parisons of gamma globulin prepared from the same plasma are needed to establish this.

Gaines and Landy (*5*), employing a hemagglutinin technique, demonstrated antibodies in normal human serums against a *Pseudomonas* antigen; higher titers were present in older age groups. Various investigators have demonstrated small amounts of *Staphylococcus* antitoxin in the serums of man and animals, and the antibacterial properties of normal serum have been widely investigated for many years (*3*). The role of these *in vitro* properties of serum in its protective action against lethal infections in animals remains to be established. However, Cameron (*6*) has demonstrated protective action from gamma globulin derived from several domestic animals against experimental infections with *Pasteurella multocida, Salmonella choleraesuis* and *Brucella suis.*

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Interrelationship between Certain Bacteria and the Rumen Ciliate Dasytricha ruminantium

The cattle and sheep ciliates of the genera *Isotricha* and *Dasytricha* have been shown to possess considerable fermentation ability (*1, 2*). Soluble carbohydrates are rapidly utilized by the protozoans, with the production of acids and gas and with concomitant deposition of food reserve amylopectin. The nature of the nitrogen source for the holotrich ciliates has been elusive (*3*). The holotrich protozoans do not easily lend themselves to microscopic observation of bacterial feeding. In well-fed ciliates the entire organism is loaded with reserve food granules, making it difficult to detect either the presence of bacteria within the ciliates or bacteria actually being ingested by the protozoans.

Growth experiments with the holotrich protozoan *Isotricha intestinalis* have been reported (*2*). The ciliates grew during a short period in an anaerobic medium containing ground alfalfa, wheat, and untreated rumen fluid in inorganic saline. Counts of the protozoans during this time indicated division every 48 hours. The smaller, more abundant holotrich found in the rumen, *Dasytricha ruminantium,* did not survive in these experiments, indicating a difference in cultural requirements. It was of interest to find what these differences were and also what factors would stimulate growth of *D. ruminantium* in laboratory cultures.

Starvation of *D. ruminantium* for 48 to 72 hours decreased the number of amylopectin reserve granules and facilitated the search for bacterial ingestion by the protozoans. The starved ciliates were placed on a slide with a small amount of 0.5 percent saline and a mixture of bacteria obtained from fresh rumen fluid by centrifugation. A cover glass, ringed with Vaseline, excluded air from the entire suspension. With this arrangement the protozoans remained active and could be observed for several hours, with the use of a phase microscope. *Dasytricha ruminantium* selected and ingested small cocci, 0.5 to 0.8 μ in diameter, from the mixture. The bacteria agglutinated at the mouth located at the posterior of the organism and were gradually ingested. Occasionally, a ciliate was observed which swam about trailing many agglutinated bacteria. Small rod-shaped bacteria, 1.0 × 1.5 μ in size, were seen also within food vacuoles in the ciliates. In several of the experiments, exposure of the bacterial suspension to triphenyltetrazolium chloride allowed more distinct observation of bacterial ingestion by the protozoans. Bacteria which stained red as a result of the deposition of the insoluble formazan were seen localized near the mouth and in the interior of the ciliate in food vacuoles.

Several bacterial strains were isolated from rumen contents in order to test their growth-promoting effect on *Dasytricha.* Following the initial isolation on a starch-alfalfa extract agar, the bacteria were grown in liquid medium of the same type and used in the ciliate cultures. Small vessels with a flat upper surface and an opening fitted with a rubber stopper allowed observation of the protozoans without exposure to air. With the aid of a dissecting microscope, the response and fate of the ciliates in each experiment could be followed easily. With cooling of the cultures from 39°C to room temperature, most of the protozoans decreased their swimming activity and settled to the bottom of the vessel. The stale supernate was then removed with a capillary pipette, while anaerobic conditions were preserved by passing carbon dioxide into the vessel opening. Fresh 0.5 percent buffered saline containing substrate and bacteria was added every 24 hours. Streptomycin and penicillin, plus washing techniques, were used to free *Dasytricha* of most of the associated rumen bacteria.

Dasytricha ruminantium was cultured for 2 weeks in the presence of two species of rumen bacteria and the following components, given in percentage: 0.05 ground alfalfa, 0.005 cornstarch, 0.5 autoclaved rumen fluid, 0.005 glucose, and a mixture of ten vitamins. Cysteine hydrochloride, 0.01 percent, was added to insure reduced conditions. Numerous dividing ciliates were observed each day. Parallel control cultures which omitted the bacteria were dead at 96 hours. The bacterial strains which stimulated growth of *Dasytricha* were a small coccus, 0.8 μ in diameter, having a large mucoid colony, and a rod 1.0 × 1.5 μ in size, possessing a filamentous type colony. The larger holotrich ciliates of the genus *Isotricha* were also observed feeding on rod-shaped bacteria (*4*). It is concluded that the holotrich rumen ciliates derive at least some of their nitrogenous requirements from the ingestion of associated bacteria.

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Rapid Chemical Changes in Reconstituted Dry Milk

Dry milk is usually reconstituted into an aqueous system before use as a food, and many of the laboratory analyses of dry milk involve reconstitution with water as a step in sample preparation. Recent study of certain chemical properties of reconstituted nonfat dry milk indicated a variability in analytic results which was correlated with the age of the reconstituted samples. The purpose of this report is to illustrate some rapid chemical changes which may occur in freshly reconstituted dry milk in order to bring the phenomenon of change to the attention of those interested in the

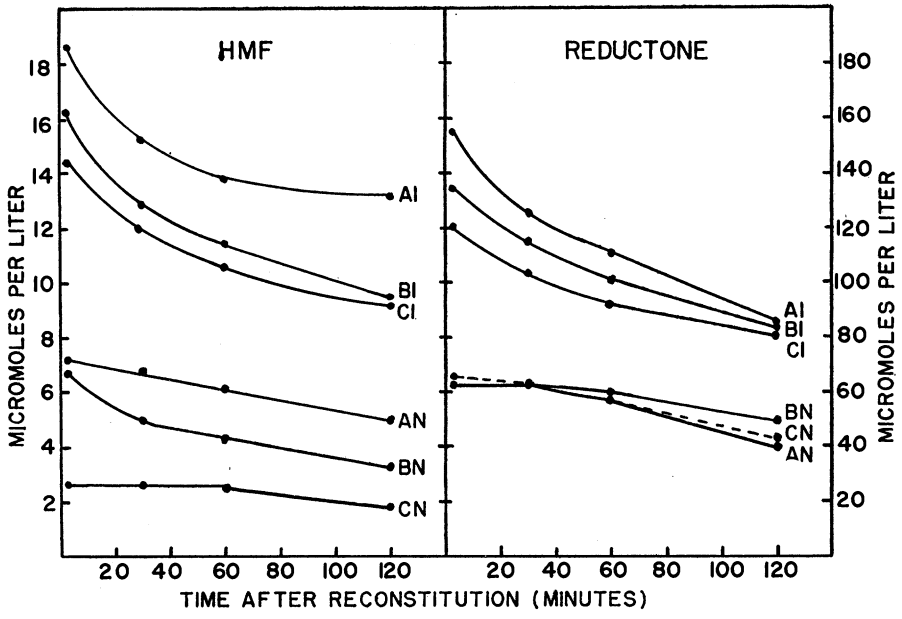

Fig. 1. Changes in certain chemical properties of reconstituted nonfat dry milk as functions of age. The letters *A, B,* and *C* designate different batches of dry milk from the same manufacturer; *I* and *N* designate the milks as instant (*6*) or noninstant products. The instant milks were prepared from the corresponding noninstant milks (for example, *AI* was prepared by instantizing *AN,* and so forth).

evaluation of either dry milk or dehydrated foods in general (*1*).

Figure 1 illustrates this effect as applied to two different chemical analyses on six samples of reconstituted nonfat dry milk. The samples were analyzed for 5-hydroxymethylfurfural (HMF) (*2*) and for indophenol-reducing substances (*3*). The 5-hydroxymethylfurfural analyses did not measure free 5-hydroxymethylfurfural in the milks but rather the amount formed under standardized digestion conditions. The quantity of 5-hydroxymethylfurfural formed under these conditions is believed to be correlated with the intensity of Maillard-type browning (*4*) reaction in the samples. The results of the indophenol titrations are expressed as molar concentration of diolreductone rather than as the conventional weight equivalent of ascorbic acid (*5*). The samples were prepared rapidly by shaking 10 g of milk powder with 100 ml of distilled water in a 250-ml erlenmeyer flask. Aliquot portions of the reconstituted samples (stored at 24°C) were removed at specified times for chemical analyses, the first samples being removed 3 minutes after the start of reconstitution.

The results shown in Fig. 1 definitely indicate that some rapid chemical changes were taking place in the freshly reconstituted instant (*6*) milks. The changes in the noninstant milks appeared to be less pronounced and more variable. Furfurals and reductones are well-known products of the browning reaction in foods containing sugar and protein (*4*). The higher levels of 5-hydroxymethylfurfural and reductones in the instant milks, as compared with their noninstant counterparts, indicate that the instantizing process used on these milks caused browning-type reactions to take place. Kumetat *et al.* (*7*) found that the ferricyanide-reducing values were generally higher in instant, as compared with noninstant, nonfat dry milks and concluded that instantizing promotes the browning reaction.

It would appear that certain characteristic products of the browning reaction accumulate in dehydrated foods and may be rapidly converted to other characteristic products upon hydration. A study of the chemistry of the browning reaction in model systems led Hodge (*4*) to the conclusion that the sugar in sugar-amine browning reactions can be dehydrated in two ways. (i) In neutral or acidic aqueous systems, furfurals are formed; (ii) in the dry state, reductones are formed. The Hodge conclusion may be an oversimplification of the situation; however, it does provide a well-defined example of the influence of water on the course of the browning reaction. Milk may be considered to be a representative sugar-protein food; therefore, the foregoing manifestations may be encountered in many other dehydrated foods.

In light of the growing market for dehydrated foods and the anticipated increase of interest in the study of manifestations of Maillard-type browning in such foods, it is suggested that the dynamic chemical state of freshly reconstituted products be carefully considered in the development of investigational procedures.

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References and Notes

1. Scientific article No. A626, contribution No. 2808, of the Maryland Agricultural Experiment Station, Dairy Department. Research was undertaken in cooperation with the Quartermaster Food and Container Institute for the Armed Forces and assigned No. 768 in the series of papers approved for publication. The views or conclusions contained in the report are those of the authors and are not to be construed as reflecting the views or endorsements of the Department of Defense.
2. A detailed report on this method of analysis is in preparation. Brief details are as follows. (i) Mix 10 ml of milk with 10 ml of 40 percent trichloracetic acid and incubate the mixture at 70°C for 25 minutes; (ii) cool and filter through Whatman No. 42 paper; (iii) add 1 ml of 0.05*M* 2-thiobarbituric acid to 4 ml of filtrate and incubate the mixture at 40°C for 50 minutes; (iv) determine absorbance at 443 mμ against blank substituting water for milk; (v) calculate 5-hydroxymethylfurfural from absorbance by reference to standard curve. The formula used on the milks was as follows: (absorbance − 0.062) × 76 = micromoles per liter of 5-hydroxymethylfurfural in milk.
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6. The recently developed instant dry milks are manufactured by the wetting of conventional spray dried milk powder with wet steam to moisture levels of 10 to 20 percent. The wet mass is then dried in hot air to approximately 3 percent moisture. The resulting product is composed of relatively large particles of low density, which disperse readily in water.
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