dry whole milk, dried cream, dried ice cream mix, and heated sterilized fluid milks, refining measures as employed by the fats and oils industry may be desirable. In any event, it seems certain that flavor defects in a number of dairy products will be inherent so long as the same form of milk fat is considered equally appropriate for all product usage.

Acknowledgment

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BROWNING REACTIONS

Mechanism of Browning of Ascorbic Acid–Citric Acid–Glycine Systems

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To determine whether Strecker degradation of amino acids occurred in the browning of orange juice, the browning of ascorbic acid in citrate-buffered solutions containing radioactive glycine was studied. Neither Schiff bases nor volatile aldehydes were detected. Less than 3% of the carbon dioxide evolved was derived from glycine-C_1; less than 0.1% from glycine-C_2. The data indicated that the Strecker degradation does not occur, in detectable degree, under the test conditions. The browning of this system, therefore, does not follow the usual pathways reported for the glucose-glycine system.

IN THE "BROWNING REACTION" in foods and, particularly, in model systems, a major position has been assigned to the Amadori rearrangement and the Strecker degradation (*8*, *13*, *14*, *20*). The particular amino acid or polypeptides and the particular carbohydrate constituents involved in these reactions have been established for a few products only recently (*3*). In model systems, glucose and glycine have been used most commonly (*6*, *14*, *21*). In 1935, Joslyn and Marsh (*16*) reported that amino acids play a minor role in the oxidative nonenzymatic browning of orange juice; this has been confirmed recently for orange juice and model systems composed of ascorbic acid and glycine and other amino acids (*9*, *15*). Occurrence of a Strecker degradation in solutions containing oxidized ascorbic acid and glycine, however, has been reported by Abderhalden (*1*, *2*) and Schönberg (*17*, *18*).

To investigate this point in more detail and to determine the possible mechanism of browning of products such as orange juice, the browning of solutions containing radioactive glycine labeled in both C-1 and C-2 positions in the presence of ascorbic acid under both oxidative and reducing conditions was investigated. The results of these studies indicate that the initial reaction is not the formation of a Schiff base between glycine and dehydroascorbic acid, as would be expected on the basis of studies of glucose-glycine systems, and that the Strecker degradation resulting in the decarboxylation and deamination of glycine occurs very slightly, if at all, under the conditions used. Formaldehyde was not accumulated in detectable amounts and only part of the carbon dioxide liberated originated from the carbonyl group of glycine.

Experimental

The browning of ascorbic acid and glycine in citric acid–potassium citrate buffers at pH 3.7 and 7, under nitrogen and oxygen, was followed at 37° and 50° C. The citric acid buffer was prepared by dissolving 10.5 grams of C.P. citric acid hydrate in distilled water, adjusting to pH 3.7 or 7 by addition of C.P. potassium hydroxide pellets, and making up to 500 ml. A stock solution of ascorbic acid was prepared by dissolving 1.1 grams of Merck L-ascorbic acid in 100 ml. of buffer just prior to use. The stock glycine solution was prepared by dissolving 0.563 gram of glycine in 100 ml. of buffer prior to use. Glycine-

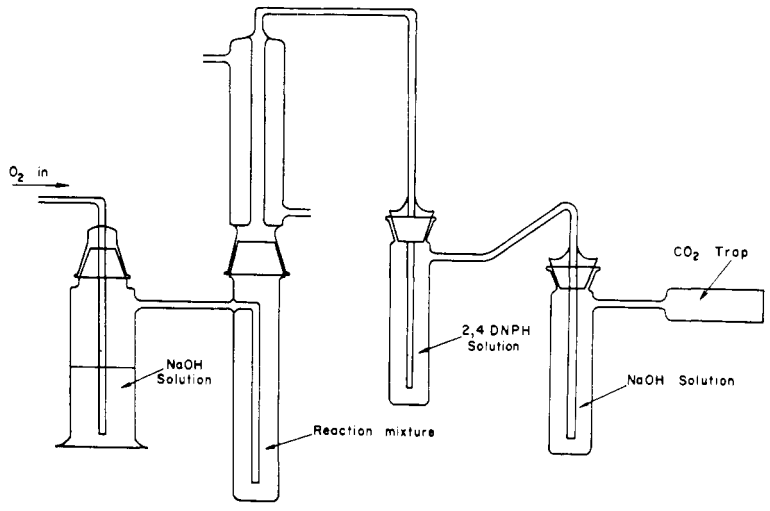

Figure 1. Absorption train

1-C^{14} (Tracerlab, 17.9 mc. per mg.), glycine-2-C^{14} (Tracerlab, 38.1 mc. per mg.), and inactive Eastman Kodak glycine were used. The chemicals were not purified to remove trace elements, although it was recognized that the presence of iron, copper, and other impurities might affect the reaction (*5*). In the nonradioactive mixtures, ascorbic acid, glycine, and citric acid buffer were mixed in the volumetric ratio, 4 : 4 : 2, to give a solution 0.25 m*M* in ascorbic acid, 0.3 m*M* in glycine, and 1.1 m*M* in citric acid. In the radioactive mixtures, 0.5 to 1.0 mg. of radioactive glycine was weighed out and added to 4 ml. of ascorbic acid solution and 3.8 to 3.9 ml. of inactive glycine solution were added to make the total amount of glycine 22.5 mg. The volume was then brought to 10 ml. with buffer solution. The volume of the active mixture was 10 ml. in all experiments, but both 10 and 50 ml. of the inactive mixture were used.

Reaction vessels were of about 70-ml. capacity, provided with side arms extending nearly to the bottom. During the experiment, oxygen or nitrogen was allowed to bubble through the reaction mixture. The oxygen was first washed by bubbling through sodium hydroxide solution; nitrogen was washed by passing first through concentrated sodium hydroxide solution and then through a saturated alkaline solution of pyrogallol. Reflux condensers were connected to the reaction vessels to prevent loss of water during incubation. The reaction vessels were maintained at 37° or 50° ± 0.5° C. by immersion in a thermostatically controlled water bath.

The effluent gases from the reaction vessels, after passing through the reflux condenser, were bubbled into a sodium hydroxide trap for carbon dioxide collection. In experiments designed to detect volatile aldehydes, a second trap was added to the train, containing either a saturated solution of 2,4-dinitrophenylhydrazine in 2*N* hydrochloric acid or a 1% dimedone solution in 80% ethyl alcohol. The reaction vessels and absorption train used are shown in Figure 1. The reaction was allowed to proceed for 2 to 3 days at 50° C. and about 7 days at 37° C.

Three-milliliter aliquots were withdrawn at intervals from the solution containing inactive glycine for color measurement and a 5-ml. aliquot from the sodium hydroxide trap for carbon dioxide determinations. When carbon dioxide evolution was low, the train with radioactive glycine was not sampled; otherwise, the sodium hydroxide trap was changed and the absorbed carbon dioxide was precipitated as barium carbonate for activity measurements.

Analyses. The absorbance of the solution containing inactive glycine was measured in a Beckman Model DU Spectrophotometer at 440 mμ, with water as a blank.

The carbon dioxide evolved was estimated by addition of barium chloride (10% solution) to the sample taken and titration of the excess alkali with 0.1*N* hydrochloric acid, with phenolphthalein as an indicator.

The carbon dioxide in the sodium hydroxide trap of the apparatus containing the radioactive mixture was precipitated as barium carbonate by addition of barium chloride. The excess alkali was neutralized with 0.1*N* hydrochloric acid and the precipitate after centrifugation was washed two or three times with distilled water and then finally with absolute alcohol. The alcohol was decanted and a portion of the precipitate plated on a previously weighed 1-inch copper plate. The activity of the barium carbonate sample was determined in a gas-flow counting chamber; a Tracerlab Autoscaler was used to total the counts. The activity measured was corrected for self-absorption and backscattering to give specific activities.

The activity of the reaction mixture was determined in triplicate at the beginning and end of each experiment. About 0.2 ml. of reaction mixture was evaporated to dryness under reduced pressure and oxidized to carbon dioxide in a Fischer microcombustion train. The carbon dioxide absorbed in sodium hydroxide solution was precipitated with barium chloride and its activity determined as above.

The reaction mixture at the beginning and end of the experiments was chromatographed on Whatman No. 1 filter paper, to determine the qualitative changes in ascorbic acid. Aliquots of 20 to 30 μl. were chromatographed one-dimensionally in butanol–acetic acid–water solvent (4 : 1 : 5) and duplicate chromatograms were sprayed with 2,6-dichlorophenolindophenol and silver nitrate solution (*4*). The chromatograms for glycine were developed two-dimensionally, first with butanol–acetic acid–water and in the other direction with water-saturated phenol and sprayed with ninhydrin.

In addition, autoradiographs were prepared by applying about 60-ml. aliquots to chromatographic papers and developing two-dimensionally for amino acids. The papers were then placed in contact with 14 × 17 inch x-ray film in exposure holders and exposed for approximately 1 month. The film was then developed with x-ray developer solutions, fixed, and washed in water.

When 2,4-dinitrophenylhydrazine was used in the trapping system, the sintered disk, through which gas entered the trap, became slightly colored but no precipitate was formed in the vessel itself. The trap in the system containing the radioactive materials was monitored with a Geiger counter to detect any activity on the sintered disk. When dimedone was used as a trap, the alcohol was evaporated and the residue dissolved in a small amount of 80% alcohol to which water was added (*1*, *2*). The solution was allowed to stand overnight and a few crystals were obtained. These, when obtained from the active train, were tested for activity with a Geiger counter.

Results

Color Formation. Initially, in the nonradioactive mixtures, pigment production and carbon dioxide evolution proceeded at a slower rate in 50-ml. samples than in the corresponding 10-ml. radioactive samples. This occurred also when 50- and 10-ml. samples of the same solutions were run simultaneously. The reaction volume of the nonradioactive mixtures therefore was reduced to

three 10-ml. portions in subsequent experiments. Even then, pigmentation was not always equivalent. The rate of color development in selected runs is shown in Figure 2; the rate of pigment production is greater at pH 7 than 3.7 and is considerably greater at 50° than 37° C. The rate of browning in the absence of oxygen is slow but is appreciably faster at pH 7 than 3.7.

The intensity of browning under aerobic conditions increases to a maximum and then decreases. No precipitation occurred in the reaction. The more rapid the initial pigment production, the more definite was this decrease.

Carbon Dioxide Production. The rate of carbon dioxide production (Figure 3) increased rapidly at first and then decreased. It was greater at pH 7 than 3.7 and higher at 50° than 37° C. Carbon dioxide evolution, however, paralleled browning only at 37° and not at 50° C. This is shown in Figure 4, in which the data are replotted. Carbon dioxide production increased regularly with absorbance at 37° but not at 50° C. At 50° C. the carbon dioxide production initially was more rapid than the increase in browning and then was less rapid until the maximum of browning, after which it again was more rapid.

Formaldehyde Production. According to Abderhalden (*1*, *2*), formaldehyde should have been produced in the reaction studied. However, it could not be detected in any of the traps used, even when the concentration of the reactants was increased thirty fold. The only differences between the test conditions of Abderhalden and those reported here were the presence of higher concentrations of citric acid buffer and lower ratios of glycine to ascorbic acid. Under the conditions used, formaldehyde, if formed, was not released from the reaction mixture. Formaldehyde is a reactive compound, a known inhibitor for the browning reactions, and accordingly it may have reacted with some of the components present in the reaction mixture and thus never entered the trapping system.

Activities. If all of the active carbon were evolved as carbon dioxide during the browning reaction, the activity of the barium carbonate in the traps would be equal to the specific activity of the active carbon in the reaction mixtures. The measured activity and the calculated percentage values for the carbon dioxide produced are given in Tables I and II. The carbon dioxide from carboxyl-labeled glycine was, with one exception, less than 2%; from the methylene carbon of glycine, less than 0.1%. These values show that glycine does not contribute appreciably to the carbon dioxide evolved in the reaction studied. The activities of the reaction mixtures at the end of the experiments were also determined (Table III).

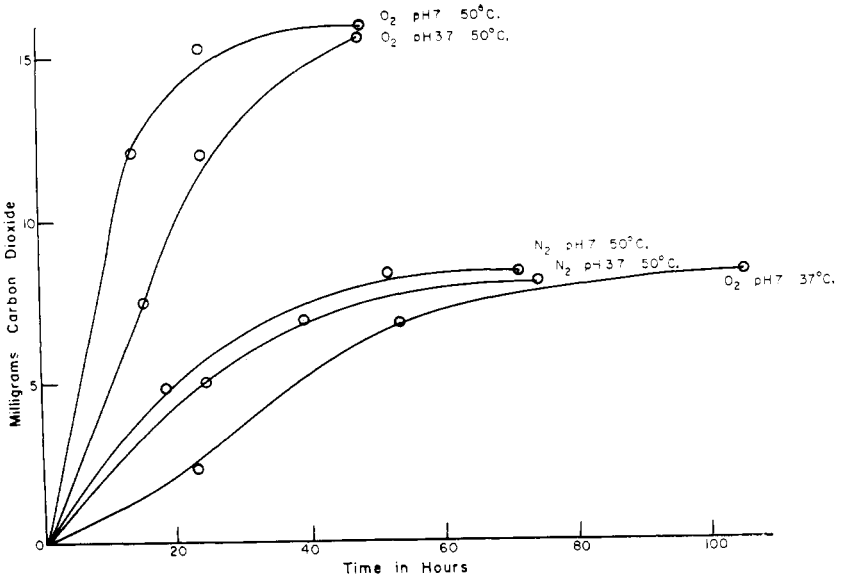

Figure 2. Rate of increase in absorbance

Table I. Measured and Specific Activities of Initial Reaction Mixtures

(Expressed as counts per minute per mg. of barium carbonate)

Expt. No.	Atmosphere	pH	Temp., ° C.	Glycine	Measured Activity (Av.)	Specific Activity of Active Carbon
1	N_2	7	50	1-C^{14}	1160	34,706
2	N_2	3.7	50	1-C^{14}	3033	89,206
3	O_2	7	50	1-C^{14}	1030	30,294
4	O_2	3.7	50	1-C^{14}	3660	107,941
5	N_2	7	50	2-C^{14}	3212	94,470
6	N_2	3.7	50	2-C^{14}	2100	61,765
7	O_2	7	50	2-C^{14}	4895	144,000
8	O_2	3.7	50	2-C^{14}	4782	140,647
9	O_2	7	37	2-C^{14}	5588	164,353

Table II. Activity of Carbon Dioxide in Relation to Source

(Carbon dioxide originating from C-1 or C-2 labeled glycine at different periods during browning reaction)

Expt. No.	Period[a]	Measured Activity	Labeled Carbon	% CO_2 Derived from Labeled Carbon
1	0–48	429	1	1.2
2	0–48	784	1	0.9
3	0–11	274	1	0.9
3	11–24	342	1	1.1
3	24–48	940[b]	1	3.1
4	0–19	1486	1	1.4
4	19–32	1167	1	1.1
4	32–48	1665	1	1.5
5	0–72	13	2	0.01
6	0–71	1	2	0.002
7	0–13	200	2	0.1
7	13–24	50	2	0.04
7	24–48	175	2	0.1
8	0–16	36	2	0.03
8	16–48	44	2	0.03
9	0–108	109	2	0.07

[a] As each experiment included a separate trap, values represent average activities for carbon dioxide formed while trap was in use—e.g., 11–24 means that trap was replaced after 11 hours and removed 24 hours after incubation started.

[b] During this period carbon dioxide evolution was minimal and amount of barium carbonate obtained, 1.2 mg., was too small to allow accurate measurements; the value, 940 counts, is probably too high.

Table III. Measured Activities of Reaction Mixtures at Beginning and End of Experiments

Expt. No.	Measured Activity, Counts/Min./Mg. Barium Carbonate			
	At Beginning		At End	
1	1173	1153	1174	1121
2	3312	2751	3283	2017
3	968	1098	1135	1278
4	3619	3706	3572	3740
5	3165	3258	2977	3060
6	1999	2207	2143	2350
7	4733	5056	6626	7237
8	4681	4884	4605	4557
9	4895	5282	4491	5192

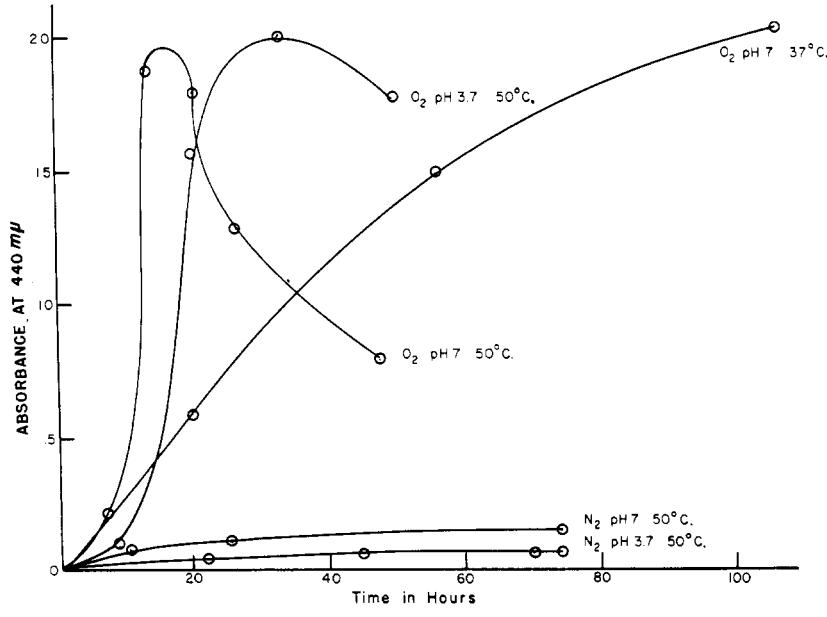

Figure 3. Carbon dioxide production relative to browning

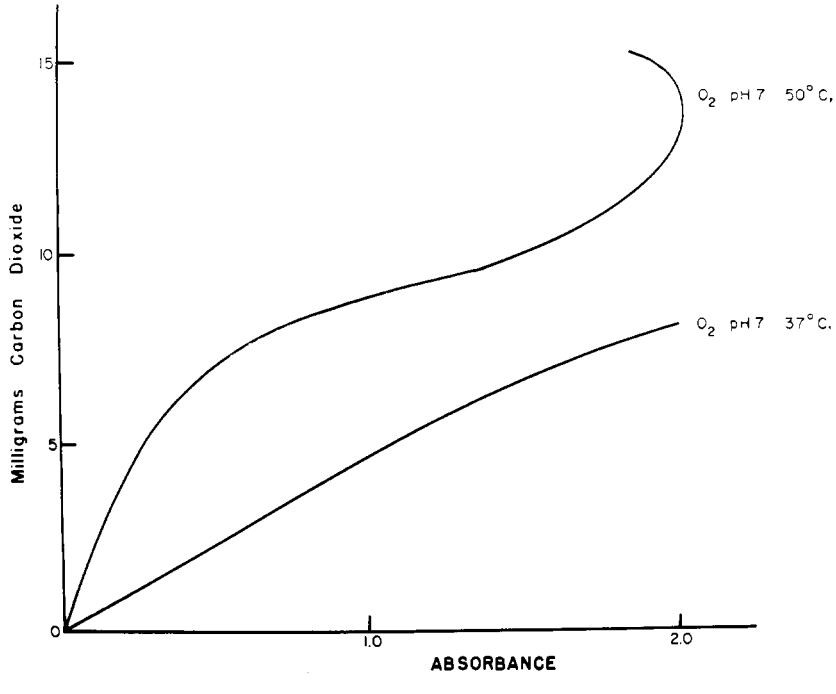

Figure 4. Rate of carbon dioxide production

Chromatograms and Autoradiograms. The chromatographic studies indicated that in nitrogen, even at the end of the experiments, reduced ascorbic acid was still present, as development with either 2,6 - dichlorophenolindophenol or silver nitrate showed. At the end of the experiments, conducted in an oxygen atmosphere, ascorbic acid was not found, but dehydroascorbic acid was observed in small amount. In each case when two-dimensional chromatograms were sprayed with ninhydrin only a glycine spot was observed.

On short exposures, the autoradiographs showed only the presence of glycine in the reaction mixtures at the end of the incubation. Upon longer exposures the autoradiographs indicated appreciable incorporation of the glycine methylene carbon into pigments, and at least three nonvolatile compounds containing the methylene carbon of glycine were found. Their total activity was about 5% of the activity of the mixture. The experiment using glycine 1-C^{14} showed very little labeling in anything except the original glycine.

Discussion

In the browning reaction between sugars and amino acids, Strecker degradation of the amino acid is the main source of carbon dioxide (*6*, *21*). This is not the case in the browning reactions studied. Less than 3% of the carbon dioxide originates from the carboxyl group of glycine. The methylene group of the glycine does not contribute to the carbon dioxide. The source of the carbon dioxide is, therefore, one of the other constituents of the mixture. Curl (*7*) found that addition of ascorbic acid to orange juice increased the gas produced considerably. Euler and Hasselquist (*10*, *11*) reported that 2,3-diketogulonic acid, formed from dehydroascorbic acid upon standing in aqueous solutions, evolves carbon dioxide when heated. Hasselquist (*12*) also isolated a trianilino compound of diketogulonic acid and found that it has the same tendency to give off carbon dioxide. Proctor (*20*, page 351) found that at 60° C. from 5 ml. of 50% citric acid containing 0.5 gram of ascorbic acid, the molecular ratio of the carbon dioxide evolved to the ascorbic acid lost was about 0.74. If less ascorbic acid was used, less carbon dioxide was produced. On the other hand, when citric acid concentration was increased over the range of 1 to 50%, the carbon dioxide production was increased. In the present study 10 ml. of reaction mixture contained 44 mg. of ascorbic acid. This amount would give rise to 11 mg. of carbon dioxide by decarboxylation of the diketogulonic acid. More carbon dioxide was produced (Figure 2) under

aerobic conditions. Thus decarboxylation of ascorbic acid or related compounds cannot be the only source of the carbon dioxide production. The carbon dioxide production under anaerobic conditions was higher than would be expected from the pigment production, if these two were related. After 48 hours under anaerobic conditions, the mixtures still contained appreciable amounts of ascorbic acid.

The absorbance of the solution after it first reached a maximum decreased in experiments conducted at 50° C. under aerobic conditions. Reactions in the other experiments proceeded so slowly that no maximum was reached during the time the reaction was followed. Seaver and Kertesz (*19*) found maximum color production when ascorbic acid was heated in the presence of glycine and at times when ascorbic acid was heated alone. The reason for this is not known. Joslyn (*15*) more recently found that the concentration of ascorbic acid in browning systems determined whether or not the color production went through a maximum. At low concentrations of ascorbic acid the color increased continuously with time. The same relation was observed at high concentrations of ascorbic acid, but at intermediate levels the color production went through a maximum.

In sugar-glycine browning reaction considerable amounts of carboxyl carbon from glycine become associated with the brown pigment produced. This was not the case in the ascorbic acid–glycine–citric acid browning reaction studied. No carboxyl carbon was found associated with the pigment, and in the experiments with 1-C^{14} glycine very little labeling was found in anything except the original glycine. Most of the methylene carbon of glycine also remains in the original compound, although it was detected in at least three other compounds; of these the major one was the pigment. Formaldehyde production could not be detected, but as some carbon dioxide was derived from glycine the glycine was apparently degraded to a small degree.

The relation between the amount of carbon dioxide produced and the pigment production differs markedly at 37° and 50° C. This is in agreement with the findings of Joslyn that a change in reaction mechanism may occur in ascorbic acid systems between 30° and 50° C. (*15*). At lower temperatures the carbon dioxide and pigment production are linearly related. At higher temperatures a more complex relationship exists. Pigment may be formed at higher temperatures by a separate mechanism or course of reaction than at the lower temperatures, or a second pigment-producing reaction may be activated at the higher temperatures. This striking difference is shown in Figure 4.

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FOOD EMULSIFIERS

Effect of Nonionic Emulsifiers on Experimental Dietary Injury of the Liver in Rats

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The intestinal flora may influence experimental and clinical injury of the liver. Because it has been claimed that emulsifiers may change the intestinal flora, their effect on experimental hepatic injury was studied. Emulsifiers of the polyoxyethylene series, and monoglycerides in doses of 1% of total food intake, had no influence on development of experimental hepatic necrosis. Even in doses up to 10% no effect was noticed on experimental cirrhosis of the liver. Some emulsifiers in doses of 5 and 10% slightly retarded production of experimental hepatic necrosis. It is improbable that in the doses used in practice these emulsifiers have a deleterious effect on the liver.

SOME NONIONIC FOOD EMULSIFIERS, after prolonged ingestion, may produce changes in the intestinal flora (*14, 16*). Reduction in the numbers of the intestinal flora of rats has been reported after feeding of high levels (25%) of sorbitan and polyoxyethylene sorbitan esters of lauric, stearic, and oleic acids and of polyoxyethylene esters of stearic acids. This effect was thought to be causally related to the reduced growth rates of the experimental animals (*4*). On the other hand, Ely (*8*) reported that several surfactants given in small doses stimulated growth of chicks in 10- to 12-