of 1959 peas are shown in Table IV. The acid composition is relatively simple. Formic acid is the primary constituent with small amounts of acetic and isovaleric acids also present. Formic acid has been detected previously in canned peas (*1*).

The steam distillate of peas has the pleasant, characteristic odor of cooked peas. If the compounds responsible for the odor can be identified, a substantial part of pea flavor would be understood. Earlier work in this field has indicated that carbonyl compounds are an important part of pea odor (*2*). Mixtures of the carbonyl compounds identified in this study do not approach the cooked pea odor. Work on the isolation and identification of the odorous components of pea steam distillate is being continued.

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LACTOSE BINDING IN MILK

Free and Bound Lactose in Milk

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By using both isotope dilution methods and a conventional protein precipitation technique, it was demonstrated that in unheated milk 0.54% lactose is associated with milk protein(s). The association is apparently weak, as all lactose is removed from unheated milk by dialysis for 72 hours. The results suggest an equilibrium, free lactose ⇄ bound lactose, favoring a free-bound ratio of about 8 to 1. Binding of lactose by heated proteins, which is apparently a carbonyl-amino reaction, was investigated at 80° and 100° C. The activation energy of the reaction, probably the first step in the browning reaction, is of the order of 11 × 10^3 calories per mole.

WHILE lactose binding by the proteins of heated milk (browning reaction) has been the object of much research and the subject of a comprehensive review (*10*), little attention has been directed to bound lactose in unheated milk.

Various carbohydrates (glucose and galactose among others) have been reported to be associated with casein (*7, 8*), with some minor protein components (*13*), and with amino acids from tryptic hydrolyzates (*1*). Goulden, studying freeze-dried model systems, interpreted infrared spectra as indicating an association between casein and lactose (*3*). Other workers (*9*) disagreed with this interpretation, and Goulden subsequently attributed the irregularities in the spectra to the polymorphism of lactose (*4*). Schober and Christ (*12*) studied the binding of glucose by casein as influenced by heat, and concluded that two different types of association occurred—a reversible binding in protein that was native or subjected to low heat treatments, and at higher temperatures an irreversible binding that was a part of the browning reaction. The existence of bound lactose in unheated milk has not been definitely established nor has the extent of binding been measured.

The objects of this investigation were to determine the amount of bound lactose in unheated milk, the nature of the binding, and the energy requirements of the process in the temperature range 80° to 100° C.

Materials and Methods

Heating Trials. Aliquots (150 ml.) of fresh, standardized (3.5% fat) mixed-herd raw milk were placed in 14½-ounce evaporated milk cans. The cans were soldered closed, placed in a constant-temperature water bath for the indicated periods, and cooled in running tap water for 15 minutes. They were stored in an ice-water bath overnight and the milk was analyzed the next day.

Dialysis Trials. Separate aliquots of the milk were placed in dialysis bags and dialyzed against a pH 6.60 buffer (3.75 grams of anhydrous monohydrogen sodium phosphate and 5.41 grams of anhydrous dihydrogen potassium phosphate per liter of distilled water). The volume ratio of milk to buffer during these trials was about 1 to 10.

Lactose Determinations. Total lactose was determined by the zinc acetate–phosphotungstic acid reagent method of Grimbleby (*5*). Free lactose was determined by two methods, with excellent agreement in results.

Isotope Dilution Methods. To reduce coincidence losses, a 6-mg. portion

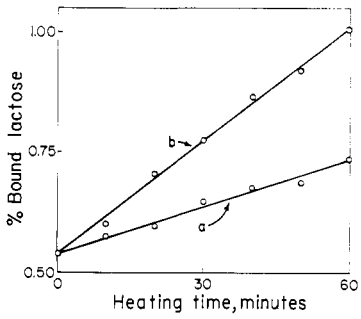

Figure 1. Influence of heating time on amount of bound lactose

a. 80°C.
b. 100°C.

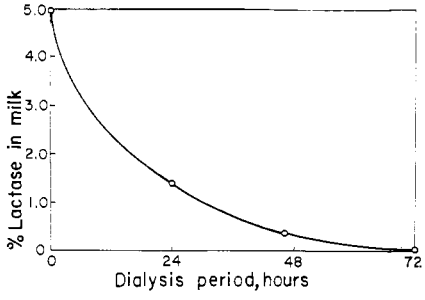

Figure 2. Amount of lactose in unheated milk as a function of dialysis time

of high - activity uniformly labeled carbon-14 lactose was added to 55 mg. of recrystallized carbon-12 lactose, and the two were completely mixed by dissolving in water and allowing slow evaporation at room temperature. When the original weight was realized, the specific activity of this "attenuated" tracer was determined. A 61-mg. portion was dissolved in 20.511 grams of fresh, standardized, raw milk, and the mixture placed in a dialysis bag for isolation of the centrifugal diffusate (*6*). The bag, suspended in a large centrifuge tube, was centrifuged 3 hours at 1500 r.p.m. in a centrifuge with a peripheral diameter of 38 cm. The centrifugal diffusate was evaporated on a steam bath until lactose crystallization occurred. The lactose was recovered by filtration and purified by paper chromatography; a mixture of methyl ethyl ketone, propionic acid, and water (75 to 25 to 30) was used as the developing solvent. After the specific activity of the recovered lactose was measured, isotope dilution was calculated by Paneth's equation as described by Willard, Merritt, and Dean (*14*).

Dialyzed Iron Method. In this simple procedure for free lactose, 15 ml. of dialyzed iron was added to 20 ml. of milk previously diluted with 20 ml. of distilled water. After standing for a few minutes the mixture was stirred well with a glass rod, made up to 100 ml., filtered, and polarized in a 2-dm. tube.

Results and Discussion

The binding of lactose at 80° and 100° C. is shown in Figure 1. From the slopes, the enthalpy of activation can be obtained with the integrated form of Arrhenius equation:

$$\log \frac{k_2}{k_1} = \frac{\Delta H^{\ddagger} (T_2 - T_1)}{2.303\, R T_2 T_1}$$

As the concentration of the reactants was the same at both temperatures, the ratio of the rate constants is equal to that of the slopes, as shown by the differential equations:

$$\left(\frac{dl}{dt}\right) 80° = k_{80°} (\text{lactose})^a (X)^b$$

$$\left(\frac{dl}{dt}\right) 100° = k_{100°} (\text{lactose})^a (X)^b$$

Therefore, as the ratio of the slopes is 2.235,

$$\log 2.235 = \frac{\Delta H^{\ddagger}}{(2.30)(1.99)(373)(353)}$$

Hence

$$\Delta H^{\ddagger} = 11 \times 10^3 \text{ calories per mole}$$

This value probably represents the energy of the first step of carbonyl-amino interaction, as there were no significant changes in color as measured by reflectance (*2*). This conclusion is supported by the fact that this value is in good agreement with the same thermodynamic quantity as determined from Patton and Flipse's data (*11*) for the binding of radioactive lactose by milk protein, and confirms Patton's results, indicating that the binding of lactose is initiated prior to color development.

In Figure 1, the intercept indicates that about 0.54% of the lactose of milk is bound before any heat treatment is applied. Grimbleby (*5*) predicted that the binding of lactose by protein could be a significant source of variation in lactose determination, as different methods of protein precipitation would liberate varying amounts of bound lactose. These data indicate how large such variations could be. It is conceivable that the glucose and galactose reported in casein hydrolyzates (*7, 8*) are actually hydrolytic products from bound lactose, particularly as the casein in question had been resuspended, reprecipitated, and washed to remove "free" impurities.

Figure 2 shows that dialysis of unheated milk for 72 hours removes essentially all lactose—both free and bound. It would seem that bound lactose in unheated milk is held through a weak linkage, probably not of carbonyl-amino nature as in heated milk. Prolonged dialysis disrupts this weak association, which may be due to hydrogen bonding, so that all lactose is dialyzable. This suggests that lactose in unheated milk occurs in a state of dynamic equilibrium:

$$\text{Bound lactose} \leftrightarrows \text{free lactose}$$

and the data indicate that the equilibrium favors a free-bound ratio of about 8 to 1.

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