TABLE I.
Effect of Ingesting Succinylsulfathiazole on Urinary Excretion of Folic Acid by the Rabbit.*
(μg excreted per rabbit per day).

Dietary regimen	Weeks on experiment						
	4	5	6	7	8	9	10
Exp. I							
Basal	2.3	1.5	2.6	3.6	10.9	4.8	8.9
Basal + S.S.	1.1	0.5	1.6	1.8	2.2	1.9	2.4
Exp. II							
Basal	2.6	3.0	7.8	16.0	—	3.8	3.4
Basal + S.S.	1.0	1.0	2.5	3.7	—	0.4	0.5

* The values are averages of 3 individual 3-day collections for the first experiment and of 6 individual collections for the second experiment.

The addition of 2% of succinylsulfathiazole markedly reduced the urinary excretion of folic acid active compounds. This effect was evident by the 4th week and although the excretion of folic acid remained at a low level for the succinylsulfathiazole group, a considerable increase in the apparent synthesis of folic acid was observed for the basal group, particularly from the 7th to 10th weeks. Although accurate measurements of folic acid in the ration were not achieved due to the low level, the estimated dietary intake of 1 μg per day approximated the amount excreted by the sulfa fed group and was much lower than the amount excreted by the basal group. It may be concluded therefore that the rabbit is capable of synthesizing sufficient folic acid, as well as pantothenic acid, riboflavin, and biotin, presumably by intestinal microorganisms to meet its needs and that the apparent synthesis of folic acid can be markedly reduced by the inclusion of 2% succinylsulfathiazole in the ration.

Other tests showed that the inclusion of succinylsulfathiazole in the ration did not reduce the hemoglobin level of the blood. Rabbit blood was found to contain folic acid conjugase;[5] however the level of apparent free folic acid in blood was very low ($<$1.0-4.0 millimicrograms per ml) and the data obtained do not permit an accurate evaluation of the folic acid blood levels of the groups receiving and not receiving the sulfa drug.

Summary. The urinary excretion of folic acid by rabbits fed purified diets with or without the addition of succinylsulfathiazole was studied. The inclusion of the drug markedly reduced the urinary excretion of folic acid. This effect was evident from the 4th to the 10th week of the experiments.

[5] Simpson, R. E., and Schweigert, B. S., *Arch. Biochem.*, 1949, **20**, 32.

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Dissociation Among Lancefield's Group "B" Streptococci of Human and Bovine Origin.*

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One hundred and thirty bovine and 26 human strains of Lancefield's group "B" streptococci were studied within one month after isolation.

Primary isolations were made on beef heart infusion agar (Difco) which contained 3%

* This investigation was conducted with the cooperation of the following institutions: San Juan City Hospital, Bayamón District Hospital and the Veterans Hospital at San Patricio.

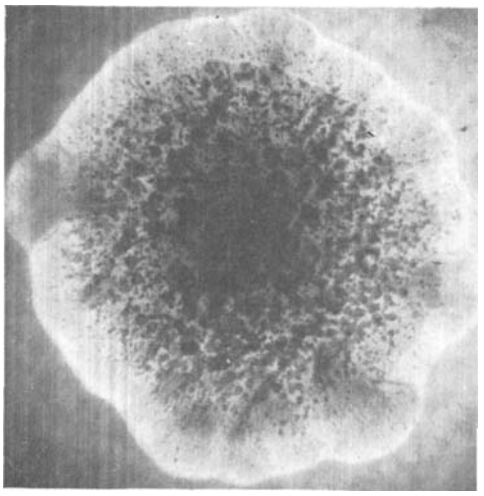

FIG. 1.
"M" colony, 60 hr, 37°C. Blood agar. × 49. Notice sectors indicating M —→ S variation. The granulations are most probably accumulations of red blood cells.

of rabbit or horse blood. The morphology of the colonies was studied on rabbit blood agar plates. The plates (streak) were incubated aerobically at 37°C and examined with a hand lens and a microscope. Only isolated colonies with ample free surrounding space were selected for study. In studying the colonial morphology by reflected light the plate had to be tilted at the proper angle in order not to miss certain details.

Four distinct morphological types were encountered among cultures recently isolated from milk or pus obtained from cows with clinical mastitis. A description of these follows:

1. Colonies slightly raised, comparatively large, about 2.5 mm after 48 to 72 hours incubation, edge entire or slightly wavy. Surface white and glossy by reflected light. With the low power of the microscope tiny white solid structures were seen scattered throughout the body of the colony giving to it the appearance of a tiny drop of slightly curdled milk. Consistency of thin paint. Semitransparent periphery and a rather opaque central area (Fig. 1). In the bacteria from some of these colonies definite capsules could be demonstrated either from young cultures on blood agar or in peritoneal exudate from mice. Broth cultures showed a homogeneous turbidity with a scanty powdery sediment. The cocci occurred singly, in pairs or in very short chains. Some strains were pathogenic and others were avirulent for mice. This description corresponds with the mucoid (M) colony of Dawson, Hobby and Olmstead.[1]

When colonies of this type were incubated at 37°C for 48 to 72 hours frequently wedge shaped, rather opaque outgrowths appeared at the periphery. (Fig. 1). These outgrowths when transplanted gave rise to rather opaque colonies which corresponded in all respects to the second type of colony encountered in primary cultures which we shall now describe.

2. Colonies raised, edge entire or very slightly wavy. Surface dull and usually slightly granular. Consistency of thick paint. They could easily be broken into *soft thick* membranous portions. Relatively opaque when compared with the "M" colonies. (Fig. 2). Growth in broth was homogeneous or very finely granular. The cocci occurred mainly in short chains. No capsules could be demonstrated. This description corresponds with the smooth (S) type colony of Dawson *et al.*[1]

It is apparent that the opaque wedge-like growths of the mucoid colonies represent a mucoid to smooth variation.

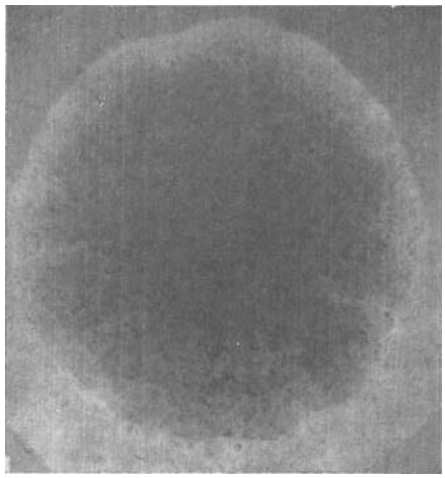

FIG. 2.
"S" colony. 48 hr at 37°C on blood agar. × 41. Granulations most probably accumulations of red blood cells.

[1] Dawson, M. H., Hobb, G. L., and Olmstead, M., *J. Infect. Dis.*, 1938, **62**, 138.

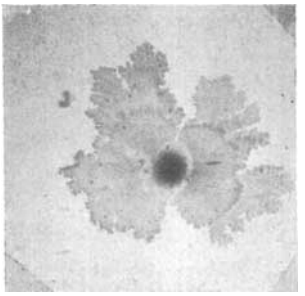

FIG. 3.
"M" colony with prominent rough outgrowths. One week on blood agar at 37°C. × 5.

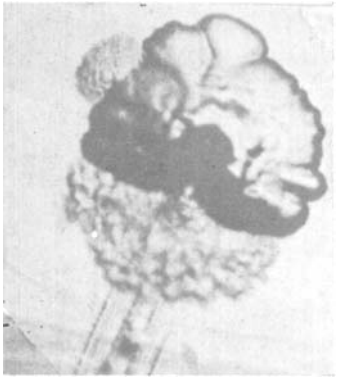

FIG. 4.
"S" colony with rough outgrowths. Four days on blood agar at 37°C. × 20.

If "S" or "M" cultures were left in the incubator from three days to one week rough, large, fan-like outgrowths usually arose from the periphery of the colonies. (Fig. 3 and 4). A large proportion of the "S" and "M" cultures produced these outgrowths. Transplants to broth from these outgrowths gave a cottony sediment with a clear supernatant as contrasted with the homogeneous turbidity obtained with "M" and "S" cultures. The cocci were larger than normal and occurred chiefly in long tangled chains. Colonies obtained from transplants of these rough sectors to 3% rabbit blood beef heart infusion agar showed a slightly raised conical central portion and a rather flat peripheral zone. Upon prolonged incubation they became flatter and flatter until their appearance was much like that exhibited by the "R" colonies obtained in primary cultures as we shall see later. This type of colony conforms in many respects with the "R" (rough) type of Dawson.[1]

3. Colonies prominently flat, composed of a thin membrane with the appearance and consistency of a spider web. Edge of colony very irregular and fimbriated. In some instances small, smooth papillae are distributed throughout the colony. (Fig. 5). Cottony sediment with clear supernatant in broth. Cocci usually larger than normal arranged in long

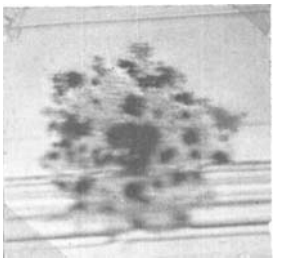

FIG. 5.
Papillated rough colony. × 15. Blood agar. Four days at 37°C.

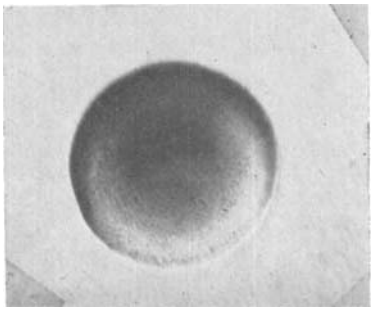

FIG. 6.
Twenty-four-hr-old precursor of smooth-ravined (SRa) colony. × 30.

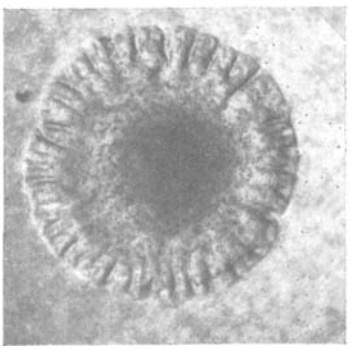

FIG. 7.
Smooth-ravined (SRa) colony. 72 hr on blood agar at 37°C. × 30.

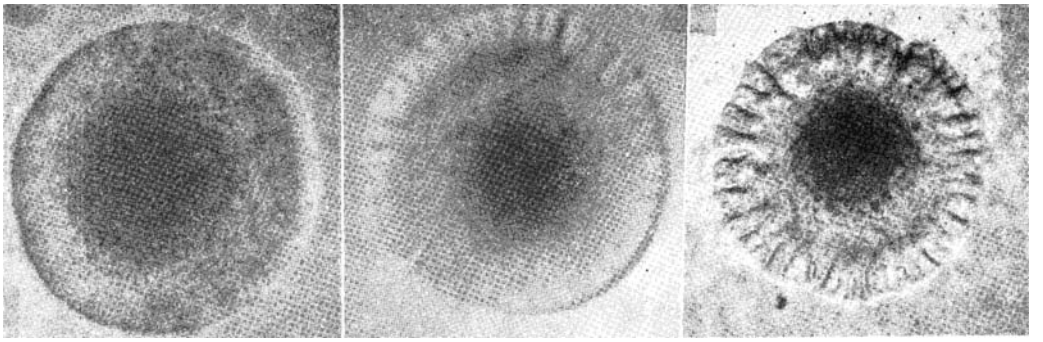

FIG. 8.
Stages in the development of the smooth-ravined (SRa) colony. Large type. Three-day-old colonies on rabbit blood agar. × 30.

tangled chains. These correspond with the rough (R) type of Dawson[1] and were very similar to the rough outgrowths already described obtained from "S" and "M" colonies.

4. Colonies were conical, with a prominently white, dense, raised central disc. The rest of the colony was much less dense. The whole colony was smooth and the surface glossy when 16 to 18 hr old and approximately 1 to 1:5 mm in diameter. (Fig. 6). A small and large type of colony were encountered. The central disc was usually of a soft consistency or exceptionally rather hard so that it could be removed with the wire without disturbing the rest of the colony which at this stage was of medium hardness and could be broken easily with an inoculating wire into thick membranous portions. Occasionally these colonies were of such consistency that they could be shifted from place to place without disturbing their characteristic morphology.

After from 48 to 72 hours incubation at 37°C the central white disc remained as described above. However, the rest of the colony showed by this time a deeply ravined glossy surface with a grayish milky appearance by reflected light. (Fig. 7). Now the colony was harder but it could be broken with the wire into thick membranous portions. The growth in broth was homogenous with scanty powdery sediment or finely granular with slightly foccular or slightly ropy sediment. The cocci occurred typically in chains of medium size. In some instances colonies in the different stages of development were encountered on the same plate (Fig. 8). So far as we know this type of colony has not been described before. We are designating it, and shall refer to it in this discussion as the "SRa" or the *smooth-ravined* colony.

If these smooth ravined cultures were left in the incubator at 37°C for 4 or 5 days some colonies showed glossy, soft pseudopod-like peripheral outgrowths. (Fig. 9). Upon prolonged continued incubation (one or two weeks) these smooth soft portions in turn gave rise occasionally to rough outgrowths similar to those already described as arising from "S" and "M" colonies.

All the morphological types described above were found in primary cultures from the mastitic udder. No rough (R) or *smooth-ravined*

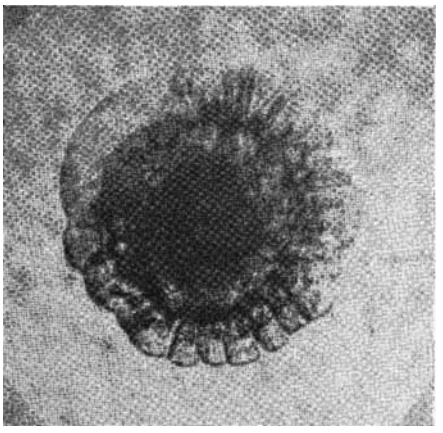

FIG. 9.
Smooth-ravined (SRa) colony with glossy outgrowth. Five days on blood agar. Large type of colony. × 30.

(SRa) types were found in primary cultures from human sources. All the human strains produced either "M" or "S" colonies. However, the S → R and M → R dissociations were produced with relative facility with human strains as described above for cultures of bovine origin.

The fermentative and other biological properties of most of these strains were tested[2] and no correlation was found between these properties and colonial morphology.

Serums prepared in rabbits with formalin treated suspensions of "M" cultures gave good precipitin reactions with formamide extracts of "M" or "SRa" organisms of human and bovine origin. Rabbit serum prepared with "S" or "SRa" suspensions reacted similarly. "M", "S" and "SRa" serums reacted negatively with formamide extracts prepared with the corresponding "R" variants.

The serums obtained from rabbits after several series of inoculations with formalin treated "R" organisms always gave negative precipitin reactions with formamide extracts of the homologous "R" suspensions or extracts prepared from the parent "M" or "S" human and bovine cultures.

These results show conclusively that the group precipitinogen extracted by Fuller's method is greatly diminished or completely lacking in the "R" phase of Lancefield's group "B" streptococci of human and bovine origin. One must keep in mind this fact in the identification of "R" variants of *Strep. agalactiae* obtained in primary cultures from the bovine udder.

Two "M" cultures which were virulent for mice (0.05 cc of 18 hr broth culture killed mice in 24 hours) gave rise to "R" variants which were completely avirulent (1 cc intraperitoneally).

All the bovine and human cultures, irrespective of their dissociative phase at the time they were tested, were resistant to streptomycin *in vitro* when tested by the filter paper disc method of Bondi *et al.*[3] All the bovine and human strains were sensitive to penicillin *in vitro* but the sensitivity varied greatly with the different strains. This variation in susceptibility to penicillin was independent of the origin and colonial morphology of the cultures.

Summary. The dissociation among 130 bovine and 26 human strains of Lancefield's group "B" streptococci has been studied. Four different morphological types of colonies (rough, smooth, mucoid and smooth-ravined) are discarded in detail. No correlation was found between colonial morphology and the biological properties and susceptibility to penicillin. All strains were resistant to streptomycin *in vitro*. Cultures from rough outgrowths of mouse virulent mucoid parent colonies were completely avirulent for mice. Formamide extracts of mucoid, smooth and smooth-ravined cultures reacted positively in precipitin tests with homologous and heterologous rabbit serums prepared with mucoid, smooth and smooth-ravined cultures and with commercial group "B" serum. Formamide extracts prepared from rough cultures always gave negative precipitin tests with good precipitating rabbit serums prepared with the corresponding mucoid, smooth and smooth-ravined parent cultures.

[2] Pomales-Lebrón, A., Morales-Otero, P., and Baralt, J., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 410.

[3] Bondi, A., Spaulding, E. H., Smith, D. E., and Dietz, C. C., *Am. J. Med. Sc.*, 1947, **213**, 221.