971.

APPENDIX IV

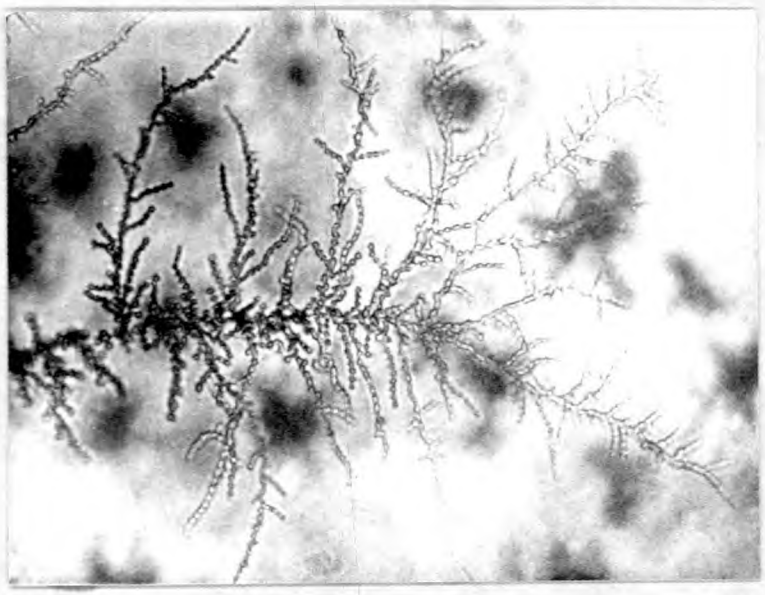
MORPHOLOGY, FERMENTATION REACTIONS AND AUXANOGRAMS OF AN UNIDENTIFIED YEAST

In the course of this investigation an unusual yeast was isolated from the vagina of a pregnant woman whose country of origin was India. A history was not available as her knowledge of English was limited. The vagina was apparently healthy but she had tuberculous cervical lymph glands.

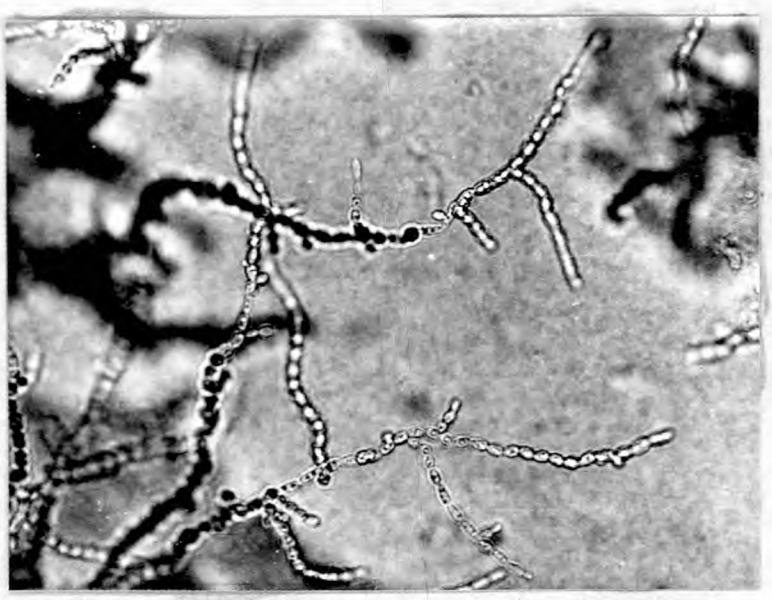
The morphology of this yeast is shown in Fig. 27. It did not produce chlamydospores on cornmeal agar or germ tubes in serum, nor did it produce ascospores on acetate medium.

The fermentation and assimilation patterns are given on pages 182 and 183. They are identical with those of C. albicans (Lodder, 1970). It differs from C. albicans in the morphology of the pseudomycelium on cornmeal agar; the inability to produce chlamydospores and germ tubes.

This yeast culture was sent to the Mycological Reference Laboratory, London and to the Centralbureau Voor Schimmelcultures, Delft, Netherlands for identification. Mr. David Yarrow of the Centralbureau rules out the identification of this yeast as C. albicans "on the grounds of absence of chlamydospores and morphology of the pseudohyphae, although the size and shape of the cells are similar to those of C. albicans as are the fermentation and assimilation characteristics". The other two possibilities suggested by him were C. parapsilosis and C. sake, "both of which



x 180



x 475

Fig. 27. Hyphae of the yeast grown on cornmeal agar with Tween 80, for 3 days at 26°C. Unstained.

Note absence of chlamydospores.

sometimes ferment maltose but not as strongly as this isolate; but neither of these species assimilates soluble starch whereas this isolate does, as does C. albicans. Moreover, C. sake assimilates β glucosides". He seems to think that this strain comes nearer to C. parapsilosis than to any of the other alternatives.

Pathogenicity tests on mice and rabbits have been carried out on this isolate by Miss B.G.S. Leask and Dr. Helen Buckley. The preliminary results indicate that this yeast is pathogenic for mice and rabbits.

The fermentation and assimilation characteristics of this yeast are identical with those of C. albicans although its morphology is quite different. It is suggested that this yeast may be a new variety of C. albicans.

Comparison of assimilation patterns

	Unidentified Yeast	C. albicans	C. parapsilosis
Glucose	+	+	+
Galactose	+	+	+
L-sorbose	+	v	v
Maltose	+	+	+
Sucrose	+	+	+
Cellobiose	-	-	-
Trehalose	+	+	+
Lactose	-	-	-
Melibiose	-	-	-
Raffinose	-	-	-
Melezitose	-	v	+
Inulin	-	-	-
Soluble starch	+	+	-
D-Xylose	+	+	+
L-Arabinose	+	v	v
D-Arabinose	-	-	-
D-Ribose	-	-	v
L-Rhamnose	-	-	-
Ethanol	+	+ or v	+
Glycerol	+	v	+
Erythritol	-	-	-
D-Mannitol	+	+	+
Methyl - D glucoside	+	+	+
Salicin	-	-	-
Lactic acid	+	+	v
Succinic acid	+	+	v
Citric acid	+	+	v
Inositol	-	-	-
Potassium nitrate	-	-	-

v = variable

Comparison of fermentation reactions

	Unidentified Yeast	C. albicans	C. parapsilosis
Glucose	+	+	+
Galactose	+ (after 17 days)	v	v
Sucrose	- (acid only)	- (gas bubble may be formed)	-
Lactose	-	-	-
Raffinose	-	-	-
Other criteria			
Germ tubes in serum at 37°C	-	+	-
Chlamydospore production on cornmeal agar + Tween 80 at 25°C	-	+	-
Sporulation on acetate medium at 25°C	-	-	-
Growth at 37°C	+	+	+