

TABLE I.

Percentage conversion of added purines to uric acid by rat tissues in 4 hours at 37.5°C. in 5 cc. of glucose Ringer's solution containing 0.5 mg. purine (10 mg./%).

	Kidney	Liver	Diaphragm	Int. Mucosa	Smooth M.	Spleen
Adenine	9	5	1.5	85	-3.5	7
Guanine	15	78	10.0	69	13.0	17
Hypoxanthine	13	113	0.0	150	21.0	57
Xanthine	54	76	10.0	112	10.0	46

It is also very active in transforming guanine, xanthine, and hypoxanthine into uric acid. The liver actively transforms all these purines except adenine. Striated muscle (diaphragm) has very little effect upon any of them, and the small conversion due to smooth muscle may be the action of small quantities of mucosa which could not be removed. The kidney and spleen were only moderately active in the conversion of xanthine and hypoxanthine. The study of the production of uric acid in these tissues from other possible precursors is being studied further.

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Morphology of *B. Acidophilus* Grown in Soy Bean Milk.

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If *B. acidophilus* be inoculated to cow's milk and to soy bean milk, the microscopic picture of the two resulting cultures after 2 or 3 days' incubation is not the same. The growth in the usual cow's milk medium needs no description. All bacteriologists are well acquainted with it. A smear preparation of the culture grown in the soy bean infusion reveals many of the expected long rods with Gram positive tinctorial reaction. In addition to these rods of usual morphology, there are vast numbers of small units, likewise of Gram positive reaction and they may be of such small size that they approach the limits of visibility under the microscope. Mingled with these are varying numbers of organisms which are somewhat swollen and distorted and which are smaller also than the commonly described form of this microorganism. In the soy bean infusion culture, then, the numbers of long slender rods apparently are less than when the same bacillus is grown in cow's milk but the observer will be under the impression that there are far more bacterial units

in the soy than there are in the latter preparation. This series of experiments was carried out in an endeavor to determine whether the small units present in the soy bean milk culture are indeed *B. acidophilus*.

We used a strain of the organism which originally had been sent from the laboratories of Dr. Kopeloff. The soy bean culture was checked most carefully both by aerobic and by anaerobic technique to assure ourselves that there was no contamination in it. A careful survey was made to select a medium among those which had been suggested for culture of *B. acidophilus* which should be most suitable for the plating of this particular strain. We chose the formula of Kulp and Rettger¹ which is entered as No. 2060 in the compendium of Levine and Schoenlein.²

Preliminary tests were made to determine by usual plating procedures whether *B. acidophilus* does multiply more rapidly in soy bean milk than it does in that from the cow. Each portion of medium was buffered by the addition of 0.5% calcium carbonate. Similar mass inoculation was made by pipette and incubation proceeded at 37°C. One representative curve from 3 done by us is now inserted.

TABLE I.

Comparative growth of *B. acidophilus* in soy bean milk and in cow's milk as determined by plating technique. Figures as millionms per cc.

Control	Soy bean	Cow's milk
(Initial)	15	12
6 hr.	15	12
8 "	225	30
20 "	900	115
32 "	845	80

Three possible methods of determining whether the small particles already described are living *B. acidophilus* occurred to us. One might endeavor to isolate a series by single cell technique. The exceeding minuteness of the forms precluded such an effort. One might be able to filter them through a Berkefeld candle but the consistency of the soy bean milk made such a procedure seem hopeless. The third possibility was to combine the technique of direct counting with plating. Breed and Brew³ years ago elaborated the practice for direct microscopic counting of milk. The method seemed to be feasible to adopt and our plans developed as follows. Soy bean milk

¹ Kulp, W. L., and Rettger, L. F., *J. Bact.*, 1924, **9**, 357.

² Levine, M., and Schoenlein, H. W., A compilation of culture media for the cultivation of microorganisms, 1930. Williams and Wilkins Co., Baltimore.

³ Breed, R. S., and Brew, J. D., *N. Y. Agr. Exp. Sta., Tech. Bull.*, 1916, **49**, 3.

and cow's milk were sterilized separately in flasks in similar amounts. Approximately equal inoculums of *B. acidophilus* were then added by pipette. After shaking, incubation at 37°C. proceeded. At the expiration of each 6 or 8 hour period for a total time of 60 hours, a series of smears for direct examination was made and at the same time interval, a series of plate dilutions was poured with the medium already designated.

From the smears made according to Breed and Brew's procedure, we computed the typically large forms of *B. acidophilus* per cc. of the different cultures at the times designated. Subsequent counting of the plates yielded information regarding the numbers of viable forms present in the same cultures regardless of the size of the organism which started the particular colony. This time consuming procedure was completed by us upon 2 different occasions. Results were similar in each instance. In Table II are shown figures obtained from one of these tests.

TABLE II.
B. acidophilus in soy bean milk. Comparison of total counts by plating and of direct microscopic counts of the large typically formed rods. Figures designate millions per cc.

hr.	Total count by plating	Large rods by direct count
24	295	145
30	310	179
36	265	179
44	570	79
52	555	106

The usual expectation when utilizing the Breed and Brew technique is that the total count of milk as indicated by plating will be fractional only of the enumeration by direct microscopic methods. The reverse is true, however, when these 2 operations are applied to *B. acidophilus* growing in soy bean milk and when the total plate results are compared to the numbers of the large supposedly typical microorganisms in that culture. We have already stated that the number of the small units in the soy infusion far exceeds the more usual morphologic type. Evidence thus is presented to indicate that the extremely microscopic form found here is also *B. acidophilus*.

Finally, we should add that when one stains a large series of the colonies appearing upon plates made from a culture of this organism growing in soy bean milk, smear preparations show only the large form usually described as *B. acidophilus*. It appears, therefore, that when the small forms grow upon the solid medium made up according to the formula indicated, they again take the usual type of morphology.