Wheelock, 1969) to investigate the effect of heat sterilization on the primary phase of rennin action.

The primary phase, as determined by the rate of release of glycopeptides soluble in 12% (w/v) trichloroacetic acid, is unaffected by the heat-sterilization process. In both the unheated control and the sterilized milks, the same sugars, *N*-acetyl-neuraminic acid, D-galactose and 2-acetamido-2-deoxy-D-galactose, are attached to these glycopeptides. The rate of release of the glycopeptides by rennin is the same in the two milks.

As these results are apparently in conflict with those obtained by Kannan & Jenness (1961), we have carried out similar experiments on heat-sterilized milks. Coagulation does not occur when milk is incubated with rennin at temperatures below 20°, although the enzyme action proceeds. It is therefore possible to compare the primary phases of two milks by incubating with rennin for periods at 16° and then determining the clotting time at 37° (Kannan & Jenness, 1961). By using this technique, the primary phase in the sterilized milk is distinctly slower than that in the control.

Our results suggest that there is more to the primary phase of rennin action in milk than just the release of glycopeptides from κ -casein. It is possible that the casein micelles may not adopt an alternative configuration after the κ -casein is hydrolysed and before coagulation. This could be instantaneous in unheated milk but retarded as a result of heating.

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A Component of Human Duodenal Juice that Affects Intrinsic Factor

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Vitamin B₁₂ is bound by intrinsic factor to form a complex essential for its active absorption (Castle, 1929). The formation of this complex is inhibited by enzymic digestion (Castle & Ham, 1936; Grasbeck, 1959) and intrinsic-factor antibodies (Schade, Abel, Shilling, Feick & Muckerheide, 1967).

During assays of human gastric juice for intrinsic factor by using the charcoal assay method, it was

found that those juices contaminated with bile gave values that were consistently lower than usual (Weir, Temperley & Collery, 1967). Further experiments confirmed that incubation of intrinsic factor with duodenal juice diminished the ability of the former to bind vitamin B₁₂. Similar inhibition could be demonstrated by using Sephadex to separate the free from the bound vitamin.

The possibility that this inhibition was due to enzymic digestion of intrinsic factor was discounted by showing that the inhibitory activity was stable to boiling for several hours. Isotopic dilution of the radioactive vitamin B₁₂ used by non-labelled vitamin B₁₂ was also discounted by showing that duodenal juice contained only trace amounts of the vitamin. Further, the inhibitory activity is eluted from Sephadex G-50 immediately after the excluded volume, suggesting a molecular weight of about 40 000.

Inhibitory activity has been found in the 100 or so duodenal juices examined. The degree of inhibition by different juices does, however, vary considerably. The rate of inactivation of vitamin B₁₂-binding activity depends on the temperature and pH of incubation and on the concentrations of duodenal juice and intrinsic factor used. Total loss of binding results by incubating intrinsic factor (200 units/ml.) with an equal volume of duodenal juice for 90 min. at pH 8.0 and 37°.

The inhibitory activity is unstable under pH 5.5 and over pH 11. It is also destroyed by treatment with papain.

More recently it has been found that duodenal juice can also dissociate the preformed intrinsic factor-vitamin B₁₂ complex, but at a rate much lower than that found for the inhibition of its formation.

Although the function of this factor is unknown, possibilities are that it might be involved in the absorption of vitamin B₁₂, possibly by removing free intrinsic factor from the ileal binding sites, or that it might be implicated in splitting the intrinsic factor-vitamin B₁₂ complex.

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