THE RELATIONSHIP OF SERUM TRANSFERRIN AND IRON TO THE RAPID FORMATION OF GERM TUBES BY *CANDIDA ALBICANS**

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The yeast cells of *Candida albicans* readily form filaments or "germ tubes" within two to three hours when incubated at 37° C. in such media as serum or plasma derived from humans and animals, human cerebrospinal fluid, raw egg white or tissue culture media 109 and 199 (1–4). Germ tubes do not develop, however, in numbers over 5 to 10 percent when the identical inoculum of yeast cells is incubated for the same time at the same temperature in distilled water, physiologic saline, 5 per cent dextrose in water, or freshly prepared Sabouraud dextrose (Bacto-peptone®) broth.

The growth of *C. albicans* may be initiated predominantly by budding, by the formation of germ tubes or by both. Germ tubes are distinguishable from buds by their longer length and narrower width (3, 4). Germ tubes appear to represent true mycelia rather than pseudomycelia since they elongate by apical growth and develop subsequent septae.

The proper interpretation of this rapid formation of germ tubes in serum by *C. albicans* has not been clarified. A principal unresolved question is whether such germination is representative of inhibition or enhancement of subsequent growth and pathogenicity of the fungus (4, 5). Observations of the growth of *C. albicans* on solid media indicate that the mycelial phase can be induced by conditions which inhibit growth without stopping it entirely but these are not necessarily pertinent. The develment of mycelia by *C. albicans* in tissues has been considered by others to be a manifestation of increased pathogenicity.

The factor in serum responsible for the rapid germ tube formation by *C. albicans* has partially been characterized as heat-stable, not removed by dialysis, and unrelated to Candida agglutinins and precipitins (1, 4). The cumulative evidence indicates that the property is distinct from classical antibody. The possibility was considered, however, that a protein in serum other than gamma globulin may influence the rapid formation of germ tubes by *C. albicans*.

Transferrins or siderophilins are glycoproteins in human plasma which migrate electrophoretically as beta$_1$ globulins and function principally in the transport of iron (6, 7). The iron found in plasma is almost all bound to transferrin. Although copper and zinc also unite with transferrin, the iron union is preferential at a physiologic pH. Serum levels of transferrin are expressed in terms of total iron-binding capacity (TIBC) which is equal to the sum of the serum iron (SI) and the unbound iron-binding capacity (UIBC). The percentage of saturation of transferrin is equal to 100 times the SI divided by the TIBC. The normal TIBC in man varies from 248 to 422 ug of iron per 100 ml of serum and the normal SI varies from 57 to 194 μg of iron per 100 ml of serum (8). In certain diseases, these values are altered. A low SI and an elevated TIBC are characteristic of patients with iron-deficiency anemia and an elevated SI and a low TIBC are characteristic of iron storage diseases.

A less well-known property of unsaturated transferrin is its non-specific antimicrobial activity against iron-dependent infectious organisms. Unsaturated transferrin has been demonstrated to inhibit the *in vitro* growth of a variety of bacteria and the inhibition has been reversed by the addition of iron in the form of ferrous ammonium sulfate in excess of the unbound iron-binding capacity (9, 10). Purified transferrin also suppresses the cytopathogenic activity of certain viruses but the addition of iron salts only slightly reversed the antiviral effect (11). Administration of small doses of purified iron-free human transferrin to rats

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and mice experimentally infected with *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* has been demonstrated to afford a slight degree of protection (12).

The availability of iron under certain circumstances has been considered to be a significant factor in the growth of *C. albicans*. The fungistatic activity of egg white on the growth of *C. albicans* has been postulated to reside, in part, in its iron-binding fraction, conalbumin, since the addition of ferrous salts to the medium containing egg white neutralized the fungistatic effect (13). The addition of iron as ferrous ammonium sulfate to media containing mouse ascites fluid or, in some instances, mouse serum, caused diminution of the inhibitory activity of these two fluids against *C. albicans* (14).

The activity of unsaturated human transferrin in the inhibition of growth of *C. albicans* has recently been demonstrated (15). The addition of excess iron as ferrous ammonium sulfate to human serum-neopeptone broth resulted in the reversal of inhibition of *C. albicans* as measured by a turbidimetric method. When undiluted serum was employed as a growth medium, inhibition of *C. albicans* occurred. Vigorous growth ensued as measured by an increase in turbidimetry and hemocytometer counts following saturation of the transferrin with iron.

The purpose of this report is to present the results of studies evaluating the role of human serum transferrin and iron in the rapid formation of germ tubes by *C. albicans*. Additional studies utilizing a quantitative plating technique were conducted in some instances to evaluate their effect on the number of colonies subsequently produced by this fungus.

MATERIALS AND METHODS

Stains of C. albicans

A strain of *C. albicans* originally isolated from a patient with paronychia and characterized previously (4) was used. In one of the experiments three other strains from the stock culture collection of this laboratory were used in addition.

Sera

Sera were obtained from fasting healthy donors and fasting medical patients without fungous infections. Some of the patients were receiving a variety of medicaments none of which, however, had any known antifungal properties. The sera were separated from the clots by centrifugation, stored at 4° C., and used within 96 hours. The equipment used in this study was specially processed to remove iron.

Other Media

Tissue culture medium 199 (Hyland Laboratories) was used at full strength.

Sabouraud broth was prepared to contain 4 per cent dextrose (Baker Analyzed with .0001 per cent iron) and 1 per cent Bacto-peptone® in distilled water.

Iron

A solution of ferrous ammonium sulfate ($FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$) in .03M acetic acid was prepared to contain 640 μg of iron per ml.

A solution of ammonium sulfate ($(NH_4)_2SO_4$) was prepared to contain the same amount of sulfate ions per ml as the ferrous ammonium sulfate solution.

The solutions were each sterilized by filtering through ultra-fine (UF) fritted discs (Pyrex).

Iron-chelating Agent

The hydrochloride form of desferrioxamine B (Ciba) was solubilized in distilled water for use.

Serum Iron and Total Iron-binding Capacity

These determinations were performed by the methods of Peters *et al.* (8). The unbound iron-binding capacity and the percentage of saturation of transferrin were then computed.

Addition of Iron to Sera or Other Media

The solution of ferrous ammonium sulfate was appropriately diluted and added to a medium to obtain the desired concentration of iron. The medium in each experiment to which iron was not added was similarly diluted with iron-free distilled water. The original medium in each instance constituted 90 per cent of the final volume. The final pH of each test medium was adjusted as necessary to 7.0 to 7.4 with iron-free saturated sodium bicarbonate.

Germ Tube Percentage Determination

The procedure, described in detail previously (4), consisted of pipetting .5 ml of the test medium into a sterile tube and inoculating with .05 ml of a fresh suspension of *C. albicans* cultured on a 4 per cent dextrose, 2 per cent agar medium. The density of the inoculum of *C. albicans* was adjusted to contain approximately 10^7 to 10^8 cells per ml so as to produce 40 to 70 per cent germ tubes when incubated in serum. Permanent slides were prepared from these test media after incubating at 37° C. for three hours. The slides were examined, 200 cells counted and the percentage of germ tubes and budding cells recorded.

Quantitative Plating Technic

The test media were distributed in .9 ml quantities into sterile tubes which were then inoculated

with .1 ml of a freshly prepared suspension of *C. albicans* containing 2×10^3 cells per ml producing a final concentration of 200 cells per ml. These were then incubated at 37° C. At intervals .05 ml were withdrawn from each tube and after suitable dilutions, cultured on Sabouraud dextrose agar plates. These plates were incubated for 48 hours at 37° C. and the number of colonies recorded.

In one experiment similar quantitative colony counts were performed with the larger inoculum employed in the germ tube determinations.

All studies of both germ tubes and colony counts were performed in duplicate and the average recorded as the result.

Statistics

The t-test was employed to compare the means and correlation coefficients to compare the different variables in experiment 1. Computations were performed at the Health Sciences Computing Facility UCLA. The sign test was used in analysis of experiment 4. A probability of occurrence of $p > .05$ was considered not statistically significant (16).

EXPERIMENTS AND RESULTS

Experiment 1.—The Effect of Saturation of Transferrin on the Formation of Germ Tubes and Buds

Sera from five normal donors and nine patients were examined before and after the addition of sufficient iron to produce 100 per cent and the equivalent of 200 per cent saturation of transferrin. In all instances more germ tubes developed in the sera with 100 per cent saturation (mean = 60 per cent) than in the sera with the unsaturated transferrin (mean = 46 per cent) (Figure 1). This finding is significant at $p < .005$. No significant additional increase occurred with further saturation of the transferrin to 200 per cent. The percentage of germ tubes and buds developing in the individual sera and the determination of the iron variables are presented in Table I. The difference in percentage of buds in the unsaturated and the 100 per cent saturated sera is not statistically significant, but the difference between the unsaturated and 200 per cent saturated sera is significant at $p < .05$. No significant correlation was found between either the percentage of germ tubes developing in any of the sera or the difference in germ tubes in the unsaturated and the 100 per cent saturated sera and the SI, TIBC, UIBC, SI/TIBC or SI/UIBC. A significant inverse correlation ($p < .05$) was found between the percentage of germ tubes and the percentage of buds in the unsaturated sera. No significant correlation was found between the absolute percentage of buds in the unsaturated sera and any of the iron variables.

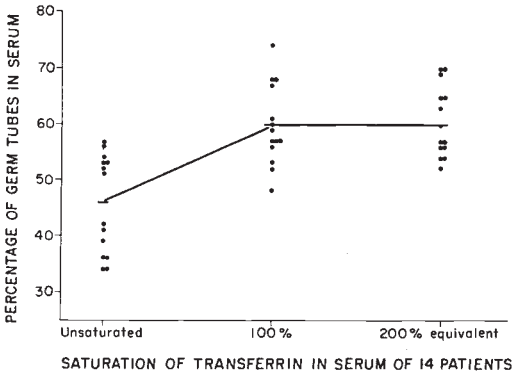


FIG. 1. Germ tube formation by *Candida albicans* in serum before and after the addition of iron to sera to produce 100 percent and the equivalent of 200 percent saturation of transferrin.

The addition of ammonium sulfate to six sera to a concentration of sulfate ions equivalent to that in the ferrous ammonium sulfate solution added to produce 100 per cent and 200 per cent saturation of transferrin did not result in any increase in percentage of germ tubes.

The increased germ tube formation in sera with saturated tranferrin compared to that in sera with unsaturated transferrin was also demonstrable with the three other strains of *C. albicans*.

Experiment 2.—The Effect on the Formation of Germ Tubes of the Addition of Graded Amounts of Iron to Sera from Two Normal Donors Before and After the Oral Administration of Two Grams of Ferrous Sulfate

The percentage saturation was increased from 18 per cent (SI/TIBC = 61/314) to 82 per cent (279/342) in one donor and from 31 per cent (99/324) to 100 per cent (326/326) in the other five hours after the ingestion of ferrous sulfate. The amount of iron required to be added directly to serum for maximum germ tube formation was dependent on the quantity necessary to achieve 100 per cent saturation (Figure 2). Since the post-ingestion sera of both donors were almost or completely saturated, maximum germ tube formation occurred

TABLE I

The Formation of Germ Tubes and Buds by Candida albicans *in Serum Before and After the Addition of Iron to Produce 100 percent and the Equivalent of 200 percent Saturation of Transferrin*

Source of Serum	SI	TIBC	UIBC	SI/TIBC × 100	Percentage of Germ Tubes in Sera with Iron to a Saturation of			Percentage of Buds in Sera with Iron to a Saturation of		
	μg per 100 ml				*unsat.*	*100%*	*200% equivalent*	*unsat.*	*100%*	*200% equivalent*
Normal Donor	95	346	251	27	53	68	65	21	22	20
Normal Donor	98	297	199	33	56	67	70	27	20	21
Normal Donor	95	283	188	34	34	59	58	37	26	23
Normal Donor	120	314	194	38	36	57	54	32	24	23
Normal Donor	162	324	162	50	34	57	57	35	22	19
Patient	67	321	254	20	54	57	54	19	25	20
Patient	57	236	179	24	51	68	69	21	16	16
Patient	72	274	202	26	39	52	52	25	20	21
Patient	230	289	59	80	42	60	65	24	19	17
Patient	48	274	226	18	53	62	63	23	20	18
Patient	131	297	166	44	41	48	56	22	19	17
Patient	80	307	227	26	52	56	56	20	24	18
Patient	76	361	285	21	36	53	57	23	23	20
Patient	28	213	185	13	57	74	70	17	18	19
Mean	97	295	198	32	46	60	60	25	21	19
Std. Deviation	52	39	54	17	9	7	6	6	3	2

SI = Serum iron. TIBC = Total iron-binding capacity. UIBC = Unbound iron-binding capacity. SI/TIBC × 100 = Percentage of saturation.

with the addition of less iron than that necessary in the respective pre-ingestion sera.

Experiment 3.—The Effect on the Formation of Germ Tubes of the Total Iron in Three Sera with Different Total Iron-binding Capacities

Three sera with total iron-binding capacities of 213, 272, and 310 μg per 100 ml respectively were studied. The addition of iron to each serum resulted in maximum germ tube formation after sufficient iron had been added to achieve 100 per cent saturation of the transferrin rather than at any specific total serum iron level (Figure 3). The addition of sufficient iron to total a serum iron of 220 μg per 100 ml resulted in saturation and maximum germ tube formation in the first serum but not in the other two. The addition of more iron to total 284 ug per 100 ml resulted in saturation and maximum germ tube formation in the first and second sera and further addition to total 328 μg per 100 ml resulted in saturation and maximum germ tube formation in all three sera.

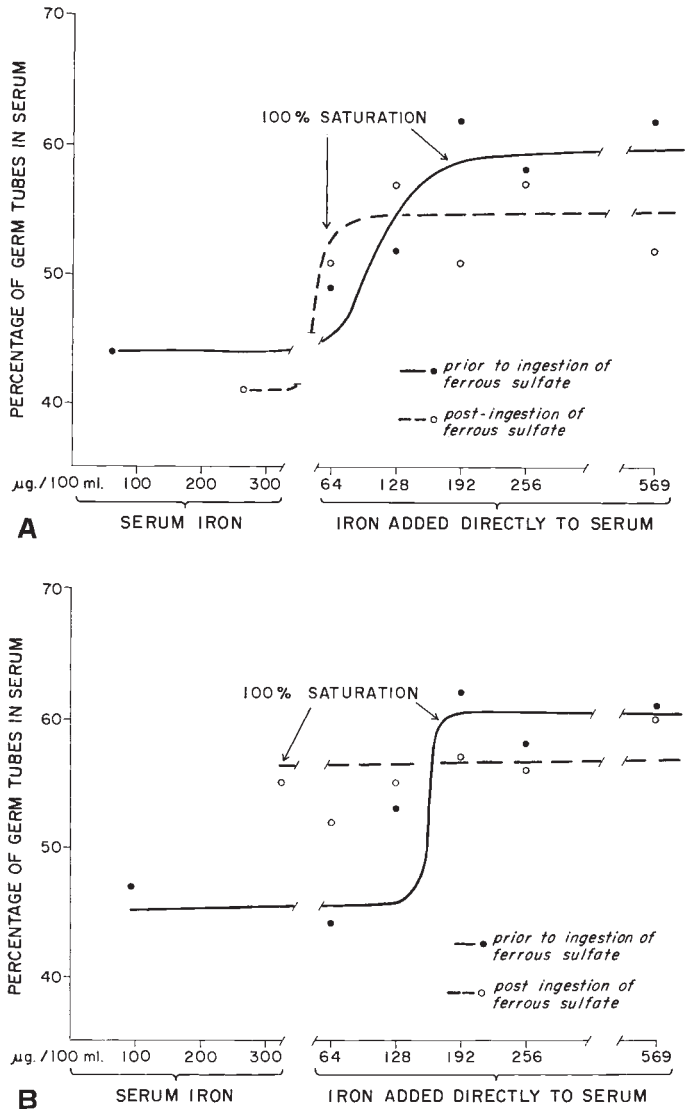
The addition of more iron to total the equivalent of 300 percent and 500 percent saturation did not alter the maximum percentage of germ tubes formed.

Experiment 4.—The Effect of Saturation of Transferrin on the Number of Colonies of C. albicans

Sera from six individuals were studied before and after the addition of iron to produce 100 percent and the equivalent of 200 percent saturation of their transferrin. An increased number of colonies was obtained in each instance from the sera with 100 per cent saturated transferrin when compared with the number of colonies from the sera with unsaturated transferrin. This is significant at $p < .05$. No increase in number of colonies occurred with the further addition of iron. The mean results are presented in Figure 4.

The addition of ammonium sulfate to three of these sera to a concentration of sulfate ions equivalent to that in the ferrous ammonium sulfate solution added to produce 100 percent and 200 percent saturation of transferrin did not result in any increase in number of colonies.

Partial saturation of transferrin did not pro-



FIGS. 2A and 2B. Germ tube formation by *Candida albicans* in serum after the addition of iron to sera from two patients before and five hours after the ingestion of two grams of ferrous sulfate.

duce any increase in number of colonies compared to that in serum with the original level of unsaturated transferrin and addition of iron to an equivalent of 500 per cent saturation produced no increase beyond that which occurred in serum saturated to 100 percent (Figure 5).

No difference between the number of colonies in three sera with saturated or unsaturated transferrin was found at three or at 24 hours when the larger inoculum employed for the germ tube procedure was used.

Experiment 5.—The Effect of Dilution of Sera with Unsaturated and Saturated Transferrin on Germ Tube Formation and Number of Colonies

The increase in germ tube formation and number of colonies in sera with 100 percent saturated transferrin compared to that in sera with unsaturated transferrin also occurred after dilution of the sera to one-third of their original volumes with iron-free distilled water. A slight increase in both germ tube formation and num-

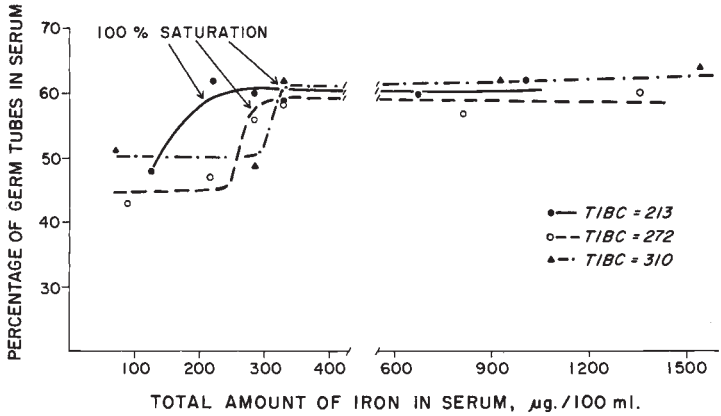


FIG. 3. Germ tube formation by *Candida albicans* in three sera with different total iron-binding capacities.

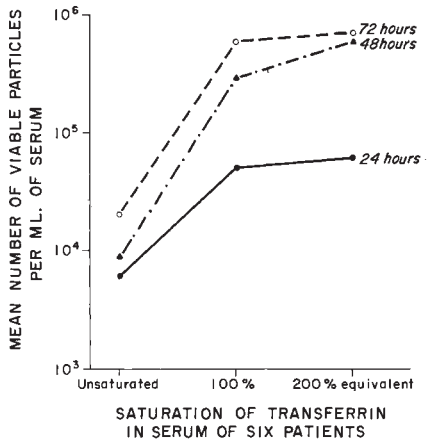


FIG. 4. The numbers of colonies of *Candida albicans* from sera before and after the addition of iron to produce 100 percent and the equivalent of 200 percent saturation of transferrin.

ber of colonies was observed in diluted sera compared to that in undiluted sera. These results are presented in Figure 6.

Experiment 6.—The Effect of the Addition of Iron to Medium 199 on Germ Tube Formation and Number of Colonies

No increase in either germ tube formation or number of colonies occurred when iron was added to the medium. Germ tube formation in medium 199, unsupplemented and supplemented with 64 µg, 320 µg and 640 µg of iron per 100 ml was 67, 65, 70 and 68 per cent, respectively and the number of colonies developing after 72 hours in this medium was 1.2×10^6, 1.7×10^6, 1.7×10^6 and 3.6×10^6 per ml, respectively.

Although iron is not included as a constituent in medium 199, 6 µg per 100 ml were found in the medium used in this study.

Experiment 7.—The Effect of the Addition of Desferrioxamine B to Serum on Germ Tube Formation

Desferrioxamine B was added to sera with saturated and unsaturated transferrin to a concentration of 5.4 mg per 100 ml. This amount theoretically has the capacity to bind 502 µg of iron (17). The addition of this chelating agent to serum with unsaturated transferrin did not affect the percentage of germ tubes. However, when added to serum with 100 percent saturated transferrin the percentage of germ tubes was reduced to that in the sera with unsaturated transferrin. The addition of this agent to serum containing iron to total 1160 µg per 100 ml did not alter the percentage of germ tubes. These results are presented in Table II.

Experiment 8.—The Relationship between Germ Tubes, Buds, and Number of Colonies

These three parameters of development of *C. albicans* were determined in two studies in which the fungus was incubated in distilled water, serum with unsaturated transferrin, serum with saturated transferrin, medium 199 and Sabouraud broth (Table III). Neither the percentage of germ tubes nor the percentage of buds formed in a medium was a satisfactory indicator of the number of colonies which subsequently developed. The percentage of

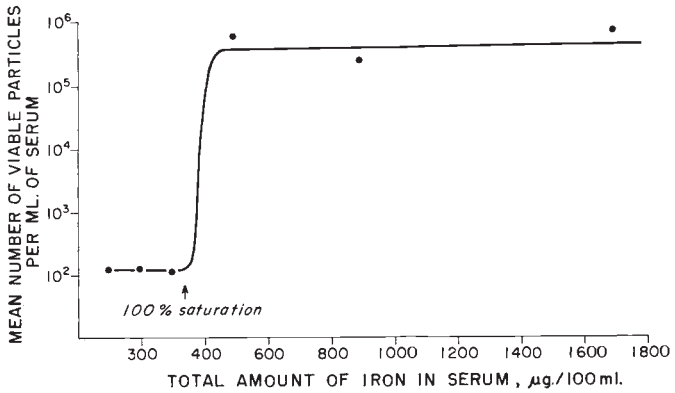


FIG. 5. The number of colonies of *Candida albicans* from a serum after the addition of graded amounts of iron.

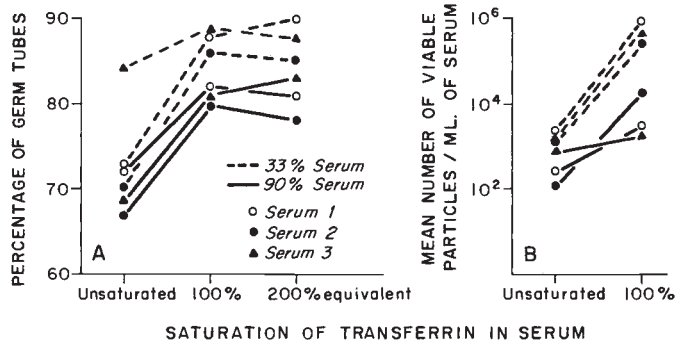


FIG. 6. Germ tube formation by and number of colonies of *Candida albicans* from three different sera with unsaturated and saturated transferrin after dilution with distilled water.

germ tubes formed in medium 199 and in the serum with saturated transferrin was approximately the same but the number of colonies obtained from the former was much greater. A very low percentage of germ tubes developed in distilled water and Sabouraud broth but the number of colonies obtained was low from the former and very high from the latter. The highest numbers of colonies were obtained from medium 199 and Sabouraud broth, but few buds were formed in the former and many in the latter.

DISCUSSION

This study demonstrates that the addition of iron as ferrous ammonium sulfate to human serum with unsaturated transferrin is followed by an increase in both the percentage of germ tubes and the total number of colonies formed by *C. albicans* subsequently incubated in this serum. The addition of ammonium sulfate alone failed to achieve this effect implying that iron is the essential element. Supporting this is the observation that ferrous sulfate administered orally to produce saturation of transferrin also increased the percentage of germ tubes formed. The quantity of iron required to achieve the maximum percentage of germ tube formation and number of colonies is related to the amount necessary to produce 100 per cent saturation of transferrin in each serum but not to the absolute amount of iron added to serum or to the total amount of iron present in serum. The addition of iron to essentially iron-free culture medium 199 produces no change in the percentage of germ tubes or number of colonies formed suggesting that it does not directly stimulate the development of *C. albicans*. The increases in addition cannot be attributed to enhancing activity of a transferrin-iron complex since dilution of serum to one-third of its original volume did not decrease germ tube or colony production.

The mechanism responsible for the increase

TABLE II

The effect on germ tube formation by Candida albicans *of the addition of desferrioxamine-B to two sera with unsaturated and saturated transferrin*

	Percentage of Germ Tubes	
	Serum A	Serum B
Serum with unsaturated transferrin	52	50
Serum with unsaturated transferrin + DFOM	53	48
Serum with 100 percent saturated transferrin	69	67
Serum with 100 percent saturated transferrin + DFOM	47	48
Serum with transferrin saturated in excess*	72	68
Serum with transferrin saturated in excess + DFOM	72	72

DFOM—Desferrioxamine B added to a concentration capable of theoretically binding 502 μg of iron per 100 ml. Serum A—SI/TIBC = 105/342. Serum B—SI/TIBC = 115/340. SI—Serum iron in μg per 100 ml. TIBC—Total iron-binding capacity in μg per 100 ml.

* Iron added to total 1160 μg per 100 ml.

in both the percentage of germ tubes and in number of colonies observed after the addition of iron to serum is not based on any active enhancement but rather appears to be due to the removal of an inhibitory factor. The factor is presumably the unbound iron-binding capacity of unsaturated transferrin which preferentially binds the iron ordinarily available for the metabolism and growth of *C. albicans*. This concept of antimicrobial activity of human unsaturated transferrin is supported by studies (9, 10, 12) concerned with bacteria and by others (14, 15) utilizing different technics to evaluate this activity on *C. albicans*.

The iron requirement of *C. albicans* was not determined in this study but must be extremely low since both germ tubes and colonies successfully developed in medium 199 which contained approximately 6 μg of iron per 100 ml. Concentrations of iron over 1500 μg per 100 ml did not adversely affect the percentage of germ tubes or the number of colonies formed. The percentage of buds, however, was significantly reduced by the addition of iron equivalent to 200 percent saturation of transferrin which averaged a total of 600 μg of iron per 100 ml.

A flavoprotein-metal complex, possibly containing ferrous ion, has been postulated to be essential for cellular division by budding of *C. albicans* grown on solid media (18). The dissociation of this complex is thought to favor filament formation. This observation is not consistent with the finding that lowered germ tube production occurs in the serum with unsaturated transferrin compared to that in serum with saturated transferrin if the unsaturated transferrin has any effect on the flavoprotein-metal complex. The metabolic pathway of cellular division, however, may differ in serum from that in solid media (19).

The possibility that unsaturated transferrin exerts its inhibitory activity on *C. albicans* by binding a metallic ion other than iron has not been entirely excluded but is unlikely. Iron alone, of ten vitamin factors and thirty-one elements tested, was demonstrated (20) to overcome the antimicrobial activity of conalbumin, the iron-binding component of raw egg white which has certain similarities to transferrin.

TABLE III

The relationship between germ tubes, buds and number of colonies in various media

Media	Percentage of Germ Tubes	Percentage of Buds	Number of Colonies per ml After 72 Hours	Iron in Medium
				μg per 100 ml
Study A				
Distilled water	3	28	1.4×10^3	0
Serum (Unsat. transferrin)	55	12	6×10^2	69
Serum (100% Saturated)	68	6	1.1×10^4	320
Medium 199	67	7	1.2×10^6	6
Sabouraud broth	6	81	6×10^6	33
Study B				
Distilled water	2	42	4×10	0
Serum (Unsat. transferrin)	50	11	5×10	105
Serum (100% Saturated)	62	12	1×10^2	342
Medium 199	68	7	2×10^6	6
Sabouraud broth	6	62	1.3×10^7	33

The data obtained with desferrioxamine B and serum with saturated transferrin provide supporting evidence that removal of inhibition is the mechanism by which the addition of iron to serum produces an increase in percentage of germ tubes. Since desferrioxamine B can remove iron from transferrin (17), its effect on *C. albicans* may have been mediated either directly or indirectly via transferrin. The failure of the addition of desferrioxamine B to serum with unsaturated transferrin to reduce the percentage of germ tubes implies that a maximum inhibition through this mechanism has already been achieved.

Iron as ferrous ion was employed in this and other studies (9, 14, 15) to saturate transferrin but ferric ion has also been used in some instances (10). The iron actually combined with transferrin and with desferrioxamine B is in the ferric state (6). Ferrous ion, however, when added to serum is rapidly autooxidized to ferric ion (6).

Some of the other constituents of serum must be inhibitory for the development of germ tubes and of colonies since dilution of serum with distilled water with either unsaturated or saturated transferrin resulted in increases. Preliminary studies in this laboratory have indicated the importance of the choice of a diluent since dilution of serum with physiologic saline produces a decrease in percentage of germ tubes formed.

The apparent parallel relationship in serum between the percentage of germ tubes and the number of colonies, both of which increased after saturation of transferrin, can not be extended to *C. albicans* incubated in other media. A high number of colonies developed, for example, in Sabouraud broth compared to a low percentage of germ tubes. Similarly the percentage of buds can not be directly related to the total number of colonies. The results of the experiment with other media do suggest, however, that a medium in which a high number of colonies develops, will also support the production of a high percentage of either germ tubes or buds. The growth of *C. albicans* can probably satisfactorily proceed by either germ tube or bud production.

This study does not explain why germ tubes rather than buds are preferentially formed in serum and medium 199. Although the occurrence of germ tubes in nonprotein medium 199 indicates that protein is not always essential for this process a role for protein in germ tube formation in serum has not been excluded. Furthermore, the mechanism by which germ tube formation occurs in serum and medium 199 may not be identical.

Unsaturated transferrin probably constitutes only one of a group of factors that retard the *in vitro* growth of *C. albicans*. Its exact relationship to the various other antifungal activities of serum (21–25) previously described has not been established. A different factor is most likely involved in those instances where dialysis (21, 25) or heat inactivation (22) at 56° C. reduced inhibitory activity. In any effort to clarify the relationships between the variety of antifungal activities of human serum, particular care must be exercised to collect and test the sera with specially cleaned iron-free equipment. Conventionally processed equipment almost invariably contains traces of iron in sufficient quantities to saturate transferrin and obscure the effect of the unsaturated transferrin.

The question of major importance is whether or not the inhibitory activity of unsaturated transferrin which has been demonstrated *in vitro* is of any importance in contributing to the non-specific defenses of man. The specific level of unsaturated transferrin does not appear to be a major factor in host resistance to candidiasis since patients with hemochromatosis who have the lowest level of unsaturated transferrin are not particularly prone to develop fungous infections. Furthermore, the few patients reported with absence or near absence of transferrin from their plasma have succumbed early to hematologic disorders and have not exhibited any abnormal susceptibility to infections (7).

Transferrins, recently, have been divided on the basis of starch gel electrophoresis into at least sixteen different genetic variants (7). These proteins do not differ in their ability to bind and transport iron. They may, however, differ in their ability to suppress the growth of microorganisms. The possibility that some genetic variants of transferrin are ineffective in suppressing fungous infections and that this contributes to the increased susceptibility of certain individuals to these infections is intriguing but has not been proved.

SUMMARY

The addition of iron as ferrous ammonium sulfate to serum with unsaturated transferrin was followed by an increase in the percentage of germ tubes and number of colonies of *Candida albicans* incubated in the serum. This study suggests that the mechanism responsible for these increases is not a stimulatory effect of ferrous ammonium sulfate but rather the removal of the inhibitory activity of unsaturated transferrin by saturation of this protein with iron. The importance of the antifungal activity of unsaturated transferrin in contributing to host resistance to fungous infections is still undetermined.

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