3. LEGAL AND TOXICOLOGICAL ASPECTS

To use the LP-system in raw milk as a temporary bacterial preservative, it is necessary to supplement the thiocyanate level in the milk and to add a source of hydrogen peroxide. Although these additions are minute, they interfere with the Code of Principles concerning milk and milk products developed by the FAO and WHO, which states that the term milk means "exclusively the normal mammary secretion obtained from one or more milkings without either addition thereto or extraction therefrom". However, as such a method could be of substantial practical use in certain situations it should be evaluated in an objective way regarding its efficiency and any potential health hazards.

In Sweden, the National Food Administration has evaluated the efficiency of the method and existing toxicological data and has decided to allow the use of LP-activation in milk in situations when raw milk cannot be properly cooled (25).

To evaluate any toxic risks with the utilization of the LP-system as a temporary milk preservative, the toxicity of added hydrogen peroxide and thiocyanate as well as the oxidation products formed, have to be evaluated.

A. Hydrogen peroxide

With the traditional way of using hydrogen peroxide as a milk preservative, i.e. additions of 300-800 ppm of H_2O_2, the hydrogen peroxide persists for long periods in the milk. The milk has therefore to be treated with catalase before processing to ensure that any residual hydrogen peroxide is destroyed. At these levels of hydrogen peroxide the lactoperoxidase is destroyed. The antibacterial effect obtained in this case is thus not related to the LP-system, it is only due to the antibacterial effect of hydrogen peroxide itself. This method has been approved by the FAO/WHO (1) and thus is not considered to constitute any health hazards.

With the LP-method, the small amounts of hydrogen peroxide added to milk are rapidly utilised in the enzymatic oxidation of thiocyanate, whereby it is reduced to water. In this context, it should also be recalled that milk itself contains several enzymes capable of producing hydrogen peroxide, viz. xanthine oxidase and sulphydryl oxidase. It is therefore likely that low levels of hydrogen peroxide are produced in milk although detectable amounts are not normally found since they are continuously reduced by enzymes such as catalase or lactoperoxidase. These facts indicate that the hydrogen peroxide used to activate the LP-system in milk does not imply a health risk.

B. Thiocyanate

Thiocyanate ingested in very high concentrations has an acute toxic effect. The LD_{50} dose of orally administrated sodium thiocyanate in rats is reported to be 764 mg/kg (26). On the other hand, thiocyanate is also considered to be a normal electrolyte in mammalian blood. Human plasma levels are 2-3 ppm in non-smokers and 9-12 ppm in smokers (27). The thiocyanate is largely of exogenous origin, being derived through the ingestion of various glucosinolates such as sinigrin, glucobrassicin and neoglucobrassicin, which release thiocyanate upon hydrolysis. Common sources of these glucosinolates are plants belonging to the cruciferous family, e.g. cauliflower, cabbage and kale. Another important source of thiocyanate is the enzymatic detoxification of cyanide by the enzyme thiosulfate sulfur transferase (E.C. 2.8.1.1) (rhodanase). This enzyme is present in most mammalian tissues although the highest concentrations are found in liver, kidney, adrenals, thyroid and pancreas (28). Cyanide intake in man is essentially due to ingestion of cyanogenetic glucosides such as amygdalin and limarin, which are present in bitter almonds, linseed and cassava. In smokers, tobacco smoke is an important source of cyanide (29).

Thiocyanate is secreted by the mammary and salivary glands and by the gastric mucosa. The levels of thiocyanate found in milk are fairly variable. Levels of 10 to 15 ppm have been reported (30, 31) but normally the concentrations are in the range between 2-7 ppm (32). Saliva is rich in thiocyanate, levels between 50-300 ppm having been reported (33).

Although thiocyanate is a normal electrolyte in many secretions, it is well-established that high serum levels result in disturbances in the thyroid function (hypothyroidism) (34). It has also been suggested that thiocyanate in milk may be a factor causing goiter. Finnish investigations (35), on the other hand, have demonstrated that doses between 200 to 400 mg were necessary to give a thyrostatic effect. More recent studies of the pharmaco-kinetics of nitro-prusside, which is used in hypertension treatment, have clearly demonstrated that serum levels above 18-20 ppm of thiocyanate are necessary to cause impaired thyroid function (36).

These investigations indicate that the levels of thiocyanate utilised in LP-activated milk would not cause any disturbances of thyroid function. However, to confirm this, a clinical experiment was carried out in Sweden during 1982 in collaboration between the National Food Administration, the University of Uppsala (Department of Medicine) and the Swedish University of Agricultural Sciences, Uppsala. The results of this experiment have been submitted to the American Journal of Clinical Nutrition for publication (Dahlberg, P.A. et al., Intake of thiocyanate by way of milk and its possible effect on the thyroid function). In this trial, 43 persons were given 0.4 l milk containing 20 ppm thiocyanate per day during 3 months. The increased intake of thiocyanate was reflected in increased serum concentrations. The highest values were obtained after 4 weeks (7.8 mg/l in non-smokers; 10.7 mg/l in smokers). Thereafter the concentrations decreased and at the end of the experimental period were (7.0 mg/l and 8.9 mg/l in the non-smoking and smoking group, respectively. No apparent effect was observed on the thyroid function, i.e. no significant changes were found in the serum levels of thyroxine-4, triiodothyronine and thyrothropic hormone (TSH). These results are thus in agreement with other investigations and indicate that the levels of thiocyanate used in LP-activated milk will not cause any disturbances in thyroid function.

C. Oxidation products of thiocyanate

The antibacterial effect of the LP-system is mediated by short-lived oxidation products of thiocyanate. These intermediates are very unstable and those not reacting with bacteria decompose spontaneously or in connection with pasteurization of the milk. When the milk reaches the consumer, none of the active agents will thus be present in the milk. On the other hand, it has also to be considered whether there is any potential risk if the milk is consumed shortly after an inactivation of the LP-system and residual levels of the oxidation products of thiocyanate still are present and ingested via the milk. Any toxic risks of the intermediate oxidation products present in freshly activated LP-activated milk have to be considered as low. These oxidation products normally are present in human saliva (37). Recent studies in which certain mammalian cell types, viz. Hela cells, fibroblasts and chinese hamster ovary cells, were exposed to the complete LP-system (utilizing concentrations up to 50 ppm of thiocyanate and an equivalent concentration of hydrogen peroxide) also failed to reveal any adverse effects (J. Carlsson, personal communication). Consumption of freshly LP-activated milk would therefore not present any additional risks apart from those generally connected with consumption of unpasteurized milk.

4. EFFECTS ON THE PROCESSING PROPERTIES AND NUTRITIVE VALUE OF MILK

In contrast to the general oxidative effect of hydrogen peroxide, which is known to affect adversely the processing properties of milk, the antibacterial effect of the LP-system is much more specific. The active agents of the system react primarily with free SH-groups in proteins. Milk proteins, however, contain few free SH-groups, implying that there is little risk of negative effects.

So far the rennet coagulation and acid production of starter cultures in LP-activated pasteurized milk have been investigated (2). Negative effects could not be found in this investigation. Regarding growth of starter cultures in LP-activated milk it should, however, be pointed out that if the starter strains used produce hydrogen peroxide, as do some strains of streptococci and lactobacilli, they may show an impaired growth rate in LP-activated milk, as the hydrogen peroxide they produce completes the LP-system. Nevertheless, this can easily be overcome by using starter strains that produce no or little hydrogen peroxide.
Although effects of the LP-activation on the vitamins in milk are not to be expected, this aspect has yet to be investigated.

5. PRACTICAL USE OF THE LP-ACTIVATION UNDER FIELD CONDITIONS

Although the optimal way of applying the LP-activation has to be decided from case to case, the following principles are recommended. The raw milk should not be treated by the individual farmers but at a collecting centre or a similar place. The main reason for this is that if the activation is carried out on the farm, the dosage of the activators will be complicated as widely variable volumes of milk are to be treated. Neither should it be overlooked that usage on the farms will be difficult to control and the risks of misuse thereby enhanced. If the treatment, on the other hand, is carried out at a collecting centre, correct dosage of the activating substances wil be easier as the milk is handled in churns of a certain volume, i.e. the activators can be distributed to give the correct concentration for this volume. At a collecting centre it will also be easier to supervise the correct use of the method. Another important factor that indicates that the activation should take place at a collecting centre, is that most of the deterioration of the raw milk often occurs during the transport from the collecting centre to the dairy plant.

The practical use of the LP-activation must also always be combined with proper control measurements to ensure that the method is correctly used and that the basic hygiene is not neglected. The correct dosage of the activators can be controlled by analysing the thiocyanate concentration of the delivered milk. This can be achieved by a simple colorimetric test. If the thiocyanate level is found to be substantially higher than 15 ppm, overdosage is likely to have occurred. If the ordinary basic hygienic precautions are neglected, it will automatically be revealed in the usual quality control carried out on the delivered milk. The LP-activation causes only an inhibition of most of the raw milk flora. Consequently, if the milk had high bacterial numbers at the time of LP-activation, high bacterial numbers will also be present at delivery to the dairy plant and will be reflected in a dye reduction test or plate count.

6. CONCLUDING REMARKS

The use of the LP-system as a temporary preservative for raw milk represents a new approach to the old problem of finding a suitable temporary preservative for milk when adequate cooling is not available.

The basic biochemical mechanism of this antibacterial system, which not only has been investigated in connection with milk but also by oral bacteriologists (the system is also present in saliva) is by now fairly well documented.

Field experiments carried out in Kenya and Sri Lanka have clearly demonstrated that a substantial improvement of the hygienic quality of raw milk can be achieved by using the method. To activate the system it is necessary to supplement milk with about 10 ppm of thiocyanate followed by an addition of 8 to 9 ppm hydrogen peroxide. Any toxicological risks of these additions have to be most carefully evaluated. The main concern is the effect that the enhanced thiocyanate levels may have in milk. Clinical experiments with LP-activated milk for 3 months have indicated that the levels of thiocyanate used in LP-activated milk do not interfere with the thyroid function or give any other clinical effects. The subjects in this trial were healthy individuals with a normal iodine status. The effect of an increased thiocyanate intake should, however, also be investigated in subjects with a low iodine status. Such an experiment has recently been carried out in Sudan. The results from this trial are expected to be available in July 1983.

The use of the LP-system as a temporary milk preservative seems to offer several advantages over the use of high concentrations of hydrogen peroxide:

- the LP-system has a much more specific antibacterial effect than the general oxidative effect of hydrogen peroxide. This will minimize the risks of any negative effects on the processing and nutritive properties of the milk;
- the effect of the LP-system on the bacterial flora in raw milk is largely of a bacteriostatic nature. Therefore, it is not possible to "improve" the hygienic quality of an initially low quality milk by use of the LP-system. This is a most important aspect as good basic hygiene is thus a prerequisite for successful use of the LP-system;
- the residual thiocyanate makes it possible to monitor the usage of the method; overdosage, etc. can easily be revealed by excessively high thiocyanate levels in the milk;
- the practical application of the method is simple as no liquid substances are required.

An alternative method to refrigeration as a way of preserving raw milk would be beneficial in many countries where adequate cooling facilities cannot always be provided, either for economic or practical reasons. Although the long-term goal in these countries is to establish complete cooling facilities throughout the milk handling system, it must be realised that this will take a considerable time to accomplish. Until then, there is an urgent need for an alternative method of preserving raw milk, primarily during collection and transportation to processing centres. If such a method becomes available, it will be possible to collect milk from more remote areas without risking an inferior hygienic quality upon arrival at the dairy plant. In turn, this would not only increase the intake of milk for the dairy industry, but also lead to a more rapid development for many small-scale dairy farmers.

The use of the LP-system as an alternative method seems to offer several advantages over the use of concentrated hydrogen peroxide. It is therefore the view of Group F19 that IDF should encourage further research and development in this area and that the FAO/WHO should be recommended to evaluate the use of the LP-system as a temporary preservative for milk when adequate cooling facilities are not available.

REFERENCES

1. FAO. 1957. Report on the meeting of experts on the use of hydrogen peroxide and other preservatives in milk. Rome. Doc. 57/11/8655.
2. Björck, L. 1978. Antibacterial effect of the lactoperoxidase system on psychrotrophic bacteria in milk. J. Dairy Res., 45 : 109.
3. Björck, L. 1979. Enzymatic stabilisation of milk utilization of the milk peroxidase for the preservation of milk. Proc. IDF Annual Sessions, Montreux.
4. Wright, R. & Tramer, J. 1958. Factors influencing the activity of cheese starters. The role of milk peroxidase. J. Dairy Res., 25 : 104.
5. Reiter, B., Pickering, A. & Oram, J.D. 1964. An inhibitory system-lactoperoxidase-thiocyanate-peroxide in raw milk. In Microbial inhibitors in food. Ed. by N. Molin, Almqvist & Wiksell.
6. Zeldow, B. 1963. Studies on the antibacterial action of human saliva. III. Cofactor requirement of a lactobacillus bactericidin. J. Immunol., 90 : 12.
7. Hoogendoorn, H. 1974. The effect of lactoperoxidase-thiocyanate-hydrogen peroxide on the metabolism of carciogenic micro-organisms *in vitro* and in the oral cavity. Thesis. Mouton, Den Haag, Netherlands.
8. Adamson, M. & Carlsson, J. 1982. Lactoperoxidase and thiocyanate protect bacteria from hydrogen peroxide. Infect. Immun., 35 : 20.
9. Reiter, B. 1978. Antimicrobial systems in milk. J. Dairy Res., 45 : 131.
10. Reiter, B. 1978. Review of non-specific antimicrobial factors in colostrum. Ann. Rech. Vet., 9 : 205.
11. Korhonen, H. 1980. A new method for preserving raw milk the lactoperoxidase antibacterial system. World Animal Review, 35 : 23.
12. Oram, J. & Reiter, B. 1966. The inhibition of streptococci by lactoperoxidase, thiocyanate and hydrogen peroxide. The oxidation of thiocyanate and the nature of the inhibitory compound. Biochem. J., 100 : 382.
13. Hogg, D. & Jago, G.R. 1970. The antibacterial action of lactoperoxidase. The nature of the natural inhibitor. Biochem. J., 117 : 779.
14. Hoogendoorn, H., Piessens, J.P., Scholtes, W. & Stoddard, L.A. 1977. Hypothiocyanite ion: the inhibitor formed by the system lactoperoxidase-thiocyanate-hydrogen peroxide. Caries Res., 11 : 77.
15. Aune, T.M. & Thomas, E.L. 1977. Accumulation of hypothiocyanite ion during peroxidase catalysed oxidation of thiocyanate ion. Eur. J. Biochem., 80 : 209.
16. Björck, L. & Claesson, O. 1979. Correlation between concentration of hypothiocyanite and antibacterial effect of the lactoperoxidase system against *Escherichia coli*. J. Dairy Sci.
17. Pruitt, K., Tenovno, J., Andrews, R.W. & McKane, T. 1982. Lactoperoxidase-catalysed oxidation of thiocyanate: Polarographic study of the oxidation products. Biochemistry, 21 : 562.
18. Thomas, E.L. & Aune, T.M. 1978. Lactoperoxidase, peroxidase, thiocyanate antimicrobial system; correlation of sulphydryl oxidation with antimicrobial action.
19. Jago, G.R. & Morrison, M. 1962. Antistreptococcal activity of lactoperoxidase. Proc. Soc. Exptl. Biol. Med., 111 : 585.
20. Oram, J.D. & Reiter, B. 1966. The inhibition of streptococci by lactoperoxidase, thiocyanate and hydrogen peroxide. The effect of the inhibitory system on susceptible and resistant group N streptococci. Biochem. J., 100 : 373.
21. Björck, L., Rosén, C-G., Marshall, V. & Reiter, B. 1975. Antibacterial activity of the lactoperoxidase system in milk against Pseudomonads and other gram-negative bacteria. Appl. Microbiol., 30 : 199.
22. Marshall, V., Reiter, B., Björck, L. & Rosén, C-G. 1976. Non-specific bactericidal activity of the lactoperoxidase-thiocyanate-hydrogen peroxide system of milk against *Escherichia coli* and some gram-negative pathogens. Infect. Immun., 13 : 800.
23. Björck, L., Claesson, O. & Schulthess, W. 1979. The lactoperoxidase-thiocyanate-hydrogen peroxide system as a temporary preservative for raw milk in developing countries. Milchwissenschaft, 34 : 726.
24. Härnulv, G. & Kandasamy, C. 1982. Increasing the keeping quality of milk by activation of its lactoperoxidase system. Results from Sri Lanka. Milchwissenschaft, 37 : 454.
25. The National Food Administration. 1980. Document Dnr 2726/80. Uppsala, Sweden.
26. Andersson & Chen. 1940. Absorption and toxicity of Sodium and Potassium Thiocyanates. J. Amer. Pharm. Assoc., 29 : 152.
27. Maliszewski, P.M. & Bass, H.E. 1955. True and apparent thiocyanate in body fluids of smokers and non-smokers. J. Appl. Physiol., 8 : 289.

28. Reinwein, D. 1961. Die Verteilung der Thiosulfat-Schwefeltransferase und des Rhodanids im menschlichen und tierischen Organismns. Z. Physiol. Chem., 326 : 94.
29. Brunneman, K.D., Yu, L. & Hoffman, D. 1977. Chemical studies of tobacco smoke. XLIX. Gas chromatographic determination of hydrogen cyanide and cyanogen in tobacco smoke. J. Anal. Toxicol., 1 : 38.
30. Boulangé, M. 1959. Fluctuation saisonnière du taux thiocyanates dans le lait frais de vache. C.R. Soc. Biol., 12 : 2019.
31. Lawrence, A.J. 1970. The thiocyanate content of milk. In XVIII Int. Dairy Congr., vol. 1 E. 99.
32. Björck, L. Unpublished observations.
33. Densen, P.M., Davidson, B., Bass, H.E. & Jones, E.W. 1967. A chemical test for smoking exposure. Archs. Environ. Health, 14 : 865.
34. Green, W.L. 1978. Mechanism of action of antithyroid compounds. In The Thyroid (eds. S.C. Werner & S.H. Ingbar), 4th ed. pp 41-51, Harper & Rox Publishers, N.Y.
35. Vilkki, P., Kreula, M. & Piironen, C. 1962. Studies on the goitrogenic influence of cow's milk on man. Annales Academiae Scientarium Fennicae, Series A : II Chemica; 110, Helsinki.
36. Bödigheimer, K., Nowak, F. & Schoenborn, W. 1979. Pharmakokinetik und Thyreotoxizität des Nitroprussid-Natrium Metaboliten Tiocyanat. Deutsche Medizinische Wochensckrift, 104-939.
37. Thomas, E.L., Bates, K.P. & Jefferson, M.M. 1980. Hypothiocyanate ion; detection of the antimicrobial agent in human saliva. J. Dental. Res., 59 : 1466.

* * *