ENHANCEMENT OF VIRULENCE OF *MALLEOMYCES PSEUDOMALLEI*[1]

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The high mortality rate and the increasing frequency of reports of infection with *Malleomyces pseudomallei* (Alain *et al.*, 1949; Beamer *et al.*, 1954; Garry and Koch; 1951; Grant and Barwell, 1943; Gutner and Fisher, 1948; de Lajudie and Brygoo, 1953; Lewis and Olds, 1952; Mirick *et al.*, 1946; Ziskind *et al.*, 1954) have emphasized the timeliness of additional studies on the experimental infection in laboratory animals.

In earlier studies on the susceptibility of smaller animals to experimental infection, large numbers of this organism, usually in the millions, were used as inoculum but the relative susceptibility of the various species was not evaluated (Whitmore, 1912, 1913; Stanton and Fletcher, 1924, 1932; Nicholls, 1930; de Lajudie and Brygoo, 1953).

In view of the incompleteness of the information on virulence, Miller *et al.* (1948) investigated quantitatively the virulence of this organism for various species by several routes of inoculation. They found that guinea pigs, contrary to previous reports, were not uniformly susceptible since some were killed when approximately 400 organisms were inoculated while others survived very large doses with no evidence of infection. Hamsters and ferrets, on the other hand, could be regularly killed with very few organisms. Mice and white rats were reported to be only slightly and irregularly susceptible to their most virulent strain. From 2 to 30 million organisms, inoculated intraperitoneally, were required to produce 30 to 70 per cent mortality in these species.

Important factors, in addition to susceptibility, in the choice of a suitable host for studies on epidemiology, assay of therapeutic agents, and immunizing procedures, etc., are its genetic standardization (in the interest of more uniform response), availability, ease, and economy of handling. Attention was therefore directed toward enhancement of the virulence of *M. pseudomallei* for white mice in spite of the report of Miller *et al.* (1948) that this species was "relatively resistant" by the intraperitoneal route.

MATERIALS AND METHODS

The following three strains of *Malleomyces pseudomallei* were used in these studies: strain 103, obtained from the Army Medical School, Washington, D. C., labeled "CSTM[2] 294, 8 Jan 46"; strain 112, obtained from the Institute for Medical Research, Kuala Lumpur, Federation of Malaya, labeled "Str. P. W. 10.2.53, mucoid, human origin"; and strain 114, obtained from the same source as strain 112, labeled "Str. Ipoh 10.2.53, mucoid, human origin."

Stock cultures of the original strains and isolates therefrom were maintained on agar slants covered with mineral oil and stored at room temperature. Transfers were made routinely at intervals of approximately 6 months, using a dilute suspension as inoculum in order to minimize transfer of variants which may have been present in small numbers.

Albino mice (Namru strain, Garber and Hauth, 1950) and golden hamsters were used as experimental animals. The mice were 3 to 4 weeks old for intracerebral (ic) inoculation and 3 to 6 weeks old for intraperitoneal (ip) and oral inoculation. Hamsters, 6 to 8 weeks old, were used for inoculation by all routes. Only females of both species were used to avoid the possibility of ulcerative orchitis.

Broth cultures were used to initiate serial passages, while diluted infective tissue suspensions from moribund mice, selected from the group which had been inoculated with the highest dilution, were used for all subsequent passages.

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[2] CSTM: Calcutta School of Tropical Medicine.

Volume rather than weight was used as the basis for preparing the infected tissue suspension. After the volume of the tissue had been determined (by liquid displacement), the tissue (brain, spleen or liver fragments) was ground with mortar and pestle without abrasive. Ten per cent suspensions were made by adding 9 volumes of 1 per cent peptone solution to 1 volume of tissue.

The number of viable organsims in all suspensions was determined prior to inoculation, using slight modifications of the method described by Miles and Misra (1938), and expressed as organisms per ml of original tissue or culture. In order to minimize the transfer of mutants which may have developed either *in vivo* or *in vitro*, only the highest dilutions estimated to be infective were inoculated into groups of 5 to 8 unanesthetized animals.

The ic dose for both mice and hamsters was 0.03 ml and the ip dose either 0.2 or 0.5 ml. The oral dose was 0.5 ml. Virulence by the inhalatory route was determined by exposing animals to aerosols in the Leif-Krueger apparatus (Leif and Krueger, 1950). The infecting dose is expressed as the number of inhaled organisms. The observation period for all animals was 14 days. The LD_{50} for all routes of inoculation was calculated by the Linearized Mortality Grid method of Goldberg *et al.* (1954).

Broth cultures, made in 4 per cent glycerin beef extract broth (pH 6.9 $\pm$ 0.1), were incubated at 37 C for 24 hr on a reciprocating shaker. Glass beads were added to broth cultures of the rough strains to minimize aggregation. The medium for slants and plates was 4 per cent glycerin beef extract agar (pH 6.9 $\pm$ 0.1).

Since various factors were found to influence profoundly the colonial morphology of *M. pseudomallei*, careful attention was paid to the preparation, storage, inoculation, and incubation of plates. Petri plates, 100 mm in diameter, with plane-bottom surface were used exclusively and poured on a leveled surface so as to obtain uniform depth of agar, 15 to 20 ml per plate. The agar was cooled to approximately 48 C before it was poured and the plates were held at room temperature 5 to 7 days before use in order to obtain a dry surface. The plates were inoculated by the dally or drop method in order to obtain well isolated colonies, and incubated at 37 C for 3 days followed by 4 days at room temperature. Only those plates with less than 100 colonies per plate were used for determining colonial morphology since the latter was sometimes not characteristic on crowded plates. Colonial morphology was observed with a stereomicroscope and incident light.

EXPERIMENTAL RESULTS

Examination of the various stock cultures of *M. pseudomallei* available for this study revealed numerous types of colonies, varying in size, and ranging in color from orange or yellow to white or colorless and in texture, from deeply corrugated, referred to as rough (R), to entirely smooth (S) or "mucoid" (M). An example of the various types of colonies which may be found in a single stock culture is shown in figure 1. Figures 2 to 17, inclusive, show representative rough and smooth variants isolated from stock cultures.

These variations in colonial morphology were disconcerting because it is well known that differences in virulence, antigenicity, susceptibility to therapy, stability during storage, etc., are often correlated with specific colonial morphology. Obviously, any results with cultures which were not genetically homogenous would reflect only the genetic composition of the culture at the moment. Heterogeneity would present difficulties not only in interpreting results but also in obtaining reproducible results in any type of experimental work. It was thus necessary, at the outset, to select stable isolates of representative variants and work with these rather than with the stock strains in our culture collection.

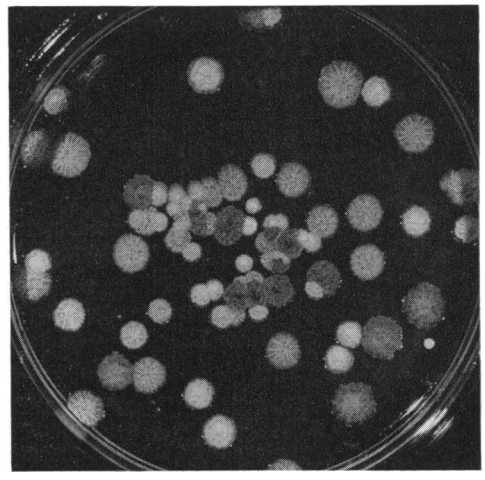
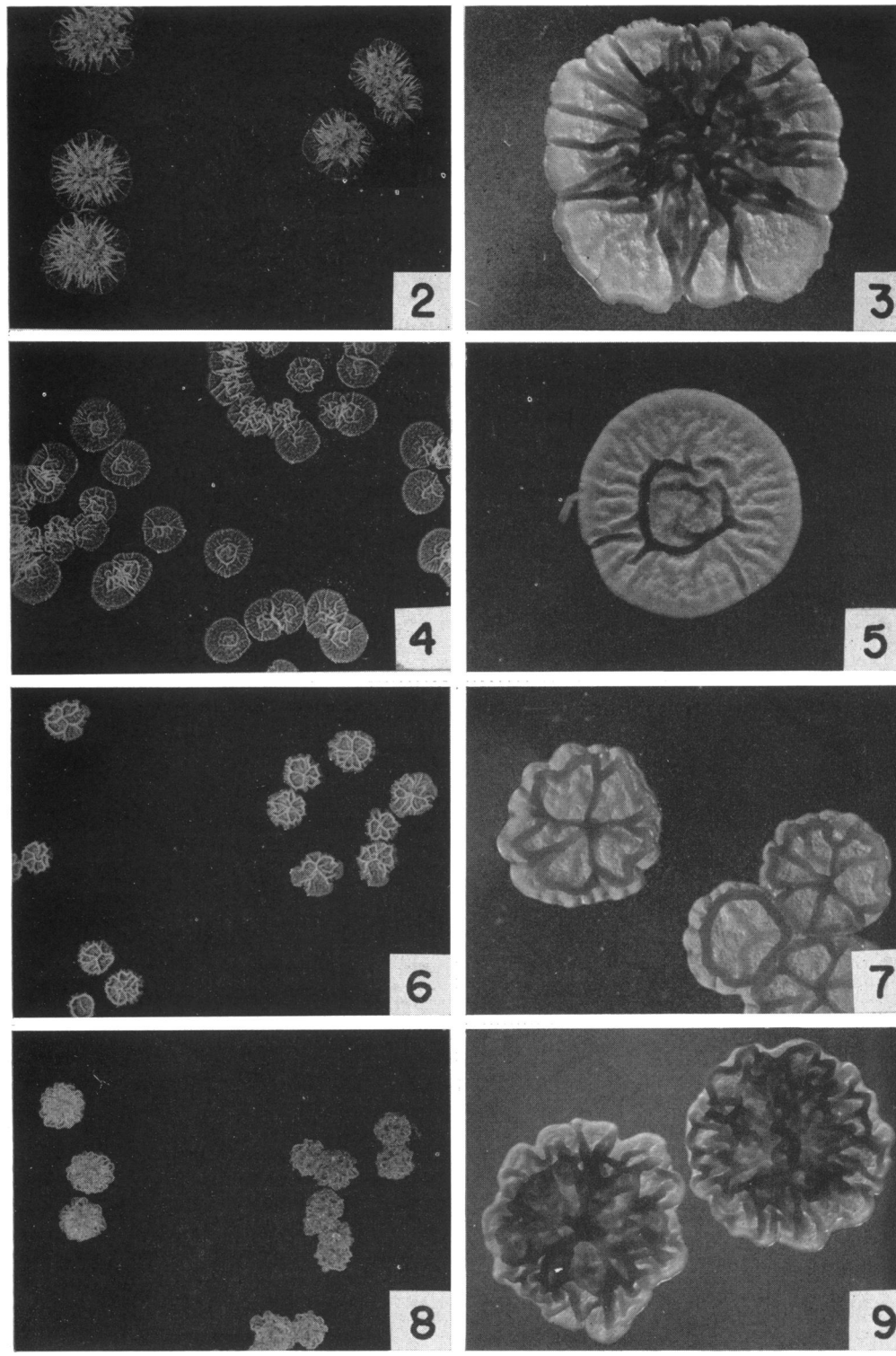


Figure 1. Malleomyces pseudomallei showing various types of colonies ranging in color from white to yellow and orange (dark colonies) and in texture from smooth (2 colonies) to rough.



Figures 2–17. Representative rough and smooth variants isolated from stock cultures of *Malleomyces pseudomallei*.

Figure 2. From strain 101. Rough yellow lacy type. 1.5 ×. *Figure 3*. From strain 101. Rough yellow lacy type. 7 ×.

Figure 4. From strain 102. Rough cream type. 1.5 ×. *Figure 5*. From strain 102. Rough cream type. 7 ×.

Figure 6. From strain 101. Rough white type. 1.5 ×. *Figure 7*. From strain 101. Rough white type. 7 ×.

Figure 8. From strain 102. Rough orange type. 1.5 ×. *Figure 9*. From strain 102. Rough orange type. 7 ×.

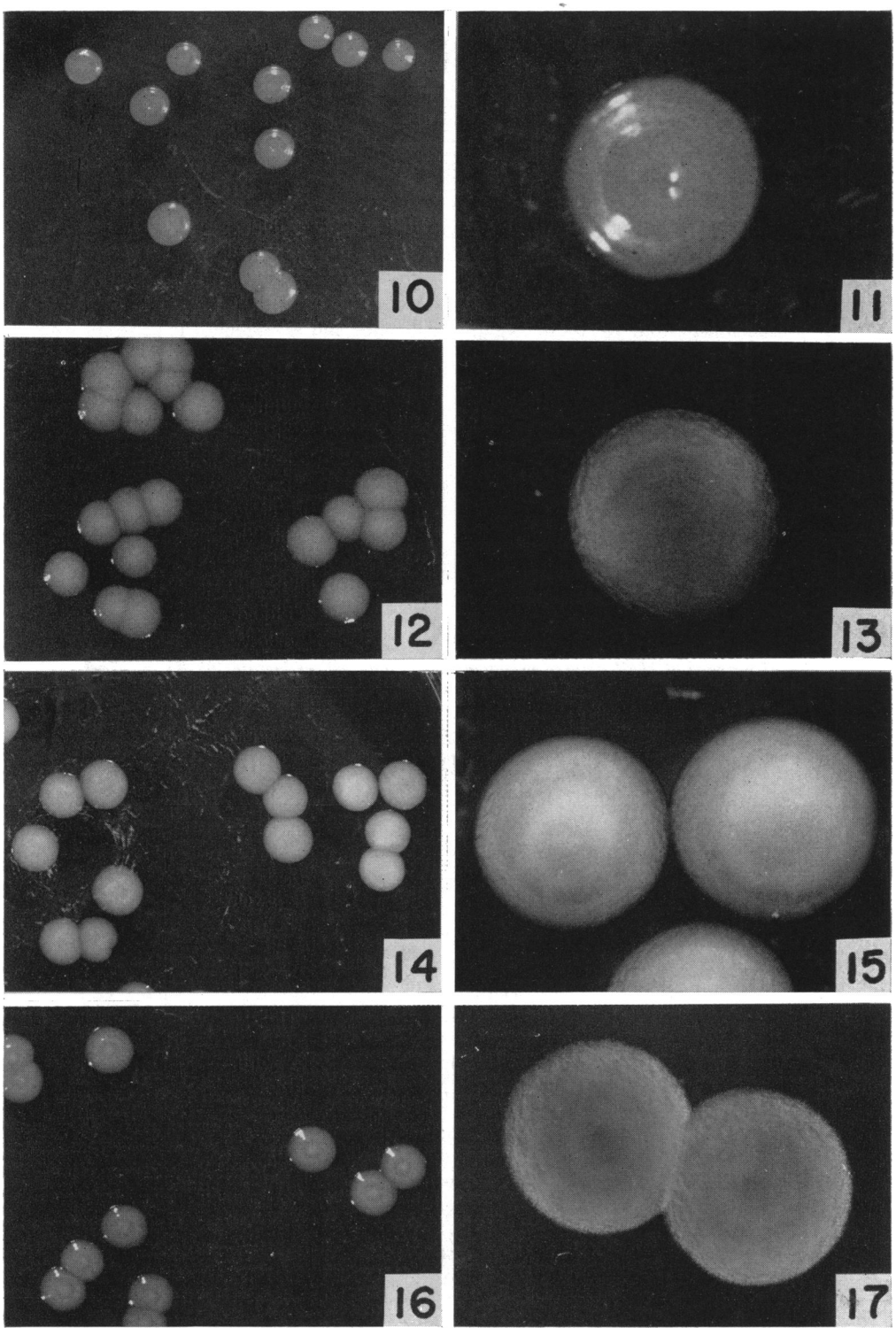


Figure 10. From strain 104–2. Smooth yellow type. 1.5 ×. *Figure 11.* From strain 104–2. Smooth yellow type. 7 ×.

Figure 12. From strain 103. Smooth cream type. 1.5 ×. *Figure 13.* From strain 103. Smooth cream type. 7 ×.

Figure 14. From strain 114. Smooth white type. 1.5 ×. *Figure 15.* From strain 114. Smooth white type. 7 ×.

Figure 16. From strain 101. Smooth white type. 1.5 ×. *Figure 17.* From strain 101. Smooth white type. 7 ×.

Over 100 representatives of the various colonial types have been isolated in this laboratory to date but many of them dissociated to such an extent during a few days' incubation that they could not be studied extensively in any one phase and the investigations were therefore limited to a few which showed a relatively low dissociation index.

In view of the reports of others that large does of *M. pseudomallei* (from 10^6 to 10^7 organisms) were required to kill mice by the intraperitoneal route, it appeared worthwhile to investigate the intracerebral route. The virulence by this route was found to be considerably higher and serial brain-to-brain passages were therefore continued in the hope of increasing the virulence to the point where small amounts of infected brain suspension would also produce fatal infection by the intraperitoneal route. It was thought that experimental infection established by the latter route would constitute a better model for the natural infection in rodents and would therefore be more satisfactory for assay purposes.

Table 1 shows the gradual enhancement of the virulence of a rough yellow isolate, 103R, during serial brain passages from 16,000 to <50 organisms per LD_{50}. The serial passages in mice were uninterrupted by culture passage except between the first and second and between the sixteenth and seventeenth ic passages when 2 or 3 culture passages intervened. Brain from the twentieth ic passage was used to initiate ip passage while spleen or liver was used for all subsequent passages by the latter route. The ip virulence continued to increase gradually from about 10^5 organisms per LD_{50} in the first ip passage to about 10 organisms from the forty-eighth through the seventy-fifth and last ip passage. Although mice were quite regularly killed with about 10 organisms in the later passages

TABLE 1

*Serial passage of Malleomyces pseudomallei (rough yellow isolate 103-R) in mice**

Route and Mouse Passage No.		Inoculum	Org/LD_{50} in Mice†				Colonial Morphology‡
Ic	Ip		Intracerebral (Ic)	Intraperitoneal (Ip)	Oral	Inhalatory (Inh)§	
		Culture	16,000				RY large
Interrupted by 2 culture passages							
2		Culture	160				RY large
9		Brain	<8			40,000	RY large
16		Brain	<11			50,000 to 64,000	RY large
Interrupted by 3 culture passages							
17		Culture	44				RY large
21	1	Brain	18	78,000			
	2	Spleen					IRY small
	8	Liver		6,000			IRY small
	10	Spleen		1,300			IRY small with occ. RY large
	17	Liver		650		580	RY large
	26	Liver				<280	IRY small
	34-47	Liver		100-1,000			IRY small
	46	Liver			8.0×10^4		IRY small
	49	Liver			6.6×10^3		IRY small
	48-75	Liver (with one exception)		ca 10			IRY small, occ. RY large

* These passages were made during a period of approximately 1 year and 8 months, from April 1, 1952 to Nov. 23, 1953.

† Organisms per LD_{50}.

‡ RY, rough yellow; IRY, intermediate rough yellow.

§ Mice were exposed to aerosols of cultures from the designated tissues and passages. The number refers to the inhaled dose.

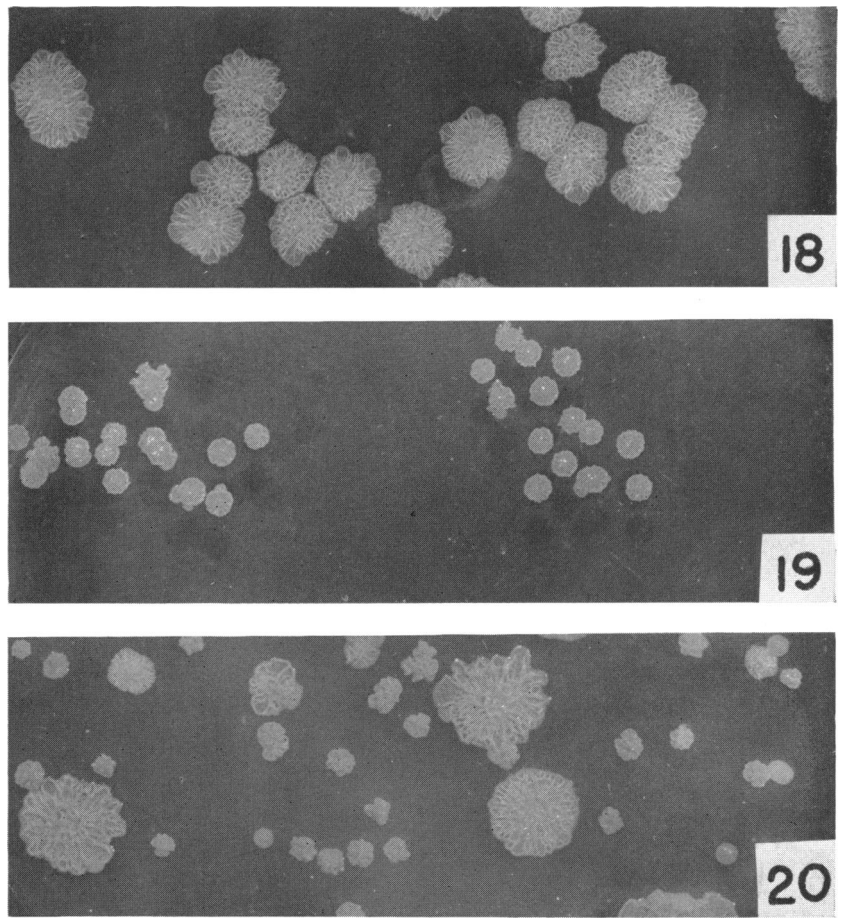


Figure 18. Strain 103R before adaptation to mice. Large lacy rough yellow colonies.

Figure 19. Strain 103R after adaptation to mice, showing pure culture of small intermediate rough yellow colonies.

Figure 20. Strain 103R, showing reversion to the parent type (in figure 18) of the small intermediate rough yellow colony (in figure 19) after several passages on agar.

by the ip route, it still required about 10^4 organisms to kill mice by the oral route. This oral LD_{50} was of interest in connection with epidemiological considerations.

The initial virulence of this strain by the inhalatory route was not determined but the LD_{50} for mice exposed to an aerosol of a 24-hr broth culture inoculated with infected brain of the ninth ic passage was 4×10^4 inhaled organisms. The inhalatory virulence had not increased by the sixteenth ic passage but had increased to <280 inhaled organisms after 20 ic passages followed by 26 ip passages.

The number of viable organisms in brain, determined at each ic passage, was usually from 10^7 to 10^8 organisms per ml, and in liver suspensions after ip inoculation from 10^7 to 10^9 organisms per ml of tissue.

A change in colonial morphology occurred during the course of serial passages of this isolate in mice. Before adaptation to mice, it produced only large lacy rough yellow (RY) colonies (figure 18) and only this type was isolated at each of the 21 ic passages, but the second ip passage showed a pure culture of a small intermediate RY (IRY) type of colony which grew slowly, attaining approximately 3 mm in diameter after 3 days' incubation (figure 19). With few exceptions the subsequent passages, through the seventy-fifth and last, yielded only this mutant, which however, retained its ability to revert to the large RY parent type on serial passage in culture

TABLE 2

*Serial passage of Malleomyces pseudomallei (rough yellow isolate 103-1) in mice**

Route and Mouse Passage No.		Inoculum	Org/LD_{50}† in Mice				Org/LD_{50}† in Hamsters			Colonial Morphology§
Ic	Ip		Intracerebral (Ic)	Intra-peritoneal (Ip)	Oral	Inhalatory (Inh)‡	Ic	Ip	Inh‡	
		Culture	ca 50,000	2×10^7		$>2 \times 10^4$	<11	<15	57	RY large lacy
2		Brain	500							RY large lacy
6		Brain	56							RY large lacy
9		Brain		5×10^6						RY large lacy
15		Brain	14							IRY large
18	1	Brain	18	1×10^5						IRY large
19		Brain	3							IRY large
20		Brain	5							IRY large
	3	Liver and spleen		<35,000						IRY large
	5	Liver		17,000						IRY large
	8	Liver				<29				IRY large
	9	Liver		<70						IRY large
	15	Liver		<200			<1	<1		IRY large
	18	Spleen		<4		<20			20	IRY large
	32	Spleen		13	6.5×10^4					IRY small
	24–57	Spleen		10–100						IRY small

* These passages were made during a period of approximately one year, from Oct. 31, 1952 to Nov. 12, 1953.

† Organisms per LD_{50}.

‡ Animals exposed to aerosols of cultures from the designated tissues and passages. The number refers to the inhaled dose.

§ RY, rough yellow; IRY, intermediate rough yellow.

media. Occasionally the colonies were completely rough, but remained characteristically small and slow-growing. The reversion to parent type is shown in figure 20. There appeared to be a correlation between the colonial morphology and virulence, since the virulence dropped as the culture reverted to the parent type. This concomitant loss in virulence was demonstrated by titrating successive culture passages of the mouse-adapted strain in mice. After 3 intervening passages in culture media, the majority of the colonies were of the large RY lacy type and the virulence dropped to an ip LD_{50} of about 100,000 organisms. A culture derived from a single colony of the reverted large RY type gave an ip LD_{50} of $>10^6$ organisms. Thus isolates, derived after reversion, were not more virulent than was the parent culture before its adaptation to mice. The small intermediate rough yellow mutant, however, retained high virulence as long as it could be maintained in this phase on culture media.

Similar experiments were then undertaken with another apparently identical RY isolate (103-1) of the same stock strain to see whether mutants like that derived from the first series of mouse passages were regularly obtained during enhancement of virulence by serial mouse passage. Table 2 shows the results with this second RY isolate. The virulence of the initiating culture was about 50,000 organisms per LD_{50} by the ic route and 2 billion by the ip route. The same culture killed hamsters by both ic and ip routes with less than 15 organisms. Upon serial ic passage in mice, using brain as inoculum, the virulence increased rapidly to 5 organisms per LD_{50} in the twentieth

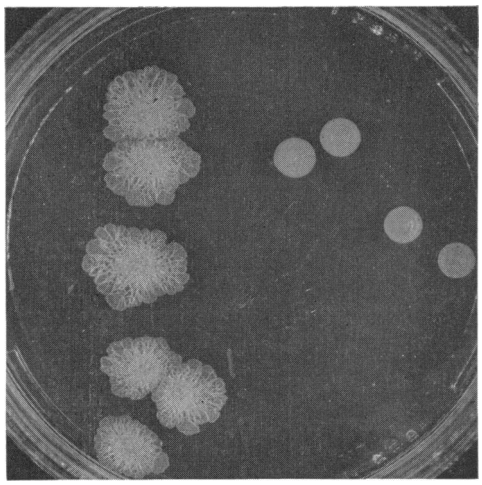


Figure 21. Strain 103-1, showing the characteristic large lacy rough yellow colonies (at the left) before adaptation to mice. The smaller intermediate rough yellow colonies (at the right) were characteristic of this strain after its adaptation to mice.

passage. At the ninth ic passage, the virulence by the ip route had increased by 1 log and at the eighteenth ic passage by 2 log. Beginning with the eighteenth ic passage, subsequent passages were made by the ip route using infected liver and/or spleen as inoculum. The virulence continued to increase to <100 organisms per LD_{50} in the ninth ip passage, and remained at this level through the fifty-seventh and last passage.

The inhalatory virulence for mice increased from $>20{,}000$ organisms per LD_{50} for the initiating culture to <20 organisms after 17 ic passages followed by 18 ip passages, while that for hamsters increased only slightly from the initial high virulence of 57 organisms to 20. The number of viable organisms in brain, determined at each ic passage, and in spleen and liver after ip inoculation, ranged between 10^7 and 10^9 organisms per ml of tissue.

This isolate (103-1) also underwent a certain change in colonial morphology. Throughout the first 11 ic passages the colonies were all of the large lacy RY parent type. With 2 exceptions the colonies of all of the subsequent ic and ip passages were less rough, designated as intermediate RY (IRY), and no longer lacy. Within the designation of IRY were included colonies showing roughness ranging from a granular appearance to slight corrugation. That this difference in colonial morphology was not referable to slight differences in plating medium was evident from figure 21 which shows colonies of both the parent (large lacy RY) and mouse adapted (IRY) strains on the same plate. The IRY colonies of the first 23 ip passages were large, while those of all subsequent passages were definitely smaller, more like those in figure 19.

The enhancement of virulence of the two RY isolates as described above suggested similar experiments with a smooth colorless isolate (103-3) derived from the same stock culture of strain 103. The results, which were very similar, are shown in table 3. After 13 serial ic passages, the virulence was enhanced to approximately 10 organisms/LD_{50}. The ip virulence at that point had also increased by 2 log, from 2×10^7 to 2×10^5 organisms. After 30 serial ip passages, both the ip and inhalatory virulence increased to <100 organisms and remained between 10 and 100 throughout the remainder of the 55 serial ip passages except for a short period when the virulence seemed to be temporarily lower. In this instance also, the initial high virulence for hamsters remained unchanged throughout the course of the mouse passages.

The number of viable organisms in brain after ic inoculation and in liver and spleen after ip inoculation ranged between 10^7 and 10^9 organisms per ml of tissue.

The colonial morphology of this isolate, unlike that of 103R and 103-1, remained unchanged, i. e., characteristically smooth and colorless like that of the original isolate, throughout the course of animal passages.

As the above studies were nearing completion, several additional strains of *M. pseudomallei*, recently isolated from human cases in Malaya, were made available to this laboratory. It was of interest to compare the virulence of these new strains before and after adaptation to mice with that of the several isolates of strain 103, which was an old laboratory stock culture of obscure history.

Two of these recently isolated strains (112 and 114) were chosen for study. While both were labelled "mucoid" upon receipt, most of the colonies of both strains were found to be smooth and colorless, although there were also a few "mucoid" colonies. The latter were very soft, of semi-fluid consistency, flowing slightly down the

TABLE 3

*Serial passage of Malleomyces pseudomallei (smooth isolate 103-3) in mice**

Route and Mouse Passage No.		Inoculum	Org/LD50† in Mice				Org/LD50† in Hamsters			Colonial Morphology§
Ic	Ip		Intracerebral (Ic)	Intraperitoneal (Ip)	Oral	Inhalatory (Inh)‡	Ic	Ip	Inh‡	
		Culture	<22,000	2×10^7		2×10^7	<23	<2	20-30	SW
2		Brain	1,000							SW
4		Brain	<580							SW
13	1	Brain	14	2×10^5						SW
16		Brain	3							SW
	2	Liver		5,000						SW
	8	Liver		110		<110				SW
	12	Liver				<200	1	<18		SW
	16	Spleen				<32			<4	SW
	30	Spleen		17	$>1.1 \times 10^6$					SW
	16-36	Spleen		10-100						SW
	40								36	SW
	37-48	Spleen		100-1000						SW
	49-55	Spleen		10-100						SW

* These passages were made during a period of one year, from Nov. 11, 1952 to Nov. 12, 1953.

† Organisms per LD_{50}.

‡ Animals were exposed to aerosols of cultures from the designated tissues and passages. The number refers to the inhaled dose.

§ SW, smooth white.

TABLE 4

*Serial passage of Malleomyces pseudomallei (strains 112 and 114) in mice**

Strain	Mouse Passage No., Ip	Inoculum	Org/LD50† in Mice			Org/LD50† in Hamsters			Colonial? Morphology§
			Intracerebral (Ic)	Intraperitoneal (Ip)	Inhalatory (Inh)‡	Ic	Ip	Inh‡	
112 (smooth)		Culture	ca 24	ca 10,000	2500	<28	<47	10	I
	2	Spleen		85					I
	4	Spleen		20					I
	7	Spleen		8					I
	14	Spleen		5			35		S
	15	Spleen		4	19			6	S and I
	16-32	Spleen		ca 10					S and I
114 (smooth)		Culture	ca 37	10^6-10^7	ca 10^5	<25	<42	9	S
	2	Liver and spleen		<550					S
	5	Spleen		60	13				I
	10	Spleen		28				26	S
	14	Spleen		6			<8		S
	17-30	Spleen		ca 10					S

* These passages were made during a period of approximately 5½ mo, beginning Sept. 4, 1953.

† Organisms per LD_{50}.

‡ Animals exposed to aerosols of cultures from the designated tissues and passages. The number refers to the inhaled dose.

§ S, smooth; I, intermediate.

agar of plates held vertically. In subsequent culture passages, a few R and IR colonies were occasionally observed but these types never reached significant proportions. The virulence studies were therefore initiated with the stock cultures of both strains rather than with isolates.

The results with both strains, summarized in table 4, differed notably from those with the 3 isolates of strain 103 in that both were immediately and highly virulent for mice by the ic route, the LD_{50} being 24 and 37 organisms for the 2 strains respectively.

The ip virulence of strain 112, before adaptation, was also higher (about 10^4 organisms per LD_{50}) than that of the strains previously studied and increased, within a few passages, to about 10 organisms. While the ip virulence of strain 114 was initially low (10^6 to 10^7 organisms per LD_{50}) like that of the 103 isolates, it was also rapidly enhanced during serial passage to <100 organisms per LD_{50} within the first few passages and to about 10 organisms in all subsequent passages.

The inhalatory virulence for mice of strain 112 increased from 2,500 organisms per LD_{50} for the initiating culture to 19 organisms at the fifteenth ip passage; that for strain 114 increased from approximately 10^5 organisms per LD_{50} to 13 organisms at the fifth ip passage.

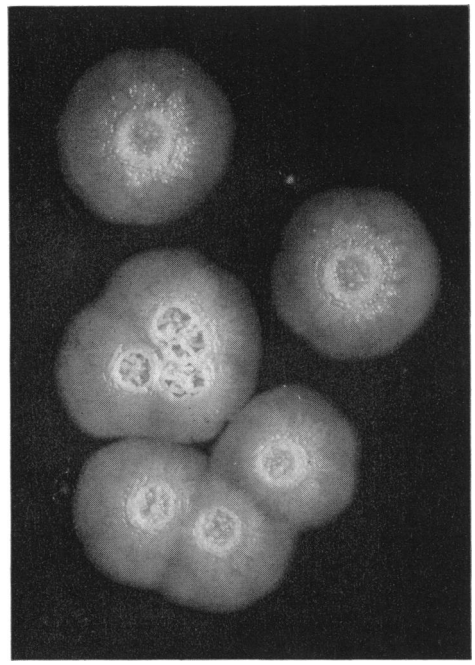


Figure 23. Strain 112. Intermediate rough white colonies which appeared in the course of serial passage through mice.

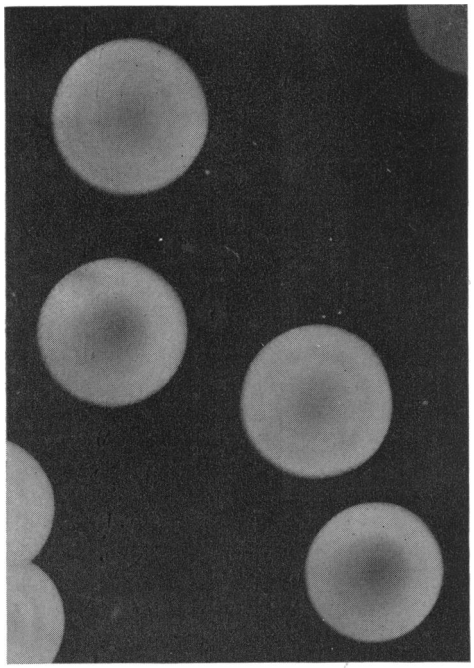


Figure 22. Strain 112. Smooth white colonies, characteristic of this strain before its adaptation to mice.

The initial virulence for hamsters of both these strains, as of those previously studied, was high by all 3 routes, namely <47 organisms per LD_{50}, and did not increase on serial passage.

The number of viable organisms in spleen and liver suspensions, determined at each passage, ranged between 10^8 and 10^9 organisms per ml of tissue.

As in the previous studies, the colonial morphology was recorded at each mouse passage for both strains. In early passages of strain 112, the colonial morphology was largely of an intermediate type (with irregular granular appearance) which however did not progress to characteristic corrugation. Beginning with the eighth passage, the colonies were usually smooth. Intermediate R colonies appeared at times but were usually absent in the succeeding passage. Typical R colonies were seen only occasionally throughout the entire course of the mouse passages. At no time were these deviations from the characteristic S colony type of the parent strain accom-

panied by any significant differences in virulence.

The characteristic S colonial morphology of strain 112 before mouse passages and the intermediate type recovered from many of the mouse passages are shown in figures 22 and 23. In other passages the colonial morphology was entirely S like that of the parent culture or a mixture of both types. Since these mixtures were observed on the same plate, they could not be accounted for on the basis of slight differences in medium or growth conditions.

The colonial morphology of strain 114 throughout the course of the mouse passages was also characterized by a low percentage of transient variations similar to those exhibited by strain 112, except that the first passages showed 90 to 100 per cent S colonies and no intermediate types were encountered after the sixteenth passage. Typical S colonies predominated at each mouse passage although a small percentage of M colonies were present in approximately half the passages. Not a single R colony was observed throughout the course of the mouse passages. In spite of these transient minimal deviations, both strains (112 and 114) could be regarded as characteristically S and the virulence observed as that of 2 smooth strains.

DISCUSSION

It was obvious from these and other studies in this laboratory that virulence was not related to a single colonial type; smooth and rough strains both before and after adaptation to mice showed comparable virulence for both mice and hamsters. However, as the more virulent organisms replaced the parent strains on serial passage, the increase in virulence was sometimes accompanied by characteristic changes in colonial morphology. This occurred with both of the rough isolates 103R and 103-1 which changed completely and quite suddenly from large lacy RY colonies to smaller IRY colonies which developed much more slowly in plate cultures than the parent strains.

The suddenness with which the parent strain could be apparently and completely replaced by a mutant of characteristically different colonial morphology, even within a single animal passage, was quite striking and may be explained by the close attention paid to the methods designed to dilute out small numbers of any mutants arising within the bacterial population *in vivo*. Animal passages were made with minimal infective doses and wherever possible from animals which had been inoculated with the highest of the several serial dilutions used for passage. The organisms seemed to multiply very rapidly as virulence increased, since mice inoculated with 1 to 10 organisms regularly showed from 10^7 to 10^9 organisms per ml of the tissue used for transfer (brain, liver or spleen) within 5 to 7 days after inoculation. The reliability of arriving at the virulence by means of viable bacterial counts on suspensions of infected tissue was borne out repeatedly by the essentially identical virulence of broth cultures of the mouse-adapted strains.

Serial passage through mice of the various strains and their increasing virulence for this host was not accompanied by any loss in virulence for hamsters. It should be mentioned that while nearly all of the strains tested were highly virulent for hamsters, an occasional isolate was encountered which could be considered avirulent, e. g., our strain 102-1, a rough isolate with an ip LD_{50} for hamsters of $>5.5 \times 10^7$ organisms.

SUMMARY

The virulence of *Malleomyces pseudomallei* could be quite readily enhanced for mice by serial passage with infected tissues to the point where 10 to 100 organisms would uniformly produce a rapidly fatal infection when inoculated intracerebrally, intraperitoneally or in the form of aerosols. The white mouse thus constitutes a highly desirable experimental animal for various types of assay if mouse-adapted or suitable freshly isolated strains are used.

REFERENCES

ALAIN, M., SAINT-ETIENNE, J., AND REYNES, V. 1949 La mélioidose: Considérations étiologiques cliniques et pathogéniques à propos de 28 cas. Méd. Trop., **9**, 119–142.

BEAMER, P. R., VARNEY, P. L., BROWN, W. G., AND MCDOWELL, F. 1954 Studies on *Malleomyces pseudomallei* isolated from melioidosis originating in the Western Hemisphere. Amer. J. Clin. Pathol., **24**, 1231–1240.

DE LAJUDIE, P., AND BRYGOO, E. R. 1953 Contribution à l'étude du pouvoir pathogène du *Bacille whitmore*. Ann. inst. Past., **85**, 99.

GARBER, E. D. AND HAUTH, F. C. 1950 A new mutation with asymmetrical expression in the mouse. J. Heredity, **41**, 122.

GARRY, M. W. AND KOCH, M. L. 1951 Chronic melioidosis: Bacteriologic and clinical correlation in diagnosis. J. Lab. and Clin. Med., **38,** 374–384.

GOLDBERG, L. J., WATKINS, H. M. S., DOLMATZ, M. S., AND SCHLAMM, N. A. 1954 Studies on the experimental epidemiology of respiratory infections. J. Infectious Diseases, **94,** 9–21.

GRANT, A. AND BARWELL, C. 1943 Chronic melioidosis: A case diagnosed in England. Lancet, **244,** 199–201.

GUTNER, L. B. AND FISHER, M. W. 1948 Chronic melioidosis: Discussion; case report and special studies. Ann. Internal Med., **28,** 1157–1169.

LEIF, W. R. AND KRUEGER, A. P. 1950 Studies on the experimental epidemiology of respiratory infections. I. An apparatus for the quantitative study of air-borne respiratory pathogens. J. Infectious Diseases, **87,** 103–116.

LEWIS, F. A. AND OLDS, R. J. 1952 Melioidosis in sheep and a goat in North Queensland. Australian Vet. J., **28,** 145.

MILLER, W. R., PANNELL, L., CRAVITZ, L., TANNER, W. A., AND ROSEBURY, T. 1948 Studies on certain biological characteristics of *Malleomyces mallei* and *Malleomyces pseudomallei*. II. Virulence and infectivity for animals. J. Bacteriol., **55,** 127–135.

MILES, A. A. AND MISRA, S. S. 1938 The estimation of the bactericidal power of the blood. J. Hyg., **38,** 732–749.

MIRICK, G. S., ZIMMERMAN, H. M., MANER, G. D., AND HUMPHRY, A. A. 1946 Melioidosis on Guam. J. Am. Med. Assoc., **130,** 1063–1067.

NICHOLLS, L. 1930 Melioidosis with special reference to the dissociation of *Bacillus whitmore*. Brit. J. Exptl. Pathol., **11,** 393–399.

STANTON, A. T. AND FLETCHER, W. 1924 Melioidosis: A disease of rodents communicable to man. Bull. Inst. Med. Research, Federation Malaya, *No. 4.*

STANTON, A. T. AND FLETCHER, W. 1932 Melioidosis: Studies from the Institute Med. Research, Federation Malaya, *No. 21.*

WHITMORE, A. 1912 An account of a Glanders-like disease occurring in Rangoon. Brit. Med. J., **2,** 1306–1308.

WHITMORE, A. 1913 An account of a Glanders-like disease occurring in Rangoon. J. Hyg. **13,** 1.

ZISKIND, J., PIZZALATO, P., AND BUFF, E. E. 1954 Characteristics of a strain of *Malleomyces pseudomallei* from chronic melioidosis. Amer. J. Clin. Pathol., **24,** 1241.