Thereafter the amount attached to these fractions increased and was still present at 48 hours (see Table). Essentially similar results were obtained in both patients.

Relative Distribution of Vitamin B_{12} Absorbed from the Gut among Serum Vitamin B_{12} Binding Proteins

Time after Oral Dose of ^{57}Co Vitamin B_{12} (Hours)	Percentage of Oral Dose per Litre of Plasma			
	Whole Plasma	Transcobalamin		Binder III
		I	II	
1	0	0	0	0
2	0	0	0	0
3	0·44	0·23	0·13	0·13
4	1·80	0·78	0·64	0·17
5	3·26	1·32	0·68	0·28
6	3·25	1·58	0·73	0·15
24	2·58	0·85	0·21	0·18

Discussion

All three vitamin B_{12} binding serum proteins took up vitamin B_{12} absorbed from the gut and all three appeared to take it up at the same time. Thus this study failed to confirm the claim that T.C. II was the first binder to take up vitamin B_{12} absorbed from the gut. This finding makes it unlikely that the delay in transport of vitamin B_{12} from gut to blood is due to synthesis of a carrier protein and more likely that in the intestinal absorption of vitamin B_{12} the free vitamin passes to the blood. The absorbed vitamin B_{12} links equally to all the carrier proteins available and the total uptake by these proteins is related to their unsaturated vitamin B_{12} binding capacity at the time.

The third vitamin B_{12} binder participates in vitamin B_{12} transport under physiological conditions. Its plasma clearance when bound to vitamin B_{12} is not very dissimilar to T.C. II (Gizis *et al.*, 1970) and it remains to be shown that it is not a polymer of T.C. II.

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MEDICAL MEMORANDA

Successful Treatment of Candidiasis with Transfer Factor

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Chronic mucocutaneous candidiasis without lethal immunological deficiencies is an infection with *Candida albicans* involving the skin, nails, scalp, and buccal and vaginal mucosa (Kirkpatrick *et al.*, 1971). Many of these patients exhibit anergy to intradermal challenge with extracts of candida, indicating a defect of cellular immunity against the organism, although humoral antibodies to the organism may be present (Turk, 1970; Chilgren *et al.*, 1967).

Of the several currently available approaches for reconstitution of cellular immune function the use of dialyzable transfer factor as described by Lawrence (1969) seems to be the most benign. The risk of graft-versus-host disease invariably accompanying bone marrow transplantation is avoided and sensitization to HL-A and other antigens is excluded, since transfer factor is dialyzable whereas tissue antigens are not (Lawrence, 1969). The reported success of Schulkind *et al.* (1971) with transfer factor and amphotericin B further highlighted its potential therapeutic usefulness.

This report describes results of intensive therapy with transfer factor in a patient previously shown to be refractory to a small dose of this preparation (Rocklin *et al.*, 1970) and to bone marrow transplantation (Meuwissen *et al.*, 1971).

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Case Report

The patient, a 9-year-old white girl, was the subject of a previous report of defects of cellular immunity associated with chronic mucocutaneous candidiasis (Rocklin *et al.*, 1970). Skin sensitization for 2,4-dinitrofluorobenzene was attempted but challenge did not lead to a positive reaction. She had never reacted to candida extract skin tests with positive delayed hypersensitivity, there was no demonstrable release of migration inhibition factor by her lymphocytes in the presence of candida antigen, and the phytohaemagglutinin response of her lymphocytes was erratic (Rocklin *et al.*, 1970). Lymphopenia had been present since the age of 4½ years, with total lymphocyte counts ranging from 400 to 1,000 cells/mm³. After her first presentation with severe cutaneous and oral moniliasis at 16 months of age several major therapeutic attempts were made. Amphotericin B 80 mg intravenously over six weeks was given at 16 months of age (body weight 8 kg). Transfer factor prepared from 3 ml of packed leucocytes was injected by Rocklin *et al.* (1970) at age 7 without indication of clinical or in-vitro lymphocytic improvement. One month later intravenous amphotericin B 330 mg was given over three weeks with definite signs of clearing of candida lesions.

Immediately after this last course a bone marrow transplant was undertaken in September 1969 with an HL-A non-identical sister as donor (Meuwissen *et al.*, 1971). Initial disappearance of candida infection after this combined treatment lasted four to six weeks. New candida lesions then appeared, and three months after the transplant a severe phase of cachexia began, characterized by diarrhoea, anorexia, raised liver enzymes, and splenomegaly, which was believed to be a graft-versus-host reaction (Meuwissen *et al.*, 1971). Severe mucocutaneous candidiasis recurred, leading to a further course of therapy with amphotericin B 230 mg intravenously over 19 days in April 1970 (body weight 14 kg). The lesions showed no sign of clearing when the drug was stopped. 5-Fluorocytosine 25 mg given by mouth in May and June was fruitless.

TRANSFER FACTOR THERAPY

In November 1970 dialyzable transfer factor was prepared after the method of Lawrence (1969). The lyophilized material was dissolved in distilled water and kept frozen at − 20°C until use. On two occasions in December transfer factor from 600 ml of blood taken from highly candida-sensitive donors was given intramuscularly. No change in lesions was noted. In June 1971 a course of amphotericin B 176 mg

intravenously over 16 days was given (body weight 15 kg). Transfer factor prepared as above was started after one week of drug therapy and continued three times a week for one week, then two times a week, and then once weekly for a further three weeks. Injections were given subcutaneously after mixing transfer factor solution with an equal volume of 1% lignocaine and 1% adrenaline. Four deposits of 0·5 ml were made each time in all four limbs. Transfer factor from 4,200 ml of blood from highly candida-sensitive donors was used in these injections; the optical density of the material injected varied from 20 to 60 O.D.$_{260}$/ml.

RESULTS

Clearing of lesions was noted after the first week of injections and was pronounced after the second week of injections. In the five months after the injections were stopped remarkable and continued clearing occurred of nail and skin lesions (Figs. 1 and 2). Nails began to grow out and the body skin and scalp became clear of candidiasis. In spite of this striking systemic improvement mucous membrane involvement regressed less impressively. There were still definite plaques of candidiasis on the tongue and buccal mucosa.

Six weeks after the start of transfer factor therapy lymphocyte counts were found to be 1,400/mm^3, and counts between 1,200 and 2,000/mm^3 were consistently seen thereafter. Lymphocyte transformation in vitro in response to candida extract was found two months after beginning transfer factor therapy (see Table). Skin test to 1/20 candida extract (Hollister-Stier) showed a 2·5 × 2·5 cm reaction at 24 and 48 hours without induration five weeks after the injections were started. The same response was obtained six weeks later with 1/100 dilution of candida extract (Hollister-Stier) but accompanied by definite induration of 7 × 7 mm at 48 hours. At the same time a 2,4-dinitrochlorobenzene challenge, 0·1% in acetone, was positive.

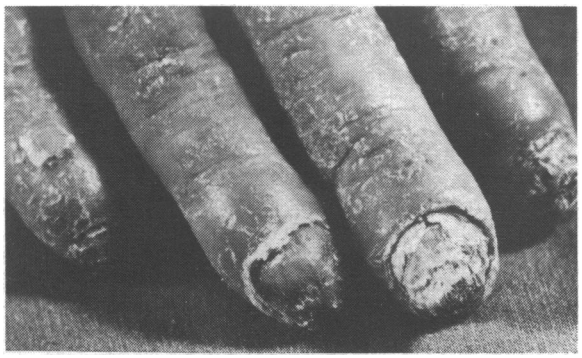

FIG. 1—Fingers of left hand immediately before treatment with dialyzable transfer factor.

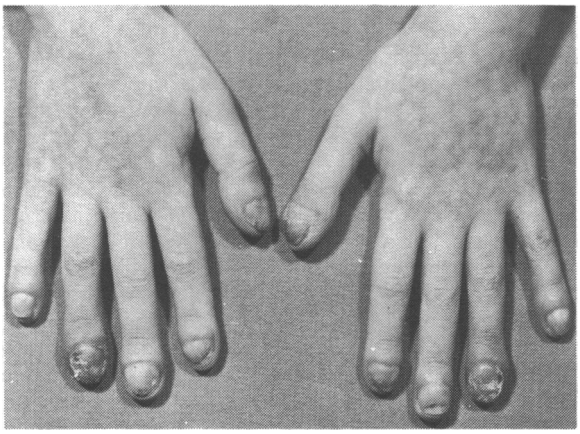

FIG. 2—Appearance of nails four months after transfer factor was stopped

Lymphocyte Response to Candida Antigen Before and After Administration of Transfer Factor

	Patient	Control
April 1971:		
Background	235	285
Candida	232	2,670
September 1971:		
Background	260	255
Candida	500	1,600

Figures are means of counts per minute of triplicate lymphocyte cultures; 0·0002 ml of candida antigen (Hollister-Stier, Spokane, Washington) was added to those marked "Candida." Cultures contained 10^5 lymphocytes each in 1 ml of TC 199 and 20% pooled AB serum. On the fifth day they were given 1 μCi of tritiated thymidine each and six hours later prepared for liquid scintillation counting.

Comment

The great clinical diversity of chronic monilia infection has been stressed in several reviews (Kirkpatrick *et al.*, 1971). It is important also to note the variability of immunological defects found in this disease (Chilgren *et al.*, 1967, Rocklin *et al.*, 1970). Probably because of these variations lymphocyte or blood transfusions or injections (Chilgren *et al.*, 1969; Valdimarsson *et al.*, 1970) have met variable measures of success. Similarly, bone marrow transplantation may exert its reported therapeutic efficacy (Buckley *et al.*, 1968) in this disease at least temporarily by an identical mechanism unless lymphocytes normally present in marrow have been specifically excluded from the inoculum. Certainly this latter form of treatment in chronic mucocutaneous candidiasis is not without grave danger, as the present case history attests (Meuwissen *et al.*, 1971).

A thorough trial of transfer factor seemed warranted in this chronically disabled patient because of her lack of cellular immunity to candida, as shown previously by Rocklin *et al.* (1970) and again in our hands before the use of transfer factor by the failure of in-vitro transformation of lymphocytes with specific antigen and by the absence of delayed skin reactivity to candida. Our rationale in using massive amounts of transfer factor was (1) that the adverse effect of the persistent and profound lymphopenia in the patient might have been overcome by "activating" all available lymphocytes, and (2) even in normal subjects it has been shown that suboptimal doses of transfer factor may produce only local skin reactivity (Lawrence, 1969; Levin *et al.*, 1970), a limitation of the efficacy of transfer factor which could have been more pronounced in this severely cachectic patient.

Although Lawrence (1969) extensively investigated transfer factor in healthy subjects clinical experience of other workers is poorly documented. Levin *et al.* (1970) reported clinical and in vitro success in a case of Wiskott-Aldrich syndrome. It is clear from this early experience with transfer factor by others and ourselves that this material is a very useful tool in the treatment of immunodeficiency. Although its mechanism of action remains to be elucidated it seems certainly worth while to utilize its known potential of improving immunodeficiency states before attempting the more onerous and far more hazardous procedure of bone marrow transplantation.

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