increased resistance. Further observations along these lines are in progress.

7765 P

Diastase in Milk.

MARIANNE KUTTNER AND MICHAEL SOMOGYI.

From the Laboratory of the Jewish Hospital, St. Louis.

Béchamp in 1883 was the first to recognize the presence of diastase in human milk; at the same time in cow's milk he found no trace of this enzyme.[1] Bouchut[2] and Moro[3–5] confirmed the findings of Béchamp.

During the last decade a number of investigators have attempted to establish a quantitative diastase test as a means of detecting whether or not a milk had been pasteurized. Namely, diastase would be entirely or partly inactivated during pasteurization, the extent of its destruction depending on the temperature and the duration of heating. The methods used by these workers, while claiming to yield quantitative results, are quite crude in comparison to the qualitative methods of Béchamp and Bouchut. The more recent workers find diastase in the milk of practically all the mammals[6] examined and are able to determine diastatic activity in the presence of lead[7–9] and even mercury salts.[10] The latter fact is characteristic of the unreliability of these methods.

We approached the problem with analytical procedures, which in the instance of blood and urine proved to be adequate for the determination of very low as well as of high diastase values.

The method is in brief as follows: a 1.5% starch paste is prepared of pure commercial corn- or rice-starch (but not of soluble starch); 10 cc. of this starch paste and 4 cc. of a 1% NaCl solution

1 Béchamp, A., *Compt. rend.*, 1883, **96**, 1508.

2 Bouchut, E., *Hygiène de la première enfance*, Paris, 1885, 102. (Quoted in Moro's work.)

3 Moro, E., *Jahrb. f. Kinderheilkunde*, 1898, **47**, 342.

4 Moro, E., *Jahrb. f. Kinderheilkunde*, 1900, **52**, 524.

5 Moro, E., *Jahrb. f. Kinderheilkunde*, 1902, **56**, 391.

6 Chrzaszcz, T., and Goralowna, C., *Biochem. Z.*, 1925, **166**, 172.

7 Rothenfusser, S., *Z. Untersuch. Lebensm.*, 1930, **60**, 94.

8 Gould, B. S., *J. Dairy Sci.*, 1932, **15**, 230.

9 Weinstein, P., *Z. Untersuch. Lebensm.*, 1934, **68**, 73.

10 Kluge, H., *Z. Untersuch. Lebensm.*, 1933, **65**, 71.

are measured into a test tube, the mixture is warmed to 40°, and then 2 cc. of diluted milk are added. The extent of the dilution (usually 1:10 to 1:40) depends on a preliminary test, which is based upon the amyloclastic activity of the milk. The test tube is stoppered, its contents mixed, and incubated in a water bath at 40°. After 30 minutes' incubation the proteins are precipitated by Somogyi's copper or zinc method,[11, 12] and the reducing matter in the filtrate determined by the Shaffer-Hartmann-Somogyi method.[13] From the reduction value thus obtained the original lactose content of the milk is subtracted.

Our results confirm, at least from the qualitative point of view, the observations of Béchamp, *i. e.*, human milk contains considerable quantities of diastase, while in cow's milk this enzyme is entirely absent. We have examined cow's milk in all stages of lactation beginning with colostrum and extending over all stages of milk secretion, with findings always in the negative. Human milk, on the contrary, contains surprisingly large amounts of diastase, in fact, far larger quantities than could be anticipated from data available in literature. According to Moro 100 cc. of milk are able to convert 25 gm. of starch into reducing matter in 24 hours. We have had 59 cases under our observation, of which nearly one-fifth produced more than 20 gm. of reducing matter in a single hour; this calculated for 24 hours means 480 gm., as against Moro's 25 gm. The most active milk in our series converted 52 gm. of starch into reducing sugar in one hour.

In the majority of the cases 100 cc. of milk formed from 6 to 15 gm. of reducing matter per hour. In one instance only was there a transitory absence of diastase; in several determinations in the course of 10 days following delivery the reducing sugar produced by 100 cc. of milk varied between zero and one gm. per hour. The blood diastase, which in others of our cases was normal, also showed an abnormally low level in this woman.

The values here reported are obtained under the standard conditions outlined above; we found, however, that the amount of sugar which milk diastase is able to produce is increased by 30%, if the temperature is raised to the optimum of about 53°, and another increase of about 50% is effected by adjusting the pH of the reaction mixture to the optimum of 6.8. This means that the most active milk in our series, which under our convenient standard conditions

[11] Somogyi, M., *J. Biol. Chem.*, 1930, **86**, 655.

[12] Somogyi, M., *J. Biol. Chem.*, 1931, **90**, 725.

[13] Shaffer, P. A., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 695.

produced 52 gm. of reducing sugar per hour, would under the optimum conditions produce approximately 100 gm. of sugar in one hour.

The level of the diastase content is subject to continuous and rapid changes at the beginning of lactation. In the colostrum stage we find invariably higher diastase contents than in the advanced stages of lactation; about the 6th to the 8th day of lactation the diastase content attains a rather permanent level.