THE FECAL FLORA OF VARIOUS STRAINS OF MICE. ITS BEARING ON THEIR SUSCEPTIBILITY TO ENDOTOXIN

By RUSSELL W. SCHAEDLER, M.D., and RENÉ J. DUBOS, Ph.D.

(*From the Rockefeller Institute*)

Plate 123

(Received for publication, February 21, 1962)

Earlier publications have described some of the characteristics of a new mouse colony (NCS), originally derived from the so called standard Swiss colony (SS) of albino mice formerly maintained at The Rockefeller Institute. (1–4) The techniques used in the development of the new colony had been designed to eliminate a number of common mouse pathogens—in particular, the virus of chronic respiratory disease, the pleuropneumonia (PPLO) organisms responsible for conjunctival disease, bacteria of the *Salmonella* species, and various endo- and ectoparasites (1). An unexpected effect of these techniques was that they also eliminated from the intestinal flora of NCS mice certain Gram-negative bacteria which are present in other mouse colonies—for example, *Escherichia coli*, *Proteus vulgaris*, and *Pseudomonas*.

The NCS colony has now been in production for over three years at the Rockefeller Institute. The high level of sanitation at which it has been maintained is illustrated by the fact that in the course of many thousands of stool and intestine cultures carried out on normal animals of various ages over these three years, we have never obtained any growth of *E. coli*, *Proteus*, or *Pseudomonas* except in a very few cases after the mice had been kept for several months in the general animal room. In fact, the management of the NCS colony has been so successful that the original SS colony has now been discontinued at The Rockefeller Institute. It seems worthwhile, therefore, to state briefly some of the differences which have been consistently observed in many comparative experiments between the new NCS colony and the SS colony from which it was initially derived.

(*a*) The average litter size of the NCS animals is larger than that of the SS. Measured in terms of numbers of weaned mice per female per week, the average production rate over a period of two years was 0.9 for the new colony as against 0.35 for the old (1).

(*b*) At 4 weeks of age, the NCS animals weigh 16 to 20 gm, *i.e.* approximately 2 to 4 gm more than did the SS mice (2).

(*c*) The NCS mice gain weight more rapidly than did the SS animals; their weight at 6 weeks of age ranges from 24 to 30 gms (2).

(*d*) In contrast to the SS mice, the NCS mice gain weight on diets containing

wheat gluten (15 per cent) as the sole source of protein, even though gluten is low in lysine and threonine. The fact that NCS mice have been maintained successfully for many weeks on corn grain (autoclaved) as the sole source of food, illustrates still further their unusual efficiency in the utilization of food (unpublished results).

(*e*) NCS mice respond by inhibition of water intake and weight loss to the injection of small doses of endotoxins (4). However, they are remarkably resistant to the lethal effect of these substances (3). On the other hand, their susceptibility to the lethal effect of endotoxins can be readily increased, and indeed rendered equal to that of SS mice by exposure to the latter animals during early life or by prior injection of killed Gram-negative bacilli (3).

(*f*) In experiments conducted two years ago, it was found that NCS mice were more susceptible than SS mice to experimental infection with *Staphylococcus aureus* and *Klebsiella pneumoniae* (type C) (2).

These profound biological differences between NCS and SS mice were observed at a time when the two colonies were maintained under identical environmental conditions, a fact which suggested at first that these differences had a genetic basis. In reality, however, experimental evidence indicates that at least some of the factors responsible for the differences between the two animal colonies reside not in their genetic make-up, but in the change in indigenous flora which was brought about by the techniques used in the development of the NCS colony (2, 3). In order to further investigate this hypothesis we have carried out a comparative study of the intestinal flora and some biological characteristics of various strains of mice. The results to be reported in the present paper describe some of the bacterial species isolated from mice fed pasteurized commercial pellets and drinking water kept essentially free of bacterial contaminants.

Materials and Methods

Mice.—

Mice of approximately the same age were obtained from the following colonies.

1. Princeton S—a non-"Swiss" albino mouse colony; maintained by Dr. Nelson at the Rockefeller Institute (1).

2. Swiss L—originally derived from the S colony, but obtained from the Jackson Memorial Laboratory (Bar Harbor, Maine) by Dr. C. Lynch; maintained by Dr. Lynch at The Rockefeller Institute (5).

3. C57Bl—obtained from the Jackson Memorial Laboratory by Dr. C. Lynch and maintained by her at The Rockefeller Institute (5).

4. RFVL—derived from the S colony; maintained in Dr. Max Theiler's department in The Rockefeller Foundation Virus Laboratory (located at The Rockefeller Institute).

5 and *6.* CFW—derived from the S colony; and CF_1, a non-"Swiss" albino mouse; both maintained and produced by Carworth Farms, Pearl River, New York (supplied by Mr. M. Cummings of Carworth Farms).

7. NCS; maintained at The Rockefeller Institute, as stated above (1–4).

Maintenance of Animals.—

The animals were maintained under two different conditions of housing: (*a*) in groups of 5 in metal cages, autoclaved wood shavings being used as litter; (*b*) in individual cages on wire grids.

All animals received *ad lib.* D & G commercial pellets (supplied by Dietrich and Gambrill, Frederick, Maryland). This product is claimed by the manufacturer to be essentially free of living bacteria, a claim which has been confirmed in our laboratory.

Tap water was given *ad lib.*, to it was added enough HCl (approximately 0.01 N) to lower its pH to 2.8. This acidity was sufficient to maintain the water essentially free of bacteria for periods of 3 days.

Stool Cultures.—

At 9 a.m. clean papers were placed in the collecting tray of individual cages for stool collection. If the animals were housed in groups, they were placed in individual cages on clean paper for the stool collection and then returned to their cages. Stools were collected at 1 p.m. An amount of 0.2 to 0.3 gm of stool was placed in a sterile tube containing 4 ml of sterile tap water. The stools were emulsified by mechanical agitation, and appropriate dilutions were made in water containing 0.01 per cent activated charcoal. Samples of the various dilutions taken with platinum loops holding approximately 0.005 ml were streaked on the surface of one of the four solid culture media to be described in the following section. The bacterial population was calculated from the numbers of colonies developing after incubation at 37°C.

Culture Media.—

Four different types of culture media were used in order to facilitate the selective cultivation and identification of several of the groups of bacteria present in the stool samples.

1. Tergitol-7 agar (Difco) supplemented with tetrazolium, was used for the differentiation and enumeration of coliform bacilli (6).

2. Chapman Stone medium (Difco) was used for the enumeration of staphylococci and other micrococci (7).

3. The so called Mitis-Salivarius agar (Difco) was used as a basis for the enumeration of enterococci (8). However, it was found that this medium also supported the growth of some Gram-negative bacilli and lactobacilli. To overcome this difficulty sodium azide was added (100 mg per liter of medium), a modification which facilitated the identification and enumeration of enterococci.

4. A new medium was developed to enumerate and isolate lactobacilli in fecal specimens. To this end comparative tests were carried out with media modified from the formulae de-described in references 9–12. On the basis of these tests Micro Assay Culture Agar (Difco) was modified in the following way which rendered it inhibitory to Gram-negative bacilli, micrococci, and enterococci.

Bacto yeast agar (Difco)	20.0 gm
Proteose peptone No. 3 (Difco)	5.0 "
Dextrose	10.0 "
KH_2PO_4	2.0 "
Sodium azide	0.2 "
l-Cystine	0.2 "
Agar	15.0 "
1 per cent oleic acid in 10 per cent tween 80	40 ml
Distilled water	1000 "

Autoclave; cool to 56°C; add 40 μg of ascorbic acid and 1.6 mg calcium pantothenate. Following surface inoculation, the agar plates were incubated for 24 hours at 37°C in a candle jar, containing approximately 6 to 10 per cent CO_2.

As will be pointed out later, one special type of lactobacilli with a rhizoid (Rhiz) colonial structure proved to be of special interest in our studies of the fecal flora. It was found that this organism (Rhiz) could be favored over the other types of lactobacilli by increasing the concentration of sodium azide (0.4 gm per liter of medium). However, since this modification reduced both the numbers and the size of the lactobacillus colonies other than those of the Rhiz type, it was used only in cases when the study was focused on the latter organism.

RESULTS

I. Comparative Prevalence of Various Bacterial Types in Several Colonies of Mice.—

Five mice of approximately the same age (4 to 6 weeks) were obtained from each of the seven different colonies described under Materials and Methods. All animals were placed in individual cages on grids and given D & G diet and acidified water *ad lib.* Stool samples were collected from individual animals 3 days after the beginning of the experiment and then again 10 days later. Appropriate dilutions of stool material were inoculated on the surface of agar media and incubated at 37°C. Table I presents in a summary form the approximate numbers of the different groups of bacteria obtained from the seven colonies of mice; the numbers correspond to approximately 1 gm of stool and are the averages for 5 mice. The results of the cultures obtained 3 days and 13 days after the beginning of the experiment were so remarkably similar that only the latter are presented in the Table.

The results presented in Table I, and those obtained in several similar experiments, illustrate the following points:

1. Lactobacilli formed a large percentage of the living bacteria in the stools of all animals. Their numbers were especially high in the NCS colony. As we shall see later several types of lactobacilli were present; furthermore, their total numbers and relative distribution changed drastically when the diet and other conditions of husbandry were changed (13).

2. The intestinal flora was characteristic for each mouse colony, even after the animals had been housed and fed under exactly the same conditions for 2 weeks. There were indications, furthermore, that each particular animal within a given colony exhibited a certain individuality with regard to its intestinal flora.

3. The NCS colony was remarkable for its very low content in enterococci, micrococci, and Gram-negative bacilli, and for the absence of *E. coli*, *Proteus*, and *Pseudomonas*.

II. The Types of Lactobacilli in the Stools of NCS Mice.—As mentioned above, several types of lactobacilli have been recognized in the stools of the various colonies of mice. Three morphological types were found to be most prevalent in the NCS mice.

(*a*) One is characterized by a rhizoid colonial morphology and very large bacillary forms (Rhiz) (Figs. 1, 1 *a*). (*b*) Another gave smaller compact, opaque

colonies with bacillary forms more slender than that of type a, and less likely to form long chains (Figs. 3, 3 *a*). (*c*) A third type gave colonies intermediate in size and morphology between those of types a and b; its bacillary morphology was identical with that of type b. (Figs. 2, 2 *a*).

All three types were Gram-positive, but types b and c rapidly lost this staining character.

As will be shown later in this paper, and again in a publication to follow (13)

TABLE I

Comparative Prevalence of Various Bacterial Types in the Stools of Mice from Seven Different Colonies

Mouse colony	Sex	Lacto-bacilli	Entero-cocci	Micro-cocci	Gram-negative bacilli			
					SLF*	*E. coli*	*Prot.*	*Pseu-dom.*
C57B1 (L)	F	$>10^8$‡	10^5‡	10^4‡	10^8‡	10^8‡	Absent	
Albino (CF_1)	F	"	10^7	10^4	10^8	10^8	Present	
" (Princeton)	M	"	10^6	10^4	10^6	$<10^3$	"	
Swiss (L)	M	"	10^6	10^4	10^8	$<10^3$	"	
" (RFVL)	M	"	10^7	10^4	10^6	10^9	"	
" (CFW)	F	"	10^6	10^4	10^9	10^6	"	
" (NCS)	M	"	10^4	0	10^4	0	Absent	

* Slow lactose fermenting coliforms.

‡ The figures correspond to the numbers of bacterial colonies recovered from 1 gm of stool, after the animals had been kept for 13 days in individual cages, and fed food (D & G pellets) and acidified water, essentially free of bacteria.

the numbers of Rhiz lactobacilli differed greatly from one animal to the other and underwent profound variations when the conditions of husbandry were changed.

It may be useful to present here some figures illustrating the relative numbers of Rhiz lactobacilli, as against the other types, found in individual mice at different intervals of time under a given set of conditions.

Five NCS male mice, 4 weeks old, were placed in individual cages and given, *ad lib.*, D & G diet and acidified water. Table II shows the numbers of colonies of lactobacilli recovered from stool cultures made at different periods of time after the beginning of the experiment.

The figures presented in Table II are characteristic of those obtained in many other experiments in which normal NCS mice were fed D & G diet. Under these conditions the numbers of colonies of lactobacilli recovered per gram of stool usually exceeded 10^8 and commonly were larger than 10^9. While it was easy to identify the Rhiz type of bacterial colonies on the agar plates, the other types were far more difficult to separate in mixed populations. For this

reason, they have been pooled under the designation "Others" in Table II as well as in other tables in the present and the following paper.

The greatest variability was observed in the numbers of Rhiz lactobacilli. In all experiments, in fact, it has been found that approximately 10 per cent of the animals had so few lactobacilli of this type that they could not be detected by ordinary techniques of cultivation. (See, for example, mouse no. 4 in Table II.) Although the significance of this finding is not yet clear, it is consistent

TABLE II

Comparative Populations of Various Types of Lactobacilli in 5 NCS Mice

Weeks on D & G diet	Type of lactobacilli	Nos.* of lactobacilli recovered from individual mice				
		1	2	3	4	5
1	Rhiz	4	2	20	0	10
	Others	40	20	20	30	10
2	Rhiz	3	80	120	0	20
	Others	180	300	150	300	190
4	Rhiz	60	10	200	0	70
	Others	300	20	500	20	50

* Figures multiplied by 3×10^6 give numbers of colonies recovered per gm of stool. Figure 0 indicates less than 1.

with the fact, to be reported later, that the Rhiz type of lactobacillus can be readily and irreversibly eliminated from the animals by a number of different treatments.

III. Effect of Caging Conditions on the Lactobacillus Flora of NCS Mice.—In all experiments reported so far, mice were kept in individual cages on wire grids. Surprisingly enough it has been found that when the NCS mice were maintained for prolonged periods of time in groups of 5 to 25 under conditions such that the wood shavings litter became heavily contaminated, their fecal flora remained nevertheless very similar to that of animals kept isolated on wire grids under conditions which prevented or minimized coprophagy. On the other hand, it has been repeatedly observed that any sudden change in caging conditions was reflected in a profound change in the lactobacillus flora. These facts are illustrated in the following experiment.

Twenty NCS male mice, 4 weeks old, were divided in four groups of 5 animals each. At the beginning of the experiment the animals in two of the groups were housed separately in individual cages on wire grids, the other two groups were maintained in two cages each containing 5 animals on wood shavings. The caging conditions were changed at two different times in the course of the experiment; some of the animals in individual cages were grouped together, 5 per cage, whereas some of the animals which had been grouped were transferred

to individual cages. The schedule of the changes is outlined in Table III. The weight of the mice and the numbers of bacterial colonies obtained from their stools, were determined almost daily for each individual animal. However, the results in Table III are presented in the form of averages for the 5 mice of each group, and only for a few of the critical periods in the experiment.

TABLE III

Effect of Changes in Caging Conditions on the Fecal Flora of NCS Mice

Date	A				B			
	Caging	Lactobacilli*		Coliforms*	Caging	Lactobacilli*		Coliforms*
		Rhiz	Others			Rhiz	Others	
12/11	Indiv.	8	10	6.0	Group‡	2	5	0.1
12/14	"	8	10	6.0	"	4	?	0.1
12/17	Group‡				"			
12/18	Group	0.5	6	10	Group	3	3	0.8
12/19	"	0.1	7	4.0	"	3	9	0.1
12/27	"	2	6	6.0	"	3	5	1.0
1/2	"	4	5	6.0	Indiv.	4	5	
1/3	Group			4.0	Indiv.	3	7	20
1/4	"	1	10	3.0	"	<.1	6	10
1/5	"			3.0	"			10
1/8	"	15	15	1.0	"	<.1	15	12

* Figures to be multiplied by 10^8 per gm of stool for the lactobacilli, and by 10^5 for the coliforms.

‡ 5 animals per cage.

The results presented in Table III illustrate that the numbers of lactobacilli recovered from the stools usually decreased when the animals were shifted from one type of caging to the other. The numbers returned to the original level only slowly, especially in the case of the Rhiz type of lactobacillus.

Unexpectedly, the numbers of Gram-negative bacilli recovered from the stools decreased progressively as the animals were kept for prolonged periods of time grouped in the larger cages with wood shavings as litter. This was the more surprising because the litter was changed only once weekly and therefore became heavily contaminated with feces, urine, and food residue. Gram-negative bacilli suddenly increased in numbers, however, when the animals were transferred to the individual cages (see Table III).

Many observations related to these phenomena have been made in the course of other experiments. For example, it has been repeatedly found that the numbers of lactobacilli recovered from the stools of NCS mice drop precipitously (within 24 hours or less) whenever the animals are placed under

somewhat stressful conditions (changes in diet, in temperature, in crowding, etc.); recovery from this experience commonly takes many days. Indeed, repeated handling of the animals, as required for weighing, collection of stools, cleaning of cages, etc. also resulted in loss of lactobacilli, and increase in the Gram-negative flora. Needless to say, this unstability of the fecal flora greatly complicates the experimental procedures, and contributes to the irregularity of the results.[1]

On the other hand, it has been found that the numbers of Gram-negative bacilli reach extremely low levels when the animals are kept for many weeks in groups of five on wood shavings, even if the litter is changed only at weekly intervals and is therefore grossly soiled. In contrast, the number of Gram-negative bacilli in the stools commonly increased when the animals were shifted to what seemed to be more "sanitary" conditions, perhaps, as mentioned above, as a result of the disturbances associated with the shift. This increase was always sudden and often very large (100 or 1000-fold).

As will be reported in a subsequent publication, similar phenomena were observed in an even more dramatic manner when the animals were treated with either penicillin, tetracycline, or chloramphenicol. Lactobacilli immediately disappeared from the stools when these drugs were administered, even for a short time (1 or a few days) and Gram-negative bacilli, as well as enterococci then became very numerous. These organisms progressively disappeared as the lactobacilli began again to increase in number. But the Rhiz type of lactobacilli either did not come back at all, or only very slowly.

While the findings outlined above are consistently reproducible, their significance is obscure. It is clear, however, that their mechanism is related to the fact that many non-specific types of disturbances can alter the physiological conditions in the digestive tract. We have already indicated, for example, that fighting among the animals, or even handling them for laboratory manipulations may cause a decrease in the numbers of lactobacilli recovered from their stools and an increase in the numbers of coliform bacilli. It must be emphasized here that the findings refer to the numbers of bacterial colonies recovered by culture and do not necessarily reflect the numbers of organisms multiplying in the digestive tract. More specifically, the techniques used in the present study gave only the bacteria surviving in the stools and did not determine whether the differences observed were to be traced to bacterial multiplication in the stomach and intestine or to death rates among these bacteria. Studies of the bacterial flora in different parts of the intestinal tract, now underway, may throw light on this important question.

IV. Susceptibility to the Lethal Effect of Endotoxin of Mice from Different

[1] In experiments not to be reported here it has been observed that the numbers of staphylococci isolated from the stools of mice infected *per os* also increases sharply during the days that follow the change from large cages to individual cages.

Colonies.—Evidence has been presented in an earlier communication that the susceptibility of mice to the lethal effect of endotoxins is conditioned by prior experience with Gram-negative bacilli (23). This fact is further documented by the results of the following experiments.

The mice from the seven different colonies used in the tests illustrated in Table I were kept for three weeks under exactly the same conditions in individual cages on wire grids, and fed the same diet (D & G diet and acidified water *ad lib.*). They then received by the intraperitoneal route 350 μg of endotoxin prepared from *E. coli.* (lot 439277, Difco and Company.)

All NCS mice survived the injection of endotoxin whereas many deaths occurred in all the other groups. A similar experiment was carried out later with NCS and CFW mice, using endotoxins prepared from three different bacterial cultures.

CFW male mice were received from Carworth Farms and placed in individual cages. They received D & G diet and acidified water. Stool cultures were carried out on all animals on three different occasions. NCS male mice of approximately the same age were maintained and studied under exactly the same conditions. All animals were challenged two weeks later by the intraperitoneal injection of either 350 or 175 μg of endotoxin prepared from one of the following three organisms: (*a*) a slow lactose fermenter (SLF) isolated from the stools of NCS mice; (*b*) *E. coli* No. 16 isolated from the stools of CFW mice; (*c*) *E. coli*, 0127, B8.

Toxins a and b were prepared in our laboratory by a modified Boivin technique. (3, 4) Toxin c was obtained from Difco laboratory (lot 439277). The results are presented in Table IV.

The results presented in Table IV illustrate once more the dramatic difference in susceptibility between NCS mice and animals obtained from other mouse colonies with regard to the lethal effect of endotoxin. It is worth emphasizing that no death occurred among NCS mice ranging from 6 weeks to 8 months of age, (fed D & G pellets and acidified water throughout) following the injection of 350 μg of endotoxins prepared from four different kinds of Gram-negative bacilli. (The tests with endotoxin from *Klebsiella pneumoniae* are not included in Table IV). As pointed out earlier the difference cannot be due entirely to genetic factors since NCS mice can be rendered susceptible by vaccination with heat-killed Gram-negative bacilli, or by contact with mice from other colonies early in life (3).

While it seems probable that colonization of the intestinal tract by Gram-negative bacilli plays a role in sensitization to endotoxin, other factors may also be of importance. For example, susceptibility to endotoxin has been observed to develop in NCS mice in which enterococci had become the dominant organisms in the intestinal tract as a result of treatment with certain antibacterial drugs. Experimental details concerning the effect of treatment with these drugs on the susceptibility of NCS mice to endotoxin will not be presented at this time. Suffice it to mention here the bearing of the present observations on the role of

allergic sensitization to endotoxin susceptibility—a hypothesis first formulated by Stetson (14), and documented experimentally in this and other laboratories (3, 4, 15).

SUMMARY

Adult mice from seven different colonies were studied with regard to (*a*) the numbers and types of bacteria that could be cultivated from their stools; (*b*) their resistance to the lethal effect of endotoxins prepared from three strains of Gram-negative bacilli.

TABLE IV

Susceptibility to Endotoxins of Mice from Two Different Colonies

Endotoxin		Deaths from injection of endotoxin	
Origin*	Dose	CFW‡ mice	NCS‡ mice
	μg		
E. coli 16	350	5/6	0
" " "	175	2/5	0
" " 0127, B8	350	6/6	0
" " "	175	2/5	0
Coliform (SLF)	350	6/6	0
"	175	4/5	0

* See text.

‡ The CFW mice used in this experiment were approximately 6 weeks old when challenged with endotoxin. Tests with NCS mice have been carried out repeatedly in the course of the past two years, always with negative results. In this particular experiment, the NCS mice used ranged from six weeks to eight months of age.

In six of the seven colonies, the stools yielded large numbers of various types of lactobacilli, enterococci, and Gram-negative bacilli. Most animals in these colonies died within 48 hours following injection of endotoxin.

The other mouse colony (NCS) has been maintained for the past three years at the Rockefeller Institute under exacting sanitary conditions; it is free of many types of common mouse pathogens. The stool flora of NCS mice yielded very large numbers of viable lactobacilli (10^9 per gm), representing at least three different morphological types. In contrast, it contained only few enterococci and Gram-negative bacilli (less than 10^6 per gm). Moreover, *E. coli*, *Proteus sp.*, and *Pseudomonas sp.* could not be recovered from the stools under normal conditions. NCS mice proved resistant to the lethal effect of endotoxins.

These characteristics of the NCS colony prevailed whether the animals were housed continuously in individual cages on wire grids, or grouped continuously in large cages with wood shavings as litter. However, the composition of the

bacterial flora could be rapidly and profoundly altered by a variety of unrelated disturbances such as sudden changes in environmental temperature, crowding in cages, handling of the animals, administration of antibacterial drugs, etc. The first effect of the change was a marked decrease in the numbers of lactobacilli and commonly an increase in the numbers of Gram-negative bacilli and enterococci. When tested 3 weeks after these disturbances some NCS animals were found to have become susceptible to the lethal effects of endotoxin.

BIBLIOGRAPHY

1. Nelson, J. B., and Collins, G. R., The establishment and maintenance of a specific pathogen-free colony of Swiss mice, *Proc. Animal Care Panel*, 1961, **11**, 65.
2. Dubos, R. J., and Schaedler, R. W., The effect of the intestinal flora on the growth rate of mice and on their susceptibility to experimental infections, *J. Exp. Med.*, 1960, **111**, 407.
3. Schaedler, R. W., and Dubos, R. J., The susceptibility of mice to bacterial endotoxins, *J. Exp. Med.*, 1961, **113**, 559.
4. Dubos, R. J., and Schaedler, R. W., The effect of bacterial endotoxins on the water intake and body weight of mice, *J. Exp. Med.*, 1961, **113**, 921.
5. Lynch, Clara B., personal communication
6. Chapman, G. H., Culture medium for detecting and confirming *Escherichia coli* in 10 hours, *Am. J. Pub. Health*, 1951, **41**, 1381.
7. Chapman, G. H., Improved Stone medium for isolation and testing of food-poisoning staphylococci, Food Research, 1948, **13**, 100.
8. Chapman, G. H., Isolation of Streptococci from mixed cultures, *J. Bact.*, 1944, **48**, 113.
9. Briggs, M., The classification of Lactobacilli by means of physiological tests, *J. Gen. Microbiol.*, 1953, **9**, 234.
10. Elliker, P. R., Anderson, A. W., and Hannesson, G., An agar culture medium for lactic acid streptococci and Lactobacilli, *J. Dairy Sc.*, 1956, **39**, 1611.
11. Petuely, F., Ein einfacher vollsynthetischer Selectivnährboden für den Lactobacillus bifidus, *Zentr. Bakt., 1. Abt., Orig.*, 1956, **166**, 95.
12. Rogosa, M., and Sharpe, M. E., Species differentiation of human vaginal lactobacilli, *J. Gen. Microbiol*, 1960, **23**, 197.
13. Dubos, R. J., and Schaedler, R. W., The effect of diet on the fecal bacterial flora of mice and on their resistance to infection, *J. Exp. Med.*, 1962, **115**, 1161.
14. Stetson, Chandler A., Jr., Symposium on bacterial endotoxins, IV. Immunological aspects of the host reaction to endotoxins, *Bact. Rev.*, 1961, **25**, 457.
15. Landy, Maurice, Symposium on bacterial endotoxins, *Bact. Rev.*, 1961, **25**, 427.

EXPLANATION OF PLATES

PLATE 123

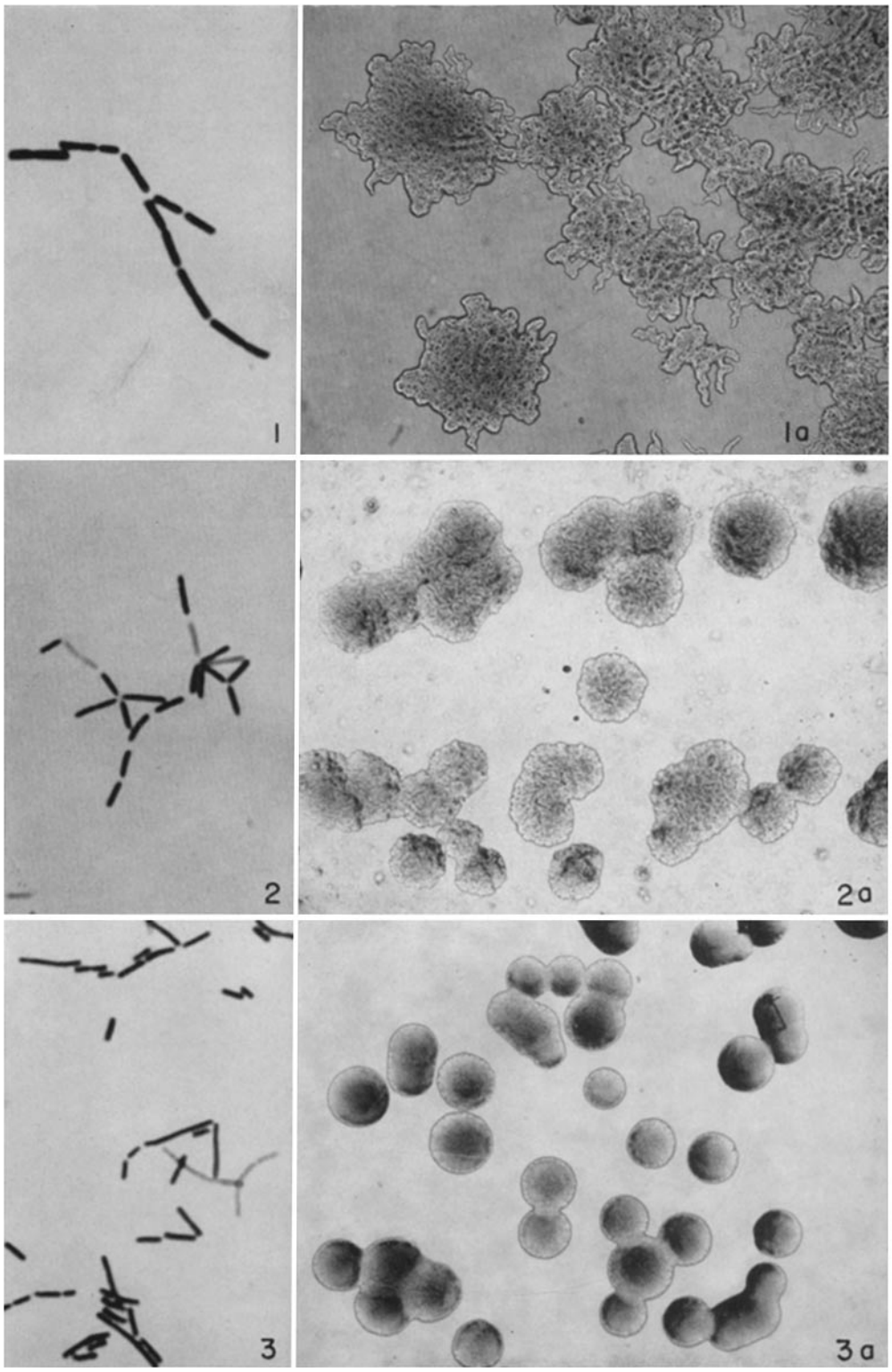
FIGS. 1, 2, 3. Stained preparations of cultures grown for 24 hours in the liquid tween oleic acid medium described in the text. (Magnification × 1500).

FIGS. 1 *a*, 2 *a*, 3 *a*. Colonial morphology of lactobacilli on the tween oleic acid medium described in the text. Surface cultures incubated for 24 hours in a candle CO_2 jar. (Magnification × 16).

FIGS. 1, 1 *a*. Rhizoid type of lactobacillus.

FIGS. 2, 2 *a*. Intermediate types of lactobacillus.

FIGS. 3, 3 *a*. Compact type of lactobacillus.



(Schaedler and Dubos: Fecal flora and endotoxin)