TABLE I

SHOWING COMPARATIVE PLATE COUNTS OF L. ACIDOPHILUS ON NEO-PEPTONE TOMATO AGAR AND BACTO-PEPTONE TOMATO AGAR

Colonies per cubic centimeter of culture plated in:		
Neo-Peptone tomato agar	Bacto-Peptone tomato agar	Percentage increase in Neo-Peptone
627 M	559 M	12.30
670 "	592 "	13.00
854 "	746 "	14.40
900 "	774 "	16.00
601 "	518 "	16.00
673 "	577 "	16.66
145 "	123 "	17.80
459 "	376 "	22.00
885 "	713 "	24.10
1,028 "	826 "	24.40
538 "	431 "	24.80
548 "	419 "	31.00
563 "	427 "	31.80
714 "	537 "	32.90
366 "	270 "	35.00
621 "	451 "	37.60
439 "	309 "	42.00
247 "	171 "	44.00
374 "	235 "	59.00
399 "	249 "	60.20
190 "	113 "	69.10
276 "	149 "	84.90
17 "	7 "	166.00

M = millions.

the colonies of *L. acidophilus* were recognized easily under hand lens magnification.

Quantitative studies were not conducted with other types of lactobacilli. However, in comparative qualitative trials it was found that strains of *L. pentoaceticus, L. delbrücki, L. fermentatae* and *L. odontolyticus* all developed larger colonies in Neo-Peptone tomato agar than in Bacto-Peptone tomato agar. Aside from this the colony characteristics remained unchanged.

The composition and the preparation of the medium is as follows:

Tomato juice	400 cc
Peptonized milk (Difco)	10 gm
Neo-Peptone (Difco)	5 gm
Water	600 cc
Agar	11 gm

The tomato juice is obtained by filtering the contents of one No. 3 can of tomatoes through filter paper. The peptone and peptonized milk are added to the tomato juice and heated gently until dissolved. The reaction is adjusted to pH 6.8. The agar is added to 600 cc of water and melted in the autoclave. The melted agar and the tomato juice-peptone-peptonized milk solution are mixed while hot and the medium tubed. Sterilization is made by autoclaving at 120° C. for 8 minutes. The medium should be removed from the autoclave as soon as possible.

The plates inoculated with material containing *L. acidophilus* and poured with Neo-Peptone tomato agar are incubated for 48 hours at 37° C. in an atmosphere containing approximately 10 per cent. carbon dioxide.

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THE CULTURING OF FRESH-WATER AMOEBAE IN THE LABORATORY

IN growing amoebae for experimental purposes in Dr. Chambers's laboratory at New York University, we have hit upon a simple method which ensures a plentiful and continuous supply.

The method consists in having a layer of agar with starch grains in the bottom of the culturing bowls. The agar serves not only to anchor the starch grains, but also offers a good surface for the amoebae to feed and crawl upon. The agar is prepared by dissolving 1½ grams in 100 cc hot water; and pouring the solution, while hot, through a filter of absorbent cotton into a thoroughly cleaned and dried finger bowl. The amount should be in sufficient quantity to form a thin layer of about 0.2 cm thick on the bottom of the bowl. Several grains of ordinary polished white rice are dropped on the layer before the agar has set.

When the agar has hardened, 10 to 15 cc of culture water, containing as large a number of amoebae as possible, is poured into the bowl and an equal quantity of distilled water then added. Each day 5 cc of distilled water is added until the bowl contains about 50 cc of liquid. In adding the water, the contents of the bowl should be agitated as little as possible, so as not to disturb the amoebae, which tend to gather about the starch grains.

The bowls should be covered to minimize evaporation, and should be kept at a temperature (17 to 18° C.) somewhat below usual room temperature. To maintain a constant temperature, the bowls may be stored in any kind of a water bath, with water from the tap constantly circulating around it.

Cultures prepared in the way described above will, within two to four weeks, develop thousands of amoebae per bowl, and they will often last for several months without subculturing.

The presence of large numbers of Chilomonas is very favorable for the cultures, as they serve as food for the amoebae. Fresh grains of rice should be added

from time to time. No harm results if the grains loosen from the agar and crumble as the culture ages. The agar frequently loosens from the bottom of the dishes, whereupon the amoebae will be found in large numbers on the agar film and on the glass bottom of the vessel. It is of interest to note that the amoebae, seldom if ever, are found attached to the under-surface of the dislodged agar films.

Syracuse watch glasses, containing about 8 cc distilled water, may be used instead of finger bowls. These dishes, with the bottom covered with agar, as described for the finger bowls, and with three grains of rice anchored in each, may be seeded with 15 to 25 amoebae from an old culture.

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SPECIAL ARTICLES

ON AN ALKALOID FROM ERGOT

DURING the past few months a new development in the chemistry and pharmacology of ergot has occurred through the almost simultaneous reports of the isolation of a new, apparently simpler and clinically more useful alkaloid from the fungus; the ergometrine of Dudley and Moir,[1] the ergotocin of Kharasch and Legault,[2] the alkaloid of Thompson,[3] and very recently the ergobasine of Stoll and Burckhardt.[4] Because of our own investigations of the structure of the ergot alkaloids, we have attempted to satisfy ourselves of the possible relationship of the new alkaloid or alkaloids to the lysergic acid group—ergotinine, ergotamine and ergoclavine—already studied by us.

The ergot powder residue which remained after it had been exhausted in the usual manner of its ether-soluble alkaloids was extracted with hot dilute acid (sulfuric or lactic acid). After neutralization of this extract with sodium carbonate and extraction with chloroform, a substance was obtained in very small yield which was characterized by its very sparing solubility in chloroform. It gave the usual color reactions of the ergot alkaloids. It was recrystallized by solution in methyl alcohol, addition of chloroform and concentration to remove the methyl alcohol. The same substance was obtained from fresh ergot powder by adhering to the exact directions as given by Dudley and Moir. Since the substance melted at 154°, it was assumed at first that we were dealing with the same alkaloid[5] described by Dudley and Moir and Kharasch and Legault. However, since further study of our material has given results which are not in agreement with the analytical results of these workers or with the rotation reported by the former, and since none of the substances of these workers was available to us for direct comparison, the question of identity must be left open for the present.

However, through the kindness of The Sandoz Company we have very recently received a small sample of the ergobasine (as the tartrate) of Stoll and Burckhardt. After isolation of the free base from this salt and crystallization from chloroform, it appeared to be identical with our own material. The former showed $[\alpha]_D^{25} = +32°$, and the latter $[\alpha]_D = +30°$ in methyl alcohol. The analytical results obtained with both samples were also in agreement. When crystallized from chloroform it separates with 1 mol of chloroform. $C_{19}H_{23}O_2N_3 \cdot CHCl_3$. Calculated C 53.98, H 5.44, Cl 23.93. Found C 54.54, H 5.83, Cl 22.83. Thus, these figures are in agreement with the formula presented by Stoll and Burckhardt, who recrystallized their alkaloid from alcohol. Because of the very limited supply of our own material, we have not employed this solvent.

Most important, however, has been the study of the hydrolysis of our alkaloid, which we had practically concluded before the publication of Stoll and Burckhardt. The results obtained appear definitely to substantiate the above formulation. On hydrolysis with 12.5 per cent. KOH in 50 per cent. alcohol, lysergic acid was obtained by our usual procedure and melted at 230°. Calculated: C 71.69, H 6.00. Found: C 71.49, H 5.60. No ammonia was produced during the hydrolysis, and no isobutyrylformic or pyruvic acids could be detected. After removal of most of the lysergic acid the material remaining in the mother liquor was subjected to hydrolysis with strong acid, a procedure heretofore employed by us where amino-acids were to be obtained and which also destroyed the residual lysergic acid. Instead, however, of an amino-acid a base resulted which was isolated as the sulfate. This salt proved to be the sulfate of an amino propanol $(C_3H_9ON)_2H_2SO_4$. Calculated: C 29.00, H 8.12. Found: C 29.25, H 8.05. This was substantiated by the preparation of a di-p-bromobenzoate which melted at 155°, $C_{17}H_{15}O_3NBr_2$. Calcu-

[1] H. W. Dudley and C. Moir, *Brit. Med. Jour.*, 1: 520, 1935.

[2] M. S. Kharasch and R. R. Legault, SCIENCE, 81: 388, 1935; *Jour. Am. Chem. Soc.*, 57: 956, 1140, 1935.

[3] M. R. Thompson, *Jour. Am. Pharm. Assoc.*, 24: 24, 185, 1935; SCIENCE, 81: 636.

[4] A. Stoll and E. Burckhardt, *Compt. rend.*, 200: 1680, 1935.

[5] The results of a preliminary study of our material which was perhaps erroneously considered by us to be ergometrine are soon appearing in the *Journal of Biological Chemistry*.