REPORT

COMMITTEE ON NUTRITION

Proteolytic Enzymes in Milk in Relation to Infant Feeding

A revival of interest in the old practice\(^1\) of treating milk with proteolytic enzymes for feeding infants has stimulated the Committee on Nutrition of the American Academy of Pediatrics to undertake an appraisal of the significance of proteolytic enzymes in human and cow's milk.

It has been known for a long time that enzymic activity may be detected in milk of humans and cows. An enzyme found in milk may be a secretory product of the mammary gland, or an excretory product which merely escaped into the milk from the blood coincident with the secretion of milk. The leukocytes and bacteria usually found in milk contain enzymes; these will contribute to the apparent enzyme content of milk, if samples are not handled properly before analysis to remove, and to prevent disintegration of, cellular and bacterial contaminants.

The simple qualitative detection of enzymic activity in milk does not provide significant information. One needs to know whether the enzymic activity is truly derived from the milk rather than a contamination. Furthermore, knowledge of the amount of enzyme present and the effect, if any, on the nutritional qualities of the milk or on the infants consuming the milk is essential before assigning a significant role to any enzyme which may be found in milk.

GENERAL CONSIDERATIONS

**Enzymes in Milk**

An impressive variety of enzymic activities has been reported to be present in human and cow's milk but only from the latter have enzymes been actually isolated and purified (xanthine oxidase and lactoperoxidase).\(^2\) The phosphatase and proteolytic activities have received most attention. This is because the destruction of phosphatase by heat is used as an index of successful pasteurization and because of the interest in the effect of proteases on curd formation. For a discussion of all the enzymic activities reported to have been identified in milk, the reader is referred to a review by McMeekin and Polis.\(^2\)

One interesting observation is that cow's milk contains considerable xanthine oxidase, but this enzyme has not been detected in human milk; indeed this difference has been proposed as a means of distinguishing human milk from other kinds of milk.\(^2\)

**Nature of Proteases in Milk**

Proteolytic activity due to a protease of trypic nature (not attributable to bacterial contamination) has been demonstrated in human and cow's milk.\(^3\) Its optimal activity is reported to be at pH 9.2. It has not been isolated and purified; the observed proteolytic activity may be the result of action of more than one protease. The proteolysis of proteins is carried beyond the peptone stage.\(^2\) The proteolytic activity of raw, untreated milk is greatly reduced by pasteurization.\(^3\)

**Proteolytic Activity in Human and Cow's Milk**

Estimation of proteolytic activity in milk has been studied mostly by measuring the amount of tyrosine liberated during incubation at 100°F (37°C), after the method of Hull.\(^4\)

Suitable quantitative data were not found in the literature which would permit a comparison of the amount of proteolytic activity in various milks in reference to a standard source of proteolytic activity such as a


pancreatic preparation.

A study by Storrs and Hull in 1956, using a modification of Anson's method, compares the proteolytic activities of human and cow's milk. The data provided are too meager and incomplete to allow a firm conclusion, but these authors state that their analyses of samples of raw human and raw cow's milk, in which bacterial growth was kept low during the incubation by antibiotics, indicate cow's milk contains about one-fifth as much proteolytic activity as human milk. (Actually, about 3 mg tyrosine per liter liberated after 6-hours incubation of raw cow's milk and 15 mg per liter in the case of human milk; with 2-hours incubation corresponding figures were about 2 mg and 7 mg, respectively.)

This is only a comparison of relative activities. Actual quantitative determination of amounts of proteases in human and cow's milk are lacking. The proteolytic activity in the samples compared may be regarded as low considering that under similar conditions 1 mg of U.S.P. pancreatin liberates approximately 560 mg of tyrosine per liter of cow's milk in 10-minutes incubation. Of course human milk has only a third the concentration of protein present in cow's milk and this along with other difficulties imposed by the use of crude substrates is a handicap in making a quantitative comparison by simple incubation.

CONSIDERATIONS RELATIVE TO ADDITION OF PROTEOLYTIC ENZYMES TO COW'S MILK FOR INFANT FEEDING

Secretion of Proteolytic Enzymes into the Duodenum in Infants

There is an abundant secretion of proteolytic enzymes in normal infants from birth. Even prematurely born infants as small as 1,000 gm in weight produce proteolytic enzymes in sufficient quantity to digest protein normally. The quantity of proteolytic enzymes secreted by normal full-term and premature infants suffices for maximal digestion of protein of cow's milk, i.e., approximately 95% absorption of ingested protein nitrogen, providing the raw milk has been treated so as to produce low curd tension. There is no evidence that additional oral administration of pancreatic enzymes will lead to greater digestion, absorption or retention of protein nitrogen by normal infants. This is in contrast to states of pancreatic deficiency, such as cystic fibrosis of the pancreas, where improved digestion and absorption of protein can be affected by pancreatic enzymes administered orally. Even in this circumstance increased retention of nitrogen does not necessarily ensue but will depend on other factors such as caloric intake, etc.

Action of Proteolytic Enzymes on Cow's Milk

Incubation of raw cow's milk with a pancreatic extract at 62.5°C (143°F) for ½ hour decreases the curd tension* from 60 to 25 gm. This degree of reduction in curd tension may also be accomplished by a variety of other processes, e.g., dilution, heat alone (180°F for 5 minutes), homogenization, and treatment with acids, alkali or rennin, all of which produce "soft-curd" milks (curd tension approximately 25 gm).

No other biologic or nutritional effects attributable to the treatment of milk with pancreatic extract or to the residual proteolytic activity have been demonstrated, as far as in-vivo actions are concerned.

The relative antigenicity of enzyme-treated milk compared to other processed milks has not been subjected to critical study.

CLINICAL EVALUATION OF MILK TREATED WITH Pancreatic Proteolytic Enzymes

The revival of the practice of treating milk with proteolytic enzymes for infant feeding dates from the proposal of an improved technique of preparation by Conquest et al. in 1938.* This has been applied commercially in the preparation of a con-
centrate of pancreatic origin* for incubation with raw cow's milk to produce an enzyme-treated milk. The resulting product has a curd tension of about 20 gm and the protein is hydrolyzed to an extent of about 1%. It has a residual proteolytic activity equivalent to that possessed naturally by human milk. It is not sterile, the bacterial counts being those generally prevailing in pasteurized cow's milk.

When milk is treated with pancreatic extract as described, the degree of protein digestion is slight, estimated as about 1% hydrolysis of the protein from the amount of tyrosine liberated. About 3% of the proteolytic activity originally present in the mixture is found to survive the heat treatment during incubation, if the enzyme-treated milk is cooled immediately at the end of the period of incubation.

In-vitro experiments, in which various forms of cow's milk were subjected to proteolysis by gastric enzymes (U.S.P. pepsin) or pancreatic extracts, showed that milk treated with pancreatic enzymes was more readily "digested" than pasteurized or homogenized milk, but heat-treated milks (e.g., evaporated milk, boiled milk or dried milk) were "digested" as well as the milk pretreated with pancreatic enzymes. Under the conditions of the test, human milk was "digested" more readily than any of the various forms of cow's milks.

Only two studies have been published on the results of clinical use of such a product in infant feeding. That of Blatt et al. in 1940 employed two groups of infants: Group I, full-term infants seen during the first 4 weeks of life and followed varying periods up to 36 weeks of age; Group II, premature infants followed from birth (duration of observations not stated). Each group was subdivided into two divisions of "like ages." In each group one division was fed the enzyme-treated milk and the other received human milk, boiled pasteurized, unboiled pasteurized and evaporated milks. In Group I, 75 infants received the enzyme-treated milk and 38 were given one of the other milks. In Group II, 71 infants were observed while fed enzyme-treated milk and 71 received other milks.

No adverse effects were observed from feeding the enzyme-treated milk without further sterilization. Blatt concluded that the infants fed the enzyme-treated milk grew as well as those fed other milks and in accordance with "normal growth expectancy." He observed less frequency of stools and lower incidence of respiratory infections in the infants fed the enzyme-treated milk but this cannot be accepted as satisfactory evidence of a consequence of feeding the enzyme-treated milk. The numbers of subjects, short periods of observation, selection which occurred merely from not being able to follow the infants the same or a sufficient length of time, and the vague descriptions and generous definitions of "diarrhea" and "infections," all subtract from the significance of the limited observations.

A recent study accumulated similar observations on groups of full-term babies receiving evaporated, enzyme-treated, or human milk. Much the same shortcomings subtract from the significance of this paper as was the case with Blatt's. All that could be concluded was that no one group appeared to surpass the others in growth or well-being.

The requirements of a proper appraisal of a food for infant nutrition were certainly not met in either of these studies, and the latter contains much irrelevant digression.

**COMMENTS**

The benefit achieved for infant feeding by reducing curd tension of raw cow's milk, first pointed out by Brennemann in 1913, represents one of the most significant advances in the artificial feeding of in-
fants. It would appear that the most that can be said at present for the treatment of milk with pancreatic proteolytic enzymes (Enzy!ac Powder) is that it is one among many methods of reducing the curd tension of raw cow's milk. Lacking is evidence for a beneficial effect of any residual proteolytic activity or other property of enzyme-treated milk which would justify advocating its use on any basis except curd tension.

It is recommended in one of the clinical studies and in current promotional literature for enzyme-treated milk that it be fed undiluted and without addition of carbohydrate. Many of the claims in behalf of enzyme-treated milk are based on vague and irrelevant allusions to the greater quantities of nutrients provided by whole cow's milk—which the product essentially is, if fed undiluted and unmodified. However, no other product or formula is being advocated at the present time as the sole food for infants that offers less than adequate amounts of the nutrients to be obtained from milk. There is no evidence that enzyme treatment "enhances the liberation of all milk nutrients," as claimed for Enzylac Milk, any more than other treatments which reduce curd tension.

Considerations which apply to the feeding of undiluted, unmodified cow's milk (processed to reduce curd tension) also apply to enzyme-treated milk, e.g., the necessity of meeting the increased water requirement imposed by prolonged increased environmental temperature, febrile states or renal insufficiency.

SUMMARY

Treatment of raw cow's milk with pancreatic proteolytic enzymes reduces curd tension to levels comparable to those achieved by many other methods suitable for the preparation of soft-curd milk. No other biologic or nutritional benefits have been shown to result from enzyme treatment of milk.

No evidence is available for assigning any benefit in infant nutrition to the proteolytic activity naturally occurring in human milk or persisting in enzyme-treated cow's milk after pasteurization.

Argument based on the mere existence of proteolytic enzymes in human milk cannot justify enthusiastic claims for use of enzyme-treated milk in infant feeding.

The subject of enzymes in milk and their potential role in infant feeding has received scant attention; further study may reveal information which will call for reappraisal in the future.

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REFERENCES

* Medical Dairy Specialties, Chicago, Illinois.


Three other sections complete the volume. The section on neoplastic diseases is relatively brief. The author’s personal interest is reflected in the more elaborate coverage of inflammatory disorders; parasitic and granulomatous disease; collagen disturbances; metabolic disturbances; and vascular disorders. A section on traumatic disorders, foreign bodies and effects of drugs conclude the presentation. Throughout, there is a strong clinical approach which supports the use of this volume as a tool in making a working diagnosis under conditions of relative urgency.

The text is in two columns of easily legible print and the illustrations are clearly reproduced and labeled. An adequate subject index is provided and the addition of an alphabetized index of authors increases usefulness as a reference tool.

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