of one hour to a more marked degree and persisted over a 24-hour period.

We had already noted the effect described by Walker,[7] which makes N/10 HCl necessary to dissolve the red blood cells in doing routine white blood counts following the infusion of acacia and we assumed that the acacia coated the cells and hindered normal blood cell respiration. Since fragility tests were constantly normal, we decided to attempt to resaturate the red blood cells by exposure to air. The results of this are shown in Table II.

It will be noted that it was possible to resaturate the red blood cells to a point which would preclude any possibility that the low oxygen saturation of the venous blood following intravenous acacia was not due to coating of the red blood cells.

7812

Nutritional Factors of S. Lactis.

C. S. MUDGE AND F. R. SMITH.

From the Division of Dairy Industry, College of Agriculture, University of California.

Whenever one is dealing with 3 variables, the trilinear chart is often found to be useful. Such a chart is based upon the thesis that in an equilateral triangle the sum of the perpendiculars from any point to the sides is equal to the altitude of the triangle. If this altitude is designated as 100%, then the position of the point within can be expressed in terms of per cent since $x + y + z = 100$.*

In our studies with *S. lactis* we have used 1% Difco peptone, 1% Difco yeast extract and water as variables. These solutions are prepared and adjusted electrometrically to pH 6.5. Media 1, 21, and 16 (see Fig.) are these 3 solutions. Each of these media contained 0.5% lactose. By properly mixing these solutions, 18 other media are made which are represented in the figure by the various numbers. Each medium varies from the other in some small degree. Thus the media in the base line (16 to 21) have no peptone. This is the zero line for peptone.

If the altitude of the triangle is now divided into 5 equal parts and lines drawn parallel to the base through these points a series of

[7] Walker, M. A., *Am. J. Clin. Path.*, 1932, **2**, 347.

* For further discussion of the theory see Haskell: How to Make and Use Graphic Charts. Codex Book Company. Page 30.

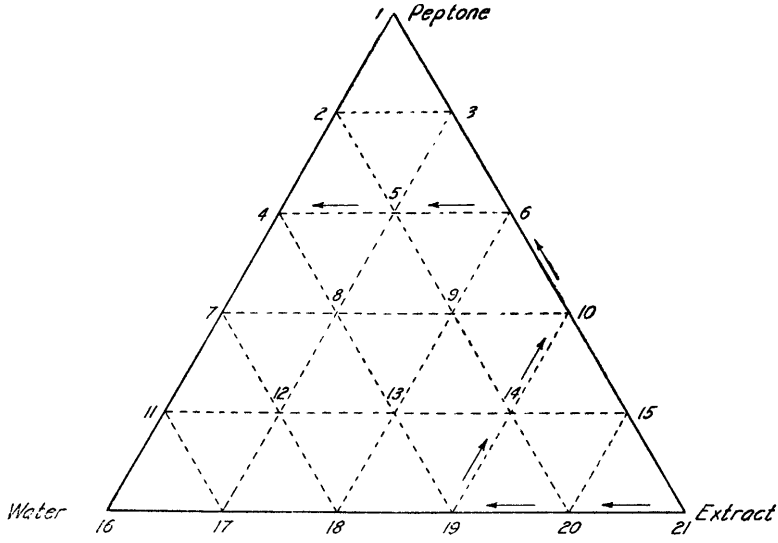

FIG. 1.
Showing the zones of similar growth in media with varying constitutions.

triangles is obtained. The media on line 11-15 will all have 0.2% peptone (20% of the original 1% solution). Also, all media on line 7-10 will have 0.4% peptone. Medium 1, of course, has 1% peptone (100% of the 1% solution). The same reasoning can be used for the extract. In the case of the water, it is merely a diluent of the media in which it appears.

With this method, some interesting results have been obtained with *S. lactis.*

These 21 media were each inoculated with 0.1 cc. of a 10^{-4} dilution of an 18-hour-old milk culture of *S. lactis.* Since the media has been distributed in tubes in 10 cc. amounts, the final dilution of the culture in the tubes was 10^{-6} These tubes had been sterilized in the autoclave and rechecked as to reaction. Little or no change was noted. After 24 hours' incubation at 30°C., the growth was recorded as amount of turbidity using ++++, +++ to designate the degree of turbidity.

Media 21 to 19 showed ++++ growth, but medium 18 gave only ++ growth. These vary only by dilution and evidently the concentration of something present in 19 is lessened in 18 to a considerable degree. However, following the "iso extract" line to medium 10 (see arrows), a ++++ growth is observed.

Medium 6, too, has +++ growth. Something must be supplied by the peptone which is not supplied by the water. Following the "iso peptone" line to medium 4 shows no growth in this latter. If

peptone supplied the necessary nitrogen, then medium 4 should support growth as well as medium 6. This has not been found to be the case. It occurred to us that perhaps the excellent growth in medium 6 as compared to medium 18 (the extract being constant) might be due to the buffer action of the peptone rather than to its nutritional value. Accordingly we made a Sörensen citrate buffer of pH 6.5 diluted 1 to 10 and substituted this for the peptone. This buffer behaved exactly as the peptone. In fact the differences between media 18 and 6 were even more marked.

Although citrate is a good buffer and might replace the peptone for this reason, it is also an excellent source of carbon. This could explain our observations. To rule out this possibility, we next made a N/100 $NaHCO_3$ solution and used that in place of the citrate. Our results were exactly the same and we were forced to the conclusion that for *S. lactis* at least the peptone serves largely, if not entirely, as a buffer. How general this is remains to be seen. It is obvious, too, that the possibilities in the use of this device are almost limitless.

7813 P

Effect of a Diet Low in Magnesium on the Rat.*

DAVID M. GREENBERG AND ELMA V. TUFTS.

From the Division of Biochemistry, University of California Medical School, Berkeley.

Feeding a diet containing only 0.18 mg. of magnesium per 100 gm. of food, Kruse, Orent and McCollum[1] have discovered a syndrome in the rat which they term magnesium tetany. In this condition, which is characterized by a hyperexcitability manifested by spontaneous or induced convulsive seizures, these authors found the plasma magnesium content to be reduced to a low level of 1 mg. % or less.

A considerably different sequence was observed by the present writers on a diet containing between 1 and 2 mg. of magnesium per 100 gm. of dry food which was composed of casein, sucrose, vegetable fat, vitamin supplements, and a purified salt mixture. On this

* Aided by a grant from the Christine Breon Fund of the Medical School.

[1] Kruse, H. D., Orent, E. R., and McCollum, E. V., *J. Biol. Chem.*, 1932, **96**, 519; 1933, **100**, 603.