THE RELATIONSHIP BETWEEN THE DOWNY AND GRANULAR FORMS OF *TRICHOPHYTON MENTAGROPHYTES**

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Mycologists and practicing dermatologists frequently have designated as distinct species the downy forms of *Trichophyton mentagrophytes* that are commonly isolated from chronic ringworm of the feet or toenails and the granular forms of this fungus usually isolated from suppurative ringworm of the beard or scalp. The chief difference between the two types of culture is one of gross colonial form. Microscopic structures appear to be identical, although the granular form produces a relatively higher percentage of spores, microconidia and macroconidia, than the downy form. Differences in pathogenicity towards experimental animals have also been noted in studies with cultures of these two colonial types. In general, it is easier to produce characteristic ringworm lesions in the guinea pig with a granular than with a downy strain.

A number of mycologists, Weidman 1926 (1), Catanei 1929 (2), and Emmons 1932 (3) have presented evidence of the instability of the cultural forms of *T. mentagrophytes* and the existence of numerous intermediates between the downy and granular type cultures. If these colonial forms are considered merely as varieties of a single species then the name *T. mentagrophytes* (Robin) Blanchard, 1896 has priority, and therefore validity.

In spite of these observations a number of workers, largely of the European school, persist in characterizing the several colonial forms of this fungus as distinct species. Those following the classification of Langeron and Milochevitch (4) even place the granular form in the genus *Ctenomyces* (a genus of the Ascomycetes) because the numerous spores, spirals, nodular bodies, and antler-like structures produced by that variety are suggestive of similar structures formed by certain species of *Ctenomyces*. The significant absence of ascospores in *T. mentagrophytes* however, does not appear to them to be an important factor in their classification. Although many mycologists have not accepted this use of a perfect genus for one of the dermatophytes, the use of distinct species names is still commonly employed for the downy and granular cultures, viz. *T. interdigitale*, *T. gypseum* var. *"C" Hodges*, *T. Kaufmann-Wolf*, *T. persicolor* for the downy cultures and *T. mentagrophytes*, *T. gypseum*, *T. asteroides*, *T. granulosum*, *T. radiolatum*, *T. lacticolor*, *T. farinulentum* for the granular cultures with *T. niveum* serving for the fluffy (nearly pleomorphic) form.

During a recent investigation of ringworm infections by this laboratory both the downy and granular types of *T. mentagrophytes* were obtained. It was observed that all in a series of 70 strains of *T. mentagrophytes* isolated from feet and toenail scrapings produced colonies of the downy type. Only one of the cultures developed a small amount of granular growth on the edge of the colony. In contrast, 50 isolates of *T. mentagrophytes* from cases of ringworm of the beard and scalp seen in farmers and their families in northeastern Michigan were coarsely granular (5). Although such findings are commonly observed, the reason why two distinct colony types are selectively isolated from these two forms of clinical infection is not understood.

It should be pointed out that this correlation of colony type with a clinical form of ringworm is certainly not absolute. For example, in a recent series of papers by Sanderson and Sloper (6, 7, 8) on skin diseases in personnel of the British Army stationed in S. E. Asia,

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Received for publication April 16, 1954.

analysis of 96 dermatophytes isolated from lesions of the feet of 85 Europeans revealed 39 strains of *T. mentagrophytes* (the species name they designate for the granular type) and only 11 strains of *T. interdigitale* (the species name they designate for the downy type). It is interesting to note, however, that in this group of cases a large number of the individuals had widespread ringworm infections caused by *T. mentagrophytes* on other parts of the body as well as on the feet, and that a number of cases showed a tendency toward deep involvement of the hair follicles, leading to the development of boggy, indurated, kerion-type lesions. These were commonest in the bearded areas, on the scalp, and sometimes on the arms and legs. Both cultural forms were never isolated simultaneously from the same patient, but in several patients, "*T. interdigitale*" infection was followed by *T. mentagrophytes* infection. These findings will be reviewed in the light of the author's experimental results in the discussion which follows.

Also in contrast to the usual observations, Epstein (9) states that his records show 2 cases of deep trichophytosis of the scalp and beard from which he had isolated downy-type cultures. Further evidence that one colonial type may produce several clinical forms of ringworm was presented by Dowding and Orr, "Three Clinical Types of Ringworm Due to *Trichophyton gypseum*" (10) in which no correlation was found between the clinical type of ringworm caused by the fungus, and the variation which it exhibited on Sabouraud's medium.

The first comprehensive study of this problem was reported by Epstein in 1938 in his "Presentation of the Hypothesis that *Trichophyton interdigitale* is a degenerated *Trichophyton gypseum*" (9). In producing experimental ringworm in guinea pigs with downy and granular type cultures of *T. mentagrophytes*, Epstein demonstrated the following: (1). The granular type cultures regularly produced infections in the guinea pig with the development of typical acute inflammatory reactions by the 10th to 12th day, accompanied by invasion of hair follicles and hairs and the development of heavily infiltrated crusted lesions. (2). The downy type cultures, on the other hand, usually produced only superficial ring-shaped lesions with little or no inflammation and no hair invasion. A few strains, however, produced crusted infiltrated lesions by the 19th to 21st day and hair invasion could be observed in biopsy sections. (3). Serial animal passages caused marked diminution of virulence in some of the granular type cultures (*T. gypseum*), while some strains of *T. interdigitale*, the downy type culture, underwent a marked increase in virulence. Despite these changes in virulence there was no change in colony form following animal passage.

From the epidemiological point of view the problem is most interesting. Downy-type cultures are regularly isolated from *T. mentagrophytes* infections of the feet and nails whether the patient lives in the city or in the country. In the United States, tinea pedis apparently is more common among city dwellers, particularly those in schools and other institutions, than among those engaged in rural occupations. The granular type cultures, however, are isolated most frequently from suppurative *T. mentagrophytes* infections of the scalp and beard, infections which are predominantly found in farm districts. It is generally assumed that this type of suppurative ringworm is of animal origin, since in many cases contact with ringworm infected animals have been demonstrated. *T. mentagrophytes* infections occur in most of the common domestic animals as well as in a large number of wild animals. (11).

In the hopes of throwing some light on this mycological problem, the question of the interrelationship of the different cultural forms of *T. mentagrophytes* and their clinical manifestations were reopened for study by the Mycology Laboratory of the Communicable Disease Center.

MATERIALS AND METHODS

Eight downy strains of *T. mentagrophytes*, isolated from cases of ringworm of the feet (including nail infections), and 8 granular strains of *T. mentagrophytes*, isolated from suppurative ringworm of the beard or scalp, were selected for study. Spore suspensions were prepared from each of the 16 strains, and 8 to 10 single spores were isolated from each according to the technic described by Georg (12) and inoculated on separate tubes of Sabouraud dextrose agar. Typical single spore strains were selected from each group so that the final cultures represented 8 downy strains, each derived from a single spore, and 8 granular strains of single spore origin. The downy and granular cultures were characterized on Sabouraud dextrose agar as follows:

Downy cultures

(a) *Gross colony characteristics:* Colonies were generally flat but often with a central umbo or irregularly rolling to cerebriforme configuration at the center. The surface was floccose, often cottony at the center or at the edge of the colony. The periphery of the colony was smooth and entire. Old cultures showed some finely powdery areas at the center of the colony or at the extreme edges. Colonies were at first white but developed a cream to tan pigmentation at the center of the colony, with age. A few strains developed a pinkish cast over the surface. The reverse of the colonies was yellowish to rose-tan, a few becoming dark red-brown at the center.

(b) *Microscopic characteristics:* All strains produced numerous subspherical or slender and slightly clavate microconidia. They were borne in dense clusters on conidiophores branched at right angles (*en grappe*) and also borne along the sides of aerial mycelium (*en thyrse*). The tendency to form slender clavate microconidia borne *en thyrse* appeared to be marked in these downy type cultures. Macroconidia were, in general, rare on Sabouraud dextrose agar; however, their production could be induced in nearly all strains by cultivation on wort agar. When produced, the macroconidia varied considerably in size and shape among the different strains as well as within a single strain. In general, they consisted of 3 to 4 cells, were thin-walled and slightly clavate. Spirals were seen in about one-third of the strains on Sabouraud dextrose agar. Growth on wort agar enhanced the production of these structures also.

Granular cultures

(a) *Gross colony characteristics:* Colonies were generally flat and spreading. The surface was finely to coarsely granular with aggregations of granular growth in irregular ridges which show radial orientation. The edge of the colony appeared fringed by strands of the granular surface growth. The pigmentation of these colonies was much more marked than that of the downy type, the surface being cream colored to light buff at first, but later, tan at least at the center of the colony. The pigmentation on the reverse of the colony was also more intense, being rose-brown to a deep dull red in many strains. The red-brown subsurface

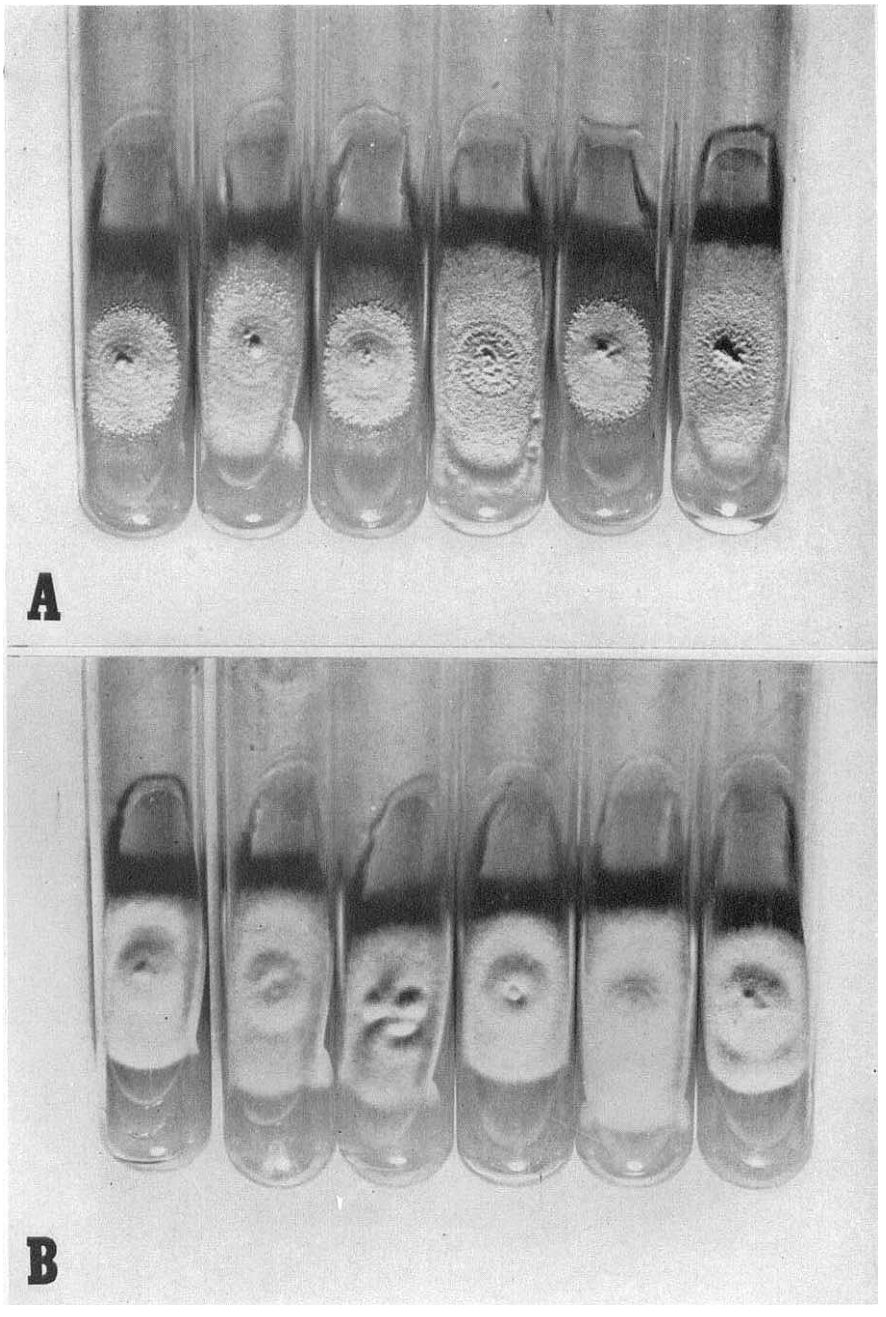

FIG. 1. (A) Granular-type colonies isolated from acute suppurative lesions of the exposed parts of the body. (B) Downy-type colonies isolated from chronic ringworm of the feet or toenails.

growth often showed as a fringe of deep red pigmentation surrounding the cream to tan surface growth.

Fig. 1 compares the colonies of the downy and granular strains.

(b) *Microscopic characteristics:* The granular surface growth was composed of masses of subspherical microconidia borne largely in clusters on conidiophores branched at right angles. Microconidia were also borne along the sides of the aerial mycelium, but this was not as common as in the downy forms. Macroconidia were found on Sabouraud dextrose agar in most strains. They were numerous in two or three strains in this group and were morphologically similar to those seen in downy cultures. Spirals were seen in most cultures.

From a microscopic point of view, the downy and granular forms were identical except in the quantity of spores produced. Fig. 2 shows similar microscopic structures from both downy and granular cultures.

Subcultures made from the selected cultures were periodically examined for the duration of the experiment and for an additional year. The following observations were made:

(1) There was little change in the downy type cultures of foot origin except that, in general, they appeared to be slightly more fluffy than originally. None of them, however, became pleomorphic (i.e., composed of sterile white, fluffy mycelium).

The granular cultures showed a marked tendency to become pleomorphic, and tufts of white fluffy aerial growth appeared constantly in these cultures. In order to prevent overgrowth of this sort, it was necessary to subculture selectively the granular growth every 10 to 14 days. One of the cultures, however, became largely pleomorphic at the end of the year in spite of constant subculturing. A few of the granular cultures gradually became somewhat downy in character after months of subculture and 2 of them after a year resembled the downy colonies so closely that they could not be distinguished from them. This gradual change of the granular culture to the downy form has been described by a number of workers. Catanei (13) in particular, described it in detail, It is important to point out, however, that although this transformation is rather commonly seen, the reverse, i.e., transformation of the downy type culture to a granular type culture has never been shown.

In view of the fact that the granular type cultures are isolated usually from lesions believed to be of animal origin (suppurative lesions on the exposed parts of the body, common only in rural areas) a study of the effect of animal passage on the strains of *T. mentagrophytes* was undertaken. Techniques similar to those of Epstein (9) were used in an attempt not only to increase the virulence of the downy type cultures through serial passage on the guinea pig, but at the same time to convert such cultures to the granular form.

ANIMAL INOCULATION STUDIES

1. *Primary guinea pig inoculations with downy and granular types cultures*

The 8 single-spore downy type strains of *T. mentagrophytes*, and the 8 single spore granular type strains of *T. mentagrophytes* were inoculated onto the backs

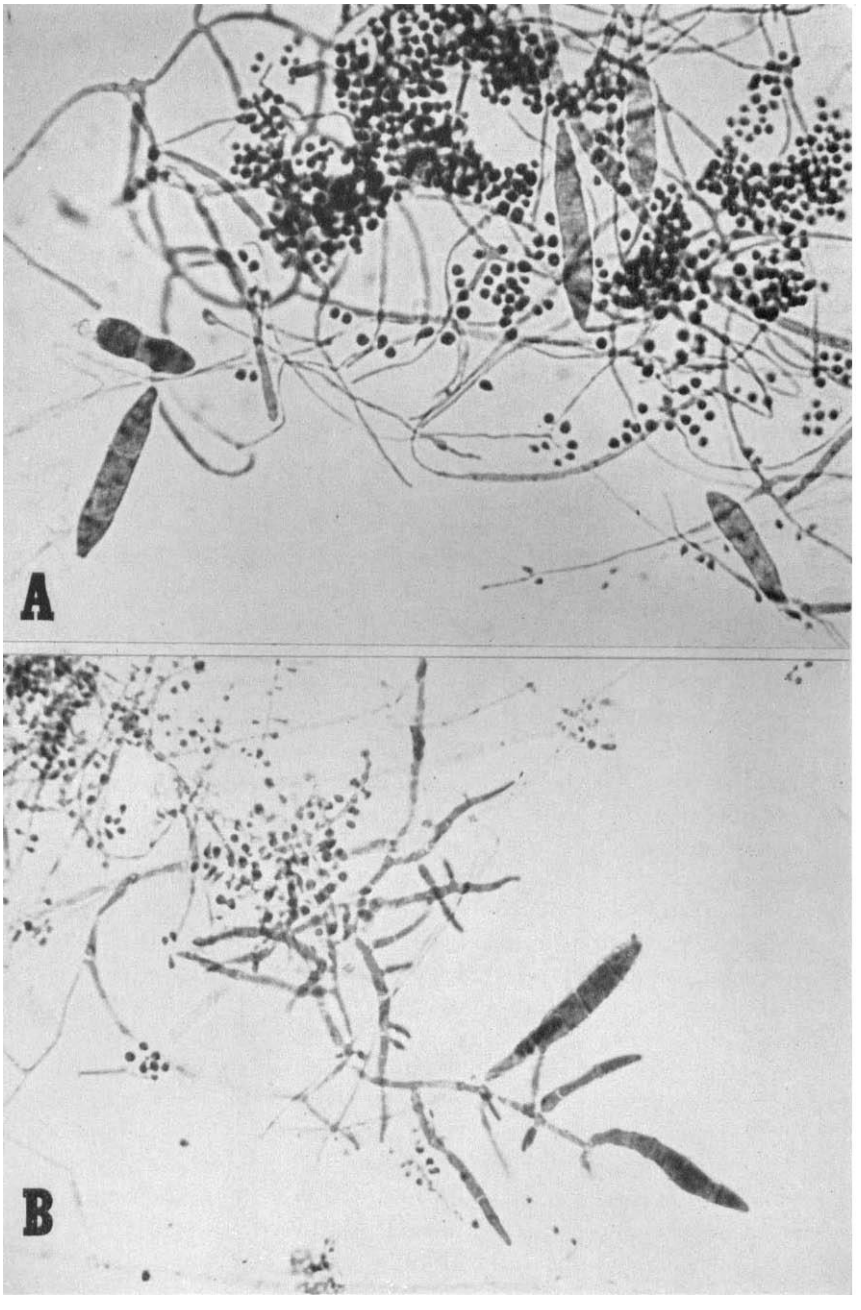

FIG. 2. (A) Microscopic morphology of granular-type cultures. (B) Microscopic morphology of downy type-cultures.

TABLE I

Cutaneous inoculations of the guinea pig with T. mentagrophytes *cultures*

CULTURE NUMBER	COLONY TYPE	7TH OR 8TH DAY					11TH TO 14TH DAY					19TH TO 21ST DAY					TYPE CULTURE RECOVERED				DURATION OF INFECTION	
		Lesion produced			Direct exam		Lesion produced			Direct exam		Lesion produced			Direct exam						Day of first negative culture	Lesion healed, days
		Erythema	Scaling	Crust	Mycelium	Hair invasion	Erythema	Scaling	Crust	Mycelium	Hair invasion	Erythema	Scaling	Crust	Mycelium	Hair invasion	7th to 8th day	11th to 14th day	19th to 21st day	30th to 35th day		
3 TM	Granular	2+	1+	0	4+	2+	4+	2+	0	4+	4+	4+	4+	2+	4+	2+	Granular	Granular	Granular	Not recovered	30th	30–35
4 TM	Granular	2+	1+	0	4+	2+	4+	4+	2+	4+	4+	2+	2+	1+	2+	2+	Granular	Granular	Granular	Not recovered	30th	25–30
5 TM	Granular	3+	4+	2+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	4+	Granular	Granular	Granular	Granular	40th	35–40
6 TM	Granular	1+	1+	0	2+	0	1+	4+	2+	4+	2+	2+	4+	4+	4+	4+	Granular	Granular	Granular	Granular	40th	35–40
7 TM	Granular	2+	2+	1+	4+	2+	4+	4+	3+	4+	2+	4+	4+	3+	4+	2+	Granular	Granular	Granular	Not recovered	30th	25–30
8 TM	Granular	1+	1+	0	2+	0+	1+	4+	2+	4+	2+	2+	4+	4+	4+	4+	Granular	Granular	Granular	Granular	40th	35–40
14 TM	Granular	2+	1+	0	4+	2+	4+	2+	0	4+	4+	4+	4+	2+	4+	2+	Granular	Granular	Granular	Not recovered	30th	30–35
20 TM	Granular	1+	1+	0	2+	0	1+	4+	2+	4+	2+	2+	4+	4+	4+	4+	Granular	Granular	Granular	Granular	40th	35–40
15 TM	Downy	1+	2+	0	2+	0	0	0	0	0	0	0	0	0	0	0	Downy	Not recovered	Not recovered	—	14th	8–15
16 TM	Downy	3+	3+	0	2+	0	0	0	0	0	0	0	0	0	0	0	—	Downy	Not recovered	—	19th	15–20
17 TM	Downy	0	1+	0	1+	0	1+	1+	0	1+	0	0	0	0	0	0	Downy	Downy	Not recovered	—	19th	15–20
43 G	Downy	±	±	0	0	0	0	0	0	0	0	0	0	0	0	0	Downy	Not recovered	—	—	11th	8–15
122 G	Downy	1+	1+	0	1+	0	0	0	0	0	0	0	0	0	0	0	Downy	Not recovered	—	—	11th	8–15
236 G	Downy	1+	1+	0	1+	0	0	0	0	0	0	0	0	0	0	0	Downy	Not recovered	—	—	11th	8–15
245 N	Downy	1+	1+	0	1+	0	0	1+	0	1+	0	0	0	0	1+	0	Downy	Downy	Downy	Not recovered	30th	20–30
258 N	Downy	0	±	0	2+	0	0	0	0	0	0	0	0	0	0	0	Downy	Not recovered	—	—	11th	8–15

of young white guinea pigs (250–450 grams) in the following manner. The hair covering the hind quarter of the guinea pigs was carefully shaved off and the area scarified with sandpaper. The inoculum was prepared by scraping the growth from the surface of an 8 to 10-day-old Sabouraud dextrose agar slant culture and grinding the mycelium with small amounts of adherent agar into a paste in a sterile mortar. The inoculum was spread thinly over the scarified area and kept in place for 3 to 4 days by means of a gauze dressing. The results of the primary inoculations of each of the 16 strains are listed in Table I. All 8 of the granular strains produced severe infections with considerable inflammatory reaction. The lesions showed some variation, but the extent and the course of the infection were quite similar in most respects. The incubation period was from 4 to 6 days, definite lesions being apparent by the 7th day and reaching their climax between the 10th to 19th days. Skin scrapings from all animals contained proliferating mycelium, and hairs invaded by fungi in the typical ectothrix pattern were seen in great numbers. Positive retro-cultures could be obtained in 4 cases up to the 21st day and in 4 cases as long as the 35th day following inoculation. All retro-cultures were of the granular type, identical in appearance to the original inoculation cultures.

The eight downy strains, in contrast to the above, only produced mild and transitory infections in the guinea pigs. It appeared doubtful that isolate 43G produced any infection at all although the culture was recovered from the guinea pig on the 7th day. In all other cases mycelium was demonstrated in skin scrapings on the 7th or 8th day and in two animals on the 11th to 14th day. Although strain 245N produced an extremely superficial lesion, mycelium was visible in skin scrapings up to the 21st day. In no case were any invaded hairs observed in direct examinations in KOH mounts or in tissue sections stained by the Hotchkiss McManus technique. All retro-cultures obtained from these animals were of the downy type similar to the inoculation cultures. (Fig. 3 shows lesions of the guinea pig produced by granular and downy type cultures of *T. mentagrophytes*). Such a demonstration of the difference in virulence for the guinea pig by the downy and granular type cultures of *T. mentagrophytes* is consistent with the findings of Catanei (13), Ota and Kawatsure (14), Epstein (9), and Emmons and Hollaender (15).

It was of interest to determine whether culture 8TM, which had been granular at the beginning of the experiment only to become downy over the course of 4 months' cultivation, had lost its original virulence for the guinea pig. The results of guinea pig inoculation indicated that this was the case, the infection being about half as intense as that produced by the original granular culture. Some hair invasion, however, was still produced by this strain. This corresponded to the findings of Catanei (13) who reported a change in virulence by an aging granular type culture of *T. mentagrophytes* (*T. gypseum radiolatum*).

2. *Serial guinea pig inoculations with downy type cultures*

Serial inoculations of guinea pigs were undertaken with the 8 downy type cultures in an effort to determine whether any increase in virulence and change of culture type could be induced. Retro-cultures from the first infected animal were

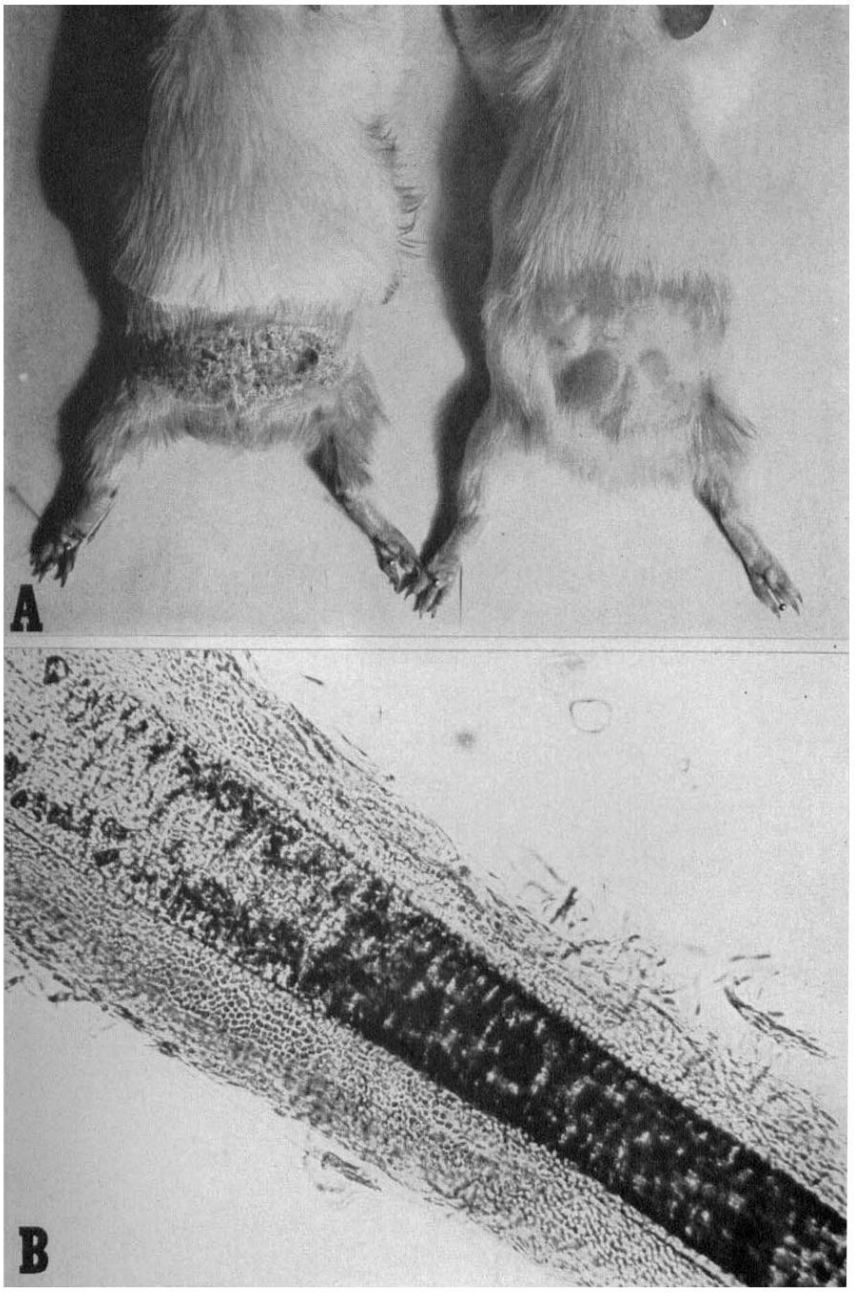

FIG. 3. (A) Lesions of the guinea pig produced by granular and downy-type cultures. Heavily crusted lesions were produced by the granular strains; while downy strains produced little evidence of infection other than slight scaling. (B) Hair invaded by granular strain shows typical sheath of spores arranged in chains. No hair invasion was produced by the downy-type cultures.

TABLE II

Serial inoculations of guinea pigs with T. Mentagrophytes (*Downy type*)

CULTURE	SERIAL INOCULATIONS IN GUINEA PIGS	LESION			DIRECT EXAMINATION		CULTURE RECOVERED	DURATION OF LESION
		Erythema	Scaling	Crust	Mycelium	Hair invasion		
122 G	Guinea pig I (inoc. with original cult.)	1+	1+	0	1+	0	Downy (passage cult. I)	8 days
	Guinea pig II (inoc. with passage cult. I)	1+	1+	0	1+	0	Not recovered	8 days
15 TM	Guinea pig I (inoc. with original cult.)	1+	2+	0	2+	0	Downy (passage cult. I)	10 days
	Guinea pig II (inoc. with passage cult. I)	2+	3+	0	2+	0	Downy (passage cult. II)	15 days
	Guinea pig III (inoc. with passage cult. II)	1+	0	0	1+	0	Downy (passage cult. III)	10 days
43 G	Guinea pig I (inoc. with original cult.)	±	±	0	0	0	Downy (passage cult. I)	10 days
	Guinea pig II (inoc. with passage cult. I)	1+	2+	0	3+	0	Granular 1+, downy 3+, (passage cult. II)	16 days
	Guinea pig III (inoc. with passage cult. II)	1+	0	0	1+	0	Downy (passage cult. III)	7 days
258 N	Guinea pig I (inoc. with original cult.)	0	±	0	2+	0	Downy (passage cult. I)	8 days
	Guinea pig II (inoc. with passage cult. I)	1+	2+	0	2+	0	Downy (passage cult. II)	15 days
	Guinea pig III (inoc. with passage cult. II)	0	1+	0	2+	1+	Downy (passage cult. III)	15 days
236 G	Guinea pig I (inoc. with original cult.	1+	1+	0	1+	0	Downy (passage cult. I)	8 days
	Guinea pig II (inoc. with passage cult. I)	2+	1+	0	1+	0	Powdery 2+, downy 2+, (passage cult. II)	15 days
	Guinea pig III (inoc. with passage cult. II)	2+	2+	0	2+	2+	Powdery 3+, Downy 1+, (passage cult. III)	20 days
16 TM	Guinea pig I (inoc. with original cult.)	2+	2+	0	2+	0	Downy (passage cult. I)	10 days
	Guinea pig II (inoc. with passage cult. I)	3+	3+	0	3+	1+	Powdery 1+, downy 3+, (passage cult. II)	20 days
	Guinea pig III (inoc. with passage cult. II)	3+	3+	1+	3+	3+	Downy (passage cult. III)	25 days

TABLE II—(*Continued*)

CULTURE	SERIAL INOCULATIONS IN GUINEA PIGS	LESION			DIRECT EXAMINATION		CULTURE RECOVERED	DURATION OF LESION
		Erythema	Scaling	Crust	Mycelium	Hair invasion		
245 N	Guinea pig I (inoc. with original cult.)	0	±	0	2+	0	Downy (passage cult. I)	8 days
	Guinea pig II (inoc. with passage cult. I)	1+	2+	1+	2+	2+	Powdery 2+, downy 2+, (passage cult. II)	15 days
	Guinea pig III (inoc. with passage cult. II)	3+	3+	4+	4+	4+	Granular (passage cult. III)	45 days
17 TM	Guinea pig I (inoc. with original cult.)	1+	1+	0	1+	0	Powdery 2+, downy 2+, (passage cult. I)	12 days
	Guinea pig II (inoc. with passage cult. I)	2+	3+	0	3+	2+	Granular 3+, downy 1+, (passage cult. II)	20 days
	Guinea pig III (inoc. with passage cult. II)	3+	3+	2+	3+	3+	Granular 3+, downy 1+, (passage cult. III)	30 days
	Guinea pig IIIa (inoc. directly from guinea pig III)	3+	4+	4+	4+	4+	Granular (passage cult. IIIa)	40 days

used for inoculation of the second animal, and so on. When infections had developed to such an extent that sufficient infected skin scrapings could be obtained, scrapings from an infected animal were used directly as inoculum for the next animal. The results of these tests are summarized in Table II. In three cases, cultures 122G, 15TM, and 43G, no striking change in virulence was obtained by animal passage. In the five remaining cases, increase in virulence was observed by the second or third guinea pig passage. In all these cases the duration of the lesion was extended from a previous average of 8 to 10 days to one persisting 15 to 45 days. Some hair invasion was observed in all cases, and with two strains, 17TM and 245N, the lesions produced in the third and fourth guinea pigs corresponded closely in appearance to those produced by granular type cultures, and large numbers of hairs were invaded in the typical ectothrix manner characteristic of *T. mentagrophytes*. (Fig. 4 shows the lesion in the guinea pig produced by 17TM, an originally downy type culture which had been passed through three guinea pigs, also a hair invaded by 17TM in guinea pig III.) With the increase in virulence there was a tendency in several of the cultures to become partially powdery or granular, and the retro-cultures from the third guinea pig of the 245N series and from the fourth guinea pig of the 17TM series were completely granular, resembling in all respects the original granular type strains studied in this series. (Fig. 5 shows the original 17TM downy type culture and 17TM after passage through four guinea pigs; also the original 245N and 245N after passage

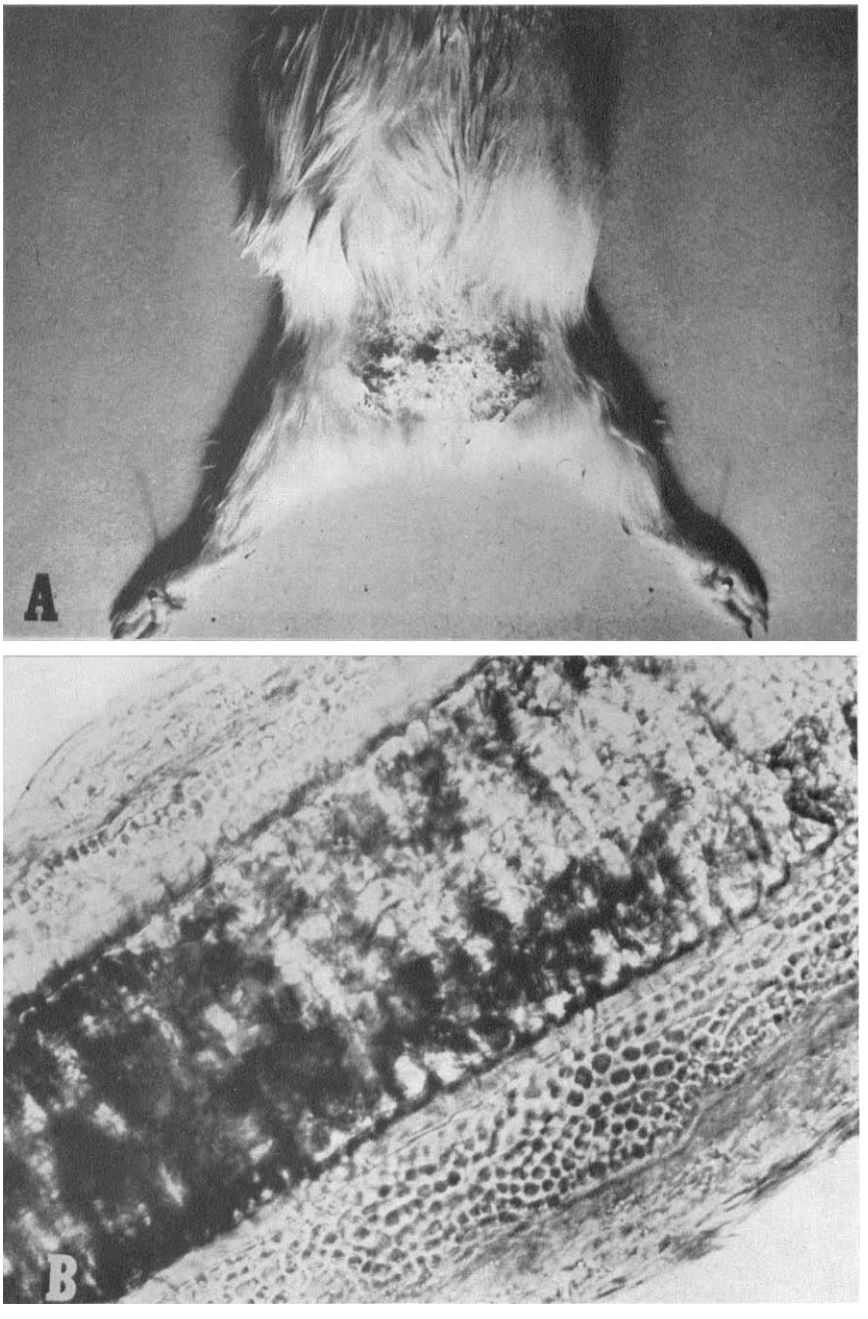

FIG. 4. (A) Lesion of the guinea pig produced by an originally downy-type culture 17TM after passage through three guinea pigs. (B) Hair invaded by 17TM in guinea pig III. Shows typical sheath of spores arranged in chains.

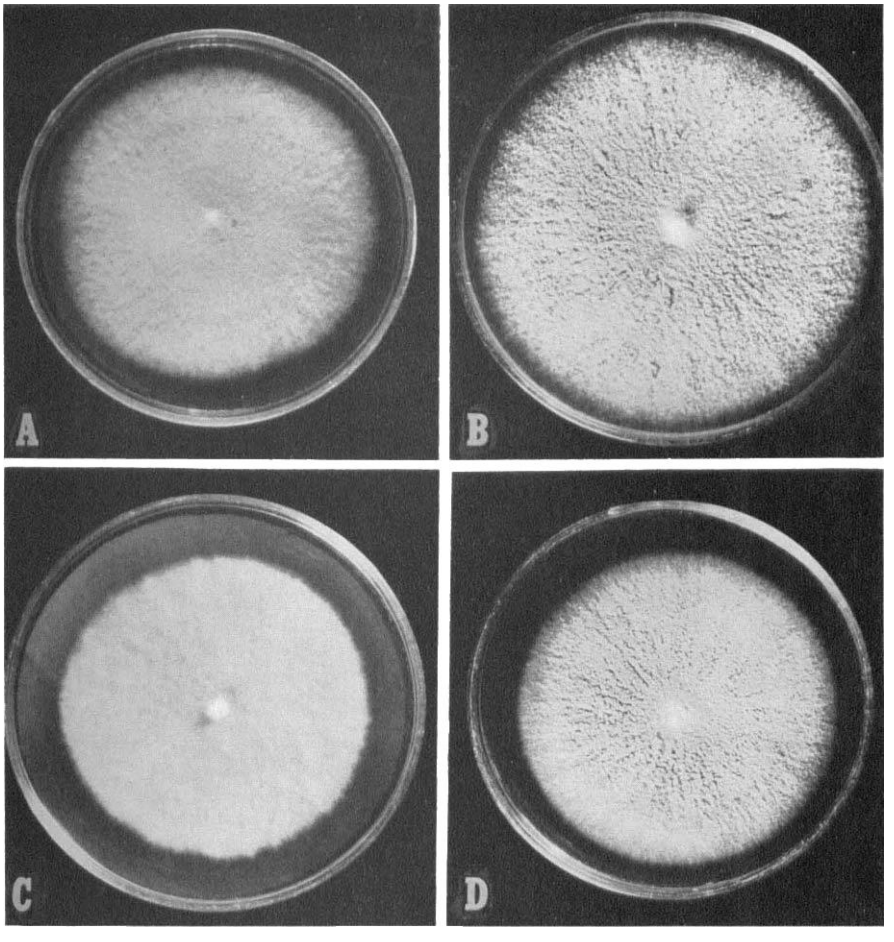

FIG. 5. (A) Original 17TM, downy-type culture, and (B) 17TM after passage through 4 guinea pigs. (C) Original 245N, downy-type culture, and (D) 245N after passage through 3 guinea pigs.

through three guinea pigs.) The increase in virulence of the downy strains by animal passage corresponds to the findings of Epstein (9). This author did not, however, succeed in converting any of his strains to the granular type by this procedure. It is interesting that Catanei was able to produce somewhat similar increase in virulence of *T. schoenleini* by serial passage in white mice (16). Artificial inoculations of the white mouse with *T. schoenleini* usually gave negative results or induced only transitory symptoms, but favus of a severe type was developed when a strain was passed directly from one mouse to another.

3. *Inoculation of dogs with downy type cultures and "guinea pig passage strains" of downy type cultures*

In an attempt to determine whether the increased virulence of *T. mentagrophytes* produced by guinea pig passage was a stable character and whether this

TABLE III

Cutaneous inoculations of dogs with T. mentagrophytes *cultures*

INOCULATED CULTURE	STATUS OF CULTURE AND COLONY TYPE	ANIMAL	7TH OR 8TH DAY					11TH TO 14TH DAY					21ST TO 28TH DAY					TYPE CULTURE RECOVERED			DURATION OF INFECTION
			Lesion produced			Direct exam		Lesion produced			Direct exam		Lesion produced			Direct exam					
			Erythema	Scaling	Crust	Mycelium	Hair Invasion	Erythema	Scaling	Crust	Mycelium	Hair Invasion	Erythema	Scaling	Crust	Mycelium	Hair Invasion	7th or 8th day	11th to 14th day	21st to 28th day	
17 TM	Original strain, downy	Dog I	0	2+	0	1+	0	0	2+	0	0	0	0	1+	0	0	0	Downy	Downy	Not recovered	15 days
245 N	Original strain, downy	Dog II	0	1+	0	1+	0	0	0	0	0	0	0	0	0	0	0	Downy	Not recovered	—	10 days
17 TM	"Guinea pig passage strain," granular	Dog III	0	3+	0	4+	0	0	3+	0	4+	2+	0	4+	3+	4+	3+	Granular	Granular	Granular	45 days
245 N	"Guinea pig passage strain," granular	Dog IV	1+	3+	0	4+	1+	2+	3+	2+	4+	2+	2+	3+	2+	4+	4+	Granular	Granular	Granular	40 days
17 TM	"Guinea pig passage strain," granular	Dog V (sick with hookworm infection)	0	3+	2+	4+	4+	(Dog died on 10th day)										Granular	Granular culture isolated from autopsy specimen—liver, spleen, bronchial lymph nodes		—
245 N	"Guinea pig passage strain," granular	Dog VI (in poor condition)	0	3+	0	3+	0	0	3+	2+	4+	2+	0	3+	3+	3+	3+	Granular	Granular	Granular culture isolated from mandibular lymph nodes	—
													(Dog very sick with hookworm infection — sacrificed)								
17 TM	"Guinea pig passage strain," granular	Dog I (reinoculated 4 weeks after test with original 17 TM culture)	0	3+	1+	3+	2+	0	2+	1+	2+	2+	0	2+	2+	3+	3+	Granular	Granular	Granular	50 days

changed virulence could be demonstrated for other animals, several dogs were inoculated with original downy strains (17TM and 245N); and later a second group of dogs was inoculated with the same strains which had been converted by guinea pig passage to granular type cultures. The dogs were 5- to 6-week-old mongrel puppies. They were inoculated on the side of the neck in a manner similar to that described for the guinea pigs. The results of these tests are summarized in Table III.

Dogs I and II, which had been inoculated with downy cultures 17TM and 245N, showed very little evidence of infection except for scaling. Direct examination after 8 days revealed some branching mycelium in skin scrapings, but there was no evidence of hair invasion. In dog I the culture was recovered on the 8th and 14th day, but in dog II it was not recovered after the 8th day. Dogs III and IV were inoculated with cultures of these same two strains which had been passed serially on guinea pigs and which had become granular. Severe ringworm infections were produced in both of these animals. The course of the infection was similar to that seen in guinea pigs which had been inoculated with granular cultures. Large numbers of branching hyphae were seen in skin scrapings; and many invaded hairs, with the typical ectothrix invasions, were found by the 14th day. The crusts formed were at first tightly adherent, but later became dry and flaky and were scratched off by the animals. Erythema was not apparent on the dark-skinned dogs but was noticeable on dog IV, a white and tan terrier.

Dogs V and VI were in poor physical condition when obtained, both being heavily infested with hookworms (*Ancylostoma caninum*). These animals were also inoculated with the granular "guinea pig passage strains" in order to determine whether the course of the infection would be changed in these animals of obviously poor physical condition. Both dogs developed very severe ringworm infections. Dog V died on the 10th day following inoculation—probably as a result of the hookworm infestation. This animal was autopsied, and aside from the large number of worms in the gut, the organs appeared normal. Cultures made from the spleen, liver, and bronchial lymph nodes, however, yielded characteristic granular type colonies of *T. mentagrophytes* identical to the inoculated strain. Dog VI became very ill in the 4th week following inoculation and was sacrificed. Positive cultures of *T. mentagrophytes* of the granular type were obtained from the mandibular lymph nodes of this animal.

In order to show that the severity of the lesions in dogs III and IV was not dependent on the particular susceptibility of these animals, dog I, which had shown only a very superficial reaction when inoculated with the original downy strain 17TM, was reinoculated 4 weeks later with the "guinea pig passage" culture of this same strain. A severe lesion was produced this time.

DISCUSSION

The gradual *in vitro* conversion of single spore strains of *T. mentagrophytes* from coarsely granular, deeply pigmented, often asteroid culture forms to a less pigmented, downy form, as well as the conversion of single-spore strains of downy

type cultures to granular type by serial guinea pig passage indicate that these two forms are closely interrelated and do not merit separate species distinction.

The factors involved in these changes are not completely understood. The gradual change toward the downy type seen in aging cultures of the granular type has been interpreted as a degenerative transformation by many mycologists. Although it is true that in the downy type of colony there is less sporulation, less pigmentation, and, in general, less virulence for the laboratory animal, this form should not be considered "pleomorphic," the term given by Sabouraud to the process of degeneration that leads a sporulating, pigmented organism to become a white, fluffy culture consisting entirely of a very fine mycelium incapable of forming spores. Nor should the downy type be considered as one of the sequential stages in the development of pleomorphism. Granular cultures frequently develop tufts of white, sterile growth without passing through a stage of downy type growth. Truly pleomorphic cultures derived from downy colonies were obtained with difficulty and were clearly distinguishable from the original type on the basis of macroscopic and microscopic characteristics.

The nature of the changes involved in the conversion from downy to granular growth are also obscure. Apparently rapid passage from one animal to another and the development of an active infection in the host tissues influences this type of change. The positive correlation of downy type cultures with low-grade chronic infections of the feet and nails and of the granular type with the more severe acute, rapidly spreading ringworm infections of the feet and other parts of the body may be more easily interpretable, however, in the light of these findings.

In ringworm infections of the feet and nails caused by *T. mentagrophytes*, the extent of tissue invasion is usually small, there is little tissue reaction, and the course of the disease is usually chronic. Cultural surveys of normal feet (17, 18) have shown that dermatophytes may persist on the skin without producing grossly visible lesions. Also it is well known that patients with cronic ringworm of the feet tend to have spontaneous remissions in periods of cold, dry weather, but that conditions of heat and high humidity, or injury to the skin or toenails often precede a recurrence or a new infection. It seems logical that one should isolate the downy, less virulent type of *T. mentagrophytes* from such lesions.

A survey by Walker (19) had indicated that "*T. interdigitale*," or the downy type culture of *T. mentagrophytes*, was consistently isolated in all cases of ringworm of the feet which she had observed in Great Britain. However, when groups of army men were sent from England to Malaya and Hong Kong, Sanderson and Sloper (6, 7, 8) reported that not only was the incidence of ringworm of the feet greatly increased, but the cultures isolated in the majority of the cases were of the granular type which they identified as *T. mentagrophytes*. Correlated with the change of cultural type were an increased severity of the lesions and a large number of cases of *T. mentagrophytes* infections of the body, face, and scalp. The lesions on these latter areas frequently showed deep involvement of the hair follicles, and suppurative reactions. The authors presented evidence that these body lesions probably had largely been acquired from active foot infections

which had spread from the patient's own feet. In a number of cases, "*T. interdigitale*" was isolated from a primary infection, but later *T. mentagrophytes* was isolated from recurrent lesions. The authors show quite clearly that the climatic conditions in southeast Asia which caused intense sweating, especially in new arrivals, was an important factor in increasing the incidence of ringworm disease among these men. In addition, the conditions of army life—communal living, poor bathing facilities, interchanges of boots and borrowing of socks—probably increased the spread of the infections from man to man. The authors suggest that "if a transformation of '*T. interdigitale*' present on the feet of these men when they left the United Kingdom, into *T, mentagrophytes* were postulated, the rapid spread of trichophytic ringworm in this unit could be more easily explained."

The experimental proof, as presented in Table II, that such a transformation can occur does aid in explaining this phenomenon. Conditions more favorable for the growth and transmission of the fungi from person to person permitted this dermatophyte to invade the tissues to a greater extent than previously and to produce more tissue reaction. Concurrently with the production of more active infections, the fungi became granular.

Another phase of the epidemiology of *T. mentagrophytes* ringworm may also be explained in the light of these experiments. Suppurative ringworm of the exposed areas of the body due to *T. mentagrophytes* is commonly encountered in rural areas. There is considerable evidence on hand that a large number of these cases are due to direct or indirect contact with ringworm infected animals, and that *T. mentagrophytes* is one of the principal causes of ringworm in domestic farm animals: cows, horses, dogs, rabbits, and others, as well as in many of the wild animals which abound in rural areas (11). Cultures from these ringworm infected animals are most commonly of the granular type, and the disease is highly contagious for man (11, 20, 21). The granular type cultures of *T. mentagrophytes* isolated from ringworm lesions of the exposed parts of the body in members of the rural human population are similar to those obtained from animals, as well as to the animal passage forms developed in this laboratory. It seems probable that the severe ringworm lesions seen in farmers and their families are caused largely by the granular, highly virulent strains which have undergone animal passage.

SUMMARY

1. The downy and granular forms of *Trichophyton mentagrophytes* have been shown to represent two cultural forms of a single species.

2. Experimental results indicate that the downy type culture has less virulence for the guinea pig and the dog. Clinical data indicate that they are also less virulent for man.

3. Virulence of the downy type culture may be enhanced by serial passage on guinea pigs. The guinea pig passage strains are also more virulent for both the guinea pig and the dog.

4. In two instances the increased virulence of the downy type culture was accompanied by a morphological change to a granular type culture.

5. The relationship of these two cultural types to the epidemiology of *T. mentagrophytes* infections is discussed. Although neither downy or granular type cultures are isolated strictly from specific clinical entities, the downy type culture is most commonly isolated from chronic, low-grade infections, and the granular type culture is most commonly isolated from acute suppurative lesions, particularly those acquired from animals. The fact that the virulence of the downy type culture can be enhanced by animal passage, and that this increase in virulence may be accompanied by a change to the granular type culture appears to explain these phenomena.

6. Evidence is presented suggesting that *T. interdigitale* should not be considered as a species distinct from *T. mentagrophytes.*

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