care, if diffusion is to be avoided.

This note describes a method to separate fractions in the single, large middle section of the Tiselius cell without extra equipment nor danger of diffusion. For example, fresh serum was diluted 1:2.5 with buffer and dialyzed one day in 2 liters of the same buffer. Electrophoresis was carried out in the usual way until the main components were separated almost completely from each other over two-thirds of the length of the channel. The middle section was moved to one side. The glass plug on the side arm of the "open" electrode vessel was removed, and all the buffer above the cell and from part of the main vessel was siphoned off. The middle section was slid back in place again; no movement of solution occurred because of the "closed" electrode vessel. The 3-way stopcock on the side arm of the "closed" electrode vessel was turned to permit continuity between the vessel and compensator syringe. The compensator motor was started and the topmost component was pushed slowly into the empty side arm of the "open" electrode vessel. The progress of this movement was followed easily by direct observation on the ground glass plate. When this first component was just completely above the glass flanges of the cell, the motor was stopped and the middle section was moved out of alignment. A long needle on a syringe was used to remove the freed component. After rinsing the side arm, the middle section was realigned, and each of the components was separated in the same manner.

The advantages of the above technic include: (1) simplicity and rapidity; (2) elimination of special extra equipment; (3) avoidance of dilution beyond that which has occurred from the migration of the respective component; (4) minimizes the danger of diffusion between components. It is true that a small amount of overlapping occurs between fractions but this factor may be eliminated by combining the respective components from several runs of whole material, followed by electrophoresis in the same manner of each of the components separately. The small amount of faster and slower moving material traveling with the main component can now be "cut" off without much difficulty.

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Influence of Incubation at 37° C on Stability of Thiamin and Riboflavin in Cow's Milk.*

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Our recent finding[1] that animal tissues destroy appreciable amounts of thiamin and riboflavin during incubation at 37°C for 24 to 48 hours suggested a study of the stability of these vitamins in cow's milk under similar treatment. Pasteurized cow's milk, obtained from the University Creamery, was used throughout this investigation. Similar results, however, were obtained with raw untreated milk.

The experiments were carried out with 20 cc samples of milk, which contained 4.95 to 7.7 μg thiamin and 29 to 32 μg riboflavin. In the first experiment, 4 20 cc samples of cow's milk, to which have been added 0 μg, 10 μg, 100 μg and 1,000 μg of thiamin and equal doses of riboflavin, were incubated at 37°C for 48 hours. It will be noted from Table I that there was considerable destruction of thiamin in cow's milk during this

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[1] Sure, B., and Ford, Z. W., *J. Nutr.*, 1943, in press.

TABLE I.
Influence of Incubation at 37°C for 48 Hours on the Stability of Thiamin and Riboflavin in 20 cc Samples of Cow's Milk.

Thiamin added, μg	Thiamin incubated, μg	Thiamin recovered, μg	% thiamin destroyed	Riboflavin added, μg	Riboflavin incubated, μg	Riboflavin recovered, μg	% riboflavin destroyed
—	7.7	2.69	65.0	—	31.67	30.85	2.6
10	17.7	7.20	59.3	10	41.67	40.83	2.0
100	117.7	82.0	30.5	100	131.67	125.25	4.9
1,000	1,017.7	690.0	32.2	1,000	1,031.67	1,032.50	0.0

TABLE II.
Influence of Previous Heat Treatment Followed by Autoclaving for 20 Minutes at 15 lbs. Pressure on Stability of Thiamin and Riboflavin to Incubation at 37° C for 22 Hours. Samples 20 cc of Milk.

Treatment	Thiamin incubated, μg	Thiamin recovered, μg	% thiamin destroyed	Riboflavin incubated, μg	Riboflavin recovered, μg	% riboflavin destroyed
Incubated	4.95	2.70	45.5	27.50	27.50	0.0
Heated to boiling and then incubated and autoclaved		2.67	46.0		27.50	0.0
Boiled 10 min. and then incubated and autoclaved		2.62	47.0		27.50	0.0
Boiled 20 min. and then incubated and autoclaved		1.23	75.7		25.00	9.1

period of incubation but that only negligible amounts of riboflavin were destroyed. These results suggested the presence in cow's milk of a factor or factors that destroyed thiamin during incubation at body temperature for a period of 2 days.

In order to determine the heat stability of such factor or factors, another series of experiments were carried out, in which the milk was brought to a boil, boiled for 10 minutes, and for 20 minutes before proceeding with analyses.

Since incubation curdled the milk, it was necessary to extract each sample and incubate with takadiastase before completing the thiamin determinations. In the first experiment (Table I) 75 cc of N/10 HCl were added to each sample, autoclaved for 20 minutes at 15 lbs pressure, incubated for 2 hours and analyzed according to the procedure of Hennessy and Cerecedo,[2] using the Merck and Co. modifications.[3] In the second experiment (Table II) the milk samples were given the same treatment but, in addition, were subjected to boiling temperature for various periods previous to autoclaving and incubation. From these results it is evident that incubation of cow's milk, containing about 5 μg thiamin and 28 μg riboflavin, at 37°C for 22 hours results in approximately 46% destruction of thiamin but that riboflavin is 100% stable under such treatment; also boiling for 10 minutes produced no change. Boiling for 20 minutes, however, produced an additional 30% destruction. From these results it would appear that the factor or factors in cow's milk responsible for thiamin destruction at 37°C is heat stable, and, therefore, probably non-enzymic in nature. On the other hand, the increased destruction of thiamin following 20 minutes of boiling is due to instability of this vitamin to heat treatment at 100°C. The latter procedure also produced small destruction of riboflavin.

Allowing 20 cc cow's milk, containing 6 μg thiamin, to stand at room temperature of 22°C for one week, produced only 6% destruction of thiamin. Allowing duplicate samples of milk to stand in a room at a tem-

[2] Hennessy, D. J., and Cerecedo, L. R., *J. Am. Chem. Soc.*, 1939, **61**, 179.

[3] Merck and Company, Inc., *Determination of Thiamin Hydrochloride by the Thiochrome Method, with the Adaptation of the Hennessy and Cerecedo Procedure*, revised June 6, 1941.

TABLE III.

Influence of Ash of Milk and pH on Stability of Thiamin to Incubation at 37°C for 22 Hours.

Thiamin, μg	Treatment	Thiamin recovered, μg	% thiamin destroyed
6	In 20 cc water, pH 5	5.73	4.5
6	In 20 cc water + ash of 20 cc cow's milk	4.93	17.83
6	In 20 cc water, pH 6	5.60	6.67
6	In 20 cc water, pH 6, + ash of 20 cc cow's milk	4.87	18.83
6	In 20 cc water, pH 7	5.33	11.17
6	In 20 cc water, pH 7, + ash of 20 cc cow's milk	4.67	22.17

perature of 31°C resulted in 16% destruction. Since incubation at 37°C for 22 hours produced 46% destruction, the critical temperature of thiamin destruction in cow's milk seems to be between 31 and 37°C.

The next point of interest that seemed worthy of investigation was to determine what contribution, if any, the minerals of milk made towards the thiamin destruction at body temperature. The results of such trials are presented in Table III. It will be noted there is only a slight destruction of thiamin in aqueous solution which increases with the increase of the pH from 5 to 7. However, the ash of milk *per se* produced a destruction of 11 to 12% thiamin, regardless of the pH. In other words, the minerals of milk are responsible for about 25% of the total thiamin destruction; therefore, the character of the factor or factors which are contributory to 75% of the destruction must be organic in character and of a nature that resist heat treatment at 100°C for 10 minutes.

Summary. Incubating cow's milk for 22 hours at 37°C produces 46% destruction of thiamin but riboflavin is entirely stable under such treatment. The critical temperature for thiamin destruction appears to be between 31 and 37°C. The factors in cow's milk responsible for thiamin destruction are stable to 100°C for 10 minutes. The minerals of cow's milk are contributory to 25% of the thiamin destroyed.

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Effect of Certain Organic Compounds on Germicidal Efficiency of Mercuric Chloride.

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In practice disinfectants are commonly used in the presence of organic matter. The value of the results obtained by any laboratory procedure may justly be questioned if the comparisons are made in the absence of organic matter. Despite the significance of this fact, which was realized and emphasized by Chick and Martin,[1] most tests for rating germicides are still performed in the absence of organic compounds.

It is well known that the type of organic matter employed may make a big difference in the final results obtained. Some compounds are without effect whereas others influence markedly the efficiency of germicides. Unless a standard organic solution or compound is incorporated in the procedure employed for the evaluation of germicides the final results will give misleading comparisons.

The present investigation was undertaken to determine which groups of organic compounds are effective in reducing the germicidal efficiency of mercuric chloride. Definite amounts of gelatin, peptone, a number of amino acids, and several carbohydrates were employed. The concentration of mercuric chloride required to kill *Staphylococcus aureus* in the presence of definite amounts of

[1] Chick, H., and Martin, C. J., *J. Hyg.*, 1908, **8**, 654.