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Effect of Milk, Lactose and Lactic Acid on Intestinal Bacteria and Protozoa.

JOHN F. KESSEL.

From the University of California at Los Angeles, and the School of Medicine, University of Southern California, and Los Angeles General Hospital.

Diet has long been recognized as a factor in producing change in the intestinal flora, and the conclusions of Metchnikoff, Rettger and Cheplin,[1] Kopeloff,[2] and others, on the significance of aciduric bacteria within the intestine, have attracted considerable attention. Only a small amount of work, however, has been reported with reference to changing the intestinal protozoa by diet, Hegner[3] and Ratcliffe[4] showing a reduction of flagellates by feeding a high protein diet, and Kessel,[5] and Kessel and Huang[6] reporting a reduction in the numbers of amoeba when an exclusive milk diet was given. Beach[7] also advocates the use of lactose or dry skim milk for the treatment of coccidiosis in chickens.

Since lactose in sufficiently large quantities may produce a similar change in flora within the intestine to that produced by a milk diet, it is reasonable to assume that the lactose of the milk is the more important factor in bringing about the change. Since the end product of lactose in the intestine is lactic acid, it further appears logical to assume that the lactic acid alone would produce identical results. With this idea in mind, it was decided to compare the effects of milk, lactose and lactic acid in producing change in pH and in intestinal bacteria and protozoa.

For the initial observations, the experimental animals were placed on a general neutral diet for a period of 2 weeks, near the close of which time the feces and colon contents, freshly obtained, were subjected to the following determinations:

1. Examination for intestinal protozoa by smear method, both

[1] Rettger, L. F., and Cheplin, H. A., The intestinal flora with special reference to the implantation of *B. acidophilus*. (New Haven, Yale Univ. press), 1921, 135.

[2] Kopeloff, N., Lactobacillus acidophilus. (Baltimore, Williams and Wilkins Company), 1926, xi, 211.

[3] Hegner, R. W., *Am. J. Hyg.*, 1923, iii, 180.

[4] Ratcliffe, Herbert L., *Am. J. Hyg.*, 1928, viii, 910.

[5] Kessel, J. F., *Univ. of Calif. Publ. Zool.*, 1924, xx, 489.

[6] Kessel, J. F., and Huang K'E-Kang, PROC. SOC. EXP. BIOL. AND MED., 1926, xxiii, 388.

[7] Beach, J. R., *Hilgardia*, 1925, i, 167.

preliminary stains in iodine-eosin, and permanent stains in iron haemotoxylin being used. In addition, the feces were cultured in Ringers-egg-serum medium.

2. Determination of pH by the colorimetric method, using standardized dilutions.

3. Routine gram stains were made of the fecal bacteria.

4. Culture for the *B. coli*-aerogenes group, using Eosin Methylene Blue plates.

5. Culture for anaerobes using Robertson's meat medium.

6. Culture for acidurics was made on Bacto-Gelactose Whey agar plates. Differentiation between *B. acidophilus,* X-type, and other aciduric bacteria was made on the basis of the fuzzy type colonies.

Three chemical and bacteriological determinations were made before feeding experiments were begun. The feeding experiments were continued for a period of from 2 to 3 weeks, the same chemical and bacteriological determinations being repeated near the close of the experiment. Protozoological examinations of the rats were made at autopsy and the monkeys were given 6 or more follow-up examinations after the conclusion of the feeding.

In the study on hospital patients it was not possible to carry on the bacteriological culture work, and the determinations were limited to the pH, the gram stains, and the protozoological examinations.

The results of investigators in the past in administering lactic acid have not met with uniformity, though the generally accepted conclusion appears to be that lactic acid given alone in food or water has a negligible effect on the contents of the colon. To check this assumption, rats were fed only lactic acid soaked bread during a period of 2 weeks. Since, at the end of that time, there was no change either in the pH of the colon contents, nor in the type of bacteria present, it was concluded that lactic acid given free is not effective in producing the changes in flora.

Lacto-kelpol, a specially prepared emulsion of lactic acid in anhydrous agar and mineral oil, was extensively employed in treating colitis and also in combination with *B. acidophilus* therapy. The claim for this product was that the lactic acid was bound in the agar so that it passed through the stomach, thus reaching the intestine unchanged. It was thought desirable to use lacto-kelpol as a means of conveying the lactic acid to the colon, and in order to determine its effectiveness, feces of 7 monkeys and 15 human cases were tested for the presence of lactic acid, using a slight modification

TABLE I.

Showing the Results of Feeding Rats, Monkeys and Man With Milk, Lactose, and Lactic Acid (Lacto-Kelpol).

	pH		Gram+		Amoeba		Tricho-monas		*B. coli aerogenes*		*B. acidophilus* (X) fuzzy		Anaerobes	
	B.	A.	B.	A.	B.	A.	B.	A.	B.	A.	B.	A.	B.	A.
Rats			%	%										
Controls	6.5	6.8	10	10	5+	5+	5+	5+	556	740	6940	6020	F++P+	F++P+
Milk	6.3	6.5	20	95	5+	1+	5++	5+	2360	615	8475	19800	F+ P+	F++P+
Lactose—.1 gm. = 4 oz. daily for man	6.5	6.6	25	45	2+	1+	5+	5+	1850	6080	10980	10020	F++P++	F++P+
Lactose—2 gm. = 2½ lbs. daily for man	6.9	6.2	10	90	3+	3+	5+	1+	936	1076	3220	47700	F+ P++	F++P+
Lactic Acid (Lacto-Kelpol 1%)	6.7	6.5	10	60	5+	2+	5+	3+	1635	870	15190	36190	F+ P+	F+ P+
Milk and Lactic Acid (Lacto-Kelpol 1%)	7.2	7.0	10	80	0	0	5+	3+	7320	1430	2580	12920	F+ P++	F++P+
Monkeys														
Controls	6.8	6.8	10	10	2+	2+	2—	2—	1000	1500	2400	2800		
Milk only	6.7	6.5	10	85	3+	2+	—	—	3766	355	9000	24133	—	
Lactose—40 gm. = 1¼ lbs. daily for man	6.8	6.6	10	70	3+	3+	3+	3+	1270	1570	2144	3150	F+ P++	F++P+
Lactic Acid (Lacto-Kelpol 1%)	6.9	6.6	10	80	3+	3+	3+	3+	2272	4234	1540	6410	F+ P+	F+ P+
Milk and Lactic Acid (Lacto-Kelpol 1%)	7.8	6.7	10	80	2+	2+	2+	2+	3250	2190	9000	72000	F+	F++
Human														
Lactic Acid (Lacto-Kelpol 1%)	7.2	6.5	G—	G+	6+	4+	7+	5+	—	—	—	—	—	
Milk only					3+	2+								

B = Before feeding experiment. A = After feeding experiment.

of Uffelman's technique employed in testing gastric contents. No acid was demonstrable in these tests. Ten of the human cases and 4 of the monkeys were then given lacto-kelpol in addition to their regular diet. A test made in 7 days demonstrated acid in the feces while the controls were negative. It was, therefore, concluded that lacto-kelpol is a satisfactory means of conveying lactic acid to the colon.

From the accompanying table, a summary of experiments conducted together with their several controls, the following observations may be made.

(1) *Changes in pH.* Cannon and McNease[8] recorded a remarkable lowering of pH in their rats which were fed on an exclusive milk diet. Rettger and Cheplin,[1] however, were unable to demonstrate such a marked change and showed that the flora was altered even though great variations in pH were not apparent. In this investigation a lowering of pH is particularly noticeable in the human series given lactic acid (lacto-kelpol) only, where there is an average reduction in pH from 7.2 to 6.5, and in the monkeys which were given a combined diet of milk and lacto-kelpol, where the drop was from 7.8 to 6.7. One would conclude that in certain instances only an increase in aciduric bacteria is associated with a corresponding decrease in pH.

(2) *Gram Positive and Gram Negative Organisms.* The gram stain gives a rough index as to the increase or decrease of aciduric bacteria, but does not give specific information as to the type of aciduric organism present. In some instances *B. acidophilus*, X-type, has not increased appreciably when gram positive organisms have, though in such instances other types of acidurics or anaerobes were present.

(3) *Protozoa.* In considering the flagellates and the intestinal amoeba, it would appear that we are dealing with two very different types of organisms, and that, in all probability, Trichomonas grows with greater facility in an acid medium than amoebae. Knowles[9] feels that *E. histolytica* grows best in a slightly acid medium, but the results of Boeck and Drbohlav[10] do not warrant this conclusion *In vitro* cultures made in this study indicate that Trichomonas will grow well in a much wider range of pH than amoebae will.

Ratcliffe[4] says that a decrease in aciduric bacteria apparently does not affect trichomonads. From my preliminary findings with pro-

[8] Cannon, P. R., and McNease, B. W., *J. Inf. Dis.*, 1923, xxxii, 175.

[9] Knowles, Robert, An introduction to Medical Protozoology. (Thacker, Spink and Company, Calcutta), 1928.

[10] Boeck, W. C., and Drbolav, J., *Am. J. Hyg.*, 1925, v, 371.

tozoa, this is exactly what one would expect, and I had hoped that the converse might be true and that an increase in aciduric bacteria might reduce the numbers of Trichomonas. This seems to be true within certain limits only, for though several of the human cases showed a reduction in the number of trichomonads, only 2 showed a complete riddance. All cases in which Trichomonads were reduced, also showed a lowering of pH, but all cases showing a lowering of pH did not give a corresponding reduction in flagellates.

The greatest reduction in amoebae has been encountered in the experiments with rats where the common species is one forming 8 nucleate cysts and, therefore, more closely related to *E. coli* than to *E. histolytica*. *E. coli* is more difficult to cultivate *in vitro* than *E. histolytica,* and the medium recommended for its cultivation is alkaline. It would, therefore, appear that *E. coli* cannot withstand as great a variation in pH as *E. histolytica*. In the rats, 8 of the 15 fed on milk, lactose, or lacto-kelpol, were cleared of their amoebae, the greatest reduction being in rats that received milk and lacto-kelpol. Only one monkey was cleared of amoebae in the series though 3 of the 9, or one-third of the human group cases given milk or lacto-kelpol, were cleared of *E. histolytica*.

(4) *B. coli-aerogenes Group.* The decrease of this group together with the change in pH, and the increase in aciduric bacteria, has not been constant, and when a reduction has occurred, the ratio of decrease has seldom been in the same proportion as the ratio of increase among the aciduric bacteria. *B. coli* does not entirely disappear as the aciduric bacteria increase, and the change in ratio is therefore due rather to the great increase of aciduric bacteria than to the decrease of *B. coli.*

(5) *Bacillus acidophilus, X-type.* Increase of this organism has been the most constant of any change noted in this study, and the same was most marked in the animals given an exclusive milk diet and in those given lactic acid (lacto-kelpol). An increase in aciduric flora among lactose fed animals, was noted only in those which were fed large amounts. Rettger and Cheplin[1] showed that 2 gm. of lactose, daily, per rat, are necessary to produce a marked increase in acidurics. This amount would approximate 2½ lbs. of lactose daily for man if body weights were compared. Rats given 0.1 gm. each, which would compare with 4 oz. daily for man, and monkeys given 40 gm. each, which would amount to 1¼ lb. daily for man, showed no increase in *B. acidophilus,* X-type, although they did show an increase in other aciduric organisms. With the exception of the rats given 2 gm. of lactose daily, the most marked and constant increase in *B. acidophilus,* X-type, was in the

animals fed lactic acid (lacto-kelpol), and in those fed a combination of milk and lacto-kelpol. Since there was no experimental implantation of *B. acidophilus*, it would appear that the predominating aciduric flora resulted merely from the addition of the lactic acid to the diet.

(6) *Proteolytic and Fermentative Anaerobes.* One may summarize the change in this group by stating that there is a slight decrease in the proteolytic anaerobes and a corresponding increase in the fermentative group, when milk, lactose, or lacto-kelpol is given.

Summary: An exclusive milk diet, lactic acid in lacto-kelpol, or large amounts of lactose added to a regular neutral diet, will usually produce a lowering of pH of the colon contents, an increase in gram positive organisms including an increase in *B. acidophilus*, X-type, and at the same time, in some instances will show a reduction in the number of intestinal trichomonads and amoebae.

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Influence of the Rate of Secretion on the Urea Concentration of Saliva.

GEORGE D. BARNETT AND ROBERT G. BRAMKAMP.

From the Division of Medicine of the Stanford Medical School.

Observations of Hench,[1] Schmitz[2] and others have shown that the concentration of urea in human saliva obtained by chewing paraffin averages about 80% of that of the blood. The published figures from which this average has been obtained show wide variations in different individuals. Those of Hench[1] vary from 50 to 130%, those of Schmitz[2] from 58 to 128%, and unpublished observations made in this laboratory in 1921 from 58 to 110%.

Specimens of saliva secreted at slow and at rapid rates were obtained from each of 16 individuals, and their urea concentrations (urea plus ammonia) compared with that of blood taken at the same time. The slow specimens were obtained without stimulation, the rapid ones by chewing paraffin. (Table I.)

All the subjects show a lower urea concentration in the rapidly secreted specimens, the average difference between slow and fast

[1] Hench, P. S., and Aldrich, M., *J. Am. Med. Assn.*, 1923, lxxxi, 1997.

[2] Schmitz, H. W., *J. Lab. and Clin. Med.*, 1922, viii, 78.