STUDIES ON THE EFFECTS OF TRANSPORT AND STORAGE ON THE BACTERIOLOGICAL QUALITY OF RAW MILK.

Part 1.—The Reduction of Methylene Blue by Raw Milk as Influenced by Time and Temperature of Storage.

By V. R. SMYTHE, B.Sc.Agr., Assistant Dairy Technologist, Dairy Research Laboratory, Division of Dairying.

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SUMMARY.

Certain aspects of Brisbane's milk supply are discussed and trends of air temperature and raw milk quality are illustrated graphically, using data obtained by testing 95,000 milk samples over a period of three years.

Methylene blue reduction tests were performed on samples of raw milk collected from 20 farms in the Brisbane milk district and incubated for various periods of time up to five hours at temperatures ranging from 5 deg. C. to 35 deg. C.

The criterion adopted for deterioration in milk quality was the fall in reduction time, which is represented by the difference between the reduction time prior to incubation and the reduction time after the application of any given treatment.

The fall in reduction time produced by a rise in temperature from 20 deg. C. to 35 deg. C. was very marked, but was not strictly linear, the steepest fall occurring when the temperature rose from 25 deg. C. to 30 deg. C. Incubation at temperatures below 20 deg. C. caused an increase in the reduction time which was noticeable immediately after the initial storage period of 30 minutes. This evidently results from the sudden lowering in temperature at the commencement of incubation, and the effect appears to be independent of the amount of temperature change.

At most temperatures an increase in storage time up to 5 hours produced marked trends, which appeared to be strictly linear. The exception occurred at 10 deg. C., at which temperature no effect resulting from the period of storage was shown. At 5 deg. C. increase in the duration of storage produced a progressive increase in the reduction time. All temperatures above 10 deg. C. resulted in a marked fall in the reduction time as the storage period increased. Gradients of deterioration have been calculated for all seven temperatures employed.

The interaction of the time factor and the reduction trends due to temperature change was positive, marked and almost linear, as was also the reciprocal action of temperature on the trends resulting from time.

As the storage temperature increased, the rate of deterioration (fall in reduction time) was greater in good quality milks than in milks of inferior quality. Furthermore, this rate of deterioration with temperature increased as storage time was prolonged and such an added deterioration was greater when milk quality was high. No correlation was obtained between the initial quality of a milk and the effect of storage time.

The shade air temperature of the day of sampling was found to exert no influence on the observed trends in the methylene blue reduction time.

INTRODUCTION.

During the three-year period 1942-1944 the raw milk supplied to Brisbane milk depots for the chilled and pasteurized milk trades was subjected to a series of quality tests in accordance with an extensive system of control. The summarized results of 95,000 tests conducted during the testing period, expressed as a percentage of all methylene blue reduction times below the 4-hour standard and plotted in Figure 1, show that a marked deterioration of quality occurs

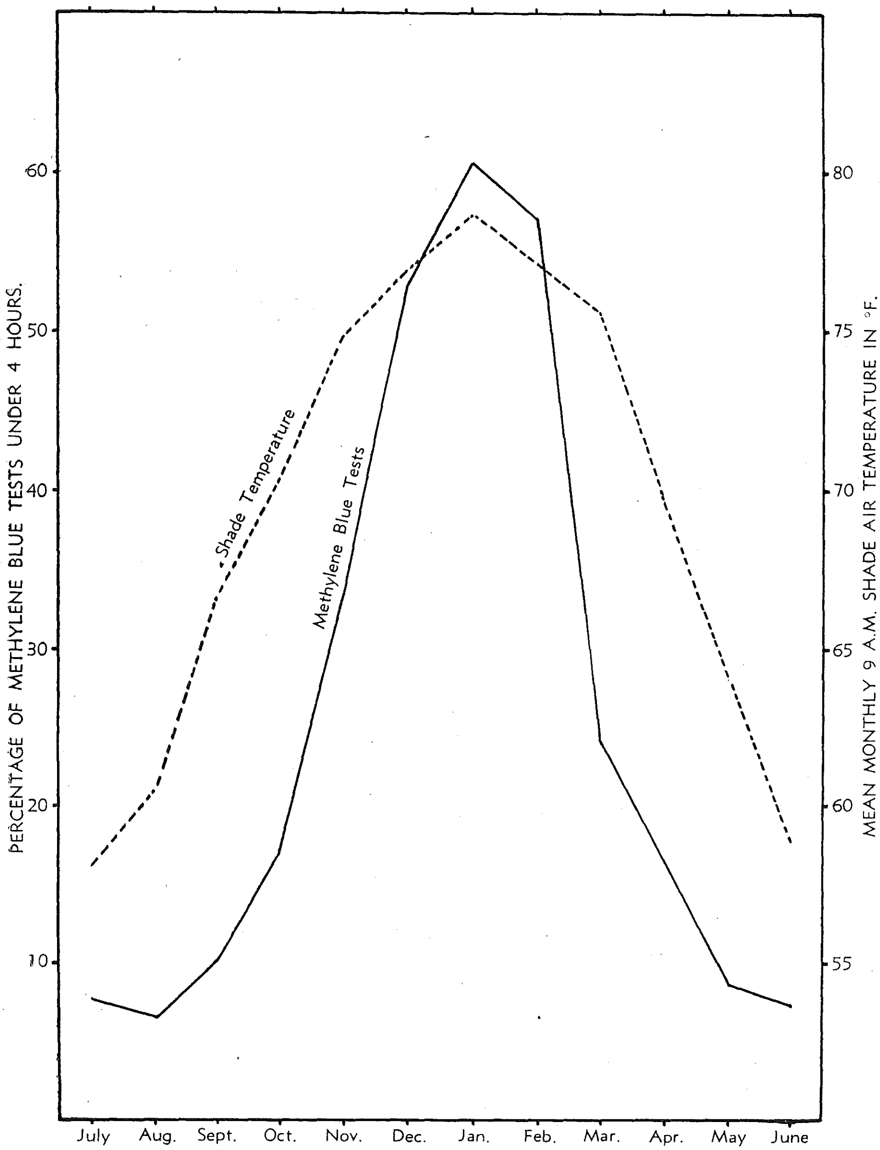

Figure 1.

Showing trends in shade temperature and raw milk quality for the period 1942-1944.

during the summer months. The pronounced rise in the percentage of low-quality milks from October to January is too great to be explained adequately in terms of increased farm contamination such as might take place as a result of increased bacterial development on improperly cleansed and imperfectly sterilized utensils at the higher temperatures prevailing. The most probable explanation of the variation in milk quality is that a considerable temperature effect operates during transport and storage of the milk following its production. This is supported by the manner in which the line representing milk quality in Figure 1 follows that representing the mean shade air temperature at 9 a.m., at which time of the day the greater part of the milk supply is in transit.

While the seasonal effect demonstrated was expected and was considered to be attributable largely to air temperature or to some factor closely dependent on this, it was obvious early in the investigation that various conditions resulting from the war accentuated changes in milk quality. An attempt was therefore made to determine to what extent these factors influence milk quality. The main factors may be stated as time and temperature of transport and time and temperature of storage at the milk depots prior to handling. Transport of raw milk in the area serving Brisbane has never been highly organized nor very efficient, and during the war years increased loads, slower trips, and delays at depots have lowered efficiency further. The increasing demand for fluid milk, and particularly for pasteurized milk, has considerably over-taxed factory equipment: in the case of some milk-pasteurizing plants the quantity of milk received has been doubled without any appreciable change in facilities for handling the supply. This has resulted in a prolonged daily receiving period, during which some milk has of necessity been stored at atmospheric temperature awaiting treatment. Procter (1943, a and b) and Rowlands and Provan, as quoted by Barkworth (1944), have commented on a deterioration of milk quality in Great Britain caused by adverse transport conditions brought about by war.

It is the purpose of this series of papers to present the results of studies on the deterioration in the quality of raw milk which occurs during transit and storage in the Brisbane area, on the effect of the milk container on milk quality, and on the changes in pasteurizability of raw milk brought about by transport and storage conditions.

Part 1.—The Reduction of Methylene Blue by Raw Milk as Influenced by Time and Temperature of Storage.

GENERAL CONSIDERATIONS.

The greater part of the raw milk delivered to Brisbane for pasteurizing and chilling is derived from the morning milking. During the winter and spring months, when production is low, some milk from the evening milking is also drawn upon, but the proportion of evening milk decreases steadily as the production increases in response to the summer rains, with the result that morning loads become progressively heavier, causing protracted delays in handling and transit. Such delays, occurring as they do during summer, coincide with high air temperatures. Furthermore, these delays mean that milk is held under a steadily increasing air temperature from hour to hour through the summer mornings. The temperature trends for each of the seasons of the year are shown in Figure 2, which has been prepared from the mean-hourly-shade-air-temperatures recorded for Brisbane for the months July, 1943, to June, 1944.

Not all milk supplies are cooled before leaving the farms, and where coolers are in use the cooling process consists merely of removing the body heat by means of water which is itself at or only slightly below atmospheric temperature. It appears from observations made that without refrigeration milk temperatures below 70 deg. F. cannot be reached during summer months. Since refrigerated transport is as yet unavailable there exists no protection for the milk against the effect of high temperatures, and during the summer months it is exceedingly common for milks to exhibit a temperature between 80 deg. F. and 90 deg. F. on arrival at the depots.

There is some evidence that the milk grading system in operation at present has been attended by considerable unfairness to milk producers. Milking is completed on practically all farms between the hours of 6 a.m. and 8 a.m., but the arrival times at depots are staggered to facilitate handling. This procedure means, in effect, that producers whose milk is handled at the depots by 9 a.m. usually obtain a satisfactory methylene blue reduction time for their article much more readily than producers whose milk is sampled several hours later. It is intended to use the information obtained in this experimental work as an aid in evolving a system of milk quality control which will obviate unfairness.

The data presented here were obtained from a study of the effects of time and temperature of storage on the methylene blue reduction time of fresh raw milk. Most attention has been paid to storage temperatures from 20 deg. C. (68 deg. F.) to 35 deg. C. (95 deg. F.) and results obtained over this temperature range have been treated separately from results obtained at lower temperatures. This has been considered desirable in view of the fact that milk quality suffers most during summer, when high temperatures of transport and storage prevail.

EFFECTS OF STORAGE TIME AND TEMPERATURE WITHIN THE TEMPERATURE RANGE 20 DEG. C.–35 DEG. C.

Some work has been published overseas showing the effect of moderate temperatures on the reduction time of raw milk, but the information is far from being complete. Newton (1942) observed the reduction time of raw milk to deteriorate markedly during summer months and particularly during long periods of transport, but no details of temperature are given. Nichols and Edwards (1936) found that an increase in temperature of 4 deg. F., within

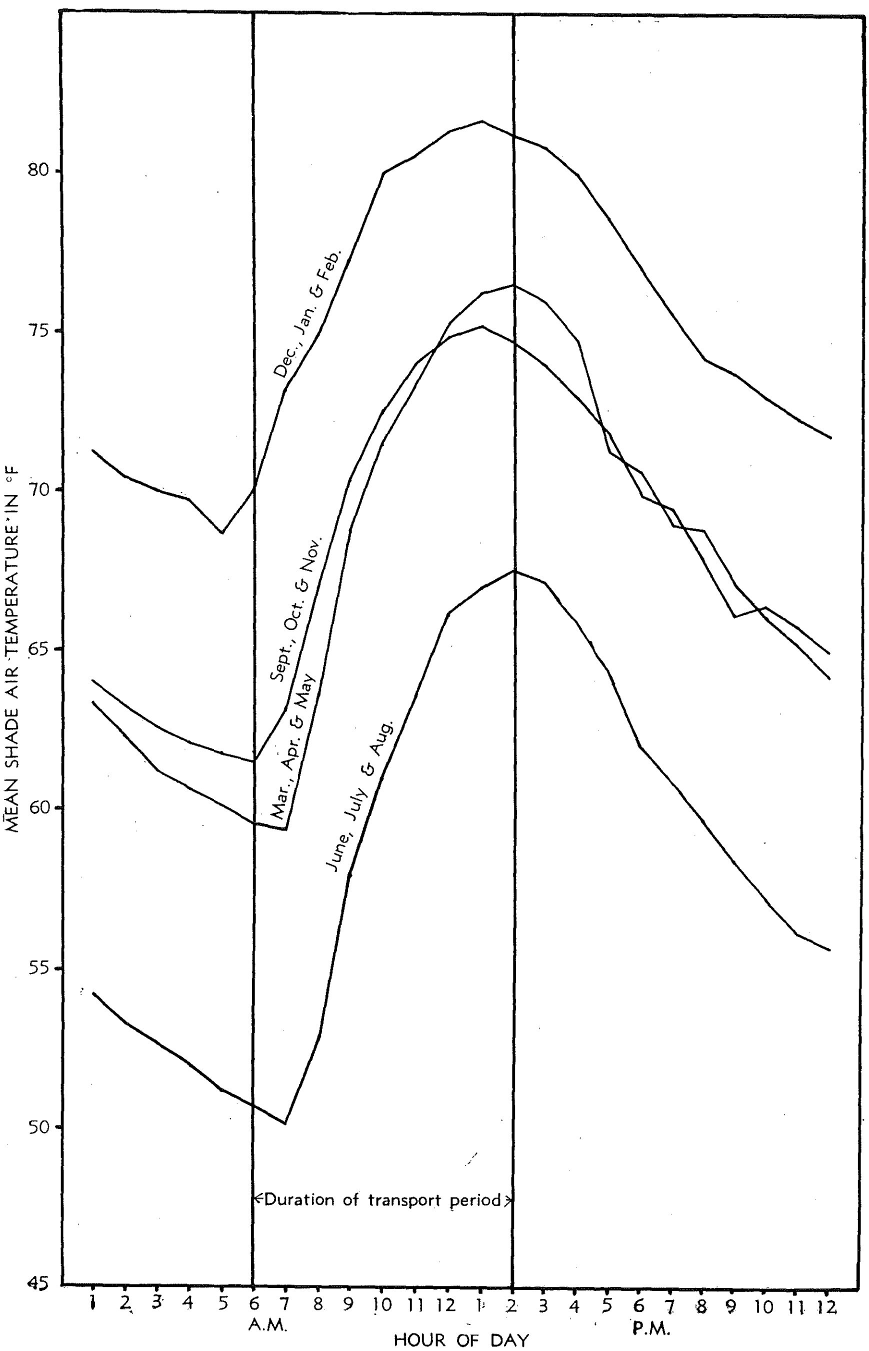

Figure 2.

Showing mean shade air temperatures at Brisbane from July, 1943, to June, 1944.

the temperature range 40 deg. F—68 deg. F., lowered the average reduction time by 70 minutes. These workers based their results on temperatures at the time of testing, which were taken as reflecting the storage temperature. Hoy and Braz (1938) concluded as a result of their work that a storage time of 18 hours at 70 deg. F. constituted drastic treatment of raw milk samples, causing reduction times of less than 4½ hours for milks considered to be of satisfactory initial quality.

Experimental Methods.

The greater part of the experimental work was done during November, 1943, December, 1943, and January, 1944, but several additional experimental samples were taken during October, 1944. Shade temperature records for the days on which tests were conducted showed that the mean daily temperature varied from 64 deg. F. to 76 deg. F.

Separate samples from 20 different farms were taken for the experimental work, and milk from the morning milking was used exclusively, as this permitted the greater portion of the work to be conducted during daylight hours. Farms were chosen at random without any warning being given that a sample was to be taken. In this way it was hoped to obtain a true sample by preventing farmers altering their normal methods of production. The samples included milks produced and treated by various methods as detailed in Table 1. Some milks were produced while the cows were being fed in stalls. The standard of hygiene on these producing farms varied considerably; in Table 1 it is given for convenience as "poor," "fair", or "good."

Because of the large number of tests and incubations which had to be performed in a limited period, it was impracticable to draw samples from more than two experimental farms on any one day.

Collection of Samples.

The producing farms were visited for the collection of samples and care was taken to secure samples as soon as practicable following the completion of milking. All the milk drawn during the milking was sampled into sterilized containers from the cans in which the milk was normally conveyed to the depot. In each case the composite sample thus obtained—approximately 1 litre—contained milk from both first- and last-milked cows in the herd. Since it was the aim to treat the milk from each farm as a separate replication unit in the experiment, while still preserving normal conditions as far as possible, this age discrepancy, which did not exceed two hours, had to be overlooked.

Treatment of Samples.

An effort was made to keep the interval between milking and commencement of laboratory tests as short and as constant as possible. This period actually varied between one and three-quarters and two and one-quarter hours (see Table 1), during which time the milk was kept under atmospheric conditions.

Icing of samples was purposely avoided, the reasons being (*a*), the desirability of keeping the samples as near as possible to normal commercial conditions, and (*b*), the uncertainty of the effect of icing on the subsequent behaviour of a sample.

The temperature of each milk sample was taken in cans at the farm and again on arrival at the laboratory; the figures are shown in Table 1.

In order to provide information concerning the quality of the experimental milk from each farm additional to that given by the methylene blue reduction tests, plate counts were performed on all farm samples before and after laboratory pasteurization. All plates were made on the tryptone-glucose-extract-milk-agar recommended by the American Public Health Association (1941), with incubation at 37 deg. C. for 48 hours. Laboratory pasteurization was at 63 deg. C. for 30 minutes, followed by immediate shock cooling in an ice and water mix. The results of the plate counts appear in Table 1.

Table 1.

RELEVANT DATE CONCERNING EXPERIMENTAL SAMPLES.

Sample No.	Records of Farm Observations.	Milk Temperature °C.		Period from Milking to Testing.	Methylene Blue Reduction Time (Control value). Hours.	Plate Count per ml. at commencement of methylene blue control test.	
		Milk in Cans at Farm.	Milk Sample at Laboratory.			Raw milk.	After Lab. Pasteurization 63°C. for 30 min.
1	Hand milked, uncooled, hygiene fair	33·5	24	2 hr. 15 min.	$5\frac{3}{4}$	10,000	500
2	Hand milked, cooled, hygiene poor	28	25	2 hr.	$4\frac{1}{2}$	110,000	7,000
3	Hand milked, uncooled, hygiene poor	34	26	2 hr.	5	58,000	430
4	Machine milked, uncooled, hygiene fair	33	26	2 hr. 15 min.	$4\frac{1}{2}$	..	..
5	Hand milked, uncooled, hygiene poor	33	27·5	2 hr. 15 min.	$3\frac{3}{4}$	160,000	7,000
6	Hand milked, uncooled, hygiene good	34	27	2 hr. 5 min.	$6\frac{1}{4}$	60,000	50
7	Hand milked, cooled, hygiene fair	29	26	1 hr. 50 min.	$5\frac{1}{4}$	140,000	200
8	Hand milked, cooled, hygiene poor	27	26	2 hr.	$4\frac{7}{8}$	82,000	1,000
9	Hand milked, cooled, hygiene poor	30	27	2 hr. 15 min.	$4\frac{1}{2}$	43,000	2,000
10	Hand milked, uncooled, hygiene poor	34	27	1 hr. 45 min.	$4\frac{1}{2}$	59,000	1,200
11	Hand milked, uncooled, hygiene poor	36	32	2 hr.	$4\frac{1}{2}$	14,000	500
12	Hand milked, cooled, hygiene good	30	29	1 hr. 45 min.	5	9,000	800
13	Hand milked, uncooled, hygiene poor	34	31	2 hr. 15 min.	$3\frac{1}{2}$	84,000	900
14	Hand milked, uncooled, hygiene good	34·5	29	1 hr. 45 min.	$6\frac{1}{4}$	30,000	200
15	Machine milked, cooled, hygiene fair	24	25	1 hr. 55 min.	$4\frac{3}{4}$	17,000	130
16	Hand milked, cooled, hygiene good	24	27	1 hr. 45 min.	$3\frac{3}{4}$	10,000	100
17	Hand milked, uncooled, hygiene fair	33	29	2 hr.	$5\frac{1}{2}$	8,000	500
18	Hand milked, cooled, hygiene good	29	24	1 hr. 45 min.	$7\frac{1}{4}$	4,000	100
19	Hand milked, cooled, hygiene good	25	23	2 hr.	$6\frac{1}{2}$	12,500	2,000
20	Hand milked, cooled, hygiene good	28	27	2 hr. 15 min.	$6\frac{1}{2}$	8,000	50

Technique of Methylene Blue Reduction Tests.

Immediately on arrival at the laboratory samples were pipetted in 10 ml. portions into sterile tubes fitted with rubber stoppers. For each milk, two such tubes were at once subjected to the modified methylene blue reduction test described by Wilson (1935), and thus served as controls, each in duplicate. The reduction time of these sub-samples is referred to subsequently as the control value (see Table 1). Twenty more tubes were incubated at each of the temperatures 20 deg. C., 25 deg. C., 30 deg. C., and 35 deg. C. for regular periods of time (half-hourly intervals) up to five hours. Water baths operating within ± 0·5 deg. C. of the desired temperature were used to obtain even temperature control. Thus incubations were performed in closed tubes without agitation during the incubation period. At half-hourly intervals duplicate tubes of milk were removed from each temperature class and also subjected to the modified methylene blue test. The methylene blue tubes were observed at 15-minute intervals and inverted at half-hourly intervals during the progress of the test.

Great care was found necessary in the reading of the methylene blue tests when tests continued past the daylight hours through twilight and into artificial light. In each such case the tube of milk appeared to hold a faint trace of blue colour. The correct end-point could be determined only by comparing the test with an obviously blue tube on one hand and with a tube long since decolourized on the other.

The experimental design, in addition to giving a control value, gave results for each milk after 40 sets of conditions of time and temperature. As each milk was regarded as a separate unit there were thus 20 replications.

Experimental Results.

The reduction times of duplicate sub-samples differed in 6·6 per cent. of cases, but in only 0·3 per cent. of cases was the difference more than 15 minutes. In every instance of variation the mean was taken as the true value. Table 2 shows the reduction times of the sub-samples of sample No. 9, which is typical in its uniformity of all 20 samples.

For the purposes of the analysis and the interpretation of results the deterioration in quality suffered by a sample of milk under a particular treatment is taken as the criterion of the effect of that treatment. This deterioration is given directly by the value of the fall in reduction time (f), which is the difference between the control reduction time and the reduction time after treatment. The value is usually positive, and is expressed in the tables in eighths of an hour. The choice of the fall in reduction time was quite arbitrary. The ultimate reduction time after treatment could probably have been used to express effects equally well, but most trends would then have been negative. Storage time (t)

Table 2.

REDUCTION TIMES OBTAINED WITH SAMPLE NO. 9.

Storage Temp. °C.	Storage Period in Hours.									
	½	1	1½	2	2½	3	3½	4	4½	5
20 ..	5 5	4¾ 4¾	4½ 4½	4½ 4½	4¼ 4¼	4 4	3¾ 3¾	3½ 3½	3½ 3½	3¼ 3¼
25 ..	4½ 4½	4½ 4½	4¼ 4¼	4 4	3¾ 3¾	3½ 3½	3¼ 3¼	2¾ 2¾	2½ 2¼	2 2
30 ..	4½ 4½	4¼ 4¼	3¾ 3¾	3½ 3½	3 3	2½ 2½	2¼ 2¼	1¾ 1¾	1½ 1½	1¼ 1¼
35 ..	4¼ 4¼	4 4	3½ 3½	3 3	2½ 2½	1¾ 2	1½ 1½	1¼ 1¼	1 1	½ ½

Control reduction time : 4½ hours.

is recorded in hours, and temperature (T) in degrees Centigrade. The observed values of f averaged over all samples are set out in Table 3, together with the respective mean values of f for all temperatures and times and regression coefficients or gradients of fall. The trends shown by these means of time and temperature are obvious, but the data have been subjected to statistical analysis to enable an accurate interpretation of the results to be made.

Table 3.

SUMMARY—MEAN VALUES OF f (IN EIGHTHS OF AN HOUR) AND REGRESSION COEFFICIENTS (GRADIENTS).

Storage Temp. °C.	Storage Time (t) in hours.										Means.	Regression Coefficients.
	½	1	1½	2	2½	3	3½	4	4½	5		
20 ..	−0·2	−0·1	1·2	2·6	3·1	4·7	6·0	7·2	7·9	9·0	4·14	1·099
25 ..	0·8	2·0	3·4	4·7	7·1	8·6	10·2	12·3	14·5	16·7	8·02	1·773
30 ..	2·7	5·0	7·5	10·7	13·5	15·8	18·8	21·3	23·5	26·2	14·51	2·650
35 ..	4·2	8·0	11·9	15·2	18·7	21·2	24·5	27·4	29·8	32·4	19·32	3·119
Means ..	1·86	3·71	6·01	8·28	10·60	12·58	14·88	17·04	18·91	21·10	11·50	2·160
Regression Coefficients	1·515	2·725	3·605	4·380	5·320	5·710	6·410	6·945	7·455	7·980	5·204	0·694

Mean values of f may be converted to hours by multiplying by 0·125.

It will be seen from the analysis of variance (Table 4) that the major portions of the variations between time and temperature means are accounted for by the linear components. Thus the greater part of the effect of time and temperature is linear. Consequently in this analysis most attention is paid to these linear trends. As there are marked variations in the components of error the three items corresponding to the main linear effects are shown separately. In calculating the standard errors of regression coefficients the separate errors have been used. The components of error shown as "remainder" are homogeneous.

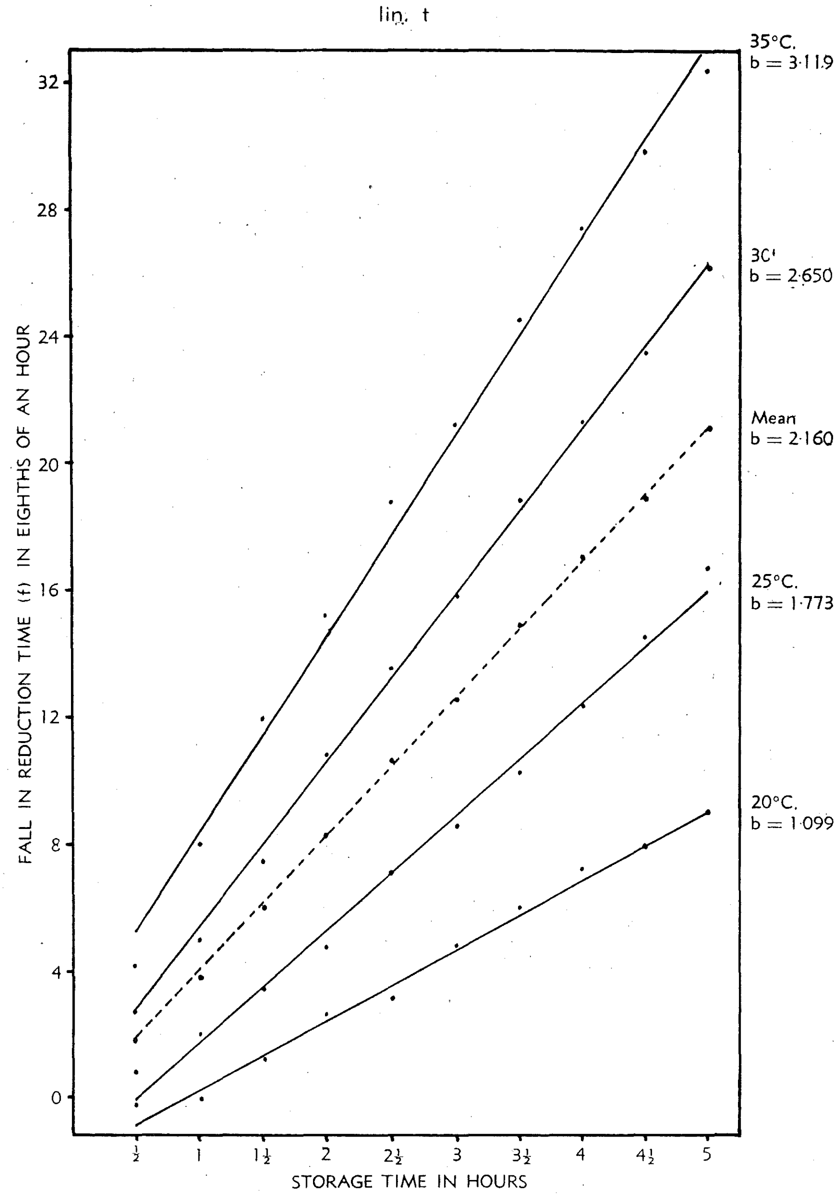

Figure 3.

Showing the effect of increased storage time on the fall in reduction time (f) at four temperatures.

Table 4.

ANALYSIS OF VARIANCE—FALL IN REDUCTION TIME.

Source of Variation.	D.F.	Sum of Squares.	Mean Square.
Suppliers	19	3,078·114	162·006
Times (t)			
lin. t	1	30,799·440	30,799·440
dev. t	8	13·761	1·720
	9	30,813·201	3,423·689
Temperature (T)			
lin. T	1	27,086·820	27,086·820
dev. T	2	226·394	113·197
	3	27,313·214	9,104·405
Times x Temperatures			
lin. t x lin. T	1	3,971·068	3,971·068
lin. t x dev. T	2	47·916	23·958
dev. t x lin. T	8	80·744	10·093
dev. t x dev. T	16	57·996	3·625
	27	4,157·724	153·990
Error Components			
Suppliers x lin. t	19	818·712	43·090
Suppliers x lin. T	19	1,113·085	58·583
Suppliers x lin. t x lin. T	19	558·744	29·408
Remainder	684	1,539·196	2·250
Total	799	69,391·989	

The terms lin. t, lin. T, and lin. t $\times$ lin. T are all very highly significant and these terms account for the major portions of the variations between the time and temperature means. Dev. T, lin. t $\times$ dev. T, and dev. t $\times$ lin. T are also very highly significant, indicating that the linear specification, especially as regards storage temperature, is not completely adequate. This is shown clearly in Figures 4, 5 and 6.

Effects of Storage Time.

At all four temperatures there is a very marked increase in the value of f as the storage time increases (Figure 3). Linear relations between f and t were estimated for each temperature in the form

$$f - \overline{f_T} = 2\ b_T\ (t - 2{\cdot}75),$$

where $\overline{f_T}$ is the mean value of f for the particular temperature, and t is the storage time in hours. The quantity b_T is the regression coefficient; it measures

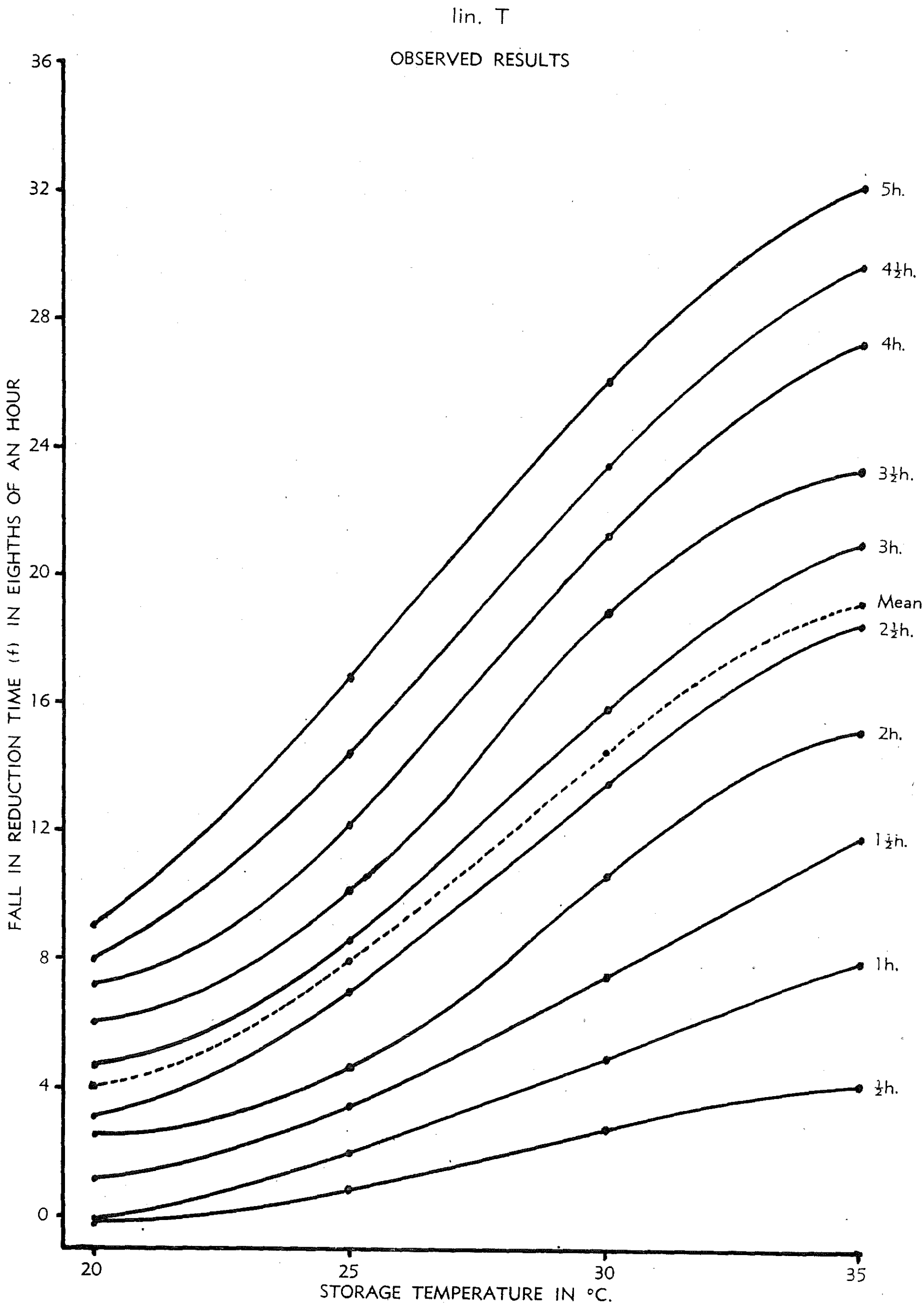

Figure 4a.

Showing the effect of storage temperature on the fall in reduction time (f).

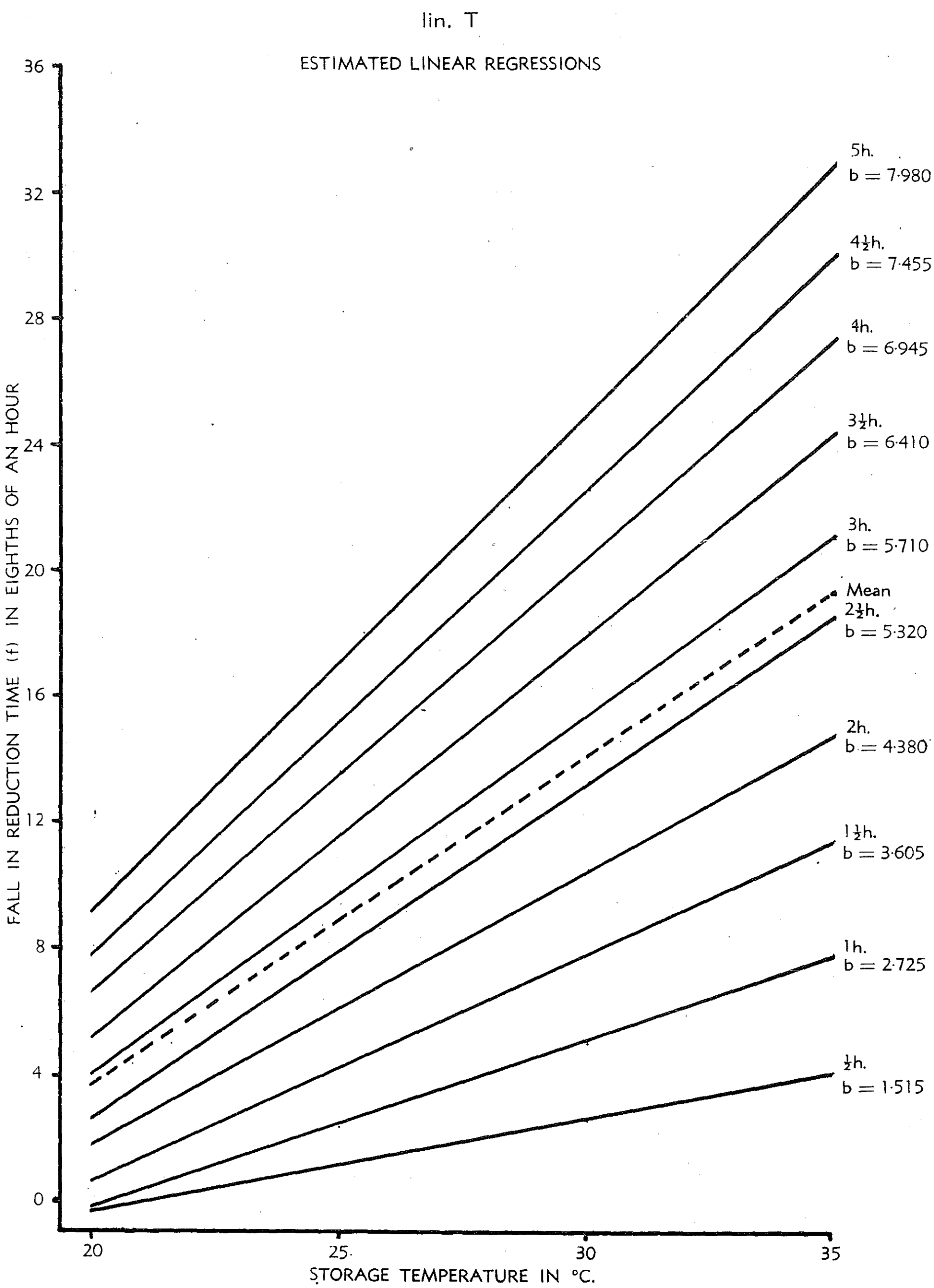

Figure 4b.
Showing the effect of storage temperature on the fall in reduction time (f).

the gradient of f on storage time for the particular temperature and represents the average change in f for each additional half-hour of storage time. The equations for the four temperatures and the mean over all temperatures are

$$
\begin{aligned}
&20°\text{C.}, && f - 4{\cdot}14 = 2 \times 1{\cdot}099\ (t - 2{\cdot}75)\ ; \\
&25°\text{C.}, && f - 8{\cdot}02 = 2 \times 1{\cdot}773\ (t - 2{\cdot}75)\ ; \\
&30°\text{C.}, && f - 14{\cdot}51 = 2 \times 2{\cdot}650\ (t - 2{\cdot}75)\ ; \\
&35°\text{C.}, && f - 19{\cdot}32 = 2 \times 3{\cdot}119\ (t - 2{\cdot}75)\ ; \\
&\text{Mean}, && f - 11{\cdot}50 = 2 \times 2{\cdot}160\ (t - 2{\cdot}75)\ ;
\end{aligned}
$$

The s.e. of each b_T is $\pm$ 0·162 and the s.e. of the mean b_T is $\pm$ 0·0808. All regression coefficients differ significantly from zero. The mean values for the various temperatures and the fitted lines are shown in Figure 3.

Effects of Storage Temperature.

For all storage times there is a very marked increase in f as the storage temperature increases (Figure 4). Linear relations between f and T for each storage time were estimated in the form

$$f - \bar{f}_t = b_t \frac{(T - 27{\cdot}5)}{5},$$

where $\bar{f}_t$ is the mean value of f for the particular storage time, and T is the storage temperature in degrees Centigrade. The regression coefficient b_t is the gradient of f on temperature for a particular storage time and represents the average increase in f for each additional change of 5 deg. C. in storage temperature. The equations for the 10 periods and for the mean over all times are

$$
\begin{aligned}
&\tfrac{1}{2}\ \text{hr.}, && f - 1{\cdot}86 = 1{\cdot}515\ \frac{(T - 27{\cdot}5)}{5}\ ; \\
&1\ \text{hr.}, && f - 3{\cdot}71 = 2{\cdot}725\ (\quad ,, \quad)\ ; \\
&1\tfrac{1}{2}\ \text{hr.}, && f - 6{\cdot}01 = 3{\cdot}605\ (\quad ,, \quad)\ ; \\
&2\ \text{hr.}, && f - 8{\cdot}28 = 4{\cdot}380\ (\quad ,, \quad)\ ; \\
&2\tfrac{1}{2}\ \text{hr.}, && f - 10{\cdot}60 = 5{\cdot}320\ (\quad ,, \quad)\ ; \\
&3\ \text{hr.}, && f - 12{\cdot}58 = 5{\cdot}710\ (\quad ,, \quad)\ ; \\
&3\tfrac{1}{2}\ \text{hr.}, && f - 14{\cdot}88 = 6{\cdot}410\ (\quad ,, \quad)\ ; \\
&4\ \text{hr.}, && f - 17{\cdot}04 = 6{\cdot}945\ (\quad ,, \quad)\ ; \\
&4\tfrac{1}{2}\ \text{hr.}, && f - 18{\cdot}91 = 7{\cdot}455\ (\quad ,, \quad)\ ; \\
&5\ \text{hr.}, && f - 21{\cdot}10 = 7{\cdot}980\ (\quad ,, \quad)\ ; \\
&\text{Mean}, && f - 11{\cdot}50 = 5{\cdot}204\ (\quad ,, \quad).
\end{aligned}
$$

The s.e. of each b_t is $\pm$ 0·765 and the s.e. of the mean b_t is $\pm$ 0·242. All regression coefficients, except that for $t = \frac{1}{2}$ hour, differ significantly from zero. The mean values for the various times and the fitted lines are shown graphically in Figure 4.

The significance of the deviation term (dev. T) in the analysis of variance (Table 4) shows that the variation of f with temperature is not adequately represented by a straight line. Both the quadratic and the cubic terms are significant. However, since the main effects are linear, there seems ample justification for treating the results in a linear fashion.

Interaction of Time and Temperature.

The mean values of f, representing the mean fall in reduction time averaged over all temperatures, show a progressive increase with storage time which is adequately described by a linear law (see mean line in Figure 3). The gradients on temperature (b_t) also show a progressive increase with time. Figure 5 shows these gradients plotted against time. The calculated linear relation is

$$b - 5{\cdot}204 = 2 \times 0{\cdot}694\ (t - 2{\cdot}75),$$

indicating that the mean increase in the gradient of f on temperature is $0{\cdot}694 \pm 0{\cdot}0597$ for each half-hour change in storage time.

lin. T × lin. t

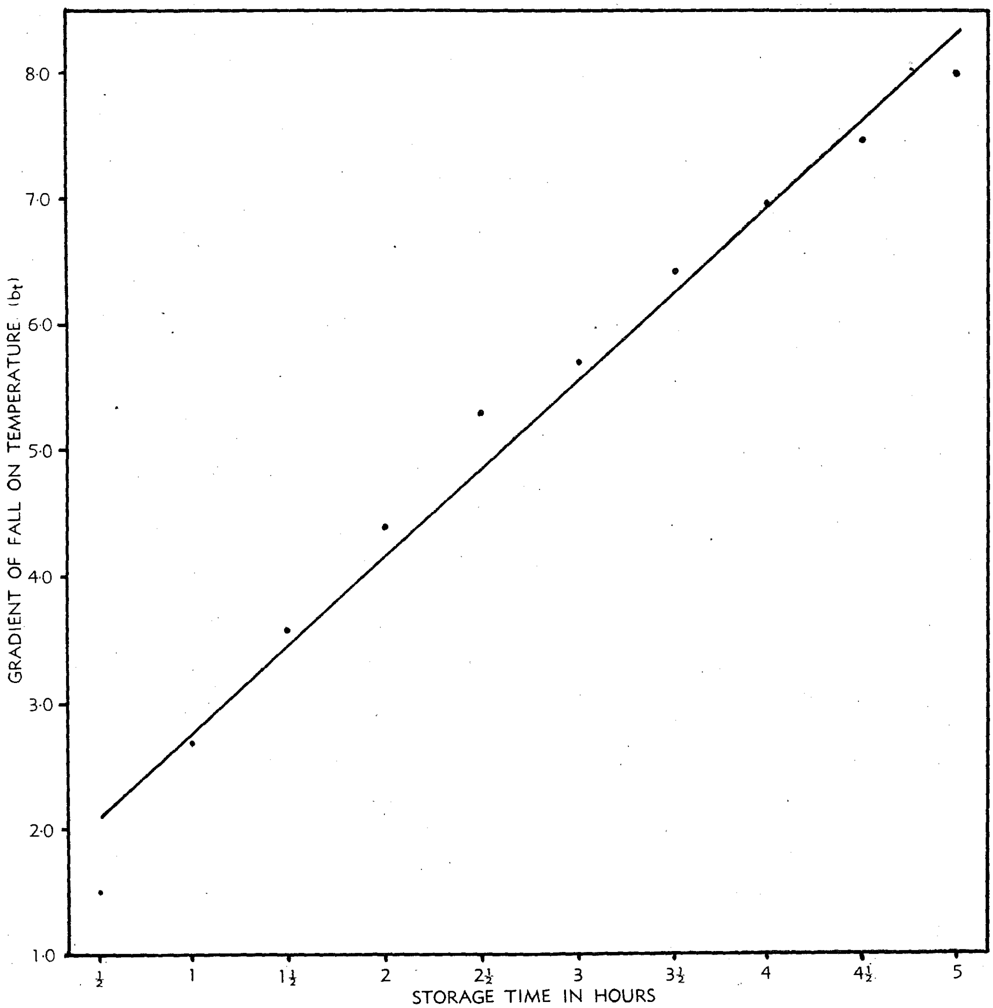

Figure 5.
Showing the effect of storage time on the gradient of fall in reduction time over the temperature range 20° C. to 35° C.

The significance of the dev. t $\times$ lin. T term in the analysis of variance indicates that the rate of change of the temperature gradient with time (Figure 5) is not strictly linear, the quadratic term showing that the regression of b_t on time changes at the rate of $-$ 0·0406 per half-hour.

The mean values of f averaged over all storage times show a progressive increase with temperature. This same positive trend with temperature increase is shown by the gradients on time, which are shown in Figure 6. The calculated linear relation is

$$b_T - 2{\cdot}160 = 0{\cdot}694 \times \frac{(T - 27{\cdot}5)}{5},$$

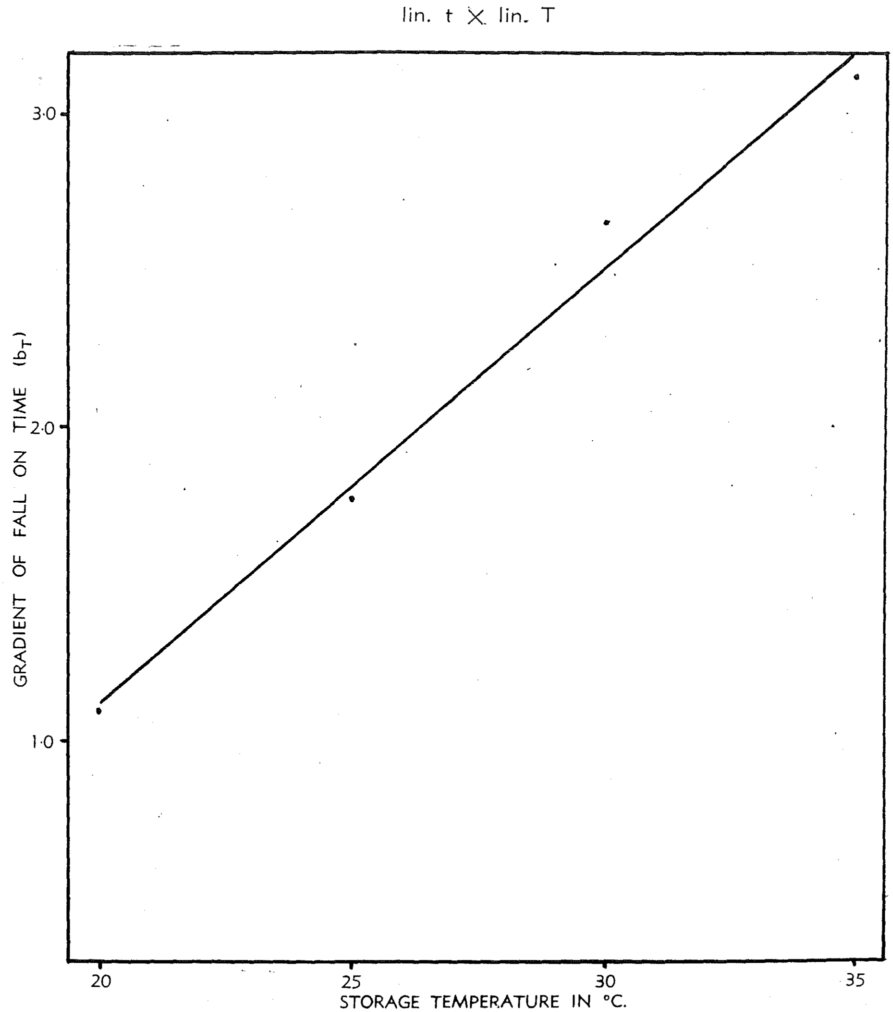

Figure 6.

Showing the effect of storage temperature on the gradient of fall in reduction time with storage time up to 5 hours.

indicating that the mean increase in the gradient of f on time is $0{\cdot}694 \pm 0{\cdot}0597$ for each 5 deg. C. change in storage temperature. Here again there appears a significant deviation from the linear, which is shown by the significance of the lin. t $\times$ dev. T term in the analysis of variance.

CORRELATION OF CONTROL VALUE AND SHADE TEMPERATURE WITH TIME AND TEMPERATURE EFFECTS.

In addition to studying the deterioration in reduction time of raw milk caused by adverse conditions of time and temperature, it was found possible to obtain from the same data some indication of the effect of the initial quality of a milk on its subsequent behaviour. The control reduction time of milk tested two hours after the completion of milking was taken as a measure of initial quality. In addition, the data were examined with a view to locating any trends which may have resulted from differences in air temperature from one day of testing to another.

Hourly mean shade temperature records commencing at 1 a.m. on the days on which the tests were conducted were obtained from the Brisbane Meteorological Observatory. Since this observatory is situated no further than 25 miles from experimental farms and there are no climatic barriers, these records were taken as indicative of temperatures prevailing on the farms. Air temperatures were recorded at several farms at the time of sampling (approximately 7 a.m.). The maximum difference between these observations and official Brisbane recordings at the same hour was 6 deg. F. and the mean difference 1 deg. F. Since air temperatures in dairy houses can be affected by a great many factors which are largely excluded by the use of a suitably constructed and properly situated shade temperature cabinet, these results do not form an accurate check. Nevertheless they serve to indicate that comparable temperature conditions prevailed at both localities.

Experimental Results.

In the analysis of the results obtained, the control value and the mean daily shade temperature were treated as independent variables and an analysis of covariance was carried out for each of the following quantities calculated for each of the 20 suppliers:—

(*a*), Mean fall in reduction time averaged over all times and temperatures;

(*b*), The regression on time averaged over all temperatures;

(*c*), The regression on temperature averaged over all times; and

(*d*), The regression on time of the regression on temperature.

The calculated values of these four variables are given in Table 5, together with the observed values of controls and shade temperatures.

In each case the fitted equation was of the form

$$y - \bar{y} = b_1 (x_1 - \bar{x}_1) + b_2 (x_2 - \bar{x}_2),$$

where x_1 represents the control value expressed in eighths of an hour and x_2 the mean daily shade temperature in degrees Fahrenheit.

The analysis of covariance for each of the four calculated variables is given in Table 6.

In no case is there any evidence of significant correlation of any of the four variables with shade temperature. Control values, however, show significant correlations with regressions on storage temperature and with lin. t $\times$ lin. T, but not with regression on storage time.

Table 5.

EFFECT OF CONTROL VALUE AND SHADE TEMPERATURE.

Sample No.	Total Fall in reduction time in eighths of an hour. (y_1).	Regression on Time. (y_2).	Regression on Temperature. (y_3).	lin. t x lin. T. (y_4)	Control Value in eighths of an hour. (x_1).	Shade Temperature °F. (x_2).
1	455	1,539	535	1,323	46	73·8
2	542	1,538	366	474	36	71·1
3	516	1,442	428	958	40	70·2
4	476	1,412	378	798	36	72·5
5	431	1,407	339	427	30	73·5
6	549	1,335	413	691	50	72·1
7	550	1,864	514	1,452	42	74·9
8	555	1,563	459	1,019	39	76·9
9	419	1,699	477	1,069	36	77·1
10	472	1,298	404	818	34	77·1
11	203	759	727	895	36	79·4
12	459	1,529	605	1,067	40	79·4
13	446	1,150	550	806	28	78·8
14	505	1,453	565	1,697	50	78·8
15	443	1,363	567	1,099	38	77·0
16	422	1,126	608	1,976	54	77·0
17	414	1,390	622	1,254	44	64·8
18	356	1,336	560	1,804	58	65·4
19	480	1,696	640	1,616	52	63·8
20	504	1,616	652	1,652	52	69·7

Table 6.

ANALYSIS OF COVARIANCE.

(a) Mean Fall in Reduction Time.

Source of Variation.	D.F.	Sum of Squares.	Mean Square.
Regression	2	39·424	19·712
Remainder	17	3,038,690	198·746
Suppliers	19	3,078·114	

$b_1 = 0{\cdot}00257 \pm 0{\cdot}0062$ (not significant) ;
$b_2 = -0{\cdot}0441 \pm 0{\cdot}1127$ (not significant).

(b) Regression on Time.

Source of Variation.	D.F.	Sum of Squares.	Mean Square.
Regression	2	75·595	37·798
Remainder	17	743·117	43·713
Suppliers x lin. t	19	818·712	

$b_1 = 0{\cdot}000550 \pm 0{\cdot}0114$ (not significant) ;
$b_2 = -0{\cdot}0219 \pm 0{\cdot}0194$ (not significant).

(c) Regression on Temperature.

Source of Variation.	D.F.	Sum of Squares.	Mean Square.
Regression	2	267·146	133·573
Remainder	17	845·939	49·761
Suppliers x lin. T	19	1,113·085	

F for regression = 2·684, which is not significant.
$b_1 = 0{\cdot}0724 \pm 0{\cdot}0312$ (significant at 5% level) ;
$b_2 = 0{\cdot}0572 \pm 0{\cdot}0532$ (not significant).

(d) Regression on Time of Regression on Temperature.

Source of Variation.	D.F.	Sum of Squares.	Mean Square.
Regression	2	394·467	197·234
Remainder	17	164·277	9·663
Suppliers x lin. t x lin. T	19	558·744	

F for regression = 20·41, which is highly significant.
$b_1 = 0{\cdot}0296 \pm 0{\cdot}00479$ (highly significant) ;
$b_2 = 0{\cdot}0124 \pm 0{\cdot}00816$ (not significant).

EFFECTS OF STORAGE TIME AND TEMPERATURE WITHIN THE TEMPERATURE RANGE 5 DEG. C.–15 DEG. C.

The low-temperature treatment of milk samples for methylene blue testing has been the subject of considerable investigation and discussion. Ellenberger and Moody (1930) found that milk samples iced for two hours prior to testing showed a mean reduction time 15 minutes less than samples of the same milk uniced. The effect of icing was further studied by Wilson (1935), who concluded that icing caused a lengthening of the reduction time but that the effect varied with different types of milk. Frayer (1934) found that incubating raw milk for 24 hours at 40 deg. F. prolonged the average reduction time by 5 per cent. A progressive lowering in reduction time was demonstrated by Chalmers (1938), when he held raw milk samples for 1, 7, 16 and 23 hours at 15 deg. C. A similar result was obtained by Powell, Jenkins and Thomas (1939), who observed a reduction time three and a-half hours longer with milks held overnight at 40 deg. F. than with duplicates held at 60 deg. F. Phillips and Thomas (1939) found that by holding fresh raw milk for 24 hours at three temperatures—40 deg. F., 60 deg. F. and 70 deg. F.—the reduction time of seven hours became 8·1, 5·0 and 0·9 hours for the respective temperatures.

It was considered that by the study of only four temperatures—20 deg. C., 25 deg. C., 30 deg. C. and 35 deg. C.—an incomplete picture was obtained of the response to temperature change shown by the reduction time of raw milk. Consequently, later samples of milk examined were incubated for 10 incubation periods up to five hours at 5 deg. C., 10 deg. C. and 15 deg. C. in addition to the four temperatures stated above. The experimental technique was similar to that employed in the earlier experiments. Samples were exposed to atmospheric temperature for approximately two hours between the completion of milking and the commencement of testing, and duplicate tests were again performed. Water baths operating automatically at these low temperatures were unavailable and temperature control had to be accomplished by the addition of hot or cold water whenever necessary. A laboratory refrigerator operating at 4-5 deg. C. facilitated the control of the 5 deg. C. and 10 deg. C. treatments. Care was taken that the temperature error did not exceed $\pm$ 1·0 deg. C. When laboratory air temperatures varied between 20 deg. C. and 30 deg. C. it was found necessary to inspect water bath temperatures at intervals of 10 minutes.

Experimental Results.

A summary of the results obtained over 10 periods from one half-hour to five hours at 5 deg. C., 10 deg. C. and 15 deg. C. is given in Table 7, and results of the analyses of variance in Tables 8 and 9.

Table 7.

MEANS VALUES OF f IN EIGHTHS OF AN HOUR.

Storage Temp. °C.	No. of Milks.	Storage Time in hours.										Means.	Regression Coefficients.
		½	1	1½	3	2½	3	3½	4	4½	5		
5 ..	4	−2	−2·5	−0·5	− 2	−2	−2·5	−2·5	−3	−4	−4·5	−2·55	−·288
10 ..	8	−1·5	−2·25	−2	−1·25	− 2	−1·5	−2	−1·5	−2·75	−2·6	−1·94	−·078
15 ..	8	−2·4	−1·9	−·9	−1·1	−·75	−·6	·5	−·5	− ·25	·25	−·79	·267

Table 8.

ANALYSIS OF VARIANCE.

5° C.—Data from four suppliers used.

Source of Variation.	D.F.	Sum of Squares.	Mean Square.
Suppliers	3	69·1	23·033
Times			
lin. t	1	27·348	27·348
dev. t	8	17·552	2·194
	9	44·900	4·989
Error	27	61·900	2·29
Total	39	175·900	

F for lin. t = 11·929, which is highly significant.
b = −0·288 ± 0·0834 (highly significant).

The variation between times for storage at 5 deg. C. is adequately described by the linear relation

$$f + 2{\cdot}55 = -2 \times 0{\cdot}288\,(t - 2{\cdot}75).$$

The mean fall in reduction time is $-2{\cdot}55 \pm 0{\cdot}239$ and is significantly different from zero.

Table 9.

ANALYSES OF VARIANCE (SUMMARY).

10° C. and 15° C.—Data from eight suppliers used.

Source of Variation.	D.F.	Mean Squares.	
		10° C.	15° C.
Suppliers	7	14·427	15·898
Times			
lin. t	1	4·019	47·200
dev. t	8	1·787	1·914
	9	2·035	6·946
Error	63	1·832	2·247
F for lin. t		2·194 Not significant	21·002 Highly significant

For 10 deg. C. the equation is

$$f + 1{\cdot}94 = -2 \times 0.078\ (t - 2{\cdot}75).$$

The mean value of f is — 1·94 ± 0·151 and is significantly different from zero. There is no evidence of any trend with time, the regression coefficient — 0·78 ± 0·0526 not being significantly different from zero.

For 15 deg. C. the equation is

$$f + 0{\cdot}79 = 2 \times 0{\cdot}267\ (t - 2{\cdot}75).$$

The mean fall in reduction time is — 0·79 ± 0·167 and is significantly different from zero. There is a very marked trend with time, the regression coefficient 0·267 ± 0·0584 being highly significant.

Despite the low number of replications employed—four in the case of 5 deg. C. and eight for both 10 deg. C. and 15 deg. C.—it is possible to demonstrate significant trends. The analysis of variance given in Table 8 shows that at 5 deg. C. lin. t is highly significant and is negative. In other words, there is a significant negative fall in reduction time as storage time increases. This means that at 5 deg. C. the mean reduction time of a number of milks shows a progressive increase over a 5-hour incubation period. This finding is in keeping with the observations of other workers (Chalmers, 1938; Ellenberger and Moody, 1930; Frayer, 1934; Phillips and Thomas, 1939; Powell *et al.*, 1939; Wilson, 1935). Further, this negative trend in the value of f is linear, since dev. t is not significant. The gradient of fall (b) = — 0·288 ± 0·0834 and is highly significant. The mean fall in reduction time is — 2·55 ± 0·239 and is significantly different from zero.

There is no evidence of any time trend at 10 deg. C., the regression coefficient or gradient for lin. t not being significant. It would appear from this data that the "neutral" point with respect to the effect of storage time on the reduction time lies in the vicinity of 10 deg. C. There is, as in the case of the 5 deg. C. treatment, a significant and almost spontaneous lowering of f, the mean value of this being of the order of — 1·94 ± 0·151.

A highly significant linear trend with time is found at 15 deg. C., the gradient of f being 0·267 ± 0·0587. The deviation from the linear (dev. t) is insignificant. It is to be noted that this gradient has a positive value, so that storage at 15 deg. C. results in a lowering in reduction time. Over a 5-hour period, however, there is a significant mean f value of — 0·79 ± 0·167.

The value of f averaged over all suppliers is plotted against storage time in Figure 7, in which the observed results for the four higher temperatures are also included for comparison. It will be observed that at and below 20 deg. C. a storage time of one half-hour results in a spontaneous lowering of f. This means a lengthening of the reduction time for each temperature, and is largely reflected in the mean value of f averaged over all storage periods up to five hours, and given in Table 10. This effect evidently occurs as an immediate result of the sudden change in temperature of the sample from atmospheric temperature to that of incubation, the effect being approximately equal at all three low temperatures and preceding any trends with time.

lin. t

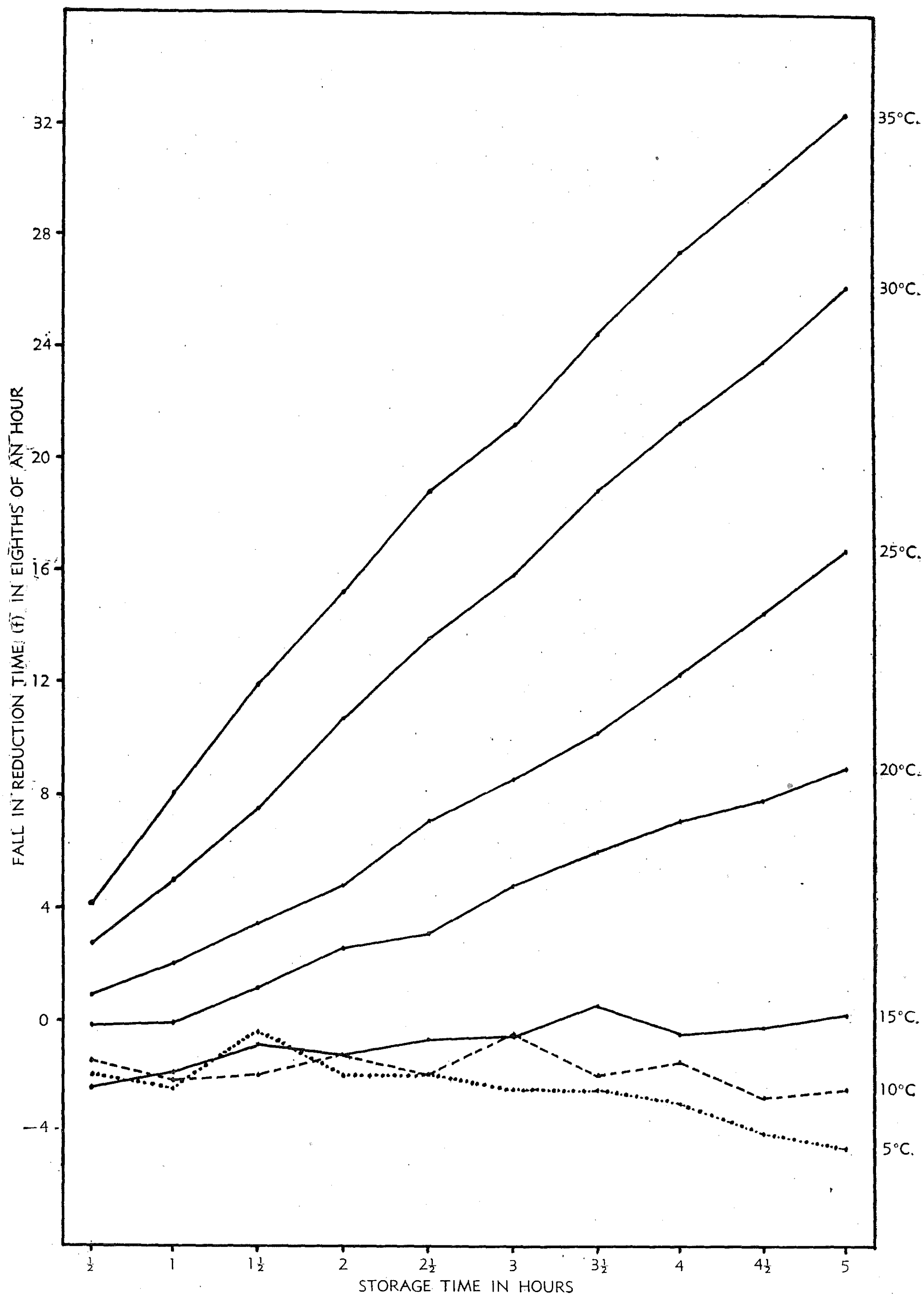

Figure 7.

Showing the effect of storage time on the fall in reduction time at seven temperatures.

Table 10.

Storage Temperature. ° C.	Mean Fall in Reduction. Time (f) over a 5-hour period.	Gradient of f in eighthsof an hour for each ½-hourly interval over a 5-hour period.
5	− 2·55 ± 0·0834	− 0·288 ± 0·239
10	− 1·94 ± 0·151	− 0·078 ± 0·0526
15	− ·79 ± 0·167	0·267 ± 0·0584
20	4·14 } ± ·165	1·099 } ± 0·162
25	8·02	1·773
30	14·51	2·650
35	19·32	3·119

DISCUSSION.

The effects of time and temperature on the reduction of methylene blue are interdependent. It is necessary, therefore, to consider these factors together when interpreting results. Nevertheless, the effect of the individual factor (i.e., time or temperature) can be studied most satisfactorily by keeping the other constant, and this has been done throughout the work. In this discussion the effects of the individual factors will be considered separately before discussing the interaction between the two.

Effects of Storage Time.

The reduction time of incubated samples shows a very marked response to increased storage time. At temperatures within the range 15 deg. C. to 35 deg. C. increased storage time prior to conducting the methylene blue test results in a progressive shortening of the reduction time. It has not been possible to demonstrate any trends resulting from increased storage time up to five hours at 10 deg. C., and it appears that in the vicinity of this temperature the effect of the time factor is negligible or absent. Low temperature storage at 5 deg. C. results in a progressive increase in the reduction time over a 5-hour period.

All trends resulting from increased storage time have been found to be strictly linear. Regression coefficients calculated from the observed fall in reduction time can be expressed for each storage temperature. Such coefficients are, in effect, the mean gradients of deterioration of the number of milks tested, and are given in Table 11. These results have been recalculated from Table 10 so as to be simply expressed in minutes instead of eighths of an hour.

Table 11.

Storage Temperature. ° C.	Deterioration Gradient (decrease in the reduction time for every ½-hour increase in storage time up to 5 hours). Minutes.
5	− 2·2
10	0
15	2·0
20	8·2
25	13·3
30	19·9
35	23·4

It will be observed that the time deterioration gradient is negative at 5 deg. C. and becomes positive at 15 deg. C. Regardless of the fact that these results are a true expression of the low temperature time gradients, they are not adequate to express fully the observed results, since temperatures of 20 deg. C. and lower cause an increase in reduction time even after a storage period as short as 30 minutes. Consequently any time trends must occur subsequent to this phenomenon and must commence with a milk possessing a reduction time longer than the control sample. Thus at 15 deg. C., five hours' storage results in a reduction time approximately equal to the initial reduction time prior to storage.

It is obvious from these results that any increase in the storage period or any delay in transport of raw milk is to be avoided when the milk temperature rises above 15 deg. C. (59 deg. F.).

Following this experimental work further information was obtained with incubation periods extending to 10 hours. The data, which are as yet unpublished, indicate that the same effects and trends can be expected over an incubation period of 10 hours as were obtained over five hours.

Effects of Storage Temperature.

The storage of raw milk at temperatures between 20 deg. C. and 35 deg. C. results in a marked shortening of the methylene blue reduction time for all storage periods up to five hours. As the temperature of storage increases the reduction time decreases in a manner which does not strictly obey the linear law. Both the quadratic and the cubic terms are significant. Figure 4 shows the curves obtained by varying the incubation temperature from 20 deg. C. to 35 deg. C. for all storage times. It will be noticed that the greatest effect on the reduction time occurs between the temperatures 25 deg. C. and 30 deg. C. All curves show a comparative decrease in gradient between 20 deg. C. and 25 deg. C. and between 30 deg. C. and 35 deg. C. It is presumed that the temperature range of greatest gradient coincides with the optimum growth temperature for most of the bacterial strains making up the floras of the various milks. Despite these deviations the greater portion of the differences between means at each temperature is for general purposes linear.

A very marked and very sudden increase in the reduction time was observed at temperatures of 5 deg. C., 10 deg. C. and 15 deg. C. This increase was noticeable after half-an-hour's storage, and is clearly shown in Figure 7. The same figure also shows that at 20 deg. C. storage for the same period caused a similar but much smaller increase to be followed by the marked linear trend of a falling reduction time. All four temperatures—5 deg. C., 10 deg. C., 15 deg. C. and 20 deg. C.—were below atmospheric temperature and were lower than the temperatures of all samples prior to commencing incubations (i.e., 23 deg. C.-29 deg. C.). It would appear from this evidence that when the temperature of raw milk samples is suddenly lowered to 20 deg. C. or lower a storage period of one half-hour at these temperatures results in a lengthing of the reduction time which is independent of the amount of temperature change. The reason for this is not clear and was not investigated. It is suggested that two factors may be involved: (*a*), the induction of a lag growth phase for at least some of the organisms making up the flora, and (*b*), the increased capacity of milk at lower temperatures for taking up oxygen. The phenomenon is of practical significance in the rapid cooling of milk on the farm.

A storage temperature of 15 deg. C. causes an initial lengthening in reduction time followed by a progressive shortening in reduction time as the storage time increases over a 5-hour period. Storage at 10 deg. C. produces a similar early lengthening in reduction time, after which there is no significant change. At 5 deg. C. the initial lengthening increases in a significant linear manner over the same storage period. These points are illustrated in Figure 7. The results obtained are comparable with those reported by Phillips and Thomas (1939) and mentioned on page 148.

Interaction of Time and Temperature.

All results indicate a high degree of interaction between time and temperature. Salient features shown by the data are that in general the effect of time increases with temperature rise; and, conversely, the effect of temperature increases with increase of time. While this finding is to be expected, one important exception occurs at 10 deg. C. At this temperature prolongation of storage up to five hours, and probably also to 10 hours, has no effect on the reduction time after the initial increases resulting from the sudden change in incubation temperature.

The mean fall in reduction time averaged over all temperatures shows a progressive increase with increased storage time, and the mean fall in reduction time averaged over all storage times increases progressively as the storage temperature increases.

At each storage temperature the curve of the fall in reduction time is linear, but it becomes progressively steeper as the storage temperature rises. This rate of gradient change is shown graphically in Figure 6. It is not strictly linear, but is nearly so.

In the same way the gradient of the fall in reduction time with temperature increase shows a steep rise as the storage time increases (Figure 5). Again the linear fit is not completely adequate, since a further examination of the results shows that this temperature gradient becomes less steep as the storage time increases, changing at the rate of — 0·3 minute per half-hour storage time.

Relationship Between Control Value and Rate of Change in Quality.

It has been found that the initial quality of a raw milk plays some part in determining its behaviour following storage at various temperatures and times. In fact, a definite correlation has been obtained between the control reduction time and the effect of storage temperature. This correlation shows that good quality milks deteriorate more rapidly in response to increased storage temperature than do low quality milks. A correlation has also been found between the control value and the rate of change of the temperature deterioration gradient with increased storage time. Thus good quality milks—i.e., milks giving long methylene blue reduction times—in addition to showing a more rapid falling off in reduction time with increasing storage temperature show a more rapid rate of such falling off with increasing storage time.

Any explanation which can be offered in respect to these observations can be only speculative, but it seems probable that good quality milks by virtue of their low bacterial populations allow greater scope for bacterial development than do those of low quality. The accumulated waste products of metabolism, and possibly the antagonism between individual members of the milk flora, would tend to retard bacterial development in those milks carrying a heavy bacterial population. Since bacterial activity is the main factor governing the rate of reduction of methylene blue, this rate may be slowed up as a consequence.

Regardless of the trends observed with milks of varying quality, it is true that, within the scope of effective testing by means of methylene blue, good quality milks possess a longer reduction time following storage than do low quality milks.

Effects of Other Factors.

The data obtained by experiment were also examined for trends resulting from shade temperatures on the days of testing, but without result. Similarly no difference could be observed between hand- and machine-milking and between farm-cooled and uncooled milk.

Bactericidal Property of Fresh Milk.

This experimental work provides no evidence of any bactericidal property in fresh milk. Any such property was without effect on the methylene blue reduction time of milk examined two hours after the completion of milking. It appears, therefore, that any bactericidal action possessed by fresh milk can be ignored in milk quality control work, at least in southern Queensland.

REFERENCES.

AMERICAN PUBLIC HEALTH ASSOCIATION. 1941. Standard methods for the examination of dairy products. 8th Edition.

BARKWORTH, H. 1944. Advisory Dairy Bacteriologists—Review of papers presented from 1925-1943. Dairy Industr. IX.: 171-180.

CHALMERS, C. H. 1938. The methylene blue reduction test and the keeping quality of milk. J. Dairy Res. 9: 351-355.

ELLENBERGER, H. B., and MOODY, R. I. 1930. Preparing samples of milk for the methylene blue reduction test. Vt. Agric. Expt. Sta. Bull. 312.

FRAYER, J. M. 1934. Light and temperature as factors in the methylene blue reduction test. Vt. Agric. Expt. Sta. Bull. 374.

Hoy, W. A., and Braz, M. 1938. The methylene blue reduction test in relation to the age and storage temperature of milk. (Abstracts) Proc. Soc. Agric. Bact. 21.

Newton, W. 1942. Links in milk improvement. Dairy Industr. VII.: 203-205.

Nichols, A. A., and Edwards, S. J. 1936. The comparative values of the plate count and the modified methylene blue reduction test as routine methods for grading milk. J. Dairy Res. 7: 258-270.

Phillips, R., and Thomas, S. B. 1939. Milk cooling on the farm. Welsh J. Agric. XV.: 150-163.

Powell, H. M., Jenkins, E., and Thomas, S. B. 1939. Methylene blue reduction by duplicate samples of raw morning's milk held overnight. Welsh J. Agric. XV.: 164-168.

Procter, F. 1943 a. A study of commercial milk in wartime. Dairy Industr. VIII.: 211-214.

————. 1943 b. Milk distribution. Dairy Industr. VIII.: 272-276.

Wilson, G. S. 1935. The bacteriological grading of milk. Spec. Rep. Ser. Med. Res. Coun., London, No. 206.

A. H. Tucker, Government Printer, Brisbane.

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