PUBLIC HEALTH REPORTS

VOL. 40 JUNE 5, 1925 NO. 23

STUDIES ON OXIDATION-REDUCTION

VIII. METHYLENE BLUE

By W. MANSFIELD CLARK, Chief of Division of Chemistry, BARNETT COHEN, Chemist, and H. D. GIBBS, Senior Chemist, Hygienic Laboratory, United States Public Health Service

CONTENTS

I. Introduction

As litmus was formerly the favorite detector of "acidity," so methylene blue is to-day the favorite indicator of reduction.

The reason for this would be difficult to see without historical perspective. The past has left no accumulation of data on oxidation-reduction comparable in type to the semi-quantitative data which led to the supremacy of litmus in differentiating "acidity" from "alkalinity." No one, to our knowledge, ever drew an artificial line of demarcation between oxidative and reductive solutions at the region of methylene blue decoloration; and while certain specific phenomena have been treated as if methylene blue were a unique reagent, there has remained a saving sense of a wider view. But when we trace significant events in the history of this interesting dye, we find that at almost the same moment it gained prestige as an invaluable staining reagent (Dreser, 1885, Ehrlich, 1886), and as an indicator of biochemical reduction (Ehrlich, 1885). As a staining reagent, the commercial grade of this dye has ever since been considered an essential of cytological equipment. As an indicator of biochemical reduction, it has held its place through a process akin to natural selection.

We are now able to show in quantitative terms what can be guessed from Ehrlich's (1885) qualitative study of the combined velocity, capacity, and intensity factors of biological reduction; namely, that the oxidation-reduction system of methylene blue stands in the scale of oxidation-reduction *intensity* distinctly beyond the position of the very easily reduced indophenols, but yet distant from the utmost limit of reduction intensity. Consequently, the decoloration of methylene blue reveals a reduction intensity unmistakably distinct, yet not extreme. Moreover, it is a much more intense tinctorial agent than the sulphonated indigoes, the potentials of which stand midway between the reductive intensity of indophenols and the extreme of hydrogen overvoltage. Thus methylene blue, among the many dyes which were products of an enthusiasm for "synthetic colors," happened without design to possess characteristics so well adapted to a first crude survey of biological reduction that it has survived.

As a staining reagent, and as an indicator of reduction, methylene blue soon became a common laboratory supply. As such it seems to have fallen into almost every conceivable use, ranging from an indicator in volumetric analysis to a therapeutic agent.

In several of these uses there can now be revealed common principles. Since these are operative in phenomena which have been cited by those who have speculated upon biological oxidation, we shall make this paper the occasion for remarks of general interest.

First we shall lay before the reader the data that we have obtained on the oxidation-reduction equilibria.

The main features of the potentiometric studies are so like those described in previous papers of this series that only special aspects need be mentioned. However, these special aspects are important for an appreciation of the more formally tabulated data; and assuming that the reader is familiar with the main features of previous papers, we shall save space by placing in perspective at the outset the difficulties attending the establishment of accurate characteristic constants for the peculiar compound, methylene blue.

II. Preparation and Analyses of Material

Preparations of methylene blue chloride, which, for brevity, we shall call methylene blue, were made by two well-known methods. An examination of the products and a consideration of the numerous side reactions which are possible, convinced us that the preparation of pure methylene blue is largely a problem of purification subsequent to synthesis. Since commercial preparations were available in the quantity required for adequate fractionation, we made use of them, drawing our supply from five different manufacturers at home and abroad.

In the following summary will be found analytical data which require a foreword. There is still disagreement upon the determination of moisture in methylene blue. Koch (1879) and Bernthsen (1885) report different results. Atack (1915) states that methylene blue is not completely dried at 105° C. and that decomposition sets in at 110° C. Wales and Nelson (1923) state that "in every case the salts (*samples of methylene blue chloride*) could be completely dehydrated by drying them at 110° for one day, thereby confirming Koch's results." Wales and Nelson used a vapor-pressure method involving drying at low pressures. *In vacuo* (2 cm. Hg.), at 100° C., our samples attained only *approximate* constancy of weight within the periods recorded below and underwent changes, presently to be mentioned, which make us skeptical regarding the significance of this and further drying.

Loss on drying sample F

	Gram	Loss, per cent.
Weight of sample before drying	0. 5008	-----
Weight of sample after 5 hours drying	. 3922	21. 69
Weight of sample after 8 hours drying	. 3914	21. 85
Weight of sample after 13.5 hours drying	. 3908	21. 96

Loss on drying sample G

Weight of sample before drying	0. 5026	-----
Weight of sample after 2 hours drying	. 3967	21. 07
Weight of sample after 7 hours drying	. 3940	21. 61
Weight of sample after 11 hours drying	. 3933	21. 75

These samples had been recrystallized from water and dried for a short time in air at laboratory temperature. For a similar preparation, Atack found, indirectly by titanium titration, 22.7 per cent moisture. This, he noted, agreed closely with the formula containing "$5H_2O$," for which the percentage moisture should be 21.99. As indicated above, our water-crystallized samples give "moistures" close to that required by five molecules of water of crystallization; but as Wales and Nelson (1923) have shown, their vapor-pressure method gives no evidence that this water is present as water of crystallization. It is not perfectly clear that Atack's experiment on drying was made with material which contained no trace of ethanol. We found that a sample containing ethanol, on heating in air at 40°, gave a "disagreeable odor," as noted by Atack. We recognized the odor of acetaldehyde.

Assuming, for purposes of comparison only, the essential correctness of the "moisture determinations," we can put our data in the form shown in Table 1.

TABLE 1.—*Comparison of estimated dye content of methylene blue samples*

Sample	"Moisture" (per cent)	Percentage dye in anhydrous material calculated from—				
		"Moisture" content	Nitrogen content	Titanium titration		
				Before drying	After drying	Loss on drying
F	22.0	[1] *100*	99.4	98.2	80.2	18.0
G	21.8	*100*	98.7	98.1	85.8	12.3
A	12.8	*100*	101.1	94.4	48.1	46.3
H	16.9	*100*		97.8	75.0	22.8
B			*100*	97.0	90.9	6.1

[1] Figures in italics are arbitrary reference values.

Samples F and G had been "air-dried" at room temperature. For these there is shown, on the one hand, a substantial although not satisfactory agreement in three different estimates of anhydrous dye, and, on the other hand, a very serious loss in titratable material on drying. Sample A had had no heat treatment so far as we know. However, the available record states that it had been "desiccated." The sensitiveness of this sample to desiccation was suggested by the first titanium titration, and was made very evident on heat treatment. Sample H was a commercial one and we know nothing of its possible previous heat treatment. In the case of A and H our heading "before drying" in Table 1 refers to treatment in our hands. A portion of sample H was also dried in air at 150° C. The resulting material was quite insoluble in water and in ethanol and could not be titrated. Data on sample B are included, although the percentage of dye is estimated on the basis of nitrogen. Progressive drying gave in sequence 90, 70, 60 per cent titratable material.

Atack's note on the effect of drying differs in detail from our observation, but the general import, which Atack had no occasion to emphasize, is the same. There is uncertainty regarding the value or even the meaning of "moisture" determinations in a scheme of analytical assay; and yet for this compound, which persistently occludes material that interferes with precise assay through chlorine and sulphur determinations, "moisture" determinations are of importance. According to Wales and Nelson the water held by methylene blue is not constitutive even to the extent of being water of crystallization. Yet its loss under the conditions that we have described is associated with loss of the essential properties of methylene blue. The effects of long desiccation at low temperature

should be investigated in detail. We suspect slight denaturation even at low temperature.

Finally, since an error of one millivolt in otherwise accurate electrometric measurements can be occasioned by 2 per cent of reactive impurity, it is evident that the *precise* definition of electrometric constants is impossible until analytical control to within at least a few tenths of a per cent is assured.

Sample A.—This was a portion of the material purified by Mr. Zoller in 1919 and used in the studies reported in Clark's (1920) preliminary paper. The detailed description of the purification has been lost, but according to the available record the material was dried at room temperature *in vacuo* over stick KOH and concentrated H_2SO_4.

We found 11.54 and 11.64 per cent nitrogen. The average indicated 88.2 per cent anhydrous dye, while moisture determination indicated 87.2 per cent and titanium titration 82.3 per cent.

Sample B.—Sixty grams were added to 500 c. c. of water containing 10 c. c. of concentrated HCl. The suspension was heated on a water bath one hour and then filtered. On cooling, a large part crystallized out. The crystals were dried in air 48 hours, and then were placed in 250 c. c. of absolute ethanol which was heated to boiling. The solution was then filtered into 300 c. c. of ether. The resulting crystals were sucked dry on a Büchner funnel and dried in a vacuum desiccator for 24 hours. When this material was heated in an air oven (temperature rising slowly to not over 40°), a strong odor of aldehyde was noticed. The crystals were therefore redissolved in water and recrystallized. The final material was sucked dry and dried in an air oven at 60° for 20 hours. Weight, 28 grams.

The percentages of components found in Sample B and anhydrous dye calculated therefrom were as follows:

	Found per cent	Average per cent	Calculated anhydrous dye, per cent
Nitrogen	11.85 11.95	11.90	90.5
Chlorine	10.59 10.51	10.55	95.2
Sulphur (Parr bomb)	10.28		
(Fusion)	10.27 10.10 10.00	10.16	101.3
Sulphate sulphur	Trace		
Ash less than	0.10		

Titration with titanium trichloride indicated 88 per cent anhydrous dye, and progressive drying, as already noted, progressively diminished the titratable material.

Sample C was crystallized from water twice. It was then dissolved in hot absolute ethanol and filtered into ether. The crystals were sucked dry and further dried over soda-lime in a vacuum desiccator at room temperature.

In sample C the percentages of components found and of anhydrous dye calculated therefrom were as follows:

	Found per cent	Average per cent	Calculated anhydrous dye, per cent
Nitrogen	11.41 11.41 11.48	11.43	87.0
Chlorine	9.87 9.82	9.85	88.8
Sulphur (Parr bomb)	9.84 9.75		
(Fusion)	9.78 9.96 9.76	9.82	97.9

Sample E.—In the preparation of this material an attempt was made to remove such excess sulphur as might be present as sulphate. Commercial, medicinal methylene blue was dissolved in acidified water containing 1 per cent barium chloride. After the solution had been heated on a steam bath it was filtered and cooled. The crystals were sucked dry and re-formed from aqueous solution. They were then dissolved in absolute ethanol and the methylene blue was precipitated with ether. The sample was dried at room temperature *in vacuo*.

The 11.50 per cent and 11.58 per cent nitrogen found, indicated 87.8 per cent,—and the moisture content, 87.6 per cent anhydrous dye. There then should have been 8.8 per cent sulphur. There was found by the Parr bomb method 8.84 and 8.44 per cent, average 8.6 per cent, and by the fusion method 8.60 and 8.32 per cent, average 8.5 per cent. On the same basis, chlorine should have been 9.7 per cent, but there was found 10.3 per cent—again an excess. Titanium titration indicated 82 per cent dye.

The material, when studied potentiometrically, behaved as though a reducing material were present in the oxidant. This was confirmed by titrating a solution of the oxidant with quinone. This reducing material probably resulted from the action of ethanol.

Sample F.—A commercial sample of "medicinal methylene blue" was dissolved in hot water, filtered, and cooled. The large crystals which formed over night were filtered with the aid of suction. This process was repeated three more times with particular care in the last two crystallizations to cool the solution very slowly. Thus large, bar crystals were formed. Finally the crystals were spread on filter paper and exposed to a gentle current of air while being turned frequently. After four hours of this drying they were bottled.

Moisture determinations indicated 78 per cent anhydrous dye. On this basis there may be calculated the quantities given below:

	Calculated, per cent	Found, per cent
Nitrogen	10.25	10.19
Chlorine	8.65	8.93
Sulphur	7.82	8.11

On titrating with titanous chloride, there was indicated 76.6 per cent anhydrous dye. The reduced solution was clear, with a slight yellow tinge.

Sample G.—This material was recrystallized four times from water exactly as was sample F, except that it was given a preliminary salting out with NaCl and particular care was taken from the first, by slow cooling, to form large crystals. The sample was air-dried at room temperature exactly as was sample F.

Moisture determinations indicated 78.3 per cent dye. On this basis there may be calculated the quantities given below:

	Calculated, per cent	Found, per cent
Nitrogen	10.28	10.15
Chlorine	8.68	8.91
Sulphur	7.84	8.03

Sample H.—An untreated commercial material.

Sample I.—A material certified by the Commission on Standardization of Biological Stains as suitable for bacteriological and general staining.

Sample J.—This was sample F after repeated extraction with cholorform and ether in a Soxhlet extractor. In the case of the chloroform, extraction was continued until little color, and that apparently methylene blue, was removed. In the case of ether extraction, it was continued until practically no color appeared in the extract. The sample was dried at room temperature *in vacuo*.

Sample K.—A commercial material which spectrophotometric measurements by Mr. French indicated to be of high purity.

In every analyzed sample of methylene blue, except sample E, there was evidence of excess sulphur. In every case there was evidence of excess chlorine even when the material had been crystallized several times from distilled water. The basis of this evidence is the nitrogen value; but if this be set aside, there still persist discrepancies in the ratios of chlorine to sulphur. These ratios should agree with theory even if there were present such impurities as undermethylated thiazines.

Spectrophotometric absorption curves, kindly made by Dr. Scott and Mr. French, of the Walter Reed Hospital Laboratory Service, show appreciable although slight differences between all the samples we have tested.

The titration curves which will be discussed later, all suggest the presence of *small* percentages of electromotively active impurity. Such impurities could be identified were it practicable to apply the method used by Sullivan, Cohen, and Clark (1923) in showing contamination of one sulphonate of indigo by another. But to apply this method it is obvious that basic data for pure materials must have been established.

Undermethylated products.—For a reason which will be made clear later, we thought measurements on an undermethylated product would be useful. Doctor Scott and Mr. French supplied us with a commercial product the absorption curve of which was indicative of a dimethyl thiazine, according to the criteria of Formanek (1908), and Doctor MacNeal (1924) gave us a beautifully crystalline preparation of his dimethyl thionin.

Lauth's violet chloride, hereinafter called Lauth's violet, was prepared by oxidizing a solution of para-phenylene-diamine and hydrogen sulphide with ferric chloride solution.

The para-phenylene-diamine was dissolved in a 10 per cent aqueous solution of hydrochloric acid, and this solution, cooled with ice, was saturated with hydrogen sulphide. The theoretical amount of ferric chloride required for the oxidation was dissolved in water, and the solution was slowly run into the mechanically agitated, cold solution of para-phenylene-diamine, while at the same time hydrogen sulphide was being continuously led in. Finally, an excess of ferric chloride was added. The black mud which separated was filtered on a Büchner funnel and extracted with hot ethanol. From this solution Lauth's violet crystallized on cooling. These crystals were purified by recrystallization from hot ethanol containing sufficient ammonium hydroxide to precipitate the iron compounds present as impurity. Excess of ethanol was removed by drying at low temperature. We have since come to suspect a slight reaction of the dye with ethanol, which may account in part for discrepancies in analysis and in electrode measurements.

Two preparations were made as described. The preparation used contained 14.39 per cent nitrogen, indicating 90.28 per cent anhydrous dye. By titanium-titration there was indicated 89.6 per cent anhydrous dye.

III. Sources of Error

In the titration of thiazines a difficulty arises which was not encountered in operating with the compounds described in our previous papers. The thiazines are bases and tend to form insoluble salts

with some of the acidic oxidizing or reducing agents previously used. For instance, it is impracticable, except for end-point work, to titrate the reductant with ferricyanide or the oxidant with leuco-indigo carmine, because in each case a salt of slight solubility is formed. We have already described in the third article of this series the objections to the titanium method used in the preliminary work of Clark (1920) and of Cohen and Clark (1921). We have, therefore, depended for the determination of an orienting value of E'_0 upon the method of mixtures and upon titrations of reductant with benzoquinone.

Of these two methods, the quinone-titration method involves a slight source of error due to the fact that the potentials of the thiazine system, on the one hand, and the potentials of the quinone system, on the other, slightly overlap near the end-point of the titration, even at the pH of the buffer used. The error, which is not large, could be quantitatively allowed for and corrected were there not evidence of several other sources of error which render corrections for any one precarious. One such source is of special interest and will now be noted very briefly.

We have described in previous papers our methods of preparing the reductant of a dye by reduction with hydrogen in the presence of platinized asbestos. When filtered from the asbestos, washed with purified nitrogen, and preserved under nitrogen without any rubber connections to the nitrogen train, such solutions have been kept for days without sign of re-oxidation. In the case of reduced methylene blue there promptly appeared re-coloration. This was not due to leakage of oxygen; it was found to be a light effect. Solutions of methylene white prepared as above noted remained perfectly colorless for 24 hours when properly protected from light.

The effect of light can be observed very nicely by the following simple procedure: A solution of methylene blue mixed with a little platinized asbestos (coarse) is placed in a separatory funnel having well-greased glass cocks. The dye is then reduced with a stream of hydrogen. After complete reduction of the dye the cocks are closed, and the asbestos is allowed to settle out in the dark. Upon irradiating the clear supernatant solution with sunlight, the solution becomes blue. If, now, the apparatus is taken into darkness and shaken, the residual hydrogen in the platinized asbestos reduces the solution, and a test of the light effect may be made again. In the absence of the reducing agent the decoloration in darkness will not take place.

We are indebted to the color laboratory of the Bureau of Chemistry for a spectrophotometric measurement showing that the blue color developed by light in a solution of methylene white is methylene blue.

If the methylene white solution and hydrogenated platinized asbestos be kept in a light-tight reservoir over mercury as displacement fluid, portions can be delivered at will through a control cock and a filter. We have used this device to deliver methylene white solution to a narrow, transparent quartz tube, where the color developing on exposure can be compared with a copper sulphate standard. This is an extremely sensitive actinometer.

An attempt was made to determine the region of the spectrum having the greatest effect. For this purpose the actinometer was modified as follows: There was blown from narrow tubing of transparent quartz an electrode vessel of the form shown in Figure 1.

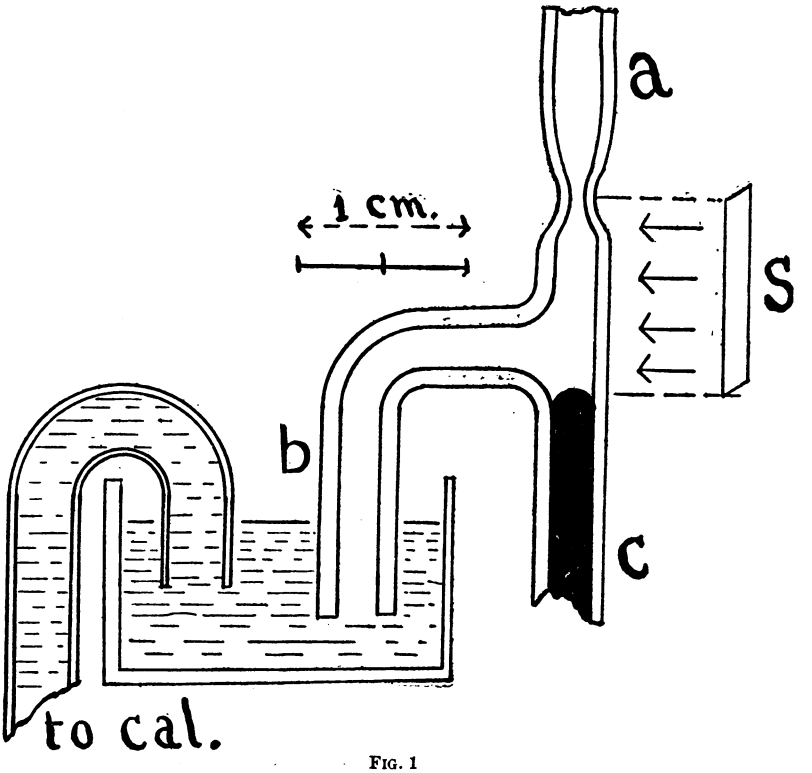


FIG. 1

Methylene white in a citrate buffer solution was delivered from the reservoir through *a*. The mercury electrode in tube *c* could have its surface renewed by wasting mercury from a reservoir into *b*. Tube *b* dipped into a saturated solution of potassium chloride through which was made liquid junction with a calomel half cell. The potential of this chain is a function of the ratio of methylene white to methylene blue. Consequently, by irradiating the methylene white from slit *s* until sufficient oxidant is formed to give a stable potential and then noting the time required on further irradiation for the po-

tential to pass between certain arbitrarily chosen values, we have an indirect measure of regenerated methylene blue which is far more delicate than visual observation of color.

The little vessel was firmly clamped against a slit s, placed at the telescope of a Hilger monochromatic illuminator having quartz optical parts.

The light source was a carbon arc operated by a 110-volt alternating current. The control of carbon feed was by clockwork in the main, but had to be supplemented by hand control in an attempt to make this powerful but unsatisfactory light source as steady as possible.

Insignificant changes in potential and no bluing of the solution visible by the light of a carbon filament electric light bulb were noted when light of the visible zones of the spectrum was streaming through the vessel. On the approach to the ultraviolet, bluing and consequent changes in potential were observed.

It is, of course, obvious that only the crudest sort of data can be obtained with the unsteady carbon arc. This is evident in Figure 2, where there are charted in μ the centers of the narrow bands of

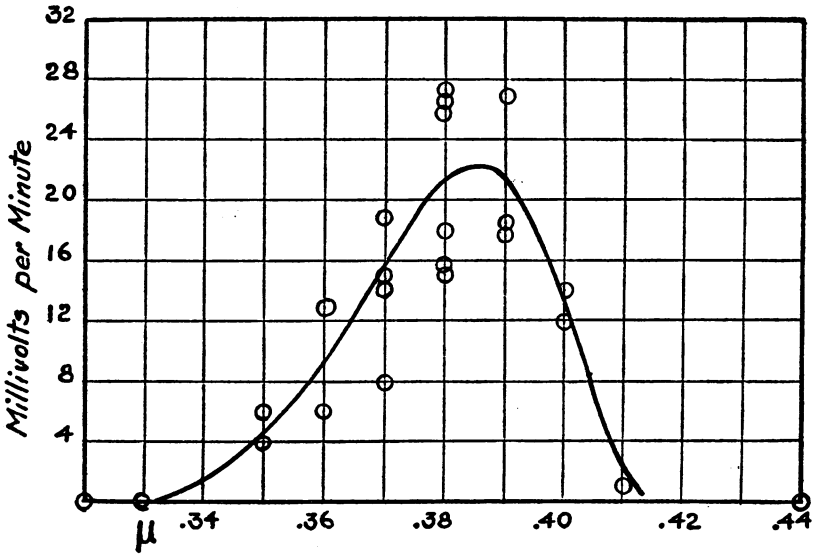


FIG. 2

wave lengths passing the slit and, as ordinates, the potential change in millivolts per minute required for the potentials to pass between two arbitrarily fixed points. The relative values of these rates are measures of relative effectiveness of the wave bands. In spite of the crudeness of the data, it is obvious that the maximum effect is centered at about 0.380 μ.

For final definition it will be necessary, of course, to operate with a more satisfactory light source. Nothing definite was gained with

the quartz-mercury vapor lamp available. Although the intense 0.365 μ line of this source falls within the zone of good effectiveness, insufficient energy passed through the illuminator. We have not studied the possible effect of the citrate buffer in screening the lower wave lengths, nor have we attempted to correct our data for the uncertain energy distribution of the carbon arc. Therefore, all we can say is that the light effect begins to be appreciable only at the edge of the visible spectrum, and this conclusion is confirmed as follows: Exposure of methylene white to daylight is much more effective when the solution is contained in quartz than in glass. Intense irradiation by monochromatic light of the visible region (e. g., yellow and green) is ineffective. Light from a carbon filament electric bulb which has little or no light of wave length shorter than 0.400 μ is ineffective, while light from a tungsten filament bulb, which has appreciable quantities of light of wave length in the zone about 0.380 μ, is effective by direct exposure.

Before it was found that methylene white is sensitive to the violet, we had been manipulating our apparatus by the light of tungsten filament lamps, and we are not sure that the absence of visible coloration can be regarded as proof that there did not take place changes too small for positive identification but large enough to have a significant part in cumulative errors. Indeed, our titration curves often have the form which would result from the presence of very small percentages of oxidant in a solution treated as if it were completely reduced.

In the preliminary paper by Clark (1920) it was shown that measurements of the methylene blue-methylene white system in neutral and alkaline solutions are rendered difficult by the slight solubility of the methylene white base. Rough estimates of the solubilities of this compound were made as follows: There was dissolved in 200 c. c. of water, 0.07 g. of methylene blue. This was filtered and then reduced with hydrogen and platinized asbestos. The reduced solution showed precipitated methylene white on the walls of the vessel and therefore must have been saturated with this compound at room temperature (about 28°). The solution was filtered into a nitrogen-protected burette and aliquots were titrated with 0.00025 molar solution of quinone. The solution in different experiments was found to be 0.00030 and 0.00035 molar. Of course, the solution prepared as above described must have been virtually acidified to a slight extent in the process of reduction.

Of more importance for present purposes are solubilities in buffer solutions. The experiment described above was repeated with proper titrating reagents, in the one case with buffer solution No. 5 as the solvent and again with buffer solution No. 22 as the solvent. In each case the temperature was about 25°. In buffer No. 5 the

acidity is such (pH 2.9) that according to our estimates of the dissociation constants, methylene white should form salts, while in buffer No. 22 (pH 8.6) only the free base could be present. The concentrations of methylene white at saturation were found to be 0.0005 to 0.0006 molar in solution No. 5 and 0.00002 molar in solution No. 22.

Such low solubilities definitely limit the range of experimental studies on homogeneous systems; and by forcing the investigator of such systems to use high dilutions, they magnify the possible effects of adsorption.

Everyone who has worked with methylene blue must have observed its very strong tendency to deposit on glass surfaces. Undoubtedly this withdrawal of oxidant from solution could be of appreciable effect in such studies as ours, but we have not investigated the matter because quantitative knowledge sufficient for our purposes would involve a study both of methylene white adsorption and of the still more difficult problem of the effects of adsorption on electrodes. We may note in passing that methylene white appears also to have a high adsorptive tendency. Recognizing the problem, we leave its quantitative significance in abeyance.

The considerable differences in the solubilities of methylene blue and methylene white at different acidities are correlated with those marked differences of these two compounds which will be discussed later. At present we shall simply note that since methylene blue must be classed as a "strong" electrolyte and methylene white as a "weak" electrolyte, we should expect to find anomalies when the data are treated by means of the classical methods. Indeed, if difficulties that we have already mentioned could be completely overcome, the system would provide excellent material for certain investigations on the difficult subject of "activities." Since the accuracy of the present measurements does not give assurance for an excursion from our elementary mode of presentation, we shall simply uncover the order of magnitude of the suggested secondary relations by showing on the one hand a "salt effect" and on the other a dilution effect.

For one experiment on the "salt effect" there were prepared solutions of the composition shown in Table 2. The pH values of the buffers diluted with water were measured and considered to be the same as those of the buffers diluted with the methylene blue-methylene white mixture. Since, as we shall show later, the potentials increase 0.0902 volt for each decrease of one unit of pH in this region, it is necessary to compare the observed electrode potentials E_h at a common pH. In this case, we took as a reference point pH 1.011. The last column of Table 2 shows the comparable values of $E_h + 0.0902 \times pH$.

TABLE 2.—*Effects of salt concentration and dilution on methylene blue potentials*

Solution	pH	Δ pH	Δ pH ×0.0902	E_h	E_h+Δ pH ×0.0902
50 c. c. C + 5 c. c. Me..	1.011			0.4409	0.4409
50 c. c. C+10 c. c. Me..	1.048	0.037	0.0033	.4335	.4368
50 c. c. C+15 c. c. Me..	1.090	.079	.0071	.4280	.4351
50 c. c. B+ 5 c. c. Me..	1.041	.030	.0027	.4363	.4390
50 c. c. B+10 c. c. Me..	1.079	.068	.0061	.4301	.4362
50 c. c. B+15 c. c. Me..	1.123	.112	.0101	.4253	.4354
50 c. c. A+ 5 c. c. Me..	1.064	.053	.0048	.4332	.4380
50 c. c. A+10 c. c. Me..	1.098	.087	.0079	.4280	.4359

A=0.1 M HCl, 0.1 M NaCl.
B=0.1 M HCl, 0.3 M NaCl.
C=0.1 M HCl, 0.6 M NaCl.
Me=partially reduced, aqueous, methylene blue (F) approximately 0.002 molecular before reduction.

It will be noticed that while there is an appreciable "salt effect," the dilution effect is much larger.

The order of magnitude of the "salt effect" here shown was confirmed by experiments with citrate solutions, and the dilution effect was shown more clearly in the following experiment. We have already mentioned a limit to the range over which concentration effects may be studied and the possibility that adsorption effects may upset calculations dealing with the very low concentrations we are forced to use. This will be remembered in considering the following remarkable data. A solution, the analysis of which proved to be 32 per cent oxidant, 68 per cent reductant, and 0.00083 molar with respect to total dye, was prepared in buffer No. 5 and added in successively increasing quantities to 50 c. c. of buffer No. 5. The potential was measured after each addition. Assuming no alteration of pH, there is a remarkable variation of potential with concentration of dye, as shown in Table 3, in which concentration is found in the first column and the averages of two closely agreeing sets of potential measurements are shown in the second column.

TABLE 3.—*Apparent effect of concentration of total dye on the potential of a fixed mixture of methylene blue and methylene white*

Concentration of total dye (molar)	Average E_h observed (volts)
0.000016	+0.2674
.000032	.2626
.000076	.2597
.000138	.2572
.000192	.2556
.000237	.2544
.000277	.2535
.000311	.2527
.000342	.2521
.000369	.2516
.000393	.2511
.000415	.2508

These data include a part of that range of concentration within which Holmes (1924) finds remarkable changes in the absorption of

light. In the paper referred to, Holmes raises several serious questions of interpretation which can not be adequately answered until several methods of study are focused upon the problem.

We had intended to include in this paper studies on various substitutions in the thiazine group of dyes. With commercial samples of toluidine blue, gentianine, and similar thiazines, we had made titanium-titrations according to the method of Clark (1920) and had reported the results at the New York meeting of the American Chemical Society (Cohen and Clark, 1921). But since materials of high purity would have to be used to obtain data on substitution comparable in accuracy with those obtained with indophenols (see previous papers, this series), it seemed hardly worth while to repeat the earlier work on various thiazines before there can be a thorough mastery of the preparation and control of this troublesome group of dyes.

On the other hand, a basic dissociation of Lauth's violet, the simplest thiazine, furnishes the key to the correlation of structure with electrode equation. Accordingly, significant data for the Lauth's violet system are presented.

IV. Buffer Solutions

In the composition of the buffer solutions some changes from the previous series were made. The new solutions are recorded in Table 4 and in subsequent tables will be referred to by number.

TABLE 4.—*Composition of buffer solutions*

Solution No.	Composition
1	250 c. c. M/5 NaCl+250 c. c. M/5 HCl+ 0 c. c. water.
2	250 c. c. M/5 NaCl+125 c. c. M/5 HCl+125 c. c. water
3	250 c. c. M/5 NaCl+ 30 c. c. M/5 HCl+220 c. c. water.
4	250 c. c. M/5 citric acid+ 50 c. c. M/5 NaOH+450 c. c. M/5 NaCl+250 c. c. water.
5	250 c. c. M/5 citric acid+125 c. c. M/5 NaOH+375 c. c. M/5 NaCl+250 c. c. water.
6	250 c. c. M/5 citric acid+210 c. c. M/5 NaOH+290 c. c. M/5 NaCl+250 c. c. water.
7	250 c. c. M/5 citric acid+300 c. c. M/5 NaOH+200 c. c. M/5 NaCl+250 c. c. water.
8	250 c. c. M/5 citric acid+400 c. c. M/5 NaOH+100 c. c. M/5 NaCl+250 c. c. water.
9	250 c. c. M/5 citric acid+500 c. c. M/5 NaOH+ 0 c. c. M/5 NaCl+250 c. c. water.
10	208 c. c. M/5 citric acid+500 c. c. M/5 NaOH+292 c. c. water.
11	185 c. c. M/5 citric acid+500 c. c. M/5 NaOH+315 c. c. water.
12	250 c. c. M/5 Na_2HPO_4+230 c. c. M/5 HCl+520 c. c. water.
13	250 c. c. M/5 Na_2HPO_4+190 c. c. M/5 HCl+560 c. c. water.
14	250 c. c. M/5 Na_2HPO_4+145 c. c. M/5 HCl+605 c. c. water.
15	250 c. c. M/5 Na_2HPO_4+100 c. c. M/5 HCl+650 c. c. water.
16	250 c. c. M/5 Na_2HPO_4+ 40 c. c. M/5 HCl+710 c. c. water.
17	250 c. c. M/5 Na_2HPO_4+ 15 c. c. M/5 HCl+735 c. c. water.
18	250 c. c. M/5 H_3BO_3+ 10 c. c. M/5 NaOH+490 c. c. M/5 NaCl+250 c. c. water.
19	250 c. c. M/5 H_3BO_3+ 16 c. c. M/5 NaOH+484 c. c. M/5 NaCl+250 c. c. water.
20	250 c. c. M/5 H_3BO_3+ 30 c. c. M/5 NaOH+470 c. c. M/5 NaCl+250 c. c. water.
21	250 c. c. M/5 H_3BO_3+ 55 c. c. M/5 NaOH+445 c. c. M/5 NaCl+250 c. c. water.
22	250 c. c. M/5 H_3BO_3+ 80 c. c. M/5 NaOH+420 c. c. M/5 NaCl+250 c. c. water.
23	250 c. c. M/5 H_3BO_3+160 c. c. M/5 NaOH+340 c. c. M/5 NaCl+250 c. c. water.
24	250 c. c. M/5 H_3BO_3+240 c. c. M/5 NaOH+260 c. c. M/5 NaCl+250 c. c. water.
25	125 c. c. M/5 Na_2HPO_4+ 40 c. c. M/5 NaOH+210 c. c. M/5 NaCl+625 c. c. water.
26	125 c. c. M/5 Na_2HPO_4+ 90 c. c. M/5 NaOH+160 c. c. M/5 NaCl+625 c. c. water.
27	125 c. c. M/5 Na_2HPO_4+150 c. c. M/5 NaOH+100 c. c. M/5 NaCl+625 c. c. water.
28	250 c. c. M/5 NaOH+250 c. c. M/5 NaCl+500 c. c. water.
29	250 c. c. M/5 NaOH+750 c. c. water.
30	250 c. c. M/5 NaOH+250 c. c. water.

V. Electrode Measurements on Lauth's Violet

In Table 5 are the results of two series of measurements on Lauth's violet by the method of mixtures. A saturated aqueous solution of Lauth's violet was filtered and divided into two portions. One was de-aerated and the other reduced with hydrogen and platinized asbestos. These two solutions were then added in the ratios shown, so that a total of 5 c. c. of oxidant and reductant was held in 50 c. c. of buffer solution. Assuming no effect of this small amount of dye solution on the pH, the pH of the dye-free buffer + 5 c. c. of water, which was found to be 2.867, was considered to be the value for the mixture.

TABLE 5.—*Mixtures of equimolecular solutions of Lauth's violet and its reduction product. In buffer of pH 2.867*

Ratio $\frac{[S_r]}{[S_o]}$	E_h	E'_o
$\frac{60.8}{39.2}$	+0.2986	0.3043
$\frac{60}{40}$	.3000	.3053
$\frac{50}{50}$	.3040	.3040
$\frac{40}{60}$	.3088	.3035
	.3091	.3038

Average, 0.3042

In Table 6 are the data on a titration of reduced Lauth's violet with benzoquinone. It will be understood from what has already been said that the end point is somewhat uncertain, that consequently the point taken is to some degree uncertain, and that corrections for change in pH can not be made with assurance.

We shall assume for pH 2.867, the value $E'_o = 0.305$.

TABLE 6.—*Titration of reduced Lauth's violet (GB) with benzoquinone at pH 2.867*

Quinone (c. c.)	Oxidation (per cent)	0.03006 log $\frac{[S_r]}{[S_o]}$	E_h	E'_o	Deviation from 0.3052
1	8.77	+0.0306	0.2771	0.3077	+0.0025
2	17.54	.0202	.2859	.3061	+.0009
3	26.32	.0134	.2920	.3054	+.0002
4	35.09	.0080	.2973	.3053	+.0001
5	43.86	+.0032	.3020	.3052	.0000
6	52.63	−.0014	.3066	.3052	.0000
7	61.40	−.0061	.3113	.3052	.0000
8	70.17	−.0112	.3169	.3057	+.0005
9	78.94	−.0173	.3235	.3062	+.0010
10	87.72	−.0257	.3336	.3079	+.0027
11	96.50	−.0433	.3566	.3133	+.0081
11.4	100.00				

In Tables 7 and 8 are summarized measurements made upon fixed mixtures of oxidant and reductant in solutions of different pH values. As has been our custom, we have reduced the data to E'_0 values for the convenience of the reader, and to do this have made measurements in each series with solution No. 5 for which at pH 2.867 we have already selected the E'_0 value of 0.305. Since it was necessary to operate with the oxidant predominating and upon the "0.09 slope" of the E_h: pH curve an experimental error is to be expected in reducing the original data to E'_0 values, and, indeed, there appears a discrepancy between Tables 7 and 8 revealed by the predominating negative deviations of Table 8. Evidently undue weight was given to one orienting value in either Table 7 or 8, and since the reduction to E'_0 values has no weight in determining K_{r2}, K_{r3}, K_{ob}, and the slopes of the several sections of the curve, we have made an arbitrary constant correction of the deviations in Table 8 which gives a fairer picture of the alignment of the experimental data (exclusive of one orienting experiment) with the calculated curve.

TABLE 7.—*Lauth's violet. Relation of E'_0 to pH. First series*

[$E'_{pH\ 0}=0.563$; $Kr_2=5\times10^{-6}$; $Kr_3=4.2\times10^{-5}$; $K_{ob}=1.88\times10^{-3}$]

Solution No.	pH	E'_0 calc.	E'_0 found	Deviation
1	1.076	+0.466	+0.465	−0.001
2	1.369	.440	.437	−.003
3	1.982	.384	.384	.000
4	2.441	.343	.339	−.004
5	2.867	.305	.303	−.002
6	3.340	.263	.261	−.002
7	3.864	.218	.218	.000
8	4.396	.177	.178	+.001
9	4.901	.144	.145	+.001
10	5.477	.115	.115	.000
11	5.896	.098	.098	.000
12	5.896	.098	.099	+.001
13	6.333	.083	.083	.000
14	6.662	.072	.072	.000
15	6.967	.063	.062	−.001
16	7.517	.046	.045	−.001
17	7.844	.036	.031	−.005
18	7.493	.047	.046	−.001
19	7.691	.041	.040	−.001
21	8.393	+.020	+.021	+.001
23	9.238	−.006	−.006	.000
26	12.115	−.127	−.123	+.004
30	12.589	−.154	−.149	+.005

TABLE 8.—*Lauth's violet. Relation of E'₀ to pH. Second series*

[$E'_{pH\ 0}=0.563$; $Kr_2=5\times10^{-6}$; $Kr_3=4.2\times10^{-5}$; $K_{ob}=1.88\times10^{-3}$]

Solution No.	pH	E'₀ calc.	E'₀ found	Deviation	Deviation corrected
1	1.073	0.466	0.457	−0.009	−0.006
3	1.978	.385	.379	−.006	−.003
5	2.872	.305	.302	−.003	.000
6	3.344	.263	.263	.000	+.003
7	3.828	.221	.222	+.001	+.004
8	4.377	.178	.180	+.002	+.005
9	4.919	.143	.142	−.001	+.002
10	5.482	.114	.114	.000	+.003
11	5.911	.098	.095	−.003	.000
13	6.351	.082	.079	−.003	.000
15	6.971	.063	.059	−.004	−.001
16	7.517	.046	.043	−.003	.000
17	7.965	.033	.030	−.003	.000
20	8.055	.030	.026	−.004	−.001
21	8.396	+.020	+.018	−.002	+.001
23	9.241	−.006	−.011	−.005	−.002
24	10.129	−.034	−.039	−.005	−.002
25	10.989	−.067	−.072	−.005	−.002
26	11.455	−.090	−.092	−.002	+.001
27	11.759	−.106	−.110	−.004	−.001
28	12.273	−.136	−.138	−.002	+.001
30	12.293	−.137	−.136	+.001	+.004

VI. Electrode Measurements on Methylene Blue

Sample A, by the method of mixtures, gave the data of Table 9, and titration with quinone of the reduced solution gave the data of Table 10. A repetition of this experiment gave essentially the same picture.

TABLE 9.—*Methylene blue (sample A). Mixtures of oxidant and reductant at pH 2.859*

[Total oxidant and reductant approximately 0.0001 molar]

First series			Second series	
Ratio $\frac{[S_r]}{[S_o]}$	E_h	E'_o	E_h	E'_o
$\frac{70}{30}$	0.2625	0.2736	----------	----------
$\frac{60}{40}$	.2681	.2734	0.2679	0.2732
$\frac{50}{50}$	.2724	.2724	.2728 .2728	.2728
$\frac{40}{60}$	.2777	.2724	.2781	.2728
$\frac{30}{70}$	.2830	.2719	----------	----------
Average		+0.2727		+0.2729
$E'_{pH\ 0}$		+.5306		+.5308

TABLE 10.—*Titration of reduced methylene blue (sample A) with benzoquinone at pH 2.859*

Quinone (c. c.)	Oxidation (per cent)	$0.03006 \log \frac{[S_r]}{[S_o]}$	E_h	E'_o	Deviation from 0.2730
1	6.45	0.0349	0.2395	0.2744	+0.0014
2	12.90	.0250	.2491	.2741	+.0011
3	19.36	.0186	.2555	.2741	+.0011
4	25.81	.0138	.2601	.2739	+.0009
5	32.26	.0097	.2640	.2737	+.0007
6	38.71	.0060	.2674	.2734	+.0004
7	45.17	+.0025	.2705	.2730	.0000
8	51.61	−.0008	.2738	.2730	.0000
9	58.06	−.0042	.2772	.2730	.0000
10	64.52	−.0078	.2807	.2729	−.0001
11	70.97	−.0117	.2845	.2728	−.0002
12	77.42	−.0161	.2888	.2727	−.0003
13	83.87	−.0215	.2942	.2727	−.0003
14	90.33	−.0292	.3013	.2721	−.0009
15.5	100				
Average				+0.2730	
$E'_{pH\ o}$				.5309	

Sample B in preliminary measurements seemed very unsatisfactory and was rejected for electrode measurements.

Sample C gave, on titration of the reduced solution with quinone, an estimated end point at 15.87 c. c. When this value was used, the E'_o values calculated from the observed potentials are those of the second column of Table 11.

TABLE 11.—*Methylene blue (sample C). E'_o values calculated from quinone-titration at pH 2.863*

[Methylene blue approximately 0.0001 molar]

Quinone (c. c.)	E'_o	Quinone (corrected) c. c.	E'_o corrected
1	0.2779	1.4	0.2736
2	.2767	2.4	.2743
3	.2759	3.4	.2743
4	.2756	4.4	.2744
5	.2752	5.4	.2742
6	.2749	6.4	.2740
7	.2746	7.4	.2739
8	.2746	8.4	.2739
9	.2745	9.4	.2739
10	.2744	10.4	.2738
11	.2743	11.4	.2738
12	.2742	12.4	.2738
13	.2747	13.4	.2743
14	.2750	14.4	.2746
15.87		16.27	
Average			0.2741
$E'_{pH\ o}$			=0.5323

Such a distribution of values is much like that which would occur were the titration begun on a solution already partially oxidized. The solution had been fairly well protected from light and appeared colorless when delivered to the faintly illuminated burette. However, if we assume about 2.5 per cent initial oxidation and correct for this by assuming that the equivalent of 0.4 c. c. quinone was already

present, we obtain the E'_o values of the last column of Table 11. Allowing for minor corrections of acidity change, which would have to be made to perfect any such series of values, the agreement seems reasonable.

A subsequent repetition of the quinone titration on sample C gave—E'_o uncorrected average 0.2756, and corrected, 0.2745, or $E'_{pH\ 0}=0.533$.

Measurements on mixtures of oxidant and reductant at pH 2.863 gave the data of Table 12.

TABLE 12.—*Methylene blue (sample C). Mixtures of oxidant and reductant at pH 2.863*

[Total oxidant and reductant approximately 0.0001 molar]

Ratio $\frac{[S_r]}{[S_o]}$	E_h	E'_o	Ratio $\frac{[S_r]}{[S_o]}$ corrected	E'_o corrected
$\frac{60}{40}$	0.2692	0.2745	$\frac{58.5}{41.5}$	0.2737
$\frac{50}{50}$	.2745	.2745	$\frac{48.75}{51.25}$	.2738
$\frac{40}{60}$	.2789	.2736	$\frac{38.8}{61.2}$	.2730

Average +0.2742 ..... +0.2735
$E'_{pH\ 0}$ = +0.5324 ..... +0.5317

In the last two columns of Table 12 are given the results of corrections for the 2.5 per cent oxidant in the reductant assumed to correct the quinone titration of the same sample.

Sample E on titration with quinone gave a series of E'_o values with graphic mid-point at 0.276, which became reasonably concordant with 0.2739 ($E'_{pH\ 0}=0.531$) (see Table 13) when an end-point at 2 c. c. less than that judged by graphic inspection of the original data was selected. This suggested the presence of reducing impurity active in the zone intermediate between the methylene blue system and the quinone system. Comparable data obtained by the method of mixtures also showed deviations which could be interpreted as due to presence of a reducing impurity in the oxidant. A quinone titration of the sample definitely disclosed the presence of a reducing substance which had, strangely enough, survived air exposure and which was sufficient to account for the above discrepancies.

TABLE 13.—*Titration of reduced methylene blue (sample E) with benzoquinone at pH 2.849*

[Methylene blue approximately 0.00009 molar]

Quinone (c. c.)	Oxidation (per cent)	$0.03006 \log \frac{[S_r]}{[S_o]}$	E_h	E'_o	Deviation from 0.2739
2	8.70	0.0307	0.2418	0.2725	−0.0014
3	13.04	.0248	.2483	.2731	−.0008
4	17.39	.0203	.2530	.2733	−.0006
5	21.74	.0167	.2568	.2735	−.0004
6	26.09	.0136	.2601	.2737	−.0002
7	30.43	.0108	.2630	.2738	−.0001
8	34.78	.0082	.2656	.2738	−.0001
9	39.13	.0058	.2679	.2737	−.0002
10	43.48	.0034	.2703	.2737	−.0002
11	47.83	+.0011	.2727	.2738	−.0001
12	52.18	−.0011	.2750	.2739	.0000
13	56.52	−.0034	.2773	.2739	.0000
14	60.87	−.0058	.2797	.2739	.0000
15	65.22	−.0082	.2821	.2739	.0000
16	69.57	−.0108	.2847	.2739	.0000
17	73.91	−.0136	.2876	.2740	+.0001
18	78.26	−.0167	.2908	.2741	+.0002
19	82.61	−.0203	.2945	.2742	+.0003
20	86.96	−.0248	.2991	.2743	+.0004
21	91.31	−.0307	.3046	.2739	.0000
22	95.65	−.0403	.3122	.2719	−.0020
23	100.00	----------	.3250	----------	----------

$E'_{pH\ o} = 0.5309$

Sample F.—By the method of mixtures at pH 2.851 (solution No. 5), there were found the relations seen in Table 14.

A titration of the reduced solution with quinone gave a series of E'_o values, varying in a more or less orderly fashion. Graphically, we estimate the mid-point of the titration curve to be 0.276, giving $E'_{pH\ o} = 0.534$. The average of the two measurements is 0.533.

TABLE 14.—*Methylene blue (sample F). Mixtures of oxidant and reductant at pH 2.851*

[Total oxidant and reductant approximately 0.00009 molar]

Ratio $\frac{[S_r]}{[S_o]}$	E_h	E'_o
$\frac{60}{40}$	0.2712	0.2765
	.2711	.2764
$\frac{50}{50}$	.2760	.2760
	.2757	.2757
$\frac{40}{60}$	.2804	.2751
	.2800	.2747

Average ---------- +0.2757
$E'_{pH\ o}$ ---------- = +0.5329

Sample G, by the method of mixtures at pH 2.851, gave the values shown in Table 15.

Quinone titration of this sample gave uniformly varying values of E'_o which we were not able to interpret. Graphically, a mid-point was estimated at 0.277, giving $E'_{pH\ o} = 0.534$.

TABLE 15.—*Methylene blue (sample G). Mixtures of oxidant and reductant at pH 2.851*

[Total oxidant and reductant approximately 0.00006 molar]

Ratio $\frac{[S_r]}{[S_o]}$	E_h	E'_o
$\frac{60}{40}$	0.2723	0.2776
$\frac{50}{50}$	.2774	.2774
$\frac{40}{60}$	.2813 .2815	.2761

Average..... 0.2770
$E'_{pH\ 0}$=..... 0.5342

Sample H was found too impure to work with.

Sample I was found by titration to be grossly impure. It should here be noted that commercial grades of methylene blue suitable for staining need not be, and perhaps are preferably not, pure methylene blue. (Compare Scott and French, 1924.)

Sample J, which was sample F extracted with chloroform and ether, was titrated with benzoquinone and gave the same type of deviation observed with sample F and a graphically estimated mid-point identical with that found for F at the same pH.

Sample K gave a peculiar titration curve difficult to interpret but surely indicative of some impurity.

In brief summary, we have the better values for the potentials of an equimolecular mixture reduced for convenience of comparison to the values at pH=0 ($E'_{pH\ 0}$) which are assembled in Table 16. Of these, the most consistent are the values for sample A. In the case of sample E the presence of the reducing impurity, of which there was direct experimental evidence, would interfere with the determination of an E'_o value by the method of mixtures, but it need not necessarily injure seriously the results of a quinone titration if the impurity becomes active only near the close of the titration and if we correct for the end-point from internal evidence. If the end-point correction (which was made solely to characterize the first, larger section of the titration) be allowed, it turns out that the constant for sample E is remarkably close to that of sample A.

TABLE 16.—*Methylene blue. Summary of values for $E'_{pH\ 0}$*

Sample	Method of mixtures		Quinone titration		
	Observed	Corrected for impurity	Graphic estimate	Calculated in detail	Corrected for impurity
A	0.5306 .5308			0.5309	
C	.5324	0.5317			0.5323
E			0.533		.5309
F	.5329		.534		
G	.5342		.534		
Average	.5322	.5317	.534	.5309	.5316

Some of the measurements were made before the effect of dilution was fully realized (see p. 1144), and consequently the data as they accumulated were data for somewhat different concentrations. However, on looking back over our notes we find that the variations in concentration were certainly not of sufficient magnitude to account for the discrepancies of Table 16.

A tempting hypothesis which we considered is this: Having shown that certain characteristic potentials for methylene blue are distinctly lower than those of Lauth's violet, we might assume that undermethylated preparations would show intermediate values. We would then expect that a pure methylene blue would give not only the most negative potential but the most uniform sets of data as are found for instance with sample A, while samples containing undermethylated products as impurity would give variable and more positive potentials as are actually found with the other samples. Against this hypothesis stands a titration we made of a commercial preparation reported to us on the basis of spectrophotometric data as a distinctly undermethylated product. In comparison with sample A, it gave distinctly more negative values. We then obtained from Dr. W. J. MacNeal a sample of his beautifully crystalline "dimethyl thionin." On titration this gave a peculiar series of deviations *suggestive* of a small percentage of some active impurity. However, the graphic mid-point at pH 2.83 was +0.268, or $E'_{pH\,0} = +0.523$, which, by inspection of Table 16, indicates again a displacement in the direction opposite to that expected from the hypothesis suggested above.

These comparative data might appear conclusively to militate against the hypothesis proposed above; but, as we have emphasized in previous papers, it is dangerous to interpret substitution effects before dissociation constants are known. We shall show presently that in acid solutions, where alone it is feasible to make measurements of the type now under consideration, we are on a "0.09-slope" of the E'_o:pH curve which is comparable to a similar slope of the indophenols. Inspection of Figure 1 of the sixth paper of this series will suggest the danger we now note. In the present instance we have not completely defined each system because we saw no use in developing the refined aspects of the subject with material of dubious purity. Consequently the hypothesis that discrepant results with different samples of methylene blue are due to undermethylated impurities must be left undecided until the effects of dissociation upon the position of the curve having the "0.09-slope" are determined.

In Tables 17 and 18 are summarized measurements made with fixed mixtures of oxidant and reductant (corrected to an equimolecular mixture) introduced into buffers of different pH values.

Here again it will be seen that the data are not so concordant as those obtained with other types of compound; but that the essential features of the relations are fairly clear will be seen from Figure 3, where the data of Table 17 are shown as dots, and supplementary data from Table 18 are shown as circles.

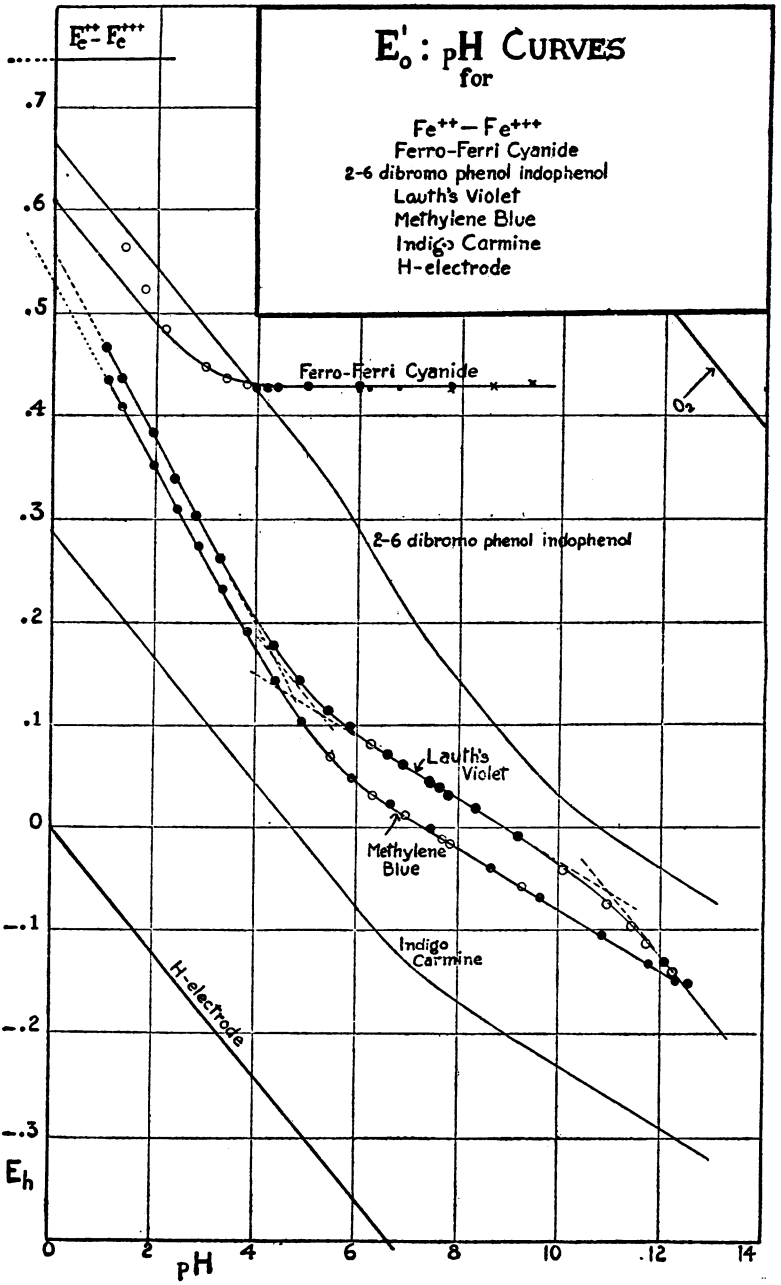


FIG. 3

In this figure we have extended the "0.09-slope" of the curve to pH = 0, since preliminary measurements at high acidities indicated that this extension could be made. There is, however, an apparent deviation in 3N HCl, which may mean either that the curve tends to return to the "0.06-slope" or that our preliminary measurements were wrongly interpreted through our failure up to this point adequately to allow for relative activities.

TABLE 17.—*Methylene blue. Relation of E'_o to pH. First series*

[$E'_{pH\ 0}$=0.532; Kr_2=1.4×10⁻⁶; Kr_3=3×10⁻⁵]

Solution No.	pH	E'_o calculated	E'_o found	Deviation
1	1.07	0.435	0.436	+0.001
2	1.37	.409	.409	.000
3	1.98	.353	.353	.000
4	2.45	.311	.311	.000
5	2.88	.273	.275	+.002
6	2.36	.230	.233	+.003
7	3.84	.188	.192	+.004
8	4.39	.144	.145	+.001
9	4.92	.105	.105	.000
11	5.92	.051	.051	.000
14	6.67	+.022	.024	+.002
16	7.48	−.004	.000	+.004
22	8.62	−.039	−.040	−.001
23 1/2	9.61	−.069	−.068	+.001
25	10.82	−.105	−.103	+.002
27	11.74	−.133	−.132	+.001
30	12.28	−.149	−.149	.000

TABLE 18.—*Methylene blue. Relation of E'_o to pH. Second series*

[$E'_{pH\ 0}$=0.532; Kr_2=1.4×10⁻⁶; Kr_3=3×10⁻⁵]

Solution No.	pH	E'_o calculated	E'_o found	Deviation
1	1.08	0.435	0.437	+0.002
2	1.37	.409	.409	.000
4	2.45	.312	.311	−.001
5	2.88	.273	.275	+.002
6	3.34	.232	.235	+.003
7	3.86	.186	.188	+.002
8	4.40	.143	.145	+.002
9	4.90	.107	.107	.000
10	5.48	.072	.071	−.001
11	5.90	.052	.050	−.002
13	6.33	.034	.033	−.001
14	6.66	.022	.024	+.002
15	6.97	+.012	+.014	+.002
16	7.49	−.005	−.003	+.002
17	7.84	−.016	−.016	.000
19	7.69	−.011	−.011	.000
23	9.24	−.058	−.057	+.001

We hope that this account of our experience with methylene blue will be helpful to someone who shall overcome the difficulties and establish more accurately the fundamental constants of the system.

After this our third series of measurements, with experience gained by studies of other systems, we are convinced that our failure to obtain concordant data of the order of agreement found in our studies of other systems is due in large measure to the inherent peculiarities of this unstable, adsorbing, polar compound, with its difference in structural type from the light-sensitive, slightly soluble reductant. Indeed not only the difficulties encountered but peculi-

arities which are suggested by experiments supplementary to the main course of experimentation, such as the dilution effect and its correlation with Holmes' observations, make it appear that methylene blue, in spite of its popularity, will ultimately be rejected from lists of oxidation-reduction indicators destined for precise use. But for the present, numerous applications of this indicator remain to be clarified, and for this purpose our data are certainly adequate.

VII. Electrode Equation

With the experimental data before us, we come to their formulation in accordance with the principles outlined in the second paper of this series.

Since the Lauth's violet system displays an inflection of the E'_o: pH curve (fig. 3) in alkaline regions which the methylene blue system does not, it furnishes the more complete picture. Therefore the following interpretation will be made with the aid of data on Lauth's violet:

The E'_o: pH curve of Lauth's violet (fig. 3) appears to have characteristics distinct from those of the dyes reported in previous papers of this series. In the acid region, the value of $\frac{-dE}{dpH}$ is 0.0902, which we shall call the "0.09-slope." While such a value was discovered among the indophenols, and was especially distinct in the case of 2, 6-dibromophenol indophenol, it had no such extension as is found in the data on the thiazines.

In Lauth's violet, the "0.09-slope" abruptly changes to a "0.03-slope" near pH 5; and since two electrons or their equivalent are concerned in the reduction process making the $\frac{RT}{nF}$ coefficient 0.03, this change of 0.06 (i. e., 2×0.03) indicates that two acid-base dissociations are encountered in this pH region. The two dissociation constants concerned are obviously not identical, because the actual inflection of the curve is not nearly so abrupt as would be the case were they identical. Do both of these constants represent ionizable groups created or destroyed in the act of reduction? If they do, we still leave unaccounted for a third group made apparent by the change from a "0.03 slope" to a "0.06-slope" at pH 11.

Since the electrometric data reveal *directly* little regarding the nature or the location of the acid-base groups encountered, it is possible to express the experimental data by a number of equations derived in accordance with the principles outlined in the second paper of this series. Without claiming to have exhausted the possibilities, we have constructed several such equations which express the experimental data well enough, but which call for bizarre chemical properties in the thiazines. But by adopting the following

rational development, we have reached a result which seems satisfactory from every viewpoint.

We shall assume that Bernthsen's (1883–1889) formula for the thiazines, supported as it is by a clever and extensive array of syntheses, is essentially correct, and we shall then write this formula in accordance with the octet theory of electronic configuration. We then have for a thiazine, Formula I, and for its reductant, Formula II, of Figure 4.

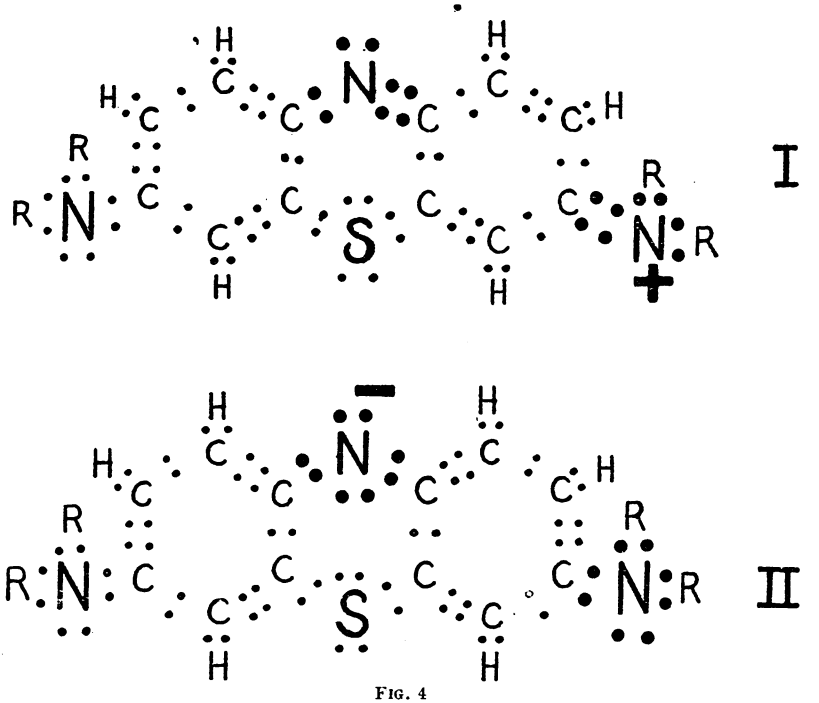


FIG. 4

It will be particularly noted that the double-bonded, terminal nitrogen of Formula I contributes but four electrons to the surrounding octet, while it has five positive charges reserved for its outer shell. Consequently this group has a distinct polar valence comparable with that of ammonium. On reduction, this polar valence is destroyed and at the same time a potential anion is created at the bridging nitrogen as in the case of the indophenols.

The oxidation-reduction process may therefore be expressed in the following form

$$Ox^+ + 2e \rightleftharpoons R\bar{e}d$$

and the corresponding electrode equation is [1]

$$E_h = C - \frac{RT}{2F} ln \frac{[R\bar{e}d]}{[Ox^+]} \qquad (1)$$

[1] See first and second papers of this series.

Next, in summing the various species of oxidant and reductant to obtain the equation embodying total oxidant, $[S_o]$, and total reductant, $[S_r]$, we shall have to take into consideration the experimental fact that three changes in steps of 0.03 are found in the slopes of the E'_o: pH curve, indicating that three dissociation constants are to be considered. In addition, there are potentially active groups which it may be well to consider.

Since we shall have to deal with basic groups and, for the sake of uniformity, desire to deal with hydrion rather than hydroxyl ion concentrations, we shall find the first section of our derivation simplified if we adopt Brönsted's (1923) extension of Michaelis' (1922) formulation of acid-base equilibria.

Brönsted unifies the representation of acid-base equilibria by the expression: acid $\leftrightharpoons$ base + H^+. Specific cases are:

$$\underset{\text{(acid)}}{\text{acetic acid}} \leftrightharpoons \underset{\text{(base)}}{\text{acetate ion}} + H^+$$

$$NH_4^+ \leftrightharpoons \underset{\text{(base)}}{NH_3} + H^+$$

The group RNH_2 may be treated as if it acquired basic properties either by addition of water and subsequent ionization of hydroxyl or by direct addition of hydrion. It is therefore immaterial to the present formalistic treatment whether we use the ordinary K_b dissociation constants or K_a constants, so long as we retain the relation $K_a = \frac{K_w}{K_b}$. We shall use either constant in accordance with convenience and shall later summarize with the customary K_b symbols.

In the following summations we shall regard each represented species as equivalent to the sum of hydrated and unhydrated molecules of the same species. For the reductant, the sum $[S_r]$ of all species is

$$[S_r] = [\overset{-}{\text{Red}}] + [\text{H Red}] + [\overset{+}{\text{H}_2\text{Red}}] + [\overset{++}{\text{H}_3\text{Red}}] \tag{2}$$

$$\frac{[\overset{-}{\text{Red}}]\,[H^+]}{[\text{H Red}]} = K_{r_1} \tag{3}$$

$$\frac{[\text{H Red}]\,[H^+]}{[\overset{+}{\text{H}_2\text{Red}}]} = K_{r_2} \tag{4}$$

$$\frac{[\overset{+}{\text{H}_2\text{Red}}]\,[H^+]}{[\text{H}_3\text{Red}^{++}]} = K_{r_3} \tag{5}$$

In the oxidant, the group $\rangle C{=}NH_2^+$ can be brought into Brönsted's formalistic scheme, but it is more realistic to treat it as a cation, adding the hydroxyl ion directly. Hence

$$[S_o] = [\overset{+}{Ox}] + [OxOH] \tag{6}$$

$$\frac{[\overset{+}{Ox}]\,[\overset{-}{OH}]}{[OxOH]} = K_{ob} \tag{7}$$

or, since we wish to use $[H^+]$

$$\frac{[\overset{+}{Ox}]\,K_w}{[H^+]\,[OxOH]} = K_{ob} \tag{7a}$$

Solving equations (2) to (7a) for $[\overset{+}{Ox}]$ and $[\overset{-}{Red}]$, substituting in (1) and collecting constants, we then have (8) in its numerical form for 30° C.:

$$E_h = E_o - 0.03006 \log \frac{[S_r]}{[S_o]} - 0.03006 \log \frac{K_{ob}[H^+] + K_w}{K_{r_1}K_{r_2}K_{r_3}[H^+] + K_{r_2}K_{r_3}[H^+]^2 + K_{r_3}[H^+]^3 + [H^+]^4} \tag{8}$$

Without further discussion, we shall assume that the bridging nitrogen fixes H^+ as was assumed for the indophenols. Consequently, Kr_1 has a value so low that the term in which it occurs can be neglected, and (8) becomes (9):

$$E_h = E_o - 0.03006 \log \frac{[S_r]}{[S_o]} - 0.03006 \log \frac{K_{ob}[H^+] + K_w}{K_{r_2}K_{r_3}[H^+]^2 + K_{r_3}[H^+]^3 + [H^+]^4} \tag{9}$$

In previous studies, the equations used for the construction of the calculated E'_o : pH curves were all of such form that when $\frac{[S_r]}{[S_o]} = 1$ and $[H^+] = 1$ normal, the neglect of second order magnitudes gave $E'_o = E_o$. On the assumption that no essential change would occur when $\frac{[S_r]}{[S_o]} = \frac{\text{normal}}{\text{normal}}$, the E_o found with *dilute* solutions under the above conditions could be called the "normal potential." In the present instance (equation 9) it will be noted that when $[H^+] = 1$ and $\frac{[S_r]}{[S_o]} = 1$, the neglect of second order values in applying the values of the constants to be given later leaves

$$E'_o = E_o - 0.03006 \log K_{ob}$$

This peculiarity arises from the fact that we have assumed both hydroxyl and hydrogen ions to be concerned, and obviously we would have met a similar situation had we continued with the same assumption and chosen to formulate the equation in terms of hydroxyl ion concentrations instead of hydrion concentrations. In short, it is necessary to remember the formalistic nature of "normal potential" and, as has frequently been noted, to define clearly the sense in which the expression is used. Indeed, had we chosen the perfectly legitimate procedure of including both hydroxyl and hydrion concentra-

tions in our equation, the term "normal potential" would become nonsense. We shall, therefore, retain our E_o in its mathematical meaning as defined by specific equations. For potentials at pH = 0 we shall use the symbol $E_{pH\,0}$, and for the half reduced solution at pH 0, the symbol $E'_{pH\,0}$.

VIII. Dissociation Constants

Since we ascribe a polar valence to the "double-bonded" terminal nitrogen and discover in Lauth's violet an inflection of the E'_o:pH curve at pH 11, we shall give to $\frac{K_w}{K_{ob}}$ a value of 10^{-11}. Tentatively accepting the value 1.88×10^{-14} for K_w at 30° as given by Michaelis (1922) we find $K_{ob} = 1.88 \times 10^{-3}$.

It will have been noted that we have left out of consideration a second group of potentially basic properties in the oxidant. Were this group active, forming the cation $Ox\overset{++}{H}$ within the experimental range of pH, we would have found at some pH-zone lower than that in which the "0.03-slope" occurs an inflection of the curve tending toward "zero slope." The inflections observed are in the opposite direction. Consequently, we can conclude that the basicity of the amino group in the oxidant is so "weak" that for all practical purposes it can be left out of account with resulting simplification of the equation. The inflections found must then be ascribed to ionizations of the two remaining groups of the *reductant.* The constants for these groups are represented by K_{r_2} and K_{r_3}.

In determining the values of K_{r_2} and K_{r_3} it is helpful to use the intersection of the projections of the so-called "0.09-" and "0.03-slopes." Those sections of the curve which are found at the region concerned are (when considered independently) determined by equations (10) to (12).

$$-E_1 = 0.03006 \log \frac{1}{K_{r_2}K_{r_3}} + 0.03006\ pH - C \qquad (10)$$

$$-E_2 = 0.0601 \log \frac{1}{K_{r_3}} + 0.0601\ pH - C \qquad (11)$$

$$-E_3 = 0.0902\ pH - C \qquad (12)$$

Equation (10) determines the "0.03-slope" and (12) the "0.09-slope"; while (11) determines the "0.06-slope" between these two limbs, which in the present case is obscured.

The intersection of (10) and (12) occurs at $E_1 = E_3$, or when $\log \frac{1}{K_{r_2}K_{r_3}} = 2 \times pH$.

Graphically we estimate the intersection to be at about pH = 4.9 (fig. 3). Hence, $\log \frac{1}{K_{r_2}} + \log \frac{1}{K_{r_3}} = 9.8$.

By subsequent trial we find that $\log \frac{1}{K_{r_2}} = 5.3$, and $\log \frac{1}{K_{r_3}} = 4.38$ (sum 9.68, intersection 4.84) fit the data fairly well. Hence we shall use $K_{r_2} = 5 \times 10^{-6}$ and $K_{r_3} = 4.2 \times 10^{-5}$.

With the values of K_{ob}, K_{r_2}, and K_{r_3} described above, $K_w = 1.88 \times 10^{-14}$, and the $E'_{pH\,0}$ previously discussed we obtain with equation (9) the calculated E'_o:pH curve shown in Figure 3 (E'_0 being the value of an equimolecular mixture at any given value of pH).

It was mentioned above that the section of the E'_o:pH curve having a "0.09-slope" is comparable to the same slope found among the indophenols. In the latter case it occurred between two "0.06-slopes" and was accounted for by two dissociations, one of the oxidant and the other of the reductant the pK values of which were distinctly different. It is now evident that the same explanation holds for the thiazines, the "0.09-slope" lying between the region of ionization of a group in the oxidant so weak that its K_b value is negligible and the region of an appreciable ionization of that same group as it appears in the reductant.

Turning now from Lauth's violet to methylene blue, we can apply the same principles, and with the exception of the new values of the constants employed, the only essential difference is the absence of an inflection of the curve in the alkaline region. This simply means that in methylene blue the value of K_{ob} is too large to permit suppression of its basic ionization by the alkaline buffers employed.

Summarizing, and using for descriptive purposes the more familiar basic ionizations shown in Table 19 rather than the corresponding acid constants employed for convenience in developing equations, we have the following concept.

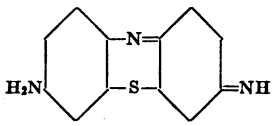
Among the thiazines, the oxidant is a strongly polar cation, comparable to a substituted ammonium, NH_4^+. As the substitution of alkyl groups for hydrogen enhances the basicity of ammonium, so we should expect methylene blue to be a stronger base than Lauth's violet. In Lauth's violet we find a color change occurring in the zone of pH 11 and correlating with the dissociation constant determined by the inflection of the E'_o:pH curve. The precipitate there formed was identified as the free base by Bernthsen (1885). On the other hand, much more intense alkalinization is required to induce a color change in methylene blue, and its free base was obtained by Bernthsen (1885) only by the use of silver oxide. We may therefore conclude that in "strength" methylene blue cation is comparable to sodium ion. Its chloride has been found by the conductivity measurements of Jaubert (1895) to compare with NaCl. Pelet-Jolivet and Wild (1908) regard it as completely dissociated in dilute solution. Hantzsch and Osswald (1900) say of the thiazines that in spite of their complex structure and high molecular weight they should be classed with the strongest bases.

TABLE 19.—*Ionization constants, inflections of E'_0: pH curves, and characteristic potentials at pH 0*

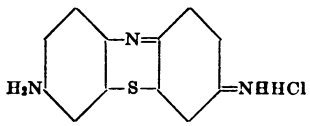
Group	Symbol of constant	Lauth's violet		Methylene blue	
		Value of constant	Inflection at pH	Value of constant	Inflection at pH
Oxidant's polar	K_{ob}	1.88×10^{-3}	11.0	Too high to measure	None.
Oxidant's amino	K_{ob2}	Negligibly small	None.	Negligibly small	None.
Reductant's bridging N	(K_{r1})	Fixes H^+	None.	Fixes H^+	None.
Reductant's 1st amino	$(K_{r2})K_{rb1}$	3.8×10^{-9}	5.30	1.35×10^{-8}	5.85
Reductant's 2d amino	$(K_{r3})K_{rb2}$	4.5×10^{-10}	4.38	6.3×10^{-10}	4.52
	$E'_{pH\,0}$	0.563		0.532	

Incidentally, the structures accorded the thiazines indicate that the salt of methylene blue base with hydrochloric acid should be termed a chloride and not a hydrochloride as has frequently been done. The curious fact that silver nitrate does not readily precipitate silver chloride from acid solutions of methylene blue chloride is not proof that the chlorine is intimately incorporated in the organic molecule, for other reagents act as if an ionic metathesis does take place (Atack, 1915). Lenz (1895) suggested a soluble silver chloride double salt as the explanation of the peculiarity noted above. Whatever the explanation, the peculiarity is not unique.

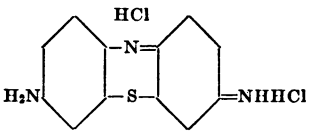
The second potentially basic group in the oxidant appears to be so weak that it forms no salt in the regions of pH we have studied. Kehrmann, Havas, and Grandmougin (1914), on the basis of spectroscopic data, believed that three salts are possible. These three salts they formulate for Lauth's violet in the following scheme:



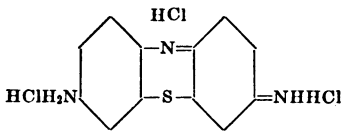
[Orange]



[Violet]



[Greenish blue]



[Yellowish green]

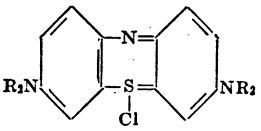
Our data show that, if more than one salt is formed, intense acidities are necessary. In conformity with this is the fact that Kehrmann, Havas, and Grandmougin required 35 per cent and 50 per cent oleum to obtain the alleged evidence of the second and third salts.

On reduction, the polar valence of the oxidant is destroyed. In the symmetrical reductant the two terminal nitrogen groups become

structurally identical, and our interpretation of the data before us is that they have distinguishable dissociation constants of the same order of magnitude, comparable in value with those of most substituted aromatic amines. This was confirmed for Lauth's violet by alkali titrations comparable with those made with oxidized and reduced indigo tetrasulfonate and described in the fourth paper of this series.

The over-all slope $\left(\frac{-dE}{dpH}\right)$ never tending to a zero value indicates that another group, presumably the bridging nitrogen, *fixes* a non-dissociating hydrogen or its equivalent; but there appears to be no evidence that there can be formed at this point a sodium salt of ordinary type as Landauer and Weil (1910) believed.

The interpretation we have given to the experimental data has allowed no place for the orthoquinoid formula, III, advanced by Kehrmann and Schaposchnikoff (1897) and Kehrmann (1902),



III

While we again emphasize the fact that the methods now under consideration can give no definite assurance to the allocation of dissociable groups, and while we might cite certain analogies as justification of Kehrmann's first formula, we consider it less probable than the Bernthsen formula, when written with the guidance of accepted principles of configuration. Although Formula III is still widely accepted and is still copied in many texts, Kehrmann himself abandoned it in 1914 as the result of investigations made with Havas and Grandmougin.

IX. General Discussion

(A) MECHANISM IN BIOLOGICAL OXIDATION-REDUCTION

Data in this and preceding papers of this series have a bearing upon certain current views of mechanism in biological oxidation-reduction.

It is of course obvious that the various schemes used to describe oxidation-reduction processes are formally interchangeable and each is legitimate for mental orientation of certain relative relations. However, there have been postulated from time to time various specific mechanisms for the operation of which one or another component of a reaction is required. In dealing with such *mechanisms* not all formal schemes of description are interchangeable. It is conceivable, for instance, that the living cell has evolved a type of catalyst

dealing with the transport of hydrogen. Unless we are prepared to reinterpret this conception in more universal terms, we must formulate oxidation-reduction processes in terms of actual hydrogen transport wherever the formulation is to conform with the postulated catalysis. It then becomes important to distinguish carefully between formalism with its legitimate uses and such quantitative data as bear upon actuality. Wieland (1922) has made an attractive case for the assumption that many important biological oxidation-reductions are essentially cases of hydrogen transport. To illustrate this thesis, Wieland cites certain reactions which have fallen within the scope of our own studies, and it is with these alone that we shall now deal.

Among Wieland's illustrations are the quinone-quinol, the indigo-indigo white, and the methylene blue-methylene white transformations. In each case two hydrogens are concerned when the *isolated* compounds are considered. In each case our own treatment has not only included the participation of these hydrogens, but has made use of electronic structures which suggest that very widely among organic systems in aqueous solutions the rule of electrical neutrality can be satisfied by the participation of the ever-present hydrions. Thus Wieland's orientation from the point of view of hydrogenation and dehydrogenation receives support from our treatment to a certain limited extent, but the nature of the limitation it is important to perceive. It becomes plain when we consider the significance of the ionizations of the compounds now under consideration.

Wieland makes the difference between quinone and hydroquinone a difference of two hydrogens, as may reasonably be done in neutral and acid solutions. The same is assumed for indigo, although we have shown (*cf.* 4th paper of this series) that in solutions of mild alkalinity only one hydrogen remains fixed in the reductant. The other hydrogen (which, in an artificial systematization on the basis of hydrogenation, can be *assumed* as one of the two *equivalents* required for reduction) can, in a generalized theory, be considered as belonging to the indigo no more than to *other constituents of the solution.* Indeed there is no experimental proof that hydrogen *per se* is essential.

The case of the methylene blue system is complicated by the variety of ways in which its reversible oxidation-reduction can be written rationally; but it is reasonable to assume that although two equivalents are required for the reduction of the discrete, free, methylene blue cation only one hydrogen, *as hydrogen,* becomes fixed and that on the bridging nitrogen. The nonpolar group created from the polar group can acquire basic properties, either by direct addition of hydrion or by addition of water and subsequent ionization

of hydroxyl, but *does not do so appreciably in neutral solution.* The balancing of the equation either as

$$MCl + H_2 = MH + HCl$$

or

$$MCl + H_2 = MHHCl$$

concerns the *solution as a whole* and may be of *entirely secondary significance for mechanisms* concerned in the conduct of an *active species* of the methylene blue molecule.

Incidentally it may be said that no one who appreciates the evolutionary nature of scientific thought would be hypercritical of the implication in Thunberg's (1922) simultaneous use of the terms "hydrogen potential" and "active hydrogen" in his adaptation of Wieland's theory to his valuable experimental work with methylene blue. At the same time it must be pointed out that if the basis of calculation previously described (Paper II, this series) be accepted, the data now available show that a half-reduced solution of methylene blue at pH 7 is *in equilibrium* with a hypothetical hydrogen pressure of only about 10^{-15} atmosphere. Likewise a half-reduced solution of 2,6-dibromo phenol indophenol at pH 7.0 in the presence of washed tissue should have a hypothetical hydrogen pressure of only 10^{-21} atmosphere. If *equilibrium conditions* have any significance, and it remains to be shown that they do, then any postulated molecular layer of hydrogen on the surface of a catalyst must have its covering ability in harmony with these calculated partial pressures.

Further discussion will be found in the fifth paper of this series.

Of course, it is perfectly easy to accommodate some of the implied demands if the *schematic* aspect of the affair is the sole consideration. If this alone is the object of Wieland's theory, then our suggestion is trivial. But it seems that Wieland has attempted to trace a mechanism, and in our conception of this problem it is of considerable importance to know whether or not hydrogen regarded as an *actual* and not as a *schematic* representative of an electrochemical equivalent is required for the transformation of any given species.

The considerations we have urged are not to be regarded as definite refutations of Wieland's theory. They are of the nature of intuitive deductions rather than of compelling necessities. However, they are of the type which, had they been appreciated earlier, might have directed speculation into a channel other than that followed by the current of the present period.

Another aspect of the Wieland theory we shall discuss in a later paper.

(B) METHYLENE BLUE IN CYTOLOGY

We come now to an aspect of methylene blue or of the thiazines which may appear at first to lie entirely outside the province of this paper. We refer to the use of thiazines as cytological staining reagents. There are two points of contact. In the first place, the conduct of thiazine as a staining reagent may be complicated by its reduction. In the second place the thiazines have been classed as basic stains without that more detailed knowledge of their "strength" as bases which we now possess.

In the voluminous literature, which may be traced through v. Möllendorff's (1920) monograph, Lee's (1921) "The Microtomist's Vade-Mecum," and Michaelis' (1902) review, there will be found frequent references to the reduction of the staining reagent as an experimental fact which sometimes complicates the interpretation of staining reactions. Furthermore, there has run through the literature from the time of Ehrlich's (1886) suggestion, a stream of speculation regarding some vaguely defined relation between the staining properties of certain tissues and their oxidation-reduction metabolism. Unna (1913) has made much of one aspect of this; and Child (1919, 1920) (*cf.* McArthur 1921) has suggested a correlation between "staining gradient" and his so-called "metabolic gradient." In all such speculations there has been a noteworthy absence of quantitative data of the type we now have to contribute as a minor but essential part of the subject.

If the interpretation long accepted and confirmed by the present studies be correct, the dissociation of methylene blue chloride itself is such that no ordinary changes in pH can affect its degree. Consequently, if we exclude from consideration phenomena which were formerly called "salt effects," changes brought about in a solution with the object of altering the "reaction" (acidity) of the cell's environment can not affect the methylene blue and any observed change in staining quality must be explained otherwise. Incidentally this conclusion has a bearing upon the attempt by Fleischer and Amster (1923) to determine whether the toxicity of methylene blue to bacteria may be modified by changes of pH in accordance with the principle of Michaelis and Dernby (1922).

But to return to the subject of vital staining, let us recall that methylene blue under certain circumstances is readily reduced by many living cells. If now a tissue maintains at its periphery a sufficient reduction intensity, its interior will have to deal with—*not* methylene blue itself but a compound of very *different type*, namely—methylene white. While this compound may still be classed as a base, its basicity is very low and, relative to the reaction of the cell as a whole and perhaps to many of its constituent chemical groups, it is a neutral substance.

The practical significance and possible application of these relations we must leave to the judgment of those who are familiar with the problems of staining. They will recognize that our remarks apply to methylene blue and not to those constituents of commercial samples which are not methylene blue but which nevertheless are the most valuable in certain staining reactions. Compare Scott and French (1924).

We had hoped that a definite potentiometric characterization of each thiazine and of related compounds would aid in the assay of different samples of these important staining reagents, but having been unable to obtain from others or by our own efforts any thiazine sufficiently free from the last traces of active impurity to establish fundamental data of requisite refinement, we have had to leave this problem unsolved.

(C) METHYLENE BLUE AS A CHEMICAL REAGENT

As a chemical reagent, methylene blue has several interesting uses. It has been employed as an end-point indicator in oxidation-reduction titrations of quinone (Knecht and Hibbert, 1910), iron (Knecht and Hibbert, 1910, Jellinek and Winogradoff, 1923), tin (Atack, 1913), molybdenum (Knecht and Atack, 1911), sugar (Lane and Eynon, 1923) and selenious acid (Moser and Prinz, 1918). Details of some of these cases are described in Knecht and Hibbert's (1918) monograph, "New Reduction Methods in Volumetric Analysis" and in Atack's (1915) review of the analytical uses of methylene blue. Methylene white in solution has also been employed as the reducing agent in volumetric analysis, as, for example, by Hibbert (1909), Atack (1913), Thornton and Elderdice (1923). See also Atack (1915) and Kikuchi (1922). The methylene white-methylene blue system has recently been employed by Spoehr (1924) as an oxygen carrier in the oxidation of carbohydrates by air.

The systematic, as contrasted with the empirical, use of such a reagent requires the quantitative data on equilibrium potentials which we have furnished. Since such data are the beginning of systematic indicator theory in the oxidation-reduction realm, it may be illuminating to chart the methylene blue system in such a way as to show its relation to a few other systems.

In Figure 3 are drawn the E'_0 : pH curves of methylene blue and Lauth's violet, 2, 6-dibromo phenol indophenol, ferricyanide, and ferric iron. The indophenol curve is drawn from data given in the sixth paper of this series; that of iron is drawn on the assumption that in the zone of pH covered the potential of an equimolecular mixture of ferrous and ferric iron does not vary from 0.73 (Abegg, Auerbach, and Luther, 1915). For the ferricyanide system Kolthoff (1920) reviewed the earlier work upon the relation of acidity to potential, and

by use of his data for acid solutions he arrived at the approximate estimate of 5×10^{-4} for the fourth dissociation constant of H_4FeCy_6. The complete E'_0 : pH curve of this system remains to be determined. Several years ago, one of us (W. M. C.) made a series of crude measurements by introducing an equimolecular mixture of potassium ferrocyanide and potassium ferricyanide into buffers of the Clark and Lubs series and measuring the differences of potential between a saturated KCl-calomel half-cell and platinum electrodes immersed in these solutions. The results are shown in Figure 3. There it will be noted that in the less acid solutions step-wise deviations appear. These are due to the well-known effect of varying cation concentration (Schoch and Felsing, 1916) upon the ferricyanide potentials. These concentrations vary in the Clark and Lubs buffer solutions in a step-wise fashion through the phthalate, phosphate, and borate systems, indicated respectively by large dots, small dots, and crosses in Figure 3. As higher acidities are approached, we should expect to encounter the region where the dissociation of the fourth hydrogen of H_4FeCy_6 is suppressed and where there is consequently an inflection of the curve. Assuming this constant to be 1×10^{-3} we should have the curve as drawn. Considering that no allowance is made for varying cation concentration, the agreement of the observed values with the calculated is fair until the higher acidities are reached. In the more acid solutions experimental errors of diffusion potentials and uncertainty regarding possible effects of the group created by reduction upon ionizations common to oxidant and reductant combine with the "salt effect" and especially with the rapid decompositions to make impossible even an approximate comparison between these crude experimental data and the elementary theory. However, the striking effects of variation in pH are clear.

With these systems charted, it now becomes clear that if the older assumption regarding the invariance of potential with change of acidity were true, an excess of ferrocyanide should reduce methylene blue at high acidities. As a matter of fact, it does not, as is clearly revealed by the chart. On the other hand, an excess of ferrocyanide can reduce the indophenol at a properly adjusted value of pH. Now, it has been stated that ferrous salts will not reduce methylene blue. We can not, of course, project our curves into the pH region of extreme acidity without encountering complications, but we may foresee the *possibility* that at very high acidities a *large excess* of ferrous iron *might* reduce methylene blue. It does.

The ferrous-ferric system at higher pH should slope toward more negative potentials in accord with the principle outlined in the second paper of this series; but in addition to the more simple effect of change in pH, there is the effect of differential solubilities of the ferrous and ferric hydroxides to be taken into consideration. In the presence of

hydroxy acids, such as citric, another complication arises—the formation of iron complexes. While definite data on these effects are lacking, the general trends are known. Since, then, the position of the methylene blue system is well established, the outline of the interaction of methylene blue and iron compounds is clearer than at the time Morgan and Quastel (1923) discussed it in its relation to biological oxidation-reduction.

In view of the well-known general characteristics of the titanous-titanic system, it is, of course, evident that it will reduce methylene blue. Knecht (1907) found that very small concentrations of titanium can be detected by the reduction of the highly colored methylene blue solution provided no other reducing agent is present.

Less amenable to systematic treatment at the present time is the use of methylene blue in testing the reducing properties of solutions such as those of the sugars and other materials (*cf.* Hasse, 1919). Ihl (1888) applied methylene blue to the detection of impurities such as invert sugar in sucrose, and several investigators (e. g., Muster and Woker, 1913, Kashahara and Hattori, 1921) have applied it to the estimation of reducing sugars in biological fluids.

Methylene blue as a cation (see p. 1161) forms several interesting salts (*cf.* Atack, 1915, Monnier, 1916, Sinnatt, 1910–1912, Rozier, 1917), some of which are of value in analytical procedures. A salt of special interest to the cytologist is the insoluble neucleinate (Feulgen, 1913). But undoubtedly the insolubility of methylene blue silicate is of most general interest, since it can be correlated with the remarkable persistence with which methylene blue solutions stain glassware.

We fail to find any *common* principle underlying the manifold uses of methylene blue as a therapeutic agent, and the nature of some of these uses leads us to wonder whether *any* principle was considered. However, the definite data on some few properties of methylene blue which we have described should be useful to the pharmacologist who will not fail to note the radical changes induced by reduction at a potential readily acquired by cells.

Among the miscellaneous applications of this remarkable and ubiquitous dye is the employment of acetone-methylene blue mixtures for measuring the intensities of ultraviolet light for physiological purposes (Webster, Hill, and Eidinow, 1924). The reaction involved is said to be the decomposition of acetone to form reducing substances which decolorize methylene blue. If so, this process must be complicated by the more direct action of light upon methylene white, which we have already discussed. At any rate the employment of electrometric methods of measuring methylene blue-methylene white ratios might be applied to a more detailed study of Webster, Hill, and Eidinow's system.

(D) ENERGY CHANGES

It is well known that from electromotive force measurements such as those here described, certain thermal data can be calculated with far greater accuracy than can be found by the calorimetric method. So far as we know, Meyerhof (1912) is the only investigator who has given any calorimetric data on methylene blue. Unfortunately, Meyerhof, in reducing his methylene blue in alkaline solution, employed a concentration which undoubtedly resulted in a partial separation of methylene white. Furthermore, he does not record the pH of the measurement. Therefore, since heats of solution and of ionization are also neglected, Meyerhof's data are inadequate to support the value for the heat of reduction at 26.5° C., which he places at 25.7 kg. calories.

To obtain the order of magnitude of the change in heat content on reduction, we made one preliminary set of measurements as follows:

A fixed mixture of methylene blue and methylene white of total concentration 0.0001 molar was found to give an E_h value of -0.0231 at 30° and of -0.0113 at 20°. At 30° the pH value was 8.62. Assuming that this borate buffer (No. 22) suffers a pH- change with change of temperature equal to that of the Sørensen buffer as given by Walbum (1920), the pH at 20° should be 8.68. Undoubtedly the slope of the E'_o:pH curve at 20° is comparable to that at 30°. Hence we can correct the E_h values at 30° and at 20° to what they would be at pH 8.62, and we then find that E_h at 20° and pH 8.62 is -0.0096. Consequently $\frac{dE_h}{dT} = -0.00135$.

From previous measurements at 30° and pH 8.62, $E'_o = -0.039$. Assuming the above temperature coefficient to be linear, E'_o at 26.5° C. (the temperature of Meyerhof's experiment) is -0.034.

From the Gibbs-Helmholtz equation

$$\Delta H = nFT \frac{dE'_o}{dT} - nFE'_o$$

$$\Delta H = -17.1 \text{ kg. calories at pH } 8.62.$$

In a similar manner at pH 10.62, we find $\Delta H = -14.4$ kg. calories. These values include the heats of reduction and of ionization at given dilutions of H^+. Somewhat different values would be obtained if the comparative data were reduced to a common dilution of OH^-.

For comparison with data on other compounds, we might add that the free energy of reduction by one atmosphere hydrogen at pH 0 and 30° C., is 25.97 kg. cal. for Lauth's violet and 24.53 kg. cal. for methylene blue. We have not determined the effect of temperature on the dissociation constants and therefore can not give several other interesting relations which it is possible to determine with

potentiometric data. We believe the quality of the materials which are available does not justify the extension of these studies at the present time.

(E) MISCELLANEOUS APPLICATIONS

It is fairly obvious that data of the type we are reporting can be of use in the investigation of a variety of problems. The following experiments are in themselves of value merely as illustrations.

In subsequent papers we hope to extend this illustrative material and furnish more definite contributions to the several problems we now only touch upon.

(1) MILK TESTS WITH METHYLENE BLUE

In the Schardinger (1902) reaction a mixture of methylene blue and formaldehyde is incubated with milk; and in milk that has not been heated, the methylene blue is soon reduced. This reduction is supposed to indicate the activity of an enzyme native to fresh milk. Bredig and Sommer (1910) simulated the Schardinger reaction with platinum as catalyst.

Since methylene blue indicates but a comparatively narrow zone of reduction intensity, we suspected that the course of the activation of formaldehyde by milk might be followed in more detail by electrode measurements. A sample of fresh whole milk was divided into four portions. One was heated in an autoclave at 15 pounds pressure for 15 minutes and then cooled. A second portion was acidified with HCl to pH 5.9. A third was alkalinized with NaOH to pH 7. The fourth portion was left at its original reaction of pH 6.5. The several portions were then warmed to 37° C., and to 100 c. c. of each there was added 5 c. c. of 1 per cent formaldehyde solution. They were placed in vessels such as *A* of Figure 5 and liquid contact with a saturated KCl calomel half-cell was made through *B*. The results of measurements are shown in Figure 6, where electrode potential reduced to the customary hydrogen scale is plotted as ordinate (E_h) and time (in minutes) of incubation at 37° is plotted as abscissa. The zones of potential within which methylene blue passes from 4 per cent to 96 per cent reduction at each pH are indicated by triangles. It is evident that this indicator reveals but a limited part of the course of reduction, that a reaction proceeds in the absence of methylene blue, and that there is a distinct pH effect both upon the rate of action (*cf.* Allemann, 1918, Virtanen, 1922) and the level of potential at which methylene blue is reduced.

This method of following the Schardinger reaction is comparable to a certain extent with Reed's (1916) method of following oxidase activity, but with the important difference that some of Reed's experiments were on depolarization phenomena and others on the "oxygen electrode," both very difficult to interpret.

Milk, when subjected to bacterial action, becomes reducing (*cf.* Duclaux, 1894). This fact has been elaborated upon in the design of the so-called methylene blue test of milk. (See references.) Owing to its practicability in factory, home, or rural district unequipped for more elaborate milk control, this simple test has been studied extensively. The opinion seems to prevail that if artificial interpretations are not stressed, the test can be of considerable public-health value. It is therefore important to establish the primary interpretation to be given to the observed fact of methylene blue reduction. Secondary correlations can then be made clearer.

In Figure 7 are shown electrode measurements made with milk subjected to the following manipulations: The sample designated "direct from cow" was delivered from the udder to a sterile tube. The sample designated "bottled" was herd milk, passed through the ordinary processes for bottling raw milk. Some of this same milk was heavily inoculated with a culture of *Bact. coli.* Each sample was placed in a bottle as shown in Figure 5, incubated at 30° C. and its electrode potential against a calomel cell measured from time to time. The potentials reduced to a hydrogen standard are plotted in Figure 7 against time in hours as abscissa.

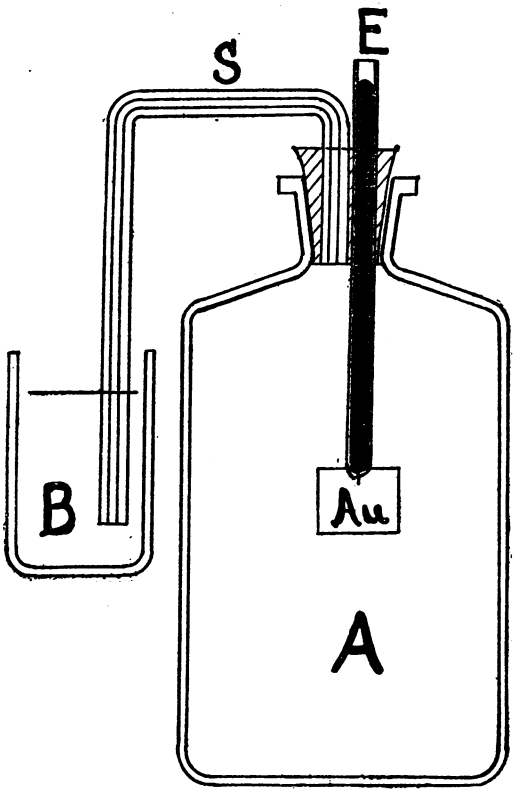


FIG. 5

We have repeatedly observed differences in the potential: time curves such as are shown in Figure 7. The differences in time required for methylene blue reduction have been repeatedly correlated by others with conditions such as were imposed in this experiment, and consequently there is nothing new in this aspect of the subject. However, we emphasize the possible advantages of obtaining for the reduction: time relations more complete histories than are

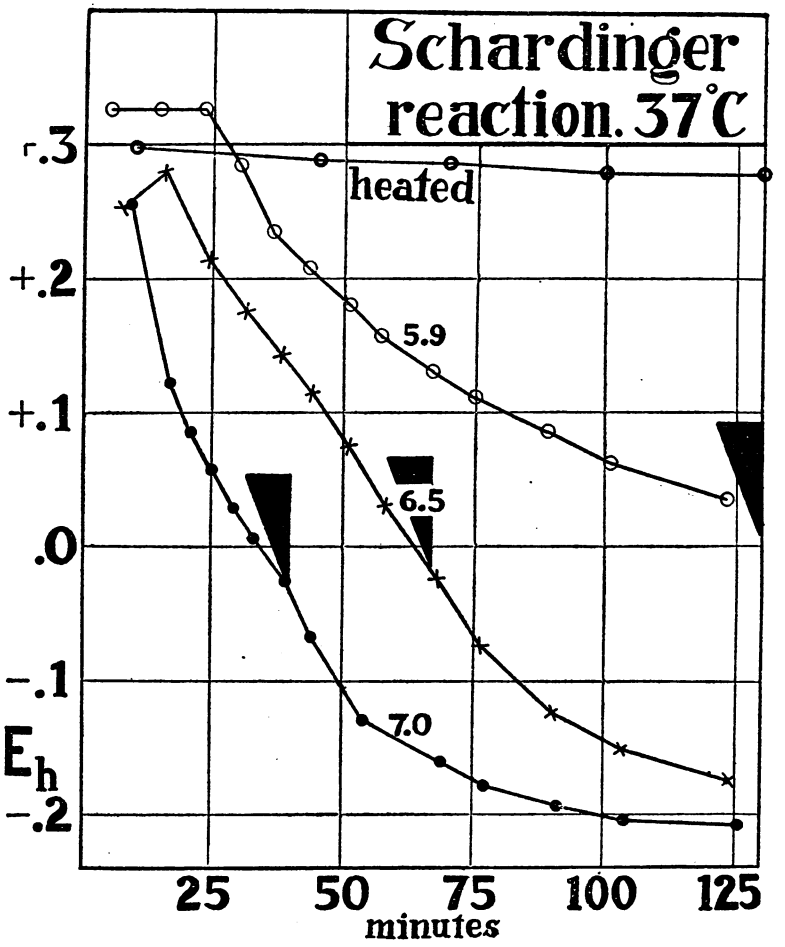


FIG. 6

revealed by methylene blue alone. It is entirely possible that an electrode study of more varieties of market milks than those available to us will show the advantage of using a more electro-positive indicator, and that this, together with simple devices, will very materially reduce the time required for the test. If given the more extensive scientific investigations it deserves, the test may well be improved.

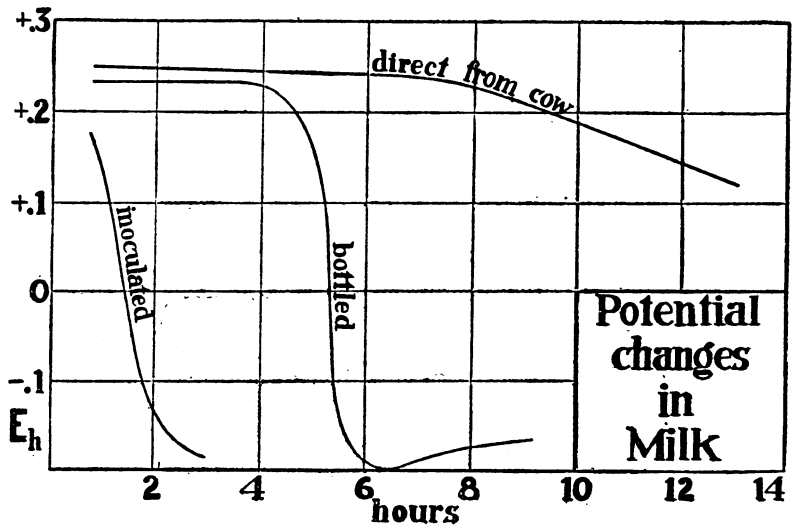


FIG. 7

(2) BACTERIAL REDUCTION

The reducing power of bacterial cultures as displayed in this test of milk is rather general and has been frequently investigated. In almost every one of these investigations methylene blue has been mentioned or has been made the specific tool. (See references under General Bacteriology.) We shall postpone an account of our general studies on bacterial reduction and recall to the reader Gillespie's (1920) demonstration that reduction by bacterial cultures is measurable by potentiometric methods. The data we report define the intensity factors controlling the reduction of methylene blue, and it is important to distinguish these from the capacity factor. Wichern (1908) was the first, we believe, who made any quantitative measurements of methylene blue reduction. He, and later Fred (1912) with bacteriâ and Strassner (1910) with tissues, allowed cells to act upon known quantities of the dye and then estimated the residual unreduced methylene blue by the titanium method of Knecht and Hibbert. They thus determined the mol fractions of dye reduced. This shows the reducing capacity which, when converted to electrochemical equivalents and multiplied by the intensity factor in volts, gives the free energy involved. The capacity factor and the intensity factor each has its unique significance. Both are of coordinate importance.

Just as different organisms are equipped to attain different levels of acid intensity (pH) under a given set of conditions, so our preliminary work has shown that different bacteria are equipped to attain different levels of electrode potential under a given set of conditions. They may now be correlated with the reducing action on dyes. One

instance is found in the observation by Sherman and Albus (1918) of the reductive abilities of milk streptococci. Having made a grouping of certain cultures on the basis of origin, morphology, and a statistical analysis of other characters, Sherman and Albus found that their *Strep. lacticus* type reduced methylene blue in milk, whereas all cultures of their *Strep. pyogenes* type failed to reduce. (Compare Avery, 1922, and Brown, 1920.) Such differences may now be expressed in numerical values for reduction intensity.

Other similar limitations in the reduction intensities attained by pure cultures might be cited.

If, however, organic material is subjected to *general* infection, there develop bacteria which are almost sure to carry the reduction potential well beyond the zone of methylene blue if the reduction be not opposed by air or other oxidations. Indeed, it is a principle emphasized by Pasteur, and now capable of reinterpretation, that with the ever-present reducing tendency of cellular life there will occur, in a general infection, a tendency for types to succeed one another in the order of their ability to endure a more and more intensely reducing environment.

(3) "RELATIVE STABILITY" OF SEWAGE

It follows, then, that a sewage, while fresh, will tend to reduce methylene blue. Recognizing this fact, Spitta and Weldert (1906) proposed the reduction of methylene blue as a test of the state of a sewage effluent.

In modern treatment of sewage it is not always practicable to effect a complete purification of the refuse-bearing water. The effluent from a sewage-treatment plant carries a residue of organic matter which is considered satisfactory if its organic content can be "burned" by the oxygen-bearing waters into which it is dumped. Therefore, following the development of the Spitta and Weldert test by Phelps and Winslow (1907), Phelps (1909) emphasized the advantages of so interpreting the test that it can indicate the condition of the effluent in relation to the degree of oxidation still required, that is, its "relative stability." Since Phelps's treatment involves some questions of general importance, we shall subject it to a brief critical examination.

There are involved the following postulates:

1. It is assumed that the bacterial activity of an effluent has already settled down to a steady state, and that lag or acceleration of growth and significant changes of flora will not occur to invalidate the following argument.

2. It is then assumed that under condition (1) the rate of disappearance of dissolved oxygen or equivalent oxidizing material will be proportional to the concentration of the oxygen or its equivalent.

In other words, the oxygen consumption while undoubtedly *not a monomolecular reaction* is postulated to have the *rate* of a monomolecular reaction. There can then be applied the familiar equation which Phelps has recast to form (A).

$$\frac{y}{a}=1-k^t \qquad (A)$$

Here a is the total amount of oxygen required to oxidize the material to a stable condition, k is a constant, and t is the time required to exhaust the available oxygen, y.

3. It is assumed that of the family of curves corresponding to equation (A) there is one having a definite value of k defining the rate for sewage.

4. It is assumed that this k can be determined by a statistical treatment of Phelps's data on the time required for reduction of methylene blue by a large number of tests, and finally,

5. It is assumed that the disappearance of available oxygen, y, at time, t, is determined by the decoloration of methylene blue.

The ratio $\frac{y}{a}$, being $\frac{\text{available oxygen}}{\text{total oxygen demand}}$, is multiplied by 100 and then called the relative stability, S.

$$S=100(1-k^t) \qquad (B)$$

The time, t, in days, required for methylene blue reduction is the only experimental datum required to determine S if k be fixed.

The following critique is an effort to revert attention to the basic phenomena which deserve investigation unembarrassed by concepts formed to meet pressing demands of a practical problem.

Starting with postulate 5, we find that the conduct of methylene blue as an oxygen-end-point indicator is of basic importance. Phelps has considered this with caution. He recalls, in the first place, the claim of H. W. Clark and Adams (1908) that indigo carmine is reduced before methylene blue. So far as interpretation of intensity is concerned, these authors must have been misled either by an inhibitory action of their sample of methylene blue, by a quantity factor, by their statistics, or by some unknown factor, because a comparison of the data in this paper and the data in the fourth paper of this series shows that indigo carmine requires a more intense reduction tendency than does methylene blue. However, the fact of a *difference* exists and was recognized by Phelps, who states that "it is possible that the end-point of methylene blue is a little too far along."

It would take us far afield if we entered into a discussion of what constitutes a theoretically good oxygen-end-point indicator. The fact of the matter is that under the conditions of the putrescibility

test there is a gradual change of potential with time, that frequently no characteristic of the time : potential curve reveals the moment of oxygen exhaustion, and that methylene blue conducts itself in the course of the potential change as an indicator of a definite level of reduction potential. For instance, consider the following experiment:

A raw Washington sewage taken from the main during a storm and therefore highly diluted, was added in 50 c. c. portions to a solution made by diluting 30 c. c. M/20 buffer to 250 c. c. with water. Both buffer solutions and distilled water had been aerated by standing a week or so at room temperature. (The oxygen contents were not determined.) The mixture was carefully siphoned into vessels of the form shown in Figure 5. The changes of potential and the pH values of the different mixtures are shown in Figure 8. Again, there are shown by means of triangles the zones of potential within which methylene blue is reduced at the different values of pH. It is obvious that the same quantities of the same sewage, diluted with equal quantities of buffers, *presumably* containing the same amounts of oxygen, require different periods of time to reduce methylene blue. Evidently, the variation in pH is *one* of the factors to be considered.

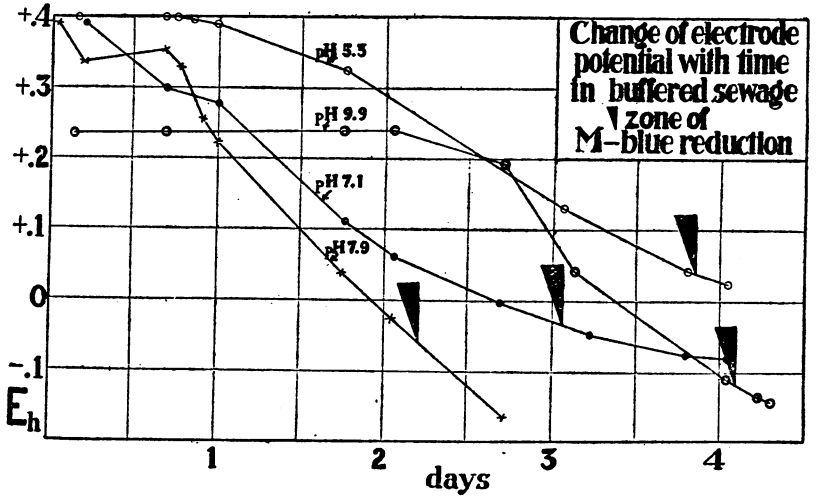


FIG. 8

Parallel experiments show that indophenols, methylene blue, and indigo carmine, with qualifications which will be discussed in a later paper, are reduced in the order named and at times predicted from the order of their reduction characteristics and from the course of potential change in the absence of the indicators. It should be noted, however, that too much indicator can produce, in addition to a poisoning action on the bacteria, a poising (see Paper I) effect with consequent delay. Compare Lederer (1914).

Other tests on sterile organic media inoculated with various bacteria show the course of the potential change to be dependent on the nature of the flora.

Of particular interest at the moment is the fact that the curves of Figure 8 give no indication of the time of oxygen exhaustion. Undoubtedly this means that the trend toward reducing potentials is not rigidly held in check by oxygen, but is delayed. Under anaerobic conditions the restraint is removed and still it is found that an appreciable time is required for the reduction of methylene blue. This has not been taken into consideration in the formal derivation of the relative stability equation. It should vary with substrate, flora, physical conditions, and amount and kind of indicator. (*cf.* Clark and Cohen, 1922.)

Let us next consider postulate 4.

For the determination of k, Phelps employed a large number of data on times required for methylene blue decoloration, but he does not describe the logic of this application. We find that without any reference whatever to mechanisms, Phelps's data can be formulated by a certain type of probability equation which finally assumes the form of the relative stability equation. This is not strange, since the law for the rate of monomolecular reaction can itself be derived from equations of probability. The important aspect is that Phelps's equation can be considered as purely descriptive of a set of data on reduction times. His extension of the equation to postulate 2 appears then to have been *intuitive*.

That the intuition was very good is suggested by Theriault's (1920) investigation of actual oxygen disappearance. Unfortunately the data reported by Theriault in this paper were incomplete; but he informs us in a private communication that recent data show not only that the rate of oxygen disappearance is that of a monomolecular reaction, but is characterized by a constant numerically very close to that deduced by Phelps for the relative stability equation.

This is so remarkable that it deserves close study. It would have seemed improbable that such variable material could be characterized by a constant in any way other than statistical. If it be true, then the relative stability equation with its statistical constant can apply only when the volume of oxygen (with its characteristic rate of exhaustion) is high with respect to the sewage demand. For we find that it certainly can not apply when the time of the anaerobic phase is large in relation to the time of the aerobic phase. Indeed this is implicit in Phelps's treatment by his rejection of all cases of low stability.

Without taking up in detail all the ramifications of this complicated problem, we believe that we have made it clear that the primary conditions revealed by the decoloration of methylene blue is of an

entirely different category from that which it was formerly possible to perceive, and that if other methods of evaluating sewages are to be correlated with the putrescibility test, the conditions under which the correlation is valid must be determined.

However, quite aside from the laborious task of establishing these conditions of correlation, there remains the inherent value of the primary fact revealed by methylene blue reduction. Coupled with extensive experience, such as Phelps and others have brought to bear, the simple test is of considerable value. However, by confining themselves to one indicator without even a quantitative evaluation of the characteristics of this one indicator, the students of the putrescibility test have been limited in their power to analyze their problem. There must have come within the view of the more experienced investigators, phenomena whose significance was obscured by the arbitrary emphasis upon the value of methylene blue. We therefore recommend that the subject be investigated with the aid of electrode measurements and without any attempt to prove or disprove preconceived ideas. Difficulties in the use of the electrode will be encountered; but we are confident that, in spite of all the difficulties, the electrode in cautious hands can contribute valuable information. We find it applicable in cases where suspended material precipitates methylene blue. It can be led to points inaccessible to ordinary methods of sampling. It can reveal a complete history of the time:reduction intensity curve. It can be used with apparatus which will furnish a *continuous* record of the reduction intensity wherever oxygen and other agents do not upset its conduct.

Thus there should be revealed characteristics of industrial wastes, the effects of materials poising the potential above and below the region of methylene blue, the oxygenation delay, the effects of prestabilized material, and, perhaps, correlations between state of reduction and flora.

Finally, we would emphasize two radically distinct aspects of the subject. In the first place, there remain to be investigated in detail those phenomena of sewage conduct which fall strictly within the category of changes in reduction intensity. Quite aside from these, but indirectly connected with them under certain circumstances, are the various problems which have entered into discussions of the putrescibility test. Therefore, in the second place, there remain to be determined the unique facts of the first category which can be correlated with those of the second.

45487°—25†——4

(4) THE RATE OF OXIDATION OF METHYLENE WHITE

Into various problems there enters the rate of methylene white oxidation by atmospheric oxygen. Atack (1915) states that leucomethylene blue may be very slow in becoming oxidized by atmospheric oxygen. The following crude experiment shows the influence of pH:

An aqueous solution of methylene blue was reduced with hydrogen and platinized asbestos. The resulting saturated solution of methylene white was filtered under nitrogen protection into a burette and aliquots of 5 c. c. were added to 50 c. c. portions of different, deaerated buffer solutions. With the same apparatus a fairly constant air stream was passed through each solution and titrations of regenerated methylene blue were made. In each case the initial concentration of methylene white in the buffer solutions was approximately 0.0001 *normal.* Instead of a special titanium solution adapted to the case at hand, a stock solution 0.018 N was used. A stop watch was used to time the aeration.

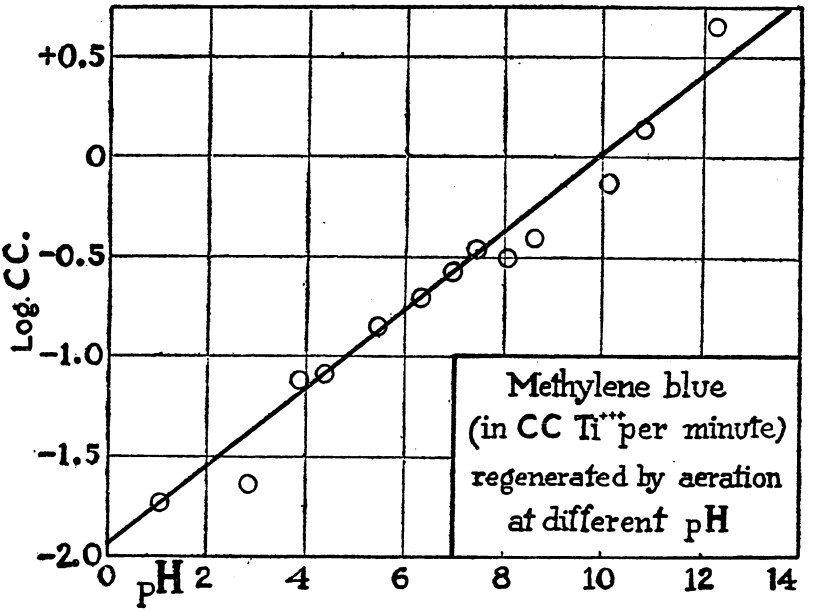


FIG. 9

The following results were obtained: In Figure 9 are plotted the pH values of the solutions and the logarithms of the rate of regeneration in terms of cubic centimeters of titanium trichloride per minute. Of course, in such an experiment, precise analysis of conditions is difficult, since even the rate of diffusion of oxygen from air bubble to methylene white is a complicated process. Nevertheless, the striking effect of pH is evident in Figure 9, and for the conditions obtaining, the rate of regeneration is roughly proportional to the fifth root of the hydroxyl ion concentration.

Since the basic dissociation constant of the oxidant is very much higher than that of the reductant and since increase of pH increases the rate of oxidation, an unbuffered solution of pure methylene white exposed to oxygen should exhibit autocatalysis.

(5) ANAEROBIOSIS

There was mentioned above the difficulty in placing end-point indicators for free oxygen upon a sound theoretical basis. The difficulty applies to the use of methylene blue as a criterion of anaerobiosis (*cf.* Hall, 1921). Here is a very real problem which, perhaps, will not be solved until the electrometric conduct of oxygen as displayed, on the one hand, in the oxygen electrode and, on the other hand, in the conduct of oxygen-combining compounds is satisfactorily described. But while this problem remains a very important one in itself, it has been suggested by Clark (1924) that the subject of anaerobiosis may be regarded from a fresh point of view which will, perhaps, leave the first problem in a position of minor significance to so-called anaerobiosis itself.

We may here again emphasize Clark's (*loc. cit.*) view that the isolation of anaerobic processes from the very confusing phenomena of aerobic life may simplify experimental attack and reveal in their elementary form phenomena which have been lost in confusion arising from the complexity of two opposing tendencies. At any rate the numerical data we furnish relieves the subject of certain speculative ideas which are rampant in the literature.

X. Conclusion

In listing the biological applications of methylene blue which can profitably be approached with a fresh and broader viewpoint, we are not overlooking a most serious difficulty which will be encountered at every turn. Briefly stated it is this: When the observational facts with their various practical uses are accumulated, what, after all, is the fundamental significance of the potentials biologically induced? The answer will be found very much more difficult than the answer to hydrogen electrode potentials. We shall discuss this more at length in a later paper. In the meantime it is pertinent to ask why it is that students of those biochemical reactions which are often called electromotively inactive or irreversible have insistently used the beautifully reversible and definitely electromotively active methylene blue system as a favorite reagent. Is it, as suggested in the introduction, merely the prestige of this ubiquitous dye or has there been an intuition of a fundamental significance? We shall not now attempt an answer, but we have furnished in this paper such answers as are implicit in the potential measurements of the methylene blue system.

Since methylene blue as an indicator of reduction has been used in a wide variety of studies which it is impracticable adequately to review, and since in many of these cases the comments we have made may be applicable, there is appended to the list of references cited in the text an incomplete bibliography which we hope will be useful.

Acknowledgments.—For chemical analyses reported in this paper we are indebted to Chemist E. Elvove and Assistant Chemist C. G. Remsburg. Mr. W. L. Hall assisted in some potentiometric measurements. We also wish to express our appreciation of the assistance rendered by Dr. J. A. Ambler and W. C. Holmes, of the Bureau of Chemistry, and by Doctor Scott and Mr. French, of Walter Reed Hospital, in spectrophotometric measurements.

XI. Summary

Methylene blue was found to be difficult to purify. Various samples carefully recrystallized contained excess chlorine and sulphur and gave evidences of small percentages of electromotively active impurities. Drying was found to destroy progressively the characteristic properties.

Methylene white solutions were found to be sensitive to light. Evidence is given that the near ultraviolet is most effective. Methylene white is soluble only to the extent of about 0.0005 molar in acid solutions and about 0.00002 molar in alkaline solutions. The rate of oxidation of methylene white solutions by air varies as the fifth root of the hydroxyl ion concentration.

Mixtures of methylene blue and methylene white give electrode potentials which vary with total concentration. Different samples behave as if there were present small quantities of active impurity.

While the limitations implied by the above facts have made impracticable a high order of accuracy in the determination of constants of the oxidation reduction equilibria, these constants have been determined sufficiently well to characterize the main features of the methylene blue and of the Lauth's violet systems. The interpretation is that methylene blue base is an extremely strong base with dissociation constant too high for measurement by the methods employed. Lauth's violet has a basic dissociation constant of 1.9×10^{-3}. In each case the nonpolar amino group has a basic dissociation constant too low to measure by the method employed.

The reductant in each case fixes one hydrion and, in addition, the two amino groups have basic dissociation constants as follows:

	K_{rb1}	K_{rb2}
Methylene white	1.4×10^{-5}	6.3×10^{-10}
Leuco Lauth's violet	3.8×10^{-9}	4.5×10^{-10}

The characteristic potentials at pH 0 and 30° C. and the corresponding free energies of hydrogenation are:

Methylene blue system 0.532 v., $\Delta F = 24.53$ kg.-cal.

Lauth's violet system 0.563 v., $\Delta F = 25.97$ kg.-cal.

An equation is developed relating these constants in convenient form with pH and with electrode potential-difference, and values calculated thereby conform satisfactorily with experimental data.

The interpretation is in harmony with the constitutional formula proposed by Bernthsen.

The peculiarities of methylene blue are such that it will be found inconvenient as a practicable reduction indicator for precise measurements.

The bearing of the concepts and of the numerical data on Wieland's theory of hydrogen transport, upon concepts used in the theory of cell staining, upon the use of methylene blue in analysis and in a variety of tests is discussed.

Experiments are described as illustrative material for the reinterpretation of methylene blue reduction in the Schardinger reaction, in the methylene blue test of milk quality, in the putrescibility test of sewage, in the differentiation of bacterial species, in the test of anaerobiosis, and in a wide variety of other applications.

XII. Bibliography

REFERENCES IN THE TEXT

Previous papers of the series, Studies on Oxidation-Reduction:

I. Introduction. W. Mansfield Clark. Public Health Reports, 1923, **38**, 443. (Reprint No. 823.)

II. An analysis of the theoretical relations between reduction potentials and pH. W. Mansfield Clark and Barnett Cohen. Public Health Reports, 1923, **38**, 666. (Reprint No. 826.)

III. Electrode potentials of mixtures of 1-naphthol-2-sulphonic acid indophenol and the reduction product. W. Mansfield Clark and Barnett Cohen. Public Health Reports, 1923, **38**, 933. (Reprint No. 834.)

IV. Electrode potentials of indigo sulphonates, each in equilibrium with its reduction product. M. X. Sullivan, Barnett Cohen, and W. Mansfield Clark. Public Health Reports, 1923, **38**, 1669. (Reprint No. 848.)

V. Electrode potentials of simple indophenols, each in equilibrium with its reduction product. Barnett Cohen, H. D. Gibbs, and W. Mansfield Clark. Public Health Reports, 1924, **39**, 381. (Reprint No. 904.)

VI. A preliminary study of indophenols: (A) Dibromo substitution products of phenol indophenol; (B) Substituted indophenols of the ortho type; (C) Miscellaneous. Barnett Cohen, H. D. Gibbs, and W. Mansfield Clark. Public Health Reports, 1924, **39**, 804. (Reprint No. 915.)

VII. A study of dichloro substitution products of phenol indophenols. H. D. Gibbs, Barnett Cohen, and R. K. Cannan. Public Health Reports, 1925, **40**, 649. (Reprint No. 1001.)

Abegg, R., Auerbach, F., and Luther, R. (1915): Messungen elektromotorischer Kräfte galvanischer Ketten. Erstes Ergänzungsheft, Halle.

Allemann, O. (1918): Beitrag zur Kenntniss der sog. Reduktase der Kuhmilch. Milchwirtschaft. Zentral., **47**, 282.

Atack, F. W. (1913): The estimation of stannous chloride and of chlorates by means of methylene blue. J. Soc. Dyers Colourists, **29**, 9.

Atack, F. W. (1913): A new method for the volumetric estimation of chromium, vanadium, and iron, in presence of one another. Analyst, **38**, 99.

Atack, F. W. (1915): Methylene blue and its use in analytical chemistry. J. Soc. Dyers Colourists, **31**, 183, 203.

Avery, R. C. (1922): Differentiation of hemolytic streptococci from human and dairy sources, particularly with methylene blue. Abstracts Bact., **6**, 31.

Bernthsen, A. (1883): Ueber das Methylenblau. Ber., **16**, 1025.

Bernthsen, A. (1883): Zur Kenntniss des Methylenblaus und verwandter Farbstoffe. Ber., **16**, 2896.

Bernthsen, A. (1884): Zur Kenntniss des Methylenblaus und verwandter Farbstoffe. Ber., **17**, 611.

Bernthsen, A. (1885): Studien in der Methylenblaugruppe. Ann., **230**, 73.

Bernthsen, A. (1889): Studien in der Methylenblaugruppe. Ann., **251**, 1.

Bredig, G., and Sommer, F. (1910): Die Schardingersche Reaktion und ähnliche enzymartige Katalysen. Z. physik. Chem., **70**, 34.

Brönsted, J. N. (1923): Einige Bemerkungen über den Begriff der Säuren und Basen. Rec. trav. chim., **42**, 718.

Brown, J. H. (1920): The cultural differentiation of beta hemolytic streptococci of human and bovine origin. J. Exp. Med., **31**, 35.

Child, C. M. (1919): The axial gradients in hydrozoa. Biol. Bull., **37**, 101.

Child, C. M. (1920): Some considerations concerning the nature and origin of physiological gradients. Biol. Bull., **39**, 147.

Clark, H. W., and Adams, G. O. (1908): Studies of incubation tests. J. Am. Chem. Soc., **30**, 1037.

Clark, W. M. (1920): Reduction potentials of mixtures of indigo and indigo white, and of mixtures of methylene blue and methylene white. J. Wash. Acad. Sci., **10**, 255.

Clark, W. M. (1924): Life without oxygen. J. Wash. Acad. Sci., **14**, 123.

Clark, W. M., and Cohen, B. (1922): Some elementary aspects of the putrescibility and similar tests. Abstracts Bact., **6**, 3 (proc.).

Cohen, B., and Clark, W. M. (1921): Oxidation-reduction potentials of certain indophenols and thiazine dyes. Science, N. S., **54**, 557.

Dreser, H. (1885): Histochemisches zur Nierenphysiologie. Z. Biol., **21**, 41.

Duclaux, E. (1894): *Le Lait.* Paris.

Ehrlich, P. (1885): Zur biologischen Verwertung des Methylenblaus. Central. med. Wissensch., **23**, 113.

Ehrlich, P. (1886): Ueber die Methylenblaureaction der lebenden Nervensubstanz. Deut. med. Wochschr., **12**, 49.

Feulgen, R. (1913): Das Verhalten der echten Nucleinsäure zu Farbstoffen. Z. physiol. Chem., **84**, 309.

Fleischer, L., and Amster, S. (1923): Ueber den Einfluss der Reaktion des Mediums auf die Desinfektionswirkung organischer Farbstoffe. Z. Immunitäts., **37**, 327.

Fred, E. B. (1912): A study of the quantitative reduction of methylene blue by bacteria found in milk and the use of this stain in determining the keeping quality of milk. Central. Bakt. Parasitenk. II, **35**, 391.

Formanek, F. (1908): *Untersuchung und Nachweis organischer Farbstoffe.* Berlin (cited).

Gillespie, L. J. (1920): Reduction potentials of bacterial cultures and of water-logged soils. Soil Sci., **9**, 199.

Hall, I. C. (1921): Chemical criteria of anaerobiosis, with special reference to methylene blue. J. Bact., **6**, 1.

Hantzsch, A., and Osswald, G. (1900): Ueber die Umwandlung von Farbbasen in Pseudoammonium-Hydrate, -Cyanide und -Sulfonsäuren. Ber., **33**, 278.

Hasse, F. (1919): Ueber die Methylenblaureduktion durch Glycin. Biochem. Z., **98**, 159.

Hibbert, E. (1909): A volumetric method for the estimation of titanium and of titanium and iron in the presence of each other. J. Soc. Chem. Ind., **28**, 189.

Holmes, W. C. (1924.): The influence of variation in concentration on the absorption spectra of dye solutions. Ind. Eng. Chem., **16**, 35.

Ihl, A. (1888): Zur Prüfung des Rübenzuckers auf seine Reinheit. Chem. Ztg., **12**, 25.

Jaubert, G. F. (1895): Ueber Beziehungen der Safranine zu den Mauveïnen und Indulinen. Ber., **28**, 1578.

Jellinek, K., and Winogradoff, L. (1923): Ersatz der iodometrischen Massanalyse durch die Eisenchlorid-Massanalyse. Z. angew. Chem., **36**, 440; Z. anorg. allgem. Chem., **129**, 15.

Kashahara, M., and Hattori, S. (1921): A rapid clinical method for the determination of the reducing substance in the cerebrospinal fluid: The methylene-blue method. Am. J. Dis. Children, **22**, 218.

Kehrmann, F. (1902): Ueber die Konstitution der Oxazin—und Thiazinfarbstoffe und ihre Beziehungen zu den Azoniumkörpern. Ann., **322**, 1.

Kehrmann, F., Havas, E., and Grandmougin, E. (1913): Zur Kenntniss der Farbsalze der Azin-Farbstoffe II. Ber., **46**, 2802.

Kehrmann, F., Havas, E., and Grandmougin, E. (1914): Konstitution und Farbe der Azin-, Azoxin- und Thiazin-Farbstoffe. III. Ber., **47**, 1881.

Kehrmann, F., and Schaposchnikoff, W. (1897): Ueberführung des Phenylphenazoniums in Phenosafranin und des Isorosindulins von Nietzki und Otto in Naphtophenosafranin. Ber., **30**, 1565.

Kikuchi, S. (1922): Uses of amalgams in volumetric analyses. V. Estimation of iron, titanium, uranium, and methylene blue. J. Chem. Soc. Japan, **43**, 544 (cited).

Knecht, E. (1907): Eine empfindliche Reaktion auf Titan. Chem. Ztg., **31**, 639.

Knecht, E., and Atack, F. W. (1911): A new method for the volumetric estimation of molybdenum. Analyst, **36**, 98.

Knecht, E., and Hibbert, E. (1910): Das Titantrichlorid in der volumetrischen Analyse. IV. Zur Bestimmung der Chinone. Ber., **43**, 3455.

Knecht, E., and Hibbert, E. (1918): *New reduction methods in volumetric analysis.* (Reissue with additions.) London.

Koch, A. (1879): Ueber den schwefelhaltigen Farbstoff aus Dimethylphenylendiamin. Ber., **12**, 592.

Koch, A. (1879): Ueber den schwefelhaltigen Farbstoff aus Paraphenylendiamin. Ber., **12**, 2069.

Kolthoff, I. M. (1920): Das Oxydationspotential einer Ferriferrocyanidlösung. Z. anorg. allgem. Chem., **110**, 143.

Landauer, P., and Weil, H. (1910): Studien über das Methylenblau. Ber., **43**, 198.

Lane, J. H., and Eynon, L. (1923): Determination of reducing sugars by means of Fehling's solution with methylene blue as internal indicator. J. Soc. Chem. Ind., **42**, 32T.

Lederer, A. (1914): A serious fallacy of the "Standard" methylene blue putrescibility test. Am. J. P. H., **4**, 241.

Lee, A. B. (1921): *The Microtomist's Vade-Mecum.* 8th ed. Philadelphia.

Lenz, W. (1895): Ueber eine eigenthümliche Schwierigkeit beim Nachweise des Chlors in Methylenblau nebst anderen Mittheilungen über Methylenblau. Z. anal. Chem., **34**, 39.

MacArthur, J. W. (1921): Gradients of vital staining and susceptibility in planaria and other forms. Am. J. Physiol., **57**, 350.

MacNeal, W. J. (1924): Report to Soc. Am. Bact. Washington meeting.

Meyerhof, O. (1912): Über scheinbare Atmung abgetöteter Zellen durch Farbstoffreduktion. Arch. ges. Physiol. (Pflüger's), **149**, 250.

Michaelis, L. (1902): *Einführung in die Farbstoffchemie für Histologen.* Berlin.

Michaelis, L. (1914): Farbenanalysche Studien. Paul Ehrlich's Festschrift., p. 40.

Michaelis, L. (1922): *Die Wasserstoffionenkonzentration.* 2nd Edition, Part I. Berlin.

Michaelis, L., and Dernby, K. G. (1922): Der Einfluss der Alkalität auf die Wirksamkeit der Chininalkaloide. Z. Immunität., **34**, 194.

Möllendorff, W. v. (1920): Vitale Färbungen an tierischen Zellen. Ergebnisse Physiol., **18**, 141.

Monnier, M. A. (1916): Sur l'emploi de sesquichlorure de titane dans les analyses volumétriques par réduction. Ann. chim. anal., **21**, 109.

Monnier, M. A. (1916): Methylene blue in chemical analysis. Arch. sci. phys. nat., **42**, 210 (cited).

Morgan, E. J., and Quastel, J. H. (1923): The reduction of methylene blue by iron compounds. Proc. Roy. Soc. [B] **95**, 61.

Moser, L., and Prinz, W. (1918): Die Massanalytische Bestimmung der Selenigen-Säure und der Selensäure. Z. anal. Chem., **57**, 277.

Muster, J. F., and Woker, G. (1913): Ueber die Geschwindigkeit der Reduktion des Methylenblaus durch Glukose und Fruktose und ihre Verwertung in der Harnanalyse. Arch. ges Physiol., **155**, 92.

Pelet-Jolivet, L., and Wild, A. (1908): État des matières colorantes en solution. Compt. rend., **147**, 683.

Phelps, E. B. (1909): Putrescibility and stability of sewage effluents. U. S. Geol. Surv., Water-Supply Paper No. 229.

Phelps, E. B., and Winslow, C.-E. A. (1907): On the use of methylene blue in testing sewage effluents. J. Inf. Dis. (Supplement No. 3), 2.

Reed, G. B. (1916): The measurement of oxidation potential and its significance in the study of oxidases. Bot. Gaz., **61**, 523.

Reed, G. B. (1916): The separation of oxidase reactions from the catalase reaction. Bot. Gaz., **62**, 303.

Reed, G. B. (1916): On the mechanism of oxidase action. Bot. Gaz., **62**, 53.

Rozier (1917): Methylene blue—a very sensitive reagent for the detection of picric acid in the urine. Bull. sci. pharmacol., March-April (cited).

Schardinger, F. (1902): Ueber das Verhalten der Kuhmilch gegen Methylenblau und seine Verwendung zur Unterscheidung von ungekochter und gekochter Milch. Z. Nahr. Genussm., **5**, 1113.

Schoch, E. P., and Felsing, W. A. (1916): The influence of the potassium ion and of the calcium ion upon the ferri-ferrocyanide potential. J. Am. Chem. Soc., **38**, 1928.

Scott, R. E., and French, R. W. (1924): Standardization of biological stains. II. Methylen blue. The Military Surgeon, **55**, 337.

Sherman, J. M., and Albus, W. R. (1918): Some characters which differentiate the lactic-acid streptococcus from streptococci of the pyogenes type occuring in milk. J. Bact., **3**, 153.

Sinnatt, F. S. (1910): The use of methylene blue as an indicator in iodimetric titrations. Analyst, **35**, 309.

Sinnatt, F. S. (1912): The use of methylene blue as an indicator in iodimetric titrations. Analyst, **37**, 252.

Spoehr, H. A. (1924): The oxidation of carbohydrates with air. J. Am. Chem. Soc., **46**, 1494.

Spitta, D., and Weldert (1906): Indikatoren für die Beurteilung biologisch gereinigter Abwässer. Mitt. Prüfungsanst. Wasserversorg-. Berlin, **6**, 161.

Strassner, W. (1910): Die reduzierenden Wirkungen des Gewebes. Biochem. Z., **29**, 295.

Theriault, E. J. (1920): A further study of the excess oxygen method for the determination of the biochemical oxygen demand of sewage and industrial wastes. Pub. Health Rep., **35**, 1087.

Thornton, W. M., Jr., and Elderdice, H. L., Jr. (1923): The volumetric estimation of small amounts of phosphorus using a standard solution of methylene blue. J. Am. Chem. Soc., **45**, 668.

Thunberg, T. (1922): Zur Frage des wasserstoffaktivierenden Vermögens der Zelien. Arch. Néerland. physiol., **7**, 240.

Unna, P. G. (1913): *Biochemie der Haut.* Jena.

Virtanen, A. I. (1922): Kolloidernas inverkan på reduktasprovet. Meddelande Nr. 229 från Centralanstalten för försöksväsendet på jordbruksområdet. Bakteriologiska Avdelningen No. 27.

Walbum, L. E. (1920): Über die Wasserstoffionenkonzentration einiger Standardlösungen bei verschiedenen Temperaturen. Biochem. Z., **107**, 219.

Wales, H., and Nelson, O. A. (1923): Adsorbed moisture and water of crystallization in certain common dyes. J. Am. Chem. Soc., **45**, 1657.

Webster, A., Hill, L., and Eidinow, A. (1924): Measurement of ultra-violet light by means of an acetone methylene-blue solution. The Lancet, **206**, 745.

Wichern, H. (1908): Zur quantitativen Bestimmung der Reduktionskraft von Bakterien und tierischen Organen. Z. physiol. Chem., **57**, 365.

Wieland, H. (1922): Über den Mechanismus der Oxydationsvorgänge. Ergebnisse Physiol., **20**, 477.

SUPPLEMENTARY REFERENCES ON THE USE OF METHYLENE BLUE IN TESTING SEWAGE

American Public Health Association (1920): Standard Methods for the Examination of Water and Sewage. Boston.

Bachmann, F. (1914): A new seal for the prevention of aeration in deaerated liquids. J. Ind. Eng. Chem., **6**, 764.

Bruckmiller, F. W. (1916): Oxygen demand of sewages. J. Ind. Eng. Chem., **8**, 403.

Bruckmiller, F. W. (1916): Nitrates and oxygen demand. J. Ind. Eng. Chem., **8**, 899.

Buswell, A. M. (1914): Modified apparatus for the putrescibility test. J. Ind. Eng. Chem., **6**, 325.

Carlson, T. (1912): Ueber die Lösung des Sauerstoffes in Wasser und sein Verhältnis bei der Selbstreinigung der Gewässer. Akad. Abh. Stockholm, p. 47 (cited).

Clark, H. W., and Adams, G. O. (1908): Studies of incubation tests. **J. Am. Chem. Soc., 30,** 1037.

Clark, W. M., and Cohen, B. (1922): Some elementary aspects of the putrescibility and similar tests. Abstracts Bact., **6,** 3 (proc.).

Dunbar (1904): Standards of purity for sewage effluents. J. Royal Sanitary Inst., **25,** Part III, 599.

Frank, L. C., and Rhynus, C. P. (1919): The treatment of sewage from single houses and small communities. Pub. Health Bull. No. 101, 11.

Fuller, G. W. (1894): Experiments upon the purification of sewage and water at the Lawrence Experiment Station. Twenty-sixth Annual Report, St. Bd. Hlth. Mass. No. 34, 447.

Fuller, G. W. (1912): *Sewage Disposal,* N. Y., p. 33.

Hale, F. E., and Melia, T. W. (1915): A comparison of methods for determining putrescibility or oxygen demand. J. Ind. Eng. Chem., **7,** 762.

Hoover, C. B. (1912): Standard tests of the efficiency of sewage treatment. Eng. News, **68,** 452.

Jackson, D. D., and Horton, W. A. (1909): Experiments on the putrescibility test for sewage and sewage effluents. J. Ind. Eng. Chem., **1,** 328.

Johnson, G. A., Copeland, W. R., and Kimberly, A. E. (1906): The relative applicability of current methods for the determination of putrescibility in sewage effluents. J. Infect. Dis. (Supplement No. 2), 80.

Kinnicutt, L. P., Winslow, C.-E. A., and Pratt, R. W. (1919): *Sewage Disposal,* 2d ed. N. Y., p. 503.

Labbé, H., Goiffon, and Nepveux (1920): L'indice d'oxydabilité comme test de putréfaction des matières fécales. Compt. rend. soc. biol., **83, 904.**

Lederer, A. (1913): The relation of the nitrates to the putrescibility of sewages. J. Infect. Dis., **13,** 236.

Lederer, A. (1914): A serious fallacy of the "Standard" methylene blue putrescibility test. Am. J. P. H., **4,** 241.

Lederer, A. (1914): The biochemical oxygen demand of sewages. J. Ind. Eng. Chem., **6,** 882.

Lederer, A. (1914): A new method for determining the relative stability of a sewage effluent, or polluted river water. J. Infect. Dis., **14,** 482.

Lederer, A. (1915): Notes on the practical application of the "Saltpeter method" for determining the strength of sewages. Am. J. P. H., **5,** 354.

Lederer, A. (1915): Determination of the biochemical oxygen demand by the "Saltpeter method" in stockyards, tannery, and corn products wastes. J. Ind. Eng. Chem., **7,** 514.

Lederer, A. (1915): "Relative stabilities" in polluted waters carrying colloids. Am. J. P. H., **5,** 740.

Mohlman, F. W. (1916): The English incubation test for the putrescibility of sewage and sewage effluents. Univ. Illinois Bull., **14,** No. 5, Water Survey Series No. 13, 315.

Mohlman, F. W. (1925): Sewage analysis and its interpretations. Am. J. Pub. Health, **15,** 10.

Ohlmüller, W., and Spitta, D. (1921): *Die Untersuchung und Beurteilung des Wassers und des Abwassers.* 4th Edition. Berlin.

Phelps, E. B. (1909): The disinfection of sewage and sewage filter effluents, with a chapter on the putrescibility and stability of sewage effluents. Water Supply Paper No. 229, U. S. Geol. Surv.

Phelps, E. B. (1913): The chemical measure of stream pollution and specifications for sewage effluents. Am. J. P. H., **3,** 524.

Phelps, E. B. (1919): Studies of methods for the treatment and disposal of sewage. Pub. Health Bull. No. 101, **7.**

Phelps, E. B., and Winslow, C.-E. A. (1907): On the use of methylene blue in testing sewage effluents. J. Infect. Dis. (Supplement No. 3), 2.

Spitta, D. (1903): Weitere Untersuchungen über Flussverunreinigung. Arch. Hyg., **46**, 64.

Spitta, D., and Weldert (1906): Indikatoren für die Beurteilung biologisch gereinigter Abwässer. Mitt. Kgl. Prüfungsanst. Wasserversorg., Berlin, **6**, 161.

Swanson, A. A., and Hulett, G. A. (1915): The determination of gases dissolved in waters and effluents. J. Am. Chem. Soc., **37**, 2490.

Theriault, E. J. (1920): A further study of the excess oxygen method for the determination of the biochemical oxygen demand of sewage and industrial wastes. Pub. Health Rep., **35**, 1087.

Theriault, E. J., and Hommon, H. B. (1918): The determination of the biochemical oxygen demand of industrial wastes and sewage. Pub. Health Bull., No. 97, 70.

SUPPLEMENTARY REFERENCES ON THE USE OF METHYLENE BLUE IN BACTERIOLOGY (EXCLUSIVE OF STAINING)

Arnold, L. (1921): Classification of streptococcus. III. Streptococci from normal and pathogenic throats, also from wounds, classified by sugar fermentation, limiting hydrogen-ion concentration, and reactions on milk mediums. J. Lab. and Clin. Med., **6**, 312.

Avery, R. C. (1922): Differentiation of hemolytic streptococci from human and dairy sources, particularly with methylene blue. Abstracts Bact., **6**, 31.

Avery, O. T., and Neill, J. M. (1924): Studies on oxidation and reduction by pneumococcus. III. Reduction of methylene blue by sterile extracts of pneumococcus. J. Exp. Med., **39**, 543.

Berner, O. (1904): On a vial for the culture of anaërobic bacteria on plates. Centr. Bakt., Parsitenk. I, **37**, 478.

Binger, C. A. L. (1919): The selective inhibitory action of methylene blue and certain other common dyes on the growth of meningococci. J. Infect. Dis., **25**, 277.

Brossa, G. A. (1923): Ueber die Agglutination von Bakterien durch Farbstoffe. Z. Immunität., I Abt., Orig., **37**, 221.

Brown, J. H. (1920): The cultural differentiation of beta hemolytic streptococci of human and bovine origin. J. Exp. Med., **31**, 35.

Cahen, F. (1887): Ueber das Reduktionsvermögen der Bacterein. Z. Hyg., **2**, 386.

Carapelle, E. (1908): Ueber die Reduktionserscheinungen der Bacterien. Centr. Bakt., Parasitenk., I Abt., **47**, 545.

Catheart, E., and Hahn, M. (1902): Über die reduzierenden Wirkungen der Bakterien Arch. Hyg., **44**, 295.

Clark, W. M. (1924): Life without oxygen. J. Wash. Acad. Sci., **14**, 123.

Douglas, S. R., Fleming, A., and Colebrook, L. (1917): On the growth of anaerobic bacilli in fluid media under apparently aerobic conditions. Lancet, **193**, 530.

Dreser, H. (1885): Histochemisches zur Nierenphysiologie. Z. Biol., **21**, 41.

Falta, W., and Richter-Quittner, M. (1921): The so-called oligodynamic action of the heavy metals and their salts. Biochem. Z. **115**, 39 (cited).

Fleischer, L., and Amster, S. (1923): Ueber den Einfluss der Reaktion des Mediums auf die Desinfektionswirkung organischer Farbstoffe. Z. Immunitäts., **37**, 327.

Fraser, C. G. (1920): Action of methylene blue and certain other dyes on living and dead yeast. J. Phys. Chem., **24**, 741.

Fraser, C. G. (1921): Methylene blue as indicator in determining the toxicity of phenol and phenol-salt solutions toward yeast. J. Phys. Chem., **25**, 1.

Fred, E. B. (1912): The use of stains in the study of bacteria. Virginia Agr. Exp. Sta. Rept., p. 202.

Fred, E. B. (1912): A study of the quantitative reduction of methylene blue by bacteria found in milk and the use of this stain in determining the keeping quality of milk. Centr. Bakt., Parasitenk., II, **35**, 391.

Fred, E. B. (1912): Eine physiologische Studie ueber die nitratreduzierenden Bakterien. Centr. Bakt., Parasitenk. II. Abt., **32**, 421.

Fremlin, H. S. (1903): A note on the cultivation of anaerobic bacteria. Lancet, **164**, 518.

Gates, F. L., and Olitsky, P. K. (1921): Factors influencing anaerobiosis, with special reference to the use of fresh tissue. J. Exp. Med., **33**, 51.

Gillespie, L. J. (1920): Reduction potentials of bacterial cultures and of water-logged soils. Soil Sci., **9**, 199.

Gózony, L., and Kramár, E. (1922): Reduktionsversuche mit Bakterien. Centr. Bakt., Parasitenk. I, **89**, 193.

Guillemont, L., and Szczawinska, W. (1908): Rôle des substances réductrices dans la culture des anaérobies en présence de l'air. Compt. rend. soc. biol., **64**, 171.

Hahn, M. (1902): Ueber die Reduktionswirkungen der Hefe und des Hefepresssaftes sowie der Bakterien. Münch. med. Wochschr., **49**, 595.

Hall, I. C. (1920): Methylene blue as a criterion of anaerobiosis. Abstracts Bact., **4**, 4.

Hall, I. C. (1921): Chemical criteria of anaerobiosis with special reference to methylene blue. J. Bact., **6**, 1.

Hammerl, H. (1901): Ein Beitrag zur Züchtung der Anaëroben. Centr. Bakt., Parasitenk. I, **30**, 658.

Harden, A., and Henley, F. R. (1921): The effect of acetaldehyde and methylene blue on the fermentation of glucose and fructose by yeast-juice and zymin in presence of phosphate and arsenate. Biochem. J., **15**, 175.

Harden, A., and Norris, R. V. (1915): The reducing enzymes of dried yeast (Lebedeff) and of rabbit muscle. Biochem. J., **9**, 330.

Harden, A., and Zilva, S. S. (1915): The reducing enzyme of *Bacillus coli communis*. Biochem. J., **9**, 379.

Hata, S. (1908): Ueber eine einfache Methode zur anërobischen Kultivierung der Anaëroben, mit besonderer Berücksichtigung ihrer Toxinproduktion. Centr. Bakt., Parasitenk. I, **46**, 539.

Jensen, V. W., and Schery, C. W. (1920): Actinomycosis treated with methylene blue and roentgen ray. J. Am. Med. Assoc., **75**, 1470.

Kabrhel, G. (1899): Zur Frage der Züchtung anaërober Bakterien. Centr. Bakt., Parasitenk. I, **25**, 555.

Kanaido, S. (1916): Reduction of methylene blue by bacteria, inhibited by immune sera. Saikin Gaku Zasshi No. 243, 169 (cited).

Kumagawa, H. (1921): Über die Einwirkung von Salzen auf die Entfärbung des Methylenblaus durch verschiedene Hefesorten. Biochem. Z., **121**, 150.

Lentz, O. (1909): Ein neues Verfahren für die Anaërobenzüchtung. Centr. Bakt., Parasitenk. I, **53**, 358.

Liefmann, H. (1908): Ein einfaches Verfahren zur Züchtung und Isolierung anaërober Keime. Centr. Bakt. I, **46**, 377.

Lvoff, S. (1913): Hefegërung und Wasserstoff. Z. Gärungsphysiol., **3**, 289.

Maassen, A. (1904): Ueber das Reduktionsvermögen der Bakterien und ueber reduzierende Stoffe in pflanzlichen und tierischen Zellen. Arb. Kais. Gesundh., **21**, 377.

Meyerhof, O. (1917): Untersuchungen zur Atmung getöteter Zellen. I. Die Wirkung des Methylenblaus auf die Atmung lebender und getöteter Staphylococcen nebst Bemerkungen über den Einfluss des Milieus, der Blausäure und Narkotika. Arch. ges. Physiol. (Pflüger's), **169**, 87.

Möllendorff, W. v. (1920): Vitale Färbungen an tierischen Zellen. Ergebnisse Physiol., **18**, 141.

Müller, F. (1899): Ueber das Reduktionsvermögen der Bakterien. Centr. Bakt., Parasitenk., Orig., **26**, 801.

Müller, F. (1899): Ueber reduzierende Eigenschaften von Bakterien. Centr. Bakt., Parasitenk, Orig., **26**, 51.

Nakayama, Y. (1920): On the toxin for leucocytes produced by streptococci (streptoleucocidin). J. Infect. Dis., **27**, 86.

Neill, J. M., and Avery, O. T. (1924): The oxidation of enzymes in sterile extracts of pneumococcus. J. Exp. Med., **40**, 405.

Neisser, M., and Wechsberg, F. (1901): Ueber das Staphylotoxin. Z. Hyg., **36**, 299.

Riemsdijk, M. van (1922): Über einen neuen, einfachen Sauerstoffindicator für die Züchtung von anaeroben Bakterien und die Kultur von Anaërobionten im Allgemeinen. Centr. Bakt., Parasitenk., Abt. I, Orig., **88**, 229.

Romanese, R. (1912): Sull'azione del bleu di metilene solo o somministrato contemporaneamente a chinino nella tripanosomiasi sperimentale. Arch. farm. sper., **13**, 27.

Rothberger, C. J. (1898): Differentialdiagnostische Untersuchungen mit gefärbten Nährböden. Centr. Bakt., Parasitenk., **24**, 513.

Rothberger, C. J. (1899): Differentialdiagnostische Untersuchungen mit gefärbten Nährböden. Centr. Bakt., Parasitenk., **25**, 1569.

Ruehle, G. L. A. (1923): The enzymic content of bacterial spores. Abstracts Bact., **7**, 7.

Sanfelice, F. (1893): Untersuchungen über anaërobe Mikroorganismen. Z. Hyg., **14**, 339.

Schnabel, A. (1922): Ueberempfindlichkeitsversuche an Bakterien. Deut. med. Wochschr., **48**, 654.

Sellards, A. W. (1904): Some researches on anaërobic cultures with phosphorus. Centr. Bakt., Parasitenk. I, **37**, 632.

Sherman, J. M., and Albus, W. R. (1918): Some characters which differentiate the lactic-acid streptococcus from streptococci of the pyogenes type occurring in milk. J. Bact., **3**, 153.

Smith, T. (1893): The fermentation tube, with special reference to anaerobiosis and gas production among bacteria. Wilder Quarter Century Book, Ithaca, N. Y., pp. 187, 220.

Smith, T. (1896): Reduktionserscheinungen bei Bacterien und ihre Beziehungen zur Bakterienzelle, nebst Bemerkungen über Reduktionserscheinungen in steriler Bouillon. Centr. Bakt., Parasitenk., **19**, 181.

Spina, A. (1887): Bacteriologische Versuche mit gefärbten Nährsubstanzen. Centr. Bakt., Parasitenk., **2**, 71.

Stange, H. (1915): Reduktion und alkoholische Gärung. Z. Gärungsphysiol., **5**, 65.

Strohecker, R. (1920): Detection of incipient putrefaction in meat. Chem. Ztg. **44**, 744 (cited).

Stüler, A. (1904): Neue Methoden zur Anaërobenkultur und Anaërokultur. Centr. Bakt., Parasitenk. I, **37**, 298.

Trenkmann (1898): Das Wachstum der anaëroben Bakterien. Centr. Bakt., Parasitenk , I, **23**, 1038.

van Steenberge, P. (1920): Les propriétés des microbes lactique; leur classification. Ann. Inst. Pasteur, **34**, 803.
Verzár, F., and Bögel, J. (1920): Weitere Untersuchungen über Stoffwechselregulierung bei Bakterien. Biochem. Z., **108**, 207.
Weigmann, and Wolff, A. (1916): Reducing action of bacteria. Centr. Bakt., Parasitenk., **44**, 164 (cited).
Wichern, H. (1908): Zur quantitativen Bestimmung der Reduktionskraft von Bakterien und tierischen Organen. Z. physiol. Chem., **57**, 365.
Wichern, H. (1910): Zur quantitativen Bestimmung der Reduktionskraft der Bakterien und tierischen Organismen. Arch. Hyg., **72**, 365.
Wilson, W. J. (1917): An easily improvised and effective method of obtaining plate cultures of anaerobes. Lancet, **192**, 724.
Wolff, A. (1902): Ueber die Reductionsfähigkeit der Bacterien einschliesslich der Anaërobien. Arb. path. Anat. Inst. zu Tübing., Brnschwg., **3**, 294.
Zinsser, H., Hopkins, J. G., and Gilbert, R. (1915): Notes on the cultivation of *Treponema pallidum*. J. Exp. Med., **21**, 213.

SUPPLEMENTARY REFERENCES ON THE USE OF METHYLENE BLUE IN GENERAL BIOCHEMISTRY

Achard, C., and Castaigne, J. (1897): Sur la décoloration du bleu de méthylène par les éléments vivants. Compt. rend. soc. biol., **4**, 1091.
Ahlgren, G. (1921): Über die spontanreduzierende Einwirkung der Muskulatur auf Methylenblau. Skand. Arch. Physiol., **41**, 1.
Ahlgren, G. (1923): Über den Angriffspunkt des Insulins. Skand. Arch. Physiol., **44**, 167.
Ahlgren, G. (1923): Gibt es einen Stoffwechsel in der Kristallinse? Skand. Arch. Physiol., **44**, 198.
Ahlgren, G. (1923): Experiments on the respiration of malignant tissues. Brit. J. Exp. Path., **4**, 196.
Aloy, J., and Valdiguié, A. (1923): Catalyses d'oxydation provoquées par les sels de cuivre et d'uranium. Bull. soc. chim., **33**, 572.
Aschmarin, P. A. (1922): Analogies between methylene blue and oxidases. J. Russ. Physiol., **4**, 283 (cited).
Atack, F. W. (1915): Methylene blue and its use in analytical chemistry. J. Soc. Dyers Colourists, **31**, 183, 203.
Battelli, F., and Stern, L. (1920–21): The mechanism of the action of the oxidizing and reducing enzymes. Arch. intern. physiol., **18**, 403; Compt. rend. soc. biol., **83**, 1544 (cited).
Bayeux, R. (1921): Le pouvoir réducteur des liquides organiques et des tissus de quelques animaux marins. Compt. rend., **172**, 878.
Beckwith, T. D. (1921): The effect of body cells and fluids on certain dyes. J. Infect. Dis., **28**, 170.
Becquerel, P. (1923): Necrobiosis of vegetable protoplasm studied by aid of a new vital reagent. Compt. rend., **176**, 601 (cited).
Child, C. M. (1919): The axial gradients in hydrozoa. Biol. Bull., **37**, 101.
Child, C. M. (1920): Some considerations concerning the nature and origin of physiological gradients. Biol. Bull. **39**, 147.
Collett, M. E. (1924): The specificity of the intracellular hydrogenases in frog's muscle. J. Biol. Chem. **58**, 793.
Dixon, M., and Tunnicliffe, H. E. (1923): The oxidation of reduced glutathione and other sulphydryl compounds. Proc. Roy. Soc., **94B**, 266.
Drury, A. N., (1914): The validity of the microchemical test for the oxygen place in tissues. Proc. Royal, Soc., **88B**, 166.

Ehrlich, P. (1885): Zur biologischen Verwertung des Methylenblaus. Centr. med. Wissensch., **23**, 113.

Ehrlich, P. (1886): Ueber die Methylenblaureaction der lebenden Nervensubstanz. Deut. med. Wochschr., **12**, 49.

Eisenhardt, W. (1920): Quantitative Blutzuckerbestimmung mit Hilfe des Methylenblaus. Münch. med. Wochschr., **67**, 1382.

Feulgen, R. (1913): Das Verhalten der echten Nucleinsäure zu Farbstoffen. Z. physiol. Chem., **84**, 309.

Grönvall, H. (1923): Untersuchungen über die Einwirkung einiger einfacher Narkotika auf die Succinodehydrogenase. Skand. Arch. Physiol., **44**, 200.

Harden, A., and Henley, F. R. (1920): The effect of pyruvates, aldehydes, and methylene blue on the fermentation of glucose by yeast juice and zymin in the presence of phosphate. Biochem. J., **14**, 642.

Harden, A., and Henley, F. R. (1921): The effect of acetaldehyde and methylene blue on the fermentation of glucose and fructose by yeast juice and zymin in presence of phosphate and arsenate. Biochem. J., **15**, 175.

Harden, A., and Norris, R. V. (1914): The enzymes of washed zymin and dried yeast (Lebedeff) II Reductase. Biochem. J., **8**, 100.

Harvey, E. N. (1922): The permeability of cells for oxygen and its significance for the theory of stimulation. J. Gen. Physiol., **5**, 215.

Hasse, F. (1919): Ueber die Methylenblaureduktion durch Glycin. Biochem. Z., **98**, 159.

Herter, C. A. (1904): On the reducing action of the animal organism under the influence of cold. Am. J. Physiol., **12**, 128.

Hopkins, F. G. (1921): On an autoxidisable constituent of the cell. Biochem. J., **15**, 286.

Hopkins, F. G., and Dixon, M. (1922): On glutathione. II A thermostable oxidation-reduction system. J. Biol. Chem., **54**, 527.

Ihl, A. (1888): Zur Prüfung des Rübenzuckers auf seine Reinheit. Chem. Ztg., **12**, 25.

Johannsen, T. (1906): Ueber die Reduktionskraft aseptisch entnommener Organe. Arb. path. Anat. Inst. zu Tübing., Brnschwg., **5**, 326.

Kashahara, M., and Hattori, S. (1921): A rapid clinical method for the determination of the reducing substance in the cerebrospinal fluid: The methylene-blue method. Am. J. Dis. Children, **22**, 218.

Knoop, F. (1922): Ueber Reduktionen und Oxydationen und eine gekoppelte Reaktion im intermediären Stoffwechsel des Tierkörpers. Biochem. Z., **127**, 200.

Lane, J. H., and Eynon, L. (1923): Determination of reducing sugars by means of Fehling's solution with methylene blue as internal indicator. J. Soc. Chem. Ind., **42**, 32T.

Lehmann, J. (1922): Über die Einwirkung verschiedener Faktoren auf Oxydationenzyme im Samen von *Phaseolus vulgaris*. Bot. Notiser. Lund.

Lvoff S. (1913): Zymase and Reduktase in ihren gegenseitigen Beziehungen. Ber. Botan. Ges. **31**, 141.

Meyerhof, O. (1912): Ueber scheinbare Atmung abgetöteter Zellen durch Farbstoffreduktion. Arch. ges Physiol. (Pflüger's), **149**, 250.

Meyerhof, O. (1918): Untersuchungen zur Atmung getöteter Zellen. II. Der Oxydationsvorgang in getöteter Hefe und Hefeextrakt. Arch. ges. Physiol. (Pflüger's), **170**, 367.

Morgan, E. J., Stewart, C. P., and Hopkins, F. G. (1922): On the anaerobic and aerobic oxidation of xanthin and hypoxanthin by tissues and by milk. Proc. Roy. Soc., **94B**, 109.

Muster, J. F., and Woker, G. (1913): Ueber die Geschwindigkeit der Reduktion des Methylenblaus durch Glukose und Fruktose und ihre Verwertung in der Harnanalyse. Arch. ges. Physiol. (Pflüger's), **155**, 92.

Neisser, M., and Wechsberg, F. (1900): Ueber eine neue einfache Methode zur Beobachtung von Schädigungen lebender Zellen und Organismen. (Bioskopie) Münch. Med. Wochschr., **47**, 1261.

Ohlsson, E. (1921): Die Abhängigkeit der Wirkung der Succinodehydrogenase von der Wasserstoffionenkonzentration. Skand. Arch. Physiol., **41**, 77.

Osterhout, W. J. V. (1918–19): An indicator method of measuring the consumption of oxygen. J. Gen. Physiol., **1**, 167.

Palladin, W. (1908): Beteiligung der Reduktase im Prozesse der Alkoholgärung. Z. physiol. Chem., **56**, 83.

Palladin, W., Hübbenet, E., and Korsakow, M. (1911): Ueber die Wirkung von Methylenblau auf die Atmung und die alkoholische Gärung lebender und abgetöteter Pflanzen. Biochem. Z., **35**, 1.

Reed, G. B. (1916): The measurement of oxidation potential and its significance in the study of oxidases. Bot. Gaz., **61**, 523.

Reed, G. B. (1916): The separation of oxidase reaction from the catalase reaction. Bot. Gaz., **62**, 303.

Reed, G. B. (1916): On the mechanism of oxidase action. Bot. Gaz., **62**, 53.

Roger, H. (1920): Le pouvoir réducteur des tissus; action des globulines et des sérines. Compt. rend. soc. biol., **88**, 1377.

Rosling, E. (1923): L'hormone pancréatique comme activateur de certaines enzymes des muscles. Compt. rend. soc. biol., **88**, 112.

Rozier (1917): Methylene blue—a very sensitive reagent for the detection of picric acid in the urine. Bull. sci. pharmacol., March-April (cited).

Sahlin, B. (1924): Untersuchungen über den Einfluss einiger Kaliumsalze auf die Succinodehydrogenase. Skand. Arch. Physiol., **46**, 64.

Schardinger, F. (1907): Verhalten von Weizen- und Roggenmehl zu Methylenblau und zu Stärkekleister. Centr. Bakt., Parasitenk. II, **18**, 748.

Schneider, H. (1914): Neue Studien zur Darstellung der Reduktions- und Sauerstofforte der Pflanzenzelle. Z. wiss. Mikr., **31**, 478.

Schumacher, J. (1922): Ueber oligodynamische Metallwirkung. Biochem. Z., **134**, 398.

Spoehr, H. A. (1924): The oxidation of carbohydrates with air. J. Am. Chem. Soc., **46**, 1494.

Strassner, W. (1910): Die reduzierenden Wirkungen des Gewebes. Biochem. Z., **29**, 295.

Thunberg, T. (1916): Ueber die vitale Dehydrierung der Bernsteinsäure bei Abwesenheit von Sauerstoff. Zentr. Physiol., **31**, 91.

Thunberg, T. (1918): Zur Kenntniss der Einwirkung tierischer Gewebe auf Methylenblau. Skand. Arch. Physiol., **35**, 163.

Thunberg, T. (1920): Zur Kenntniss des intermediären Stoffwechsels und der dabei wirksamen Enzyme. Skand. Arch. Physiol., **40**, 1.

Thunberg, T. (1922): Zur Frage des Wasserstoffaktivierenden Vermögens der Zellen. Arch. néerland. physiol., **7**, 240.

Thunberg, T. (1922): Besteht ein genetischer Zusammenhang zwischen dem eingeatmeten Sauerstoff und dem Sauerstoff der ausgeatmeten Kohleosäure? Naturwissensch. Heft. 17.

Thunberg, T. (1923): Zur Kenntniss der Stoffwechselenzyme der Nervenfaser. Skand. Arch. Physiol., **43**, 275.

Underhill, F. P., and Closson, O. E. (1905): The physiological behavior of methylene blue and methylene azure: a contribution to the study of the oxidation and reduction processes in the animal organism. Am. J. Physiol., **13**, 358.

Unna, P. G. (1913): *Biochemie der Haut.* Jena.
Unna, P. G., and Golodetz, L. (1913): Biochemie der Haut. Oppenheimer's Handb. Biochem. Menschen und Tiere. Ergänzb., 327.
Verzár, F. (1918): Contraction und Starre des quergestreiften Muskels nach Untersuchungen mit vitalen Farbstoffen. Biochem. Z., **90**, 63.
Voegtlin, C., Johnson, J. M., and Dyer, H. A. (1924): Quantitative estimation of the reducing power of normal and cancer tissue. J. Pharm. Exp. Therap., **24**, 305.
Warburg, O. (1923): Ueber die Grundlagen der Wielandschen Atmungstheorie. Biochem. Z., **142**, 518.
Widmark, M. P. (1921): Studien über die Succinodehydrogenase. Skand. Arch. Physiol., **41**, 200.
Wieland, H. (1913): Ueber den Mechanismus der Oxydationsvorgänge. Ber., **46**, 3327.
Wieland, H. (1922): Ueber den Mechanismus der Oxydationsvorgänge. Ergebnisse Physiol., **20**, 477.
Wishart, G. M. (1923): Reduction of methylene blue by tissue extracts. Biochem. J., **17**, 103.

SUPPLEMENTARY REFERENCES ON THE USE OF METHYLENE BLUE IN TESTS OF MILK

Allemann, O. (1918): Beitrag zur Kenntniss der sog. Reduktase der Kuhmilch. Milchwirtschaft. Zentr., **47**, 282.
Anon. (1898): Eine Methode zur Unterscheidung von pasteurisierten und nichtpasteurisierten Milch. Milch-Ztg., **27**, 374.
Anon. (1923): Methylene blue method of estimating the keeping quality of milk. New Hampshire Agr. Sta. Bull., **208**, 19.
Bach, A. (1911): Zur Kenntniss der Reduktionsfermente. I. Über das Schardinger-Enzym (Perhydridase). Biochem. Z., **31**, 443.
Bach, A. (1917): Non-Spécificité du ferment réducteur animal et végétal. Compt. rend., **164**, 248.
Bahr, L. (1914): Om Maelkens Evne til at affarve Methylenblaat. Skand. Veterinärtidskr., **4**, 28.
Barthel, C. (1908): Sur la valeur de l'épreuve de la réductase. Rev. gén. du lait, Lierre, **7**, 1.
Barthel, C. (1910): Die Reduktaseprobe. Milch-Ztg., **39**, 25.
Barthel, C. (1911): Die Reduktaseprobe. Z. Nahr. Genussm., **21**, 513.
Barthel, C. (1916): Ueber die Ursache der Entfärbung von Methylenblau bei der Reduktaseprobe. Molkerei-Ztg., **30**, 419.
Barthel, C. (1917): Weitere Untersuchungen über die Reduktaseprobe, sowie Vergleiche mit einigen anderen neueren milchhygienischen Untersuchungsmethoden. Z. Nahr. Genussm., **34**, 137.
Barthel, C. (1921): La valeur de l'épreuve de la réductase dans la practique laitière. Le Lait, **1**, 62.
Barthel, C., and Orla-Jensen (1912): Ueber internationale Methoden zur Beurteilung der Milch. Milchwirtschaft. Zentr., **41**, 417.
Barthel, C., and Orla-Jensen (1912): Méthodes internationales d'appréciation du lait. Rev. gén. du lait, Lierre, **9**, 227.
Bertin-Sans, H., and Gaujoux, E. (1912): Les catalases du lait de vache au point de vue de la valeur hygiénique du lait. Rev. d'hyg., Par., **34**, 1020.
Bertin-Sans, H., and Gaujoux, E. (1914): Les catalases du lait de vache au point de vue de la valeur hygiénique du lait. Rev. d'hyg., Par., **36**, 258.

45487°--25†——5

Borck, W. (1921): Vergleichende Prüfungen bakteriologischer und biochemischer Methoden zur Beurteilung der Milch. Centr. Bakt., Parasitenk. II Abt., Ref., **54**, 127.

Brand, E. (1907): Ueber die praktische Bedeutung der Reduktionsfähigkeit der Milch. Münch. med. Wochschr., **54**, 821.

Bredig, G., and Sommer, F. (1910): Die Schardingersche Reaktion und ähnliche enzymartige Katalysen. Z. physik. Chem., **70**, 34.

Burri, R., and Kürsteiner, J. (1912): Zur Klärung der Anschauungen über die reduzierenden Eigenschaften der Kuhmilch. Milchwirtschaft. Zentr., **41**, 40, 68, 101, 134, 168.

Burri, R., and Schmid, H. (1911): Die Beeinflussung des Verlaufs der sog. Schardinger-Reaktion durch die Kühlung der Milch. Biochem. Z., **36**, 376.

Butterberg, P. (1906): Zur Untersuchung der pasteurisierten Milch. Z. Nahr. Genussm., **11**, 377.

Carrieu, M. F. (1921): The aldehyde reducing power of milk (Schardinger reaction). *Le Lait*, **1**, 429 (cited).

Cooledge, L. H. (1921): The colorimetric hydrogen ion determination as a means of studying biological changes in dairy products. Mich. Agr. Exp. Sta., Tech. Bull., **52**, 20.

Dons, R. (1912): Zur Beurteilung der Reduktase- (Gärreduktase-) Probe. Centr. Bakt., Parasitenk, II Abt., **40**, 132.

Duclaux, E. (1894): *Le Lait.* Paris.

Eichwald, E. (1919): Kolloidchemische Betrachtungen zur Reduktaseprobe der Milch. Z. Nahr. Genussm., **38**, 359.

Fred, E. B. (1912): A study of the quantitative reduction of methylene blue by bacteria found in milk and the use of this stain in determining the keeping quality of milk. Centr. Bakt., Parasitenk., II, **35**, 391.

Fred, E. B., and Chappelear, G. W. (1912): Bacteriological and chemical methods for determining the quality of milk. Virginia Agr. Exp. Sta. Rept., p. 206.

Haas, P., and Hill, T. G. (1923): Reducing and oxidizing properties of milk. Pharm. J., **111**, 94; Chemist and Druggist, **99**, 146; Biochem. J., **17**, 671.

Harden, A., and Lane-Claypon, J. E. (1912): Occurrence of ferments in the sterile milk collected by milking tube from cows and goats. J. Hyg., **12**, 144.

Harvey, E. N. (1918–19): The relation between the oxygen concentration and rate of reduction of methylene blue by milk. J. Gen. Physiol., **1**, 415.

Hastings, E. G. (1919): The comparative value of quantitative and qualitative bacteriological methods as applied to milk, with special consideration of the methylene blue reduction test. J. Dairy Sci., **2**, 293.

Hastings, E. G. (1920): The methylene blue reduction test: A simple method for determining the number of bacteria in milk. Hoard's Dairyman, **60**, 280.

Hastings, E. G., and Davenport, A. (1920): The relative value of the methylene blue reduction test, the bromthymol blue test, and the bromcresol purple test in determining the keeping quality of milk. J. Dairy Sci., **3**, 353.

Hastings, E. G., Davenport, A., and Wright, W. H. (1922): The influence of certain factors on the methylene blue reduction test for determining the number of bacteria in milk. J. Dairy Sci., **5**, 438.

Hecht, A. F. (1904): Die Reduction als Lebensfunktion der Milch. Arch. Kinderheilk., **38**, 349.

Heineman, P. G. (1919): *Milk.* W. B. Saunders Co. Phila.

Höyberg, H. M. (1914): Ist die Reduktaseprobe eine wertvolle Probe im Dienste der Milchkontrolle? Z. Fleisch-Milchhyg., **24**, 107.

Jone, H. (1915): *An easy test for bacteria in milk and cream.* (Barthel's reductase test improved.) Brooklyn.

Koning, C. J. (1906): Biologische und biochemische Studien über Milch. Milchwirtschaft. Zentr., **2**, 513, 517; **3**, 41, 53.

Kufferath, H. (1919): Le controle bactériologique et hygiénique des laits. Ann. inst. Past., **33**, 462.

Lagane, L. (1914): Le pouvoir réducteur des laits (nonmicrobiens) dans la série animale. Rev. d'hyg., **36**, 222.

Lane-Claypon, J. E. (1913): Report to the Local Government Board upon the "biological properties of milk, both of the human species and of cows, considered in special relation to the feeding of infants." Repts. L. G. B. on Pub. Health and Medical Subjects, N. S. No. 76.

Lane-Claypon, J. E. (1916): *Milk and its Hygienic Relations.* Longmans Green & Co. London.

Lee, R. E., and Mellon, M. C. (1916): A study of certain ferments, with a view of determining a method for the differentiation of pasteurized milk from raw milk. J. Ind. Eng. Chem., **9**, 360.

Lind, C. (1920): The value of the methylene blue reduction test as compared with the plate method. The Milk Dealer, **9**, 24, 42.

Lind, C. (1920): Reduktaseprøven sammenlignet med andre Bakterietaelningsmethoder, isaer anvendt paa Maelken fra enkelte Køer. Maelkeritidende, **33**, 207.

Marshall, C. E., and Hood, E. G. (1920): Clarification of milk. J. Dairy Sci., **3**, 245.

Monvoisin, A. (1907): Sur quelques diastases du lait. Rev. gén. lait, Lierre, **6**, 265.

Morres, W. (1911): Beziehungen zwischen Reduktaseprobe, Säuregrad und Alkoholprobe. Milchwirtsch. Centr., **7**, 441.

Oppenheimer, S. (1908): Weiteres zur Schardingerschen Reduktionsprobe. Arb. Inst. exp. Therap. Frankf., **4**, 75.

Orla-Jensen (1906): De l'origine des catalases et réductases du lait de vache. Rev. gén. Lait, **6**, 33, 56, 85.

Orla-Jensen (1907): Ueber den Ursprung der Oxydasen und Reduktasen der Kuhmilch. Centr. Bakt., Parasitenk., II Abt., **18**, 211.

Philippe, E. (1912): Beiträge zur Frage der Verwendbarkeit der neueren Milchprüfungsmethoden. Centr. Bakt., Parasitenk., II, Ref., **33**, 365.

Race, J. (1918): *Examination of Milk for Public Health Purposes.* 1st Ed. Wiley & Sons, N. Y.

Rahn, O. (1920): Die Grenzen der Reduktaseprobe für die Milchbearbeitung, Milchwirtschaft. Zentr., **49**, 287, 299, 311.

Reed, H. S., and Reynolds, R. R. (1916): Some effects of temperature upon the growth and activity of bacteria in milk. Virginia Agr. Exp. Sta., Tech. Bull., **10**, 26.

Reinle, H. (1921): Ueber die Wirkung der Becquerel- und Röntgenstrahlen sowie des ultravioletten Lichtes auf die Peroxydase und Methylenblau-Formalin-Reduktase-Reaktion der Kuhmilch. Biochem. Z., **115**, 1.

Reis (1905): Die Katalase der Milch. Z. klin. Med., **56**, (cited).

Römer, P. H., and Sames, T. (1910): Beiträge zur Schardingerschen Reaktion der Kuhmilch. Z. Nahr. Genussm., **20**, 1.

Rullmann, W. (1910): Ueber den Enzym- und Streptokokkengehalt aseptisch entnommener Milch. Arch. Hyg., **73**, 81.

Sassenhagen, M. (1910): Ueber die biologischen Eigenschaften der Kolostral- und Mastitis-Milch. Arch. Kinderheilk., **53**, 281.

Schardinger, F. (1902): Ueber das Verhalten der Kuhmilch gegen Methylenblau und seine Verwendung zur Unterscheidung von ungekochter und gekochter Milch. Z. Nahr. Genussm., **5**, 1113.

Schern, K. (1909): Beobachtungen über die Schardinger-Reaktion der Milch. Biochem. Z., **18**, 261.

Schroeter, O. (1912): Vergleichende Prüfung bakteriologischer und biochemischer Methoden zur Beurteilung der Milch. Centr. Bakt., Parasitenk., II, **32**, 181.

Seligmann, E. (1905): Ueber den Einfluss einiger Aldehyde besonders des Formalins, auf die Oxydationsfermente der Milch und des Gummi arabicums. Z. Hyg., **50**, 97.

Seligmann, E. (1906): Ueber die Reductasen der Kuhmilch. Z. Hyg., **52**, 161.

Seligmann, E. (1907): Über die Reduktasen der Kuhmilch. Z. Hyg., **58**, 1.

Siegfeld, M. (1901): Ueber den Nachweis einer Erhitzung der Milch. Milch-Ztg., **30**, 723.

Siegfeld, M. (1903): Der Nachweis einer Erhitzung der Milch. Z. angew. Chem., **16**, 764.

Simmons, J. E. (1919): A comparison with the standard plate method of some rapid methods for bacteriologic analysis of milk. J. Inf. Dis., **24**, 322.

Skar, O. (1913): Verhalten der Leukozyten der Milch bei der Methylenblau-Reduktaseprobe. Molk. Ztg. (Berlin), **23**, 374.

Smidt, H. (1904): Ueber die Fähigkeit der Milch, Methylenblau zu reducieren. Hyg. Rundschau, **14**, 1137.

Smidt, H. (1906): Über die sog. Reduktase der Milch. Arch. Hyg., **58**, 313.

Smith, J. (1922): The reductase test of Barthel and Jensen. J. Hyg., **21**, 139.

Sommerfeld, P. (1908): Zur Frage der reduzierenden Eigenschaften der Milch. Hyg. Zentr., **4**, 1.

Tennent, R. B. (1920): Notes on the methylene blue reductase test. Queensland Agr. J. [Ser. 2] **13**, 51.

Trommsdorff, R. (1909): Zur Frage der reduzierenden Eigenschaften der Milch und der Schardingerschen Reaktion. Centr. Bakt., Parasitenk., I, **49**, 291.

Utz (1903): Nachweis von gekochter und ungekochter Milch. Milch-Ztg., **32**, 129, 417, 594.

Utz (1904): Ueber Reaktionen des oxydierenden Enzyms der Kuh- und Frauenmilch. Oesterr. Chem. Ztg., **7**, 389.

Vaudin, L. (1897): Le carmin d'indigo pour apprécier la fraîcheur du lait. Repert. pharm. [3], **9**, 538.

Virtanen, A. I. (1922): Kolloidernas inverkan på reduktasprovet. Meddelande Nr. 229 från Centralanstalten för försoksväsendet på jordbruksområdet. Bakteriologiska Avdelningen No. 27.

Wedemann, W. (1914): Über die Schardingersche Formaldehyd-Methylenblau Reaktion und einige andere Fermentreaktionen bei Ziegenmilch. Biochem. Z., **60**, 330.

Woker, G., and Maggi, H. (1917): Zur Theorie des Schardingerschen Enzyms und über wechselseitige Beeinflussung ver. Reduktoren bei einigen lichtchemischen Versuchen. Ber., **50**, 1321.

Yamazaki, E., and Kaneko, H. (1921): Chemical kinetics of the system: reductase-methylene blue. J. Chem. Soc., Japan, **42**, No. 11, p. 90.

SUPPLEMENTARY REFERENCES ON MISCELLANEOUS PHARMACOLOGICAL AND OTHER USES OF METHYLENE BLUE

Bach, A. (1911): Récherches sur les ferments réducteurs. Arch. sci. phys. nat., **32**, 27.

Bieber, W., and Dieterich, H. (1920): Lokale Behandlung Antitoxinbildung und Immunisierung mit Toxin-Antitoxin (T-A) bei Wunddiphtherie. Münch. med. Wochschr., **67**, 1381.

Bizzozero, E. (1922): Influence of bacteria on the coloration of human erythrocytes with methylene blue. Arch. sci. med., **45**, 241.

Blum (1919): Traitement de la grippe par le bleu de méthylène. Bull. Acad. med., Paris, **81**, 256.

Botelho, C. (1918): Sur une nouvelle méthode pour la mise en évidence de bleu de méthylène dans les urines. Compt. rend. soc. biol., **81**, 291.

Brauer, L. (1903): Untersuchungen über die Leber. Z. physiol. Chem., **40**, 182.

Castriota, G. (1911): Inhibition of some glucosurias by methylene blue. Chem. Abst., **5**, 3083.

Churchman, J. W. (1922): The anilin dyes in therapeutics. J. Am. Med. Assoc., **79**, 1657.

De Witt, L. M. (1913): Report on some experimental work on the use of methylene blue and allied dyes in the treatment of tuberculosis. J. Inf. Dis., **13**, 378.

Dreser, H. (1885): Histochemisches zur Nierenphysiologie. Z. Biol. (N. S.), **3**, 41.

Drew, A. H. (1920): The comparative oxygen avidity of normal and malignant cells measured by their reducing powers on methylene blue. Brit. J. Exp. Path., **1**, 115.

Editorial (1920): Methylene blue reduction test in study of problems of infection and immunity. J. Am. Med. Assoc., **75**, 940.

Ehrlich, P. (1885): *Das Sauerstoff-Bedürfniss des Organismus* (Eine Farbenanalytische Studie). Berlin. (Hirschwald.)

Ehrlich, P. (1885): Antikritische Bemerkungen über Drüsenfunctionen. Centr. med. Wissensch., **23**, 161.

Ehrlich, P. (1886): Methylenblau-reaktion der lebenden Nervensubstanz. Deut. med. Wochschr., **12**, 49.

Ehrlich, P., and Leppmann, A. (1890): Ueber schmerzstillende Wirkung des Methylenblaus. Deut. med. Wochschr., **16**, 493.

Einhorn, M. (1891): Ueber die Anwendung des Methylenblaus bei Cystitis, Pyelitis, und Carcinom. Deut. med. Wochschr., **17**, 620.

Elsner, K. (1900): Ueber die Ausscheidung des Methylenblaus. Deut. Arch. klin. Med., **69**, 47.

Fantus, B. (1915): A report of experiments to determine the antidotal value of fuller's earth in methylene blue poisoning. J. Am. Pharm. Assoc., **4**, 836.

Fleig, C. (1909): Note rectificative à propos de la récherche dans l'urine des chromogènes du bleu de méthylène par les oxydants (persels, H_2O_2) en milieu acide. Compt. rend. soc. biol., **66**, 607.

Fürst, T. (1920): Über Steigerung der Antikörperproduktion durch unspezifische Reizwirkung intravenöser Methylenblauinjektionen. Arch. Hyg., **89**, 161.

Garfounkel, I.-G., and Gautrelet, J. (1913): Contribution à l'étude de l'action des matières colorantes sur le coeur et la pression. Compt. rend., **157**, 1464.

Gautier, C. (1918): Procédé simple de récherche du bleu de méthylène dans les urines. Compt. rend. soc. biol., **81**, 496.

Gautrelet, J. (1922): Du mode d'action physiologique de certaines substances considérées comme agents anti-choc. Compt. rend. soc. biol., **87**, 150.

Genoese, G. (1919): Intorna alla prova di Boveri e ad una reazione al bleu di metilene in liquidi cefalo-rachidiani patologici. Policlin., Roma, sez. prat., **26**, 97.

Herter, C. A. (1904): Über die Anwendung reduzierbarer Farbstoffe beim Studium Verteilung von Giften und ihre Wirkungen auf die Zelltätigkeit. Z. physiol. Chem., **42**, 493.

Herter, C. A., and Richards, A. N. (1904): The influence of chloroform on intravital staining with methylene blue. Am. J. Physiol., **12**, 207.

Heymans, C. (1922): The temperature-raising property of methylene azure. Compt. rend. soc. biol., **86**, 964 (cited).

Heymans, C. (1922): Le bleu de méthylène antagoniste des excitants parasympathiques. Compt. rend. soc. biol., **87**, 396. Arch. Internat. Pharm. Therap., **27**, 257.

Heymans, C., and Maigre, E. (1921): Action du bleu de méthylène sur l'appareil cardio-inhibiteur de la grenouille. Compt. rend. soc. biol., **85**, 45.

Heymans, J. F., and Heymans, C. (1922): Hyperthermia and increase of the respiratory volume and of the elimination of carbon dioxide produced by methylene blue. Arch. intern. pharmacodyn., **26**, 443 (cited).

Heymans, J. F., and Heymans, C. (1922): Excessive loss of heat during the hyperthermia induced by methylene blue. Arch. inter. pharmacodyn., **27**, 319 (cited).

Hinman, F. (1915): Urinary antisepsis. J. Am. Med. Assoc., **65**, 1769.

Huyghebaert, E. (1923): Action hémolytique du bleu méthylène chez le chien. Compt. rend. Soc Biol., **89**, 1177 (cited).

Justin-Besancon, L. and Monceaux, R. (1923): Reducing and oxidizing power of saliva. Compt. rend. soc. biol., **88**, 1024 (cited).

Jacobi, A. (1906): Methylthionin hydrochlorid in inoperable cancer. J. Am. Med. Assoc., **47**, 1545.

Kahn, M., and Wechsler, L. (1916): A study of the diagnostic value of the Russo reaction. Med. Record, **89**, 106.

Koskowski, W., and Maigre, E. (1921): Action paralysante du bleu de méthylène sur les terminaisons nerveuses parasympathiques. Compt. rend., **173**, 397.

Koskowski, W., and Maigre, E. (1921): Origine périphérique de l'hyperthermie provoquée par le bleu de méthylène. Compt. rend., **173**, 448.

Lewis, J. H., and de Witt, L. M. (1919): The continuous injection method in the treatment of experimental tuberculosis. Am. Rev. Tuberculosis, **3**, 548.

Linden, G. v. (1912): Beiträge zur Chemotherapie der Tuberkulose. Beitr. klin. Tuberculose, **23**, 201.

Linden, G. v. (1914): Versuche zur Feststellung der Affinität und Giftigkeit von Kupfer- und Methylenblau-salzen für den Tuberkelbazillus. Munch. med. Wochschr., **61**, 586.

Linden, G. v. (1917): Experimentalforschungen zur Chemotherapie der Tuberkulose mit Kupfer- und Methylenblausalzen. Beitr. klin. Tuberculose, **34**, 1; **37**, 1.

Lipschitz, W. (1922): Forschungsprobleme und -Ergebnisse auf dem Gebiet der Zellatmung und der biologischen Oxydationen. Klin. Wochschr., **1**, 33.

Lundberg, H. (1922): Le pouvoir pharmacodynamique du bleu de méthylène. Compt. rend. soc. biol., **87**, 483.

Lundberg, H. (1924): Untersuchung über die pharmakologischen Eigenschaften des Methylenblaus. Skand. Arch. Physiol., **45**, 237.

McDroy, J. H. (1919): A report on the value of methylene blue in purulent discharge from the eye socket. Brit. Med. J., **1**, 405.

Magnus-Alsleben, E., and Hoffmann, P. (1922): Über den Einfluss der nervösen Versorgung auf die vitale Färbbarkeit der Muskeln. Biochem. Z., **127**, 103.

Maigre, E. (1919): De l'action du bleu et de l'azur de méthylène sur les cellules nerveuses medullaires: action antagoniste vis-à-vis de la toxine tétanique et de la strychnine. Compt. rend. soc. biol., **82**, 845.

Marshall, D. G. (1920): The "toxicity" of methylene blue. Lancet, **198**, 1334.

Meissen, E. (1912): Meine Erfahrungen bei Lungentuberkulose mit Jod-Methylenblau und Kupferpräparaten. Beitr. klin. Tuberculose, **23**, 215.

Mitchell, E. W. (1904–5): The therapeutic value of methylene blue. Am. Therapist, **13**, 115.

Müller, F. (1899): Ueber die Ausscheidung des Methylenblaus durch die Nieren. Deut. Arch. klin. Med., **63**, 130.

Neisser, M., and Wechsberg, F. (1900): Ueber eine neue einfache Methode zur Beobachtung von Schädigungen lebender Zellen und Organismen. Münch. med. Wochschr., **47**, 1261.

Pfeiffer, R. (1921): The excretion of methylene blue in tuberculosis. Beitr. klin. Tuberculose, **47**, 46 (cited).

Pohle, E. (1921): Der Einfluss der Wasserstoffionenkonzentration auf die Aufnahme und Ausscheidung saurer und basischer organischer Farbstoffe im Warmblüterorganismus. Deut. med. Wochschr., **47**, 1464.

Rappoport, A. P. (1904): Methylene blue and its use in psychiatric practice. Obozr. Psikhiat., Nevrol., **9**, 900 (cited).

Raubenheimer, O. (1910): The detection of methylene blue in urine. Am. Druggist, **57**, 369.

Reitler, R. (1920): Ueber kombinierte Chinin- Methylenblautherapie der Malaria. Wiener klin. Wochschr., **33**, 9.

Ricketts, H. T. (1904): The reduction of methylene blue by nervous tissue. J. Infect. Dis., **1**, 590.

Rieder, W. (1919): Zur Pharmakologie der Oxydationsmittel. Z. exp. Med., **10**, 169.

Rosenblatt, J., and Stillman, B. P. (1921): Tuberculous Pyopneumothorax treated with methylene blue. Am. Rev. Tuberculosis, **5**, 819.

Russo, M. (1905): La bleumetilenereazione, suo valore clinico. Riforma med., **21**, 507.

Saxl, P., and Scherf, D. (1922): The elimination of dyes through the gastric juice and the bile. Wiener klin. Wochschr., **35**, 128 (cited).

Schulmann, E., and Justin-Besancon, L. (1922): The determination of methylene blue circulating in the blood. Compt. rend. soc. biol., **87**, 519 (cited).

Sellei, J. (1913): Die Wirkung der Farbstoffe in Verbindung mit Giften und Arzneimitteln. Biochem. Z., **49**, 466.

Shafer, G. D. (1908): Kidney secretion of indigo carmine, methylene blue, and sodium carminate. Am. J. Physiol., **22**, 335.

Strauss, A. (1912): Meine Erfahrungen mit Jodmethylenblau und Kupferpräparaten bei äusserer Tuberkulose, speziell bei Lupus. Beitr. klin. Tuberkulose, **23**, 223.

Takemura, M. (1910): Sind Methylenblau und Hämatoxylin Antigene? Z. Immunitat., Orig., **5**, 697.

Thomas, J., and Binetti (1922): Étude de la variation du pouvoir réducteur des sérums normaux et cancéreux, en présence d'extraits de tumeurs. Compt. rend. soc. biol., **86**, 29.

Tribondeau, L. (1917): Procédé de recherche du bleu de méthylène dans les urines. Compt. rend. soc. biol., **80**, 882.

Vallot, J. (1921): Mesure de l'influence de la chaleur et de la lumière sur l'activité de réduction des tissus animaux et applications à l'héliothérapie. Compt. rend., **173**, 1196.

Weber, F. P. (1901): The occurrence of green or blue urine and its most frequent cause. Lancet, **2**, 774.

Weiss, K. (1922): Bioscopic methods for testing *in vitro* the viability of a tissue, especially mouse tumors. Centr. Bakt., Parasitenk, Abt. I, **88**, 115 (cited).

Wishart, G. M. (1923): Reduction of methylene blue by tissue extracts. Biochem. J., **17**, 103.

Woker, G., and Belencki, E. (1913): Über die Beeinflussung der Reaktionsgeschwindigkeit bei den Reduktionsproben des Traubenzuckers durch Gegenwart von Metallen im Harn. Arch. ges. Physiol., **155**, 45.

WYOMING LAW PERTAINING TO PREVENTION OF GOITER

The following is a Wyoming law (ch. 123) approved February 25, 1925, giving the board of health of that State authority to adopt regulations looking to the prevention and control of goiter:

SECTION 1. The State Board of Health of the State of Wyoming shall have authority to pass such rules and regulations as shall be necessary to regulate the sale of domestic salt or prescribe such manner of treatment as has been found practical to prevent goiter from becoming more prevalent among the citizens of the State of Wyoming.

SEC. 2. This act shall take effect and be in force from and after its passage.

DIGEST OF CURRENT PUBLIC HEALTH COURT DECISIONS

Local sanitary code held nullity because board adopting same lacked legal existence (New Jersey Supreme Court).—In 1912 the town of Nutley changed its form of government to the commission form provided for by chapter 221, laws of 1911. At that time Nutley had a board of health as provided for by the board of health act of 1887. In 1913 a law supplementing the 1911 act was passed, such supplemental act being chapter 282 of the 1913 laws. This 1913 law provided that whenever the provisions of the 1911 act had been adopted by any municipality "all boards and bodies, whether State or local municipal agencies then existing in such municipality (except the board of education and the district court or courts), shall be ipso facto abolished." The board of health created under the 1887 act continued in office and continued to function, adopting in 1914 a sanitary code. In 1917 this board of health was abolished by the board of commissioners, who, in 1924, repealed the 1914 sanitary code and adopted a new one. In 1922 the plaintiff was convicted of three separate offenses under the 1914 sanitary code. The supreme court set aside all three convictions, holding that the 1914 sanitary code never had any legal existence as a piece of municipal legislation. The court held that the board attempting to adopt it had no legal existence because by the 1913 act their offices had been abolished and thereafter they were neither de jure nor de facto officers and their acts were nullities. (Corb *v.* Board of Health of Town of Nutley et al., 127 Atl. 812.)

Liability for injury caused by consumption of food containing mouse (Massachusetts Supreme Judicial Court).—The plaintiffs, husband and wife, boarded with a certain person, who, through her agent, purchased of the defendant a raisin pie. Portions of the pie were served to the plaintiffs who partook of the same, the husband finding the body of a mouse in his portion. Both plaintiffs were made ill, and each brought action for alleged negligence. The defendant company bought the filling for its pies but made the pie crusts and baked

the pies. The lower court directed the jury to return a verdict for the defendant. The supreme court held that, upon the evidence, the jury could have found for the plaintiffs and that the case should have been submitted to the jury. The court stated that the defendant, being a manufacturer of a part of the pie, was, for that reason, responsible for the finished product. (Sullivan *v.* Manhattan Market Co., 146 N. E. 673.)

Seller of unwholesome meat held liable (Ohio Supreme Court).—The plaintiff in the lower court was made ill by eating some veal purchased at retail from the defendant. The jury found that the veal was unwholesome when sold by the defendant, such a sale being in violation of a State law. The supreme court held that such unlawful sale was negligence per se and basis for recovery of damages, provided there was no contributory negligence on the plaintiff's part. It was further held that neither lack of intent on the seller's part to violate the law or the seller's ignorance of the unwholesome condition of the meat constituted a defense. (Portage Markets Co. *v.* George, 146 N. E. 283.)

Law prohibiting use of saccharin in soft drinks held valid (Ohio Supreme Court).—Section 1089–9, Ohio General Code, prohibiting the use of saccharin in soft drinks, was held constitutionally valid and within the inherent police powers of the State. Regarding the contention that the act was discriminatory because the prohibition was confined to soft drinks, the court stated that "The constitutional validity of the act can not be attacked because its scope was not extended to cover the entire field of possible abuses." (Longbrake *v.* State, 146 N. E. 417.)

PREVALENCE OF DISEASE

No health department, State or local, can effectively prevent or control disease without knowledge of when, where, and under what conditions cases are occurring

UNITED STATES

CURRENT WEEKLY STATE REPORTS

These reports are preliminary, and the figures are subject to change when later returns are received by the State health officers

Reports for Week Ended May 30, 1925

ALABAMA

	Cases
Cerebrospinal meningitis	1
Chicken pox	32
Diphtheria	6
Dysentery	88
Influenza	71
Malaria	63
Measles	10
Mumps	27
Pellagra	40
Pneumonia	51
Poliomyelitis	1
Scarlet fever	25
Smallpox	134
Tetanus	2
Tuberculosis	53
Typhoid fever	36
Whooping cough	35

ARIZONA

Cerebrospinal meningitis	1
Chicken pox	7
Diphtheria	1
Measles	35
Mumps	2
Pneumonia	1
Scarlet fever	5
Tuberculosis	9
Typhoid fever	3
Whooping cough	3

ARKANSAS

Chicken pox	17
Diphtheria	2
Hookworm disease	4
Influenza	37
Malaria	116
Measles	16
Mumps	28
Ophthalmia neonatorum	2
Pellagra	25
Scarlet fever	2
Smallpox	1
Trachoma	2
Tuberculosis	9
Typhoid fever	19
Whooping cough	48

CALIFORNIA

	Cases
Cerebrospinal meningitis	7
Diphtheria	89
Influenza	20
Leprosy	2
Lethargic encephalitis	1
Measles	62
Poliomyelitis:	
Fresno	1
Long Beach	1
Los Angeles	2
Los Angeles County	1
Orange County	1
San Francisco	1
Santa Ana	1
Williams	1
Scarlet fever	96
Smallpox:	
Los Angeles	40
Los Angeles County	10
Monterey Park	5
Oakland	17
Riverside County	6
Sacramento	5
Scattering	23
Typhoid fever	11

COLORADO

(Exclusive of Denver)

Chicken pox	2
Diphtheria	6
Measles	3
Mumps	17
Pneumonia	1
Rocky Mountain spotted fever	1
Scarlet fever	3
Tuberculosis	19
Typhoid fever	1
Whooping cough	2

CONNECTICUT

Cerebrospinal meningitis	1
Chicken pox	34
Diphtheria	17
German measles	86
Lethargic encephalitis	2
Influenza	2

CONNECTICUT—continued	Cases
Measles	171
Mumps	13
Paratyphoid fever	2
Pneumonia:	
Broncho	15
Lobar	29
Scarlet fever	67
Tuberculosis (all forms)	28
Typhoid fever	7
Whooping cough	83
DELAWARE	
Diphtheria	1
Scarlet fever	6
Tuberculosis	5
Whooping cough	2
FLORIDA	
Cerebrospinal meningitis	1
Chicken pox	9
Diphtheria	9
Malaria	6
Measles	1
Mumps	25
Pneumonia	1
Smallpox	10
Tuberculosis	14
Typhoid fever	15
Whooping cough	3
GEORGIA	
Anthrax	1
Cerebrospinal meningitis	2
Chicken pox	42
Diphtheria	3
Dysentery	92
German measles	1
Hookworm disease	7
Influenza	49
Malaria	87
Measles	28
Mumps	78
Pellagra	12
Pneumonia	29
Rabies	2
Scarlet fever	5
Septic sore throat	11
Smallpox	30
Tetanus	1
Tuberculosis	64
Typhoid fever	54
Whooping cough	31
ILLINOIS	
Diphtheria:	
Cook County	49
Scattering	24
Influenza	50
Lethargic encephalitis—Cook County	1
Measles	1,210
Pneumonia	159
Poliomyelitis—Rock Island County	1
Scarlet fever:	
Cook County	204
Clinton County	10
Stephenson County	11
Scattering	85

ILLINOIS—continued	Cases
Smallpox	35
Tuberculosis	230
Typhoid fever	20
Whooping cough	245
INDIANA	
Chicken pox	64
Diphtheria	12
Influenza	19
Measles	124
Mumps	2
Pneumonia	11
Scarlet fever	104
Smallpox	102
Tuberculosis	90
Typhoid fever	4
Whooping cough	27
IOWA	
Diphtheria	13
Scarlet fever	26
Smallpox	25
KANSAS	
Chicken pox	71
Diphtheria	10
German measles	3
Influenza	5
Lethargic encephalitis	1
Measles	12
Mumps	115
Pneumonia	26
Scarlet fever	46
Smallpox	1
Tuberculosis	50
Typhoid fever	3
Whooping cough	51
LOUISIANA	
Diphtheria	13
Dysentery	2
Influenza	40
Malaria	14
Pneumonia	44
Poliomyelitis	1
Scarlet fever	6
Smallpox	16
Tuberculosis	21
Typhoid fever	54
Whooping cough	18
MAINE	
Cerebrospinal meningitis	1
Chicken pox	19
Diphtheria	3
Influenza	21
Mumps	34
Pneumonia	10
Scarlet fever	20
Tetanus	2
Tuberculosis	6
Typhoid fever	3
Vincent's angina	1
Whooping cough	2

MARYLAND [1]

	Cases
Cerebrospinal meningitis	1
Chicken pox	108
Diarrhea enteritis	1
Diphtheria	26
Dysentery	1
German measles	4
Influenza	15
Lethargic encephalitis	2
Malaria	1
Measles	33
Mumps	81
Pneumonia (broncho)	31
Pneumonia (lobar)	37
Scarlet fever	46
Tuberculosis	57
Typhoid fever	5
Whooping cough	116

MASSACHUSETTS

Cerebrospinal meningitis	3
Chicken pox	113
Conjunctivitis (suppurative)	33
Diphtheria	75
German measles	267
Hookworm disease	1
Influenza	10
Lethargic encephalitis	5
Measles	707
Mumps	44
Ophthalmia neonatorum	24
Pneumonia (lobar)	98
Scarlet fever	216
Trachoma	2
Tuberculosis (pulmonary)	166
Tuberculosis (other forms)	93
Typhoid fever	9
Whooping cough	118

MICHIGAN

Diphtheria	55
Measles	553
Pneumonia	98
Scarlet fever	291
Smallpox	15
Tuberculosis	303
Typhoid fever	8
Whooping cough	121

MINNESOTA

Cerebrospinal meningitis	1
Chicken pox	105
Diphtheria	41
Influenza	6
Measles	48
Pneumonia	3
Scarlet fever	239
Smallpox	21
Tuberculosis	113
Typhoid fever	8
Whooping cough	38

MISSISSIPPI

Diphtheria	6
Scarlet fever	1
Smallpox	17
Typhoid fever	21

[1] Week ended Friday.

MISSOURI

(Exclusive of Kansas City)

	Cases
Chicken pox	90
Diphtheria	59
Influenza	4
Malaria	7
Measles	22
Pneumonia	17
Scarlet fever	157
Smallpox	26
Trachoma	2
Tuberculosis	96
Typhoid fever	2
Whooping cough	45

MONTANA

Chicken pox	4
Diphtheria	1
German measles	2
Measles	7
Mumps	1
Rocky Mountain spotted fever—Forsyth R. D	2
Scarlet fever	10
Tuberculosis	2
Tularæmia—Hamilton	3

NEBRASKA

Chicken pox	20
Diphtheria	7
Measles	1
Mumps	31
Scarlet fever	10
Smallpox	20
Typhoid fever	2
Whooping cough	7

NEW JERSEY

Cerebrospinal meningitis	2
Chicken pox	138
Diphtheria	60
Influenza	3
Measles	415
Pneumonia	108
Scarlet fever	186
Smallpox	4
Typhoid fever	12
Whooping cough	165

NEW MEXICO

Chicken pox	4
Diphtheria	2
Dysentery	3
German measles	2
Measles	7
Mumps	8
Pneumonia	7
Scarlet fever	4
Tetanus	1
Tuberculosis	60
Tularæmia	1
Typhoid fever	1
Whooping cough	6

NEW YORK

(Exclusive of New York City)

	Cases
Cerebrospinal meningitis	1
Diphtheria	83
Influenza	22
Lethargic encephalitis	3
Measles	669
Pneumonia	213
Scarlet fever	186
Smallpox	9
Typhoid fever	8
Whooping cough	135

NORTH CAROLINA

Cerebrospinal meningitis	1
Chicken pox	58
Diphtheria	12
German measles	3
Measles	28
Ophthalmia neonatorum	1
Scarlet fever	10
Septic sore throat	2
Smallpox	56
Typhoid fever	16
Whooping cough	144

OKLAHOMA

(Exclusive of Oklahoma City and Tulsa)

Chicken pox	5
Diphtheria	1
Influenza	34
Measles	4
Mumps	9
Pneumonia	18
Scarlet fever	25
Smallpox	3
Typhoid fever	17
Whooping cough	25

OREGON

Cerebrospinal meningitis	1
Chicken pox	20
Diphtheria:	
Portland	23
Scattering	4
Influenza	11
Measles	1
Mumps	6
Pneumonia	[1]7
Scarlet fever	7
Smallpox	5
Tuberculosis	24
Typhoid fever	2
Whooping cough	16

SOUTH DAKOTA

Measles	2
Scarlet fever	7
Smallpox	3
Tuberculosis	2
Typhoid fever	1

TEXAS

Chicken pox	9
Diphtheria	3

TEXAS—continued

	Cases
Dysentery (epidemic)	1
Influenza	4
Measles	24
Mumps	7
Paratyphoid fever	1
Pellagra	7
Pneumonia	3
Scarlet fever	2
Smallpox	11
Tuberculosis	1
Typhoid fever	2
Whooping cough	12

VERMONT

Chicken pox	22
Measles	19
Mumps	52
Scarlet fever	7
Whooping cough	4

VIRGINIA

Smallpox:	
Franklin County	1
Henry County	2

WASHINGTON

Cerebrospinal meningitis—Tacoma	1
Chicken pox	56
Diphtheria	9
German measles	18
Measles	5
Mumps	45
Scarlet fever	21
Smallpox	29
Tuberculosis	63
Typhoid fever	2
Whooping cough	99

WEST VIRGINIA

Diphtheria	6
Scarlet fever	15
Smallpox	12
Typhoid fever	3

WISCONSIN

Milwaukee:	
Cerebrospinal meningitis	2
Chicken pox	22
Diphtheria	12
German measles	60
Measles	211
Mumps	64
Pneumonia	23
Scarlet fever	21
Smallpox	26
Whooping cough	36
Scattering:	
Chicken pox	102
Diphtheria	13
German measles	256
Influenza	81
Lethargic encephalitis	1
Measles	296
Mumps	123
Pneumonia	19

[1] Deaths.

WISCONSIN—continued

	Cases
Scattering—Continued	
Poliomyelitis	2
Scarlet fever	133
Smallpox	23
Tuberculosis	20
Typhoid fever	1
Whooping cough	94

WYOMING

	Cases
Chicken pox	14
Diphtheria	4
Influenza	1
Measles	4
Mumps	1
Scarlet fever	3
Whooping cough	13

Reports for Week Ended May 23, 1925

ALABAMA

	Cases
Cerebrospinal meningitis	1
Chicken pox	36
Diphtheria	12
Dysentery	62
Influenza	60
Malaria	59
Measles	9
Mumps	37
Pellagra	39
Pneumonia	70
Poliomyelitis	3
Scarlet fever	34
Smallpox	92
Tetanus	1
Tuberculosis	51
Typhoid fever	45
Whooping cough	72

CALIFORNIA

	Cases
Cerebrospinal meningitis:	
San Francisco	1
Diphtheria	67
Influenza	23
Leprosy:	
Los Angeles County	1
Lethargic encephalitis:	
San Francisco	1
Measles	41
Poliomyelitis:	
Alhambra	1
Los Angeles County	2
Monterey Park	1
San Francisco	2
San Gabriel	1
Scarlet fever	87
Smallpox:	
Berkeley	9
Los Angeles County	9
Oakland	17
San Diego	10
Scattering	36
Typhoid fever	8

DISTRICT OF COLUMBIA

	Cases
Chicken pox	11
Diphtheria	11
Lethargic encephalitis	1
Measles	35
Pneumonia	11
Scarlet fever	21
Smallpox	1
Tuberculosis	24
Whooping cough	19

GEORGIA

	Cases
Chicken pox	36
Diphtheria	13
Dysentery	126
Hookworm disease	3
Influenza	75
Malaria	51
Measles	15
Mumps	58
Pellagra	17
Pneumonia	45
Scarlet fever	5
Septic sore throat	15
Smallpox	31
Trichinosis	1
Tuberculosis	93
Typhoid fever	34
Whooping cough	85

INDIANA

	Cases
Cerebrospinal meningitis	2
Chicken pox	95
Diphtheria	21
Influenza	30
Measles	86
Mumps	13
Pneumonia	8
Scarlet fever:	
Clark County	9
Elkhart County	10
Marion County	8
St. Joseph County	16
Vigo County	11
Scattering	56
Smallpox	57
Tuberculosis	48
Typhoid fever	8
Whooping cough	36

MINNESOTA

	Cases
Chicken pox	117
Diphtheria	83
Influenza	14
Lethargic encephalitis	1
Measles	61
Pneumonia	3
Poliomyelitis	1
Scarlet fever	215
Smallpox	23
Tuberculosis	53
Typhoid fever	2
Whooping cough	36

MISSISSIPPI	Cases
Diphtheria	4
Scarlet fever	2
Smallpox	17
Typhoid fever	22
MISSOURI (Exclusive of Kansas City)	
Cerebrospinl meningitis	1
Chicken pox	71
Diphtheria	64
Influenza	2
Malaria	2
Measles	37
Mumps	37
Pneumonia	10
Scarlet fever	173
Smallpox	10
Trachoma	1
Tuberculosis	76
Typhoid fever	3
Whooping cough	44
MONTANA	
Cerebrospinal meningitis	1
Chicken pox	12
Diphtheria	6
German measles	28
Leprosy	1
Measles	9
Mumps	28
Rocky Mountain spotted fever:	
Lismas	1
Milltown	1
Saco	1
Scarlet fever	57
Smallpox	3
Tuberculosis	5
Typhoid fever	7
Whooping cough	8
NEBRASKA	
Chicken pox	15
Diphtheria	3
Measles	1
Mumps	3
Scarlet fever	7

NEBRASKA—continued	Cases
Smallpox	25
Tuberculosis	2
Whooping cough	13
NORTH DAKOTA	
Chicken pox	14
Diphtheria	2
German measles	2
Measles	2
Mumps	25
Pneumonia	8
Scarlet fever	32
Smallpox	4
Tuberculosis	2
Typhoid fever	1
Whooping cough	10
OKLAHOMA (Exclusive of Oklahoma City and Tulsa)	
Cerebrospinal meningitis:	
Lincoln County	1
Chicken pox	14
Diphtheria	7
Influenza	63
Measles	6
Mumps	6
Pneumonia	22
Scarlet fever:	
Washington County	10
Scattering	19
Smallpox	12
Typhoid fever	14
Whooping cough	29
WYOMING	
Chicken pox	8
Diphtheria	10
Influenza	1
Measles	2
Mumps	10
Pneumonia	3
Rocky Mountain spotted fever	10
Scarlet fever	4
Tuberculosis	2
Whooping cough	24

SUMMARY OF MONTHLY REPORTS FROM STATES

The following summary of monthly State reports is published weekly and covers only those States from which reports are received during the current week.

State	Cerebrospinal meningitis	Diphtheria	Influenza	Malaria	Measles	Pellagra	Poliomyelitis	Scarlet fever	Smallpox	Typhoid fever
March, 1925										
Tennessee	43	47	1,402	64	86	20	------	116	251	26
April, 1925										
Kansas	2	68	83	0	61	0	0	397	35	6
Mississippi	1	51	5,518	4,019	605	926	3	14	145	134
Missouri	1	264	169	5	79	0	1	1,061	61	24
Oregon	24	131	570	------	16	------	------	125	31	14
South Dakota	------	13	7	------	4	------	1	199	57	------
Virginia	3	83	3,174	86	970	22	1	106	19	70
Washington	9	105	0	0	22	0	0	119	196	13
Wyoming	2	7	1	------	53	------	------	34	0	2

PLAGUE-ERADICATIVE MEASURES IN THE UNITED STATES

The following items were taken from the reports of plague-eradicative measures from the cities named:

Los Angeles, Calif.

Week ended May 16, 1925:	
Number of rats examined	2,714
Number of rats found to be plague infected	4
Number of squirrels examined	976
Number of squirrels found to be plague infected	0
Totals, Nov. 5, 1924, to May 16, 1925:	
Number of rats examined	101,884
Number of rats found to be plague infected	186
Number of squirrels examined	13,677
Number of squirrels found to be plague infected	9

Date of discovery of last plague-infected rodent, May 26, 1925.
Date of last human case, Jan. 15, 1925.

Oakland, Calif.

(Including other East Bay communities)

Week ended May 16, 1925:	
Number of rats trapped	1,847
Number of rats found to be plague infected	0
Totals, Jan. 1 to May 16, 1925:	
Number of rats trapped	45,827
Number of rats found to be plague infected	21

Date of discovery of last plague-infected rat, Mar. 4, 1925.
Date of last human case, Sept. 10, 1919.

New Orleans, La.

Week ended May 16, 1925:	
Number of vessels inspected	305
Number of inspections made	745
Number of vessels fumigated with cyanide gas	24
Number of rodents examined for plague	6,679
Number of rodents found to be plague infected	0
Totals, Dec. 5, 1924, to May 16, 1925:	
Number of rodents examined for plague	102,987
Number of rodents found to be plague infected	12

Date of discovery of last plague-infected rat, Jan. 17, 1925.
Date of last human case occurring in New Orleans, Aug. 20, 1920.

GENERAL CURRENT SUMMARY AND WEEKLY REPORTS FROM CITIES

Diphtheria.—For the week ended May 16, 1925, 35 States reported 1,254 cases of diphtheria. For the week ended May 17, 1924, the same States reported 1,540 cases of this disease. One hundred and three cities, situated in all parts of the country and having a population of nearly 28,800,000, reported 904 cases of diphtheria for the week ended May 16, 1925. Last year, for the corresponding week,

they reported 930 cases. The estimated expectancy for these cities was 929 cases. The estimated expectancy is based on the experience of the last nine years, excluding epidemics.

Measles.—Thirty-two States reported 5,161 cases of measles for the week ended May 16, 1925, and 10,997 cases of this disease for the week ended May 17, 1924. One hundred and three cities reported 3,444 cases of measles for the week this year and 4,015 cases last year.

Scarlet fever.—Scarlet fever was reported for the week as follows: 34 States—this year, 2,971 cases; last year, 3,170; 103 cities—this year, 1,941; last year, 1,495; estimated expectancy, 973 cases.

Smallpox.—For the week ended May 16, 1925, 35 States reported 790 cases of smallpox. Last year, for the corresponding week, they reported 1,233 cases. One hundred and three cities reported smallpox for the week as follows: 1925, 252 cases; 1924, 527 cases; estimated expectancy, 104 cases. These cities reported 22 deaths from smallpox for the week this year.

Typhoid fever.—Two hundred and fifty-six cases of typhoid fever were reported for the week ended May 16, 1925, by 34 States. For the corresponding week of 1924 the same States reported 244 cases. One hundred and three cities reported 74 cases of typhoid fever for the week this year and 71 cases for the corresponding week last year. The estimated expectancy for these cities was 69 cases.

Influenza and pneumonia.—Deaths from influenza and pneumonia (combined) were reported for the week by 103 cities as follows: 1925, 764 deaths; 1924, 792 deaths.

45487°—25†——6

City reports for week ended May 16, 1925

The "estimated expectancy" given for diphtheria, poliomyelitis, scarlet fever, smallpox, and typhoid fever is the result of an attempt to ascertain from previous occurrence how many cases of the disease under consideration may be expected to occur during a certain week in the absence of epidemics. It is based on reports to the Public Health Service during the past nine years. It is in most instances the median number of cases reported in the corresponding week of the preceding years. When the reports include several epidemics or when for other reasons the median is unsatisfactory the epidemic periods are excluded and the estimated expectancy is the mean number of cases reported for the week during nonepidemic years.

If reports have not been received for the full nine years, data are used for as many years as possible, but no year earlier than 1915 is included. In obtaining the estimated expectancy, the figures are smoothed when necessary to avoid abrupt deviations from the usual trend. For some of the diseases given in the table the available data were not sufficient to make it practicable to compute the estimated expectancy.

Division, State, and city	Population July 1, 1923, estimated	Chicken pox, cases reported	Diphtheria		Influenza		Measles, cases reported	Mumps, cases reported	Pneumonia, deaths reported
			Cases, estimated expectancy	Cases reported	Cases reported	Deaths reported			
NEW ENGLAND									
Maine:									
Portland	73,129	3	2	0	2	0	0	23	2
New Hampshire:									
Concord	22,408	0	0	0	0	0	1	0	4
Vermont:									
Barre	[1] 10,008	0	0	0	0	0	0	2	0
Massachusetts:									
Boston	770,400	----	55	34	14	1	286	----	27
Fall River	120,912	6	3	3	0	0	0	0	2
Springfield	144,227	3	3	2	0	0	16	9	1
Worcester	191,927	20	4	2	0	0	49	0	3
Rhode Island:									
Pawtucket	68,799	1	1	0	0	0	1	0	1
Providence	242,378	0	11	6	0	0	4	0	9
Connecticut:									
Bridgeport	[1] 143,555	1	4	7	1	2	22	0	2
Hartford	[1] 138,036	2	6	8	1	0	2	3	3
New Haven	172,967	4	4	0	0	0	97	0	0
MIDDLE ATLANTIC									
New York:									
Buffalo	536,718	4	11	5	4	0	244	3	18
New York	5,927,625	191	256	296	24	16	187	44	165
Rochester	317,867	4	6	14	0	0	83	14	5
Syracuse	184,511	16	8	4	0	0	8	22	1
New Jersey:									
Camden	124,157	0	4	3	0	0	61	3	4
Newark	438,699	28	16	13	9	1	77	6	15
Trenton	127,390	3	4	0	0	0	6	0	1
Pennsylvania:									
Philadelphia	1,922,788	47	64	125	--------	4	372	24	45
Pittsburgh	613,442	21	19	9	--------	2	333	3	29
Reading	110,917	8	3	2	0	0	147	3	0
Scranton	140,636	1	3	4	0	0	0	0	10
EAST NORTH CENTRAL									
Ohio:									
Cincinnati	406,312	7	7	5	0	0	2	0	3
Cleveland	888,519	63	20	29	--------	5	10	2	23
Columbus	261,082	1	3	3	--------	2	4	1	4
Toledo	268,338	18	4	3	--------	2	114	1	3
Indiana:									
Fort Wayne	93,573	6	2	0	0	0	9	0	0
Indianapolis	342,718	--------	6	2	0	0	13	--------	9
South Bend	76,709	2	1	3	0	0	2	0	2
Terre Haute	68,939	4	1	0	0	0	27	0	1
Illinois:									
Chicago	2,886,121	68	102	53	12	4	652	33	70
Cicero	55,968	5	2	3	0	0	18	0	1
Springfield	61,833	3	1	0	1	0	35	36	2
Michigan:									
Detroit	995,668	38	48	28	9	3	15	15	29
Flint	117,968	8	3	4	0	0	30	1	2
Grand Rapids	145,947	0	3	2	0	1	123	2	2

[1] Population Jan. 1, 1920.

City reports for week ended May 16, 1925—Continued

Division, State, and city	Population July 1, 1923, estimated	Chicken pox, cases reported	Diphtheria		Influenza		Measles, cases reported	Mumps, cases reported	Pneumonia, deaths reported
			Cases, estimated expectancy	Cases reported	Cases reported	Deaths reported			
EAST NORTH CENTRAL—continued									
Wisconsin:									
Madison	42,519	1	0	0	0	0	3	15	1
Milwaukee	484,595	32	12	16	0	0	209	59	17
Racine	64,393	7	1	1	0	0	0	16	3
Superior	[1] 39,671		1						
WEST NORTH CENTRAL									
Minnesota:									
Duluth	106,289	2	2	0	0	0	0	0	1
Minneapolis	409,125	41	15	26		2	16	6	5
St. Paul	241,891	37	13	17	0	0	8	19	8
Iowa:									
Davenport	61,262	0	1	2	0		0	0	
Sioux City	79,662	9	1	0	0		0	8	
Waterloo	39,667		0	0	0		1	1	
Missouri:									
Kansas City	351,819	11	7	5	2	2	3	22	7
St. Joseph	78,232	0	1	0	0	0	0	2	1
St. Louis	803,853	30	39	48	1	1	10	8	
North Dakota:									
Fargo	24,841	1	0	0	0	0	0	4	0
Grand Forks	14,547	2	0	0	0		0	0	
South Dakota:									
Aberdeen	15,829	0		1	0		0	0	
Sioux Falls	29,206		0						
Nebraska:									
Lincoln	58,761	15	1	3	0	0	0	1	1
Omaha	204,382	10	4	0	0	0	0	0	2
Kansas:									
Topeka	52,555	5	1	2	0	0	0	36	2
Wichita	79,261	18	1	3	0	0	0	0	0
SOUTH ATLANTIC									
Delaware:									
Wilmington	117,728	2	1	5	0	0	4	1	0
Maryland:									
Baltimore	773,580	59	18	22	11	4	18	64	17
Cumberland	32,361	0	1	1	0	0	0	0	0
Frederick	11,301	0	0	0	0	0	0	0	0
District of Columbia:									
Washington	[1] 437,571	8	10	9	0	0	30	0	17
Virginia:									
Lynchburg	30,277	0	1	0	0	0	0	19	0
Norfolk	159,089	5	1	1	0	0	4	28	1
Richmond	181,044	10	1	1	0	0	25	1	6
Roanoke	55,502	1	1	0	0	0	16	0	3
West Virginia:									
Charleston	45,597	0	1	0	0	0	54	1	1
Huntington	57,918	0	0	0	0		0	0	
Wheeling	[1] 56,208	1	1	1	0	0	6	0	3
North Carolina:									
Raleigh	29,171	2	1	0		1	0	0	0
Wilmington	35,719	6	0	0	0	0	0	2	2
Winston-Salem	56,230	12	1	0	0	0	4	3	1
South Carolina:									
Charleston	71,245	0	0	0	0	0	0	0	0
Columbia	39,688	1	0	0	0		0	1	
Greenville	25,789	0	0	0	0	0	0	0	3
Georgia:									
Atlanta	222,963	24	1	1	12	0	0	3	10
Brunswick	15,937	0	0	0	0	0	0	0	0
Savannah	89,448	0	0	0	0	0	1	7	2
Florida:									
St. Petersburg	24,403	0	0	0	0	0	0	0	0
Tampa	56,050	1	1	1	0	0	0	0	1

[1] Population Jan. 1, 1920

City reports for week ended May 16, 1925—Continued

Division, State, and city	Population July 1, 1923, estimated	Chicken pox, cases reported	Diphtheria		Influenza		Measles, cases reported	Mumps, cases reported	Pneumonia, deaths reported
			Cases, estimated expectancy	Cases reported	Cases reported	Deaths reported			
EAST SOUTH CENTRAL									
Kentucky:									
Covington	57,877	0	1	2	0	1	0	0	3
Louisville	257,671	1	4	2	2	2	6	1	9
Tennessee:									
Memphis	170,067	11	3	1	----	3	6	5	2
Nashville	121,128	1	1	0	----	3	15	1	4
Alabama:									
Birmingham	195,901	12	1	1	14	5	2	2	7
Mobile	63,858	1	0	0	0	0	0	1	3
Montgomery	45,383	3	1	0	0	0	0	9	1
WEST SOUTH CENTRAL									
Arkansas:									
Fort Smith	30,635	0	1	0	0	----	0	2	----
Little Rock	70,916	1	1	0	0	0	0	0	1
Louisiana:									
New Orleans	404,575	2	7	7	----	3	2	0	9
Shreveport	54,590	3	----	1	0	0	0	1	1
Oklahoma:									
Oklahoma	101,150	3	1	1	2	1	1	0	2
Texas:									
Dallas	177,274	----	3	3	0	0	1	----	4
Galveston	46,877	0	0	0	0	0	0	0	0
Houston	154,970	1	3	1	0	0	0	0	0
San Antonio	184,727	2	1	0	----	1	0	0	7
MOUNTAIN									
Montana:									
Billings	16,927	0	0	0	0	0	1	15	0
Great Falls	27,787	0	1	1	0	0	6	7	0
Helena	[1] 12,037	0	0	0	0	0	0	0	0
Missoula	[1] 12,668	0	0	0	0	0	1	0	2
Idaho:									
Boise	22,806	1	0	0	0	0	0	0	0
Colorado:									
Denver	272,631	7	10	15	----	5	3	41	10
Pueblo	43,519	1	1	0	----	1	1	0	2
New Mexico:									
Albuquerque	16,648	1	1	0	0	0	0	4	0
Arizona:									
Phoenix	33,899	0	0	0	----	1	1	0	0
Utah:									
Salt Lake City	126,241	23	3	0	0	0	0	35	2
Nevada:									
Reno	12,429	0	0	0	0	0	0	0	1
PACIFIC									
Washington:									
Seattle	[1] 315,685	39	5	5	0	----	1	65	----
Spokane	104,573	6	2	5	0	----	0	0	----
Tacoma	101,731	----	1	----	----	----	----	----	----
California:									
Los Angeles	666,853	36	33	23	4	2	46	22	16
Sacramento	69,950	0	2	0	0	0	0	1	1
San Francisco	539,038	20	24	12	4	1	11	36	2

[1] Population Jan. 1, 1920.

City reports for week ended May 16, 1925—Continued

Division, State, and city	Scarlet fever		Smallpox			Tuberculosis, deaths reported	Typhoid fever			Whooping cough, cases reported	Deaths, all causes
	Cases, estimated expectancy	Cases reported	Cases, estimated expectancy	Cases reported	Deaths reported		Cases, estimated expectancy	Cases reported	Deaths reported		
NEW ENGLAND											
Maine:											
Portland	1	2	0	0	0	0	1	0	0	2	21
New Hampshire:											
Concord	0	1	0	0	0	0	0	0	0	0	11
Vermont:											
Barre	1	0	0	0	0	0	0	0	0	0	4
Massachusetts:											
Boston	52	66	0	0	0	18	2	1	0	----	216
Fall River	3	6	0	0	0	2	0	2	0	8	30
Springfield	6	22	0	0	0	0	0	0	0	14	33
Worcester	7	12	0	0	0	4	0	0	0	9	65
Rhode Island:											
Pawtucket	1	3	0	0	0	0	0	0	0	1	25
Providence	11	12	0	0	0	4	0	0	0	0	79
Connecticut:											
Bridgeport	5	10	0	0	0	0	0	0	0	4	28
Hartford	4	3	0	0	0	2	1	0	0	14	37
New Haven	5	7	0	0	0	2	1	2	0	34	42
MIDDLE ATLANTIC											
New York:											
Buffalo	18	18	0	1	0	13	1	0	0	26	142
New York	208	293	0	3	0	[1] 123	11	14	8	126	1,520
Rochester	13	40	0	0	0	3	1	0	0	14	60
Syracuse	12	5	0	0	0	2	0	0	0	3	46
New Jersey:											
Camden	3	14	0	3	1	2	0	0	0	2	32
Newark	20	23	0	0	0	10	1	0	0	63	111
Trenton	2	2	0	0	0	1	0	0	0	9	36
Pennsylvania:											
Philadelphia	74	166	0	7	1	56	5	4	1	63	536
Pittsburgh	23	81	0	0	0	14	1	0	0	15	174
Reading	2	12	0	0	0	0	0	1	0	1	24
Scranton	2	3	0	0	0	0	0	0	0	4	----
EAST NORTH CENTRAL											
Ohio:											
Cincinnati	11	17	2	0	0	6	1	1	0	8	122
Cleveland	21	26	1	1	0	12	2	0	0	41	190
Columbus	5	13	2	4	0	3	1	0	0	6	88
Toledo	14	15	4	0	0	7	1	0	0	24	74
Indiana:											
Fort Wayne	2	6	3	0	0	1	0	0	0	0	22
Indianapolis	16	6	6	7	0	6	0	0	1	----	84
South Bend	3	5	0	0	0	0	0	0	0	0	16
Terre Haute	2	6	1	2	0	0	0	0	0	0	16
Illinois:											
Chicago	70	235	2	2	0	44	3	5	0	96	642
Cicero	1	13	0	0	0	1	0	0	0	6	5
Springfield	2	8	1	1	0	1	1	0	0	1	28
Michigan:											
Detroit	76	124	9	0	0	20	3	1	0	124	247
Flint	5	4	2	0	0	1	0	0	0	10	30
Grand Rapids	6	50	1	1	0	4	0	0	0	5	35
Wisconsin:											
Madison	2	1	1	0	0	1	0	0	0	15	10
Milwaukee	28	17	1	57	13	5	1	1	0	30	128
Racine	5	7	2	1	0	0	0	0	0	1	6
Superior	2	----	2	----	----	----	1	----	----	----	9

[1] Pulmonary tuberculosis only.

City reports for week ended May 16, 1925—Continued

Division, State, and city	Scarlet fever		Smallpox			Tuberculosis, deaths reported	Typhoid fever			Whooping cough, cases reported	Deaths, all causes
	Cases, estimated expectancy	Cases reported	Cases, estimated expectancy	Cases reported	Deaths reported		Cases, estimated expectancy	Cases reported	Deaths reported		
WEST NORTH CENTRAL											
Minnesota:											
Duluth	3	19	1	0	0	0	1	0	0	1	17
Minneapolis	27	112	7	10	4	9	1	0	0	2	110
St. Paul	18	43	5	7	2	6	0	0	1	28	61
Iowa:											
Davenport	2	0	5	1			0	0		0	
Sioux City	3	0	1	0			0	0		0	
Waterloo	2	0	0	2			0	0		2	
Missouri:											
Kansas City	9	62	3	1	0	9	1	0	0	10	80
St. Joseph	2	6	0	0	0	0	0	0	0	4	27
St. Louis	30	95	2	8	0	8	1	0	0	17	212
North Dakota:											
Fargo	1	5	1	0	0	0	0	0	0	4	5
Grand Forks	1	1	0	0			0	0		0	
South Dakota:											
Aberdeen	1	3	0	0			0	0		3	
Sioux Falls	1		0				0				
Nebraska:											
Lincoln	2	0	1	0	0	0	0	0	0	9	13
Omaha	5	2	3	10	0	0	0	0	0	4	59
Kansas:											
Topeka	2	3	0	0	0	0	0	0	0	4	8
Wichita	2	2	3	0	0	0	0	0	0	31	26
SOUTH ATLANTIC											
Delaware:											
Wilmington	3	0	0	0	0	1	0	0	0	2	28
Maryland:											
Baltimore	25	43	0	2	0	19	3	2	0	103	237
Cumberland	1	0	0	0	0	1	1	0	0	0	9
Frederick	2	1	0	0	0	1	0	0	0	0	3
District of Columbia:											
Washington	17	24	2	3	0	13	1	1	0	19	125
Virginia:											
Lynchburg	1	4	0	1	0	0	0	2	0	12	4
Norfolk	1	1	0	0	0	1	0	0	0	6	
Richmond	3	0	0	0	0	3	1	1	0	9	52
Roanoke	1	0	1	0	0	0	0	0	0	0	9
West Virginia:											
Charleston	1	1	0	2	0	0	0	0	0	1	12
Huntington	1	6	0	10			0	0		0	
Wheeling	2	4	0	0	0	0	1	0	0	1	17
North Carolina:											
Raleigh	1	0	0	1	0	2	0	0	0	0	15
Wilmington	0	0	0	3	0	1	0	0	0	0	16
Winston-Salem	1	0	3	4	0	2	0	0	0	2	20
South Carolina:											
Charleston	0	0	0	0	0	6	1	2	0	0	36
Columbia	0	0	0	0			1	0		3	
Greenville	0	0	0	2	0	1	1	1	0	0	12
Georgia:											
Atlanta	3	3	6	0	0	4	0	3	0	15	83
Brunswick	0	0	0	0	0	0	1	1	0	0	3
Savannah	0	0	0	0	0	4	1	0	0	2	24
Florida:											
St. Petersburg	0	0	0	0	0	1	0	0	1	0	11
Tampa	0	0	0	0	0	0	1	0	1	0	17
EAST SOUTH CENTRAL											
Kentucky:											
Covington	1	2	0	0	0	1	0	0	0	1	19
Louisville	3	24	1	0	0	8	2	1	0	10	
Tennessee:											
Memphis	4	6	2	4	0	8	1	4	0	17	56
Nashville	1	9	1	4	0	8	1	0	0	1	46
Alabama:											
Birmingham	2	16	0	24	0	5	1	2	2	2	76
Mobile	0	0	1	0	0	0	0	3	0	0	23
Montgomery	0	0	1	1	0	0	0	1	0	0	17

City reports for week ended May 16, 1925—Continued

Division, State, and city	Scarlet fever		Smallpox			Tuberculosis, deaths reported	Typhoid fever			Whooping cough, cases reported	Deaths, all causes
	Cases, estimated expectancy	Cases reported	Cases, estimated expectancy	Cases reported	Deaths reported		Cases, estimated expectancy	Cases reported	Deaths reported		
WEST SOUTH CENTRAL											
Arkansas:											
Fort Smith	1	1	1	0			0	0		2	
Little Rock	1	1	1	0	0	2	0	1	0	0	
Louisiana:											
New Orleans	3	12	3	1	0	10	3	14	0	4	146
Shreveport		0		1	0	0		0	0	0	20
Oklahoma:											
Oklahoma	2	0	4	0	0	1	0	4	0	2	23
Texas:											
Dallas	2	2	3	1	0	4	0	0	1		40
Galveston	0	0	1	0	0	0	1	1	1	0	7
Houston	1	0	0	5	0	2	0	0	0	0	55
San Antonio	0	0	0	0	0	4	0	1	0	0	64
MOUNTAIN											
Montana:											
Billings	1	3	1	0	0	0	0	0	0	0	7
Great Falls	1	13	2	0	0	0	0	0	0	2	6
Helena	1	0	0	0	0	0	0	0	0	0	2
Missoula	1	2	0	0	0	0	0	0	0	1	9
Idaho:											
Boise	1	1	0	0	0	0	0	0	0	0	2
Colorado:											
Denver	11	13	1	0	0	12	0	0	0	12	81
Pueblo	1	2	0	0	0	2	1	0	0	0	8
New Mexico:											
Albuquerque	1	0	0	0	0	4	0	0	0	0	8
Arizona:											
Phoenix	0	3	0	0	0	6	0	0	0	0	14
Utah:											
Salt Lake City	2	3	0	0	0	1	0	0	0	3	32
Nevada:											
Reno	0	0	0	3	0	0	0	0	0	0	1
PACIFIC											
Washington:											
Seattle	7	10	3	26			0	0		98	
Spokane	4	0	5	2			0	0		24	
Tacoma	2		1				0				
California:											
Los Angeles	13	36	1	27	0	18	2		1	61	214
Sacramento	2	1	0	2	0	2	0	0	0	3	28
San Francisco	14	17	2	5	1	9	1	1	0	42	140

City reports for week ended May 16, 1925—Continued

Division, State, and city	Cerebrospinal meningitis		Lethargic encephalitis		Pellagra		Poliomyelitis (infantile paralysis)			Typhus fever	
	Cases	Deaths	Cases	Deaths	Cases	Deaths	Cases, estimated expectancy	Cases	Deaths	Cases	Deaths
NEW ENGLAND											
Massachusetts:											
Springfield	0	0	0	1	0	0	0	0	0	0	0
Rhode Island:											
Providence	1	0	0	0	0	0	0	0	0	0	0
MIDDLE ATLANTIC											
New York:											
New York	0	1	3	2	0	0	1	2	0	1	0
Pennsylvania:											
Philadelphia	0	0	1	1	0	0	0	0	0	0	0
EAST NORTH CENTRAL											
Ohio:											
Cleveland	2	1	0	0	0	0	0	0	0	0	0
Indiana:											
Indianapolis	0	2	0	0	0	0	0	0	0	0	0
Illinois:											
Chicago	2	2	0	0	1	0	0	0	1	0	0
Michigan:											
Detroit	0	0	2	0	0	0	0	1	0	0	0
Wisconsin:											
Milwaukee	1	0	0	0	0	0	0	0	0	0	0
Superior	0	0	0	1	0	0	0	0	0	0	0
WEST NORTH CENTRAL											
Missouri:											
St. Louis	1	0	0	0	0	0	0	0	0	0	0
SOUTH ATLANTIC											
Maryland:											
Baltimore	0	1	0	0	0	1	0	0	0	0	0
Virginia:											
Norfolk	0	0	0	0	1	1	0	0	0	0	0
North Carolina:											
Raleigh	0	0	0	0	0	2	0	0	0	0	0
Georgia:											
Atlanta	0	0	0	0	0	1	0	0	0	0	0
Savannah	0	0	0	0	1	1	0	0	0	0	0
EAST SOUTH CENTRAL											
Alabama:											
Mobile	0	0	0	0	0	1	0	0	0	0	0
WEST SOUTH CENTRAL											
Arkansas:											
Little Rock	0	0	0	0	1	0	0	0	0	0	0
Louisiana:											
New Orleans	0	0	1	1	1	2	0	0	0	0	0
Shreveport	0	0	0	0	0	1	-------	0	0	0	0
Texas:											
Dallas	0	0	0	0	1	1	0	0	0	0	0
Houston	0	0	0	0	0	0	0	1	0	0	0
San Antonio	0	0	0	1	0	1	0	0	0	0	0
MOUNTAIN											
Colorado:											
Denver	0	0	0	1	0	0	0	0	0	0	0
Utah:											
Salt Lake City	0	1	0	0	0	0	0	0	0	0	0
PACIFIC											
California:											
Los Angeles	0	2	0	0	0	0	0	1	0	0	0
San Francisco	0	0	0	0	0	0	0	1	0	0	0

The following table gives the rates per hundred thousand population for 105 cities for the 10-week period ended May 16, 1925. The population figures used in computing the rates were estimated as of July 1, 1923, as this is the latest date for which estimates are available. The 105 cities reporting cases had an estimated aggregate population of nearly 29,000,000, and the 97 cities reporting deaths had more than 28,000,000 population. The number of cities included in each group and the aggregate populations are shown in a separate table below.

Summary of weekly reports from cities, March 8 to May 16, 1925—Annual rates per 100,000 population [1]

DIPHTHERIA CASE RATES

	Week ended—									
	Mar 14	Mar. 21	Mar. 28	Apr. 4	Apr. 11	Apr. 18	Apr. 25	May 2	May 9	May 16
105 cities	167	167	[2] 168	177	158	160	162	158	[2] 157	[3] 164
New England	176	147	119	171	166	129	144	127	109	154
Middle Atlantic	214	196	231	241	220	228	218	213	212	238
East North Central	128	134	112	93	96	110	113	110	113	110
West North Central	201	199	247	220	226	168	187	201	278	[4] 212
South Atlantic	91	136	95	81	73	102	108	104	104	85
East South Central	40	69	57	23	34	46	40	40	11	34
West South Central	158	97	121	83	107	74	79	70	65	56
Mountain	105	143	134	124	105	239	267	115	105	153
Pacific	197	249	[3] 179	374	171	168	165	206	[3] 123	[5] 138

MEASLES CASE RATES

105 cities	449	506	[2] 507	558	531	589	645	581	[2] 627	[3] 624
New England	542	725	755	957	1,011	917	1,217	1,004	984	1,188
Middle Atlantic	518	598	633	734	680	815	782	734	797	768
East North Central	740	775	798	736	710	742	901	761	890	854
West North Central	75	93	89	77	58	91	102	79	112	[4] 80
South Atlantic	146	189	136	209	207	256	295	305	240	329
East South Central	11	69	34	69	34	97	189	200	343	166
West South Central	88	42	9	88	51	65	37	28	32	14
Mountain	763	573	38	219	57	267	219	534	181	57
Pacific	110	189	[3] 151	209	241	154	203	162	[3] 95	[5] 178

SCARLET FEVER CASE RATES

105 cities	432	427	[2] 419	409	367	342	360	309	[2] 323	[3] 352
New England	534	544	604	534	529	350	407	430	415	358
Middle Atlantic	439	417	405	436	359	343	336	323	319	331
East North Central	497	498	483	442	422	403	433	324	366	390
West North Central	719	792	755	736	647	651	692	518	618	[4] 734
South Atlantic	219	146	167	175	152	167	175	132	106	165
East South Central	355	286	286	263	280	229	257	263	263	326
West South Central	107	134	102	51	88	60	121	111	88	74
Mountain	200	429	248	277	258	315	401	334	277	353
Pacific	229	218	[3] 222	191	174	145	148	125	[3] 151	[5] 197

[1] The figures given in this table are rates per 100,000 population, annual basis, and not the number of cases reported. Populations used are estimated as of July 1, 1923.
[2] Spokane, Wash., not included. Report not received at time of going to press.
[3] Sioux Falls, S. Dak., and Tacoma, Wash., not included.
[4] Sioux Falls, S. Dak., not included.
[5] Tacoma, Wash., not included.

Summary of weekly reports from cities, March 8 to May 16, 1925—Annual rates per 1,000 population—Continued

SMALLPOX CASE RATES

	Week ended—									
	Mar. 14	Mar. 21	Mar. 28	Apr. 4	Apr. 11	Apr. 18	Apr. 25	May 2	May 9	May 16
105 cities	61	63	[2] 58	57	51	48	62	50	[2] 46	[3] 46
New England	0	0	0	12	2	0	2	0	2	0
Middle Atlantic	5	8	7	21	10	18	12	8	6	7
East North Central	39	32	33	24	22	27	39	30	44	56
West North Central	124	102	135	87	97	85	89	75	60	[4] 80
South Atlantic	59	57	67	49	43	53	79	63	45	37
East South Central	446	646	423	42	572	395	457	435	377	189
West South Central	74	107	107	46	51	14	42	32	28	37
Mountain	95	67	19	19	19	10	29	10	48	29
Pacific	247	212	[2] 191	255	148	162	264	206	[2] 176	[5] 191

TYPHOID FEVER CASE RATES

105 cities	10	12	[2] 11	9	10	12	16	18	[2] 14	[3] 13
New England	5	30	12	5	2	7	17	10	5	12
Middle Atlantic	5	8	7	4	9	11	14	22	13	10
East North Central	4	7	3	4	6	4	7	4	9	6
West North Central	10	8	6	2	2	2	6	12	2	[4] 0
South Atlantic	24	22	12	30	20	12	14	28	28	26
East South Central	34	46	57	17	17	34	80	46	46	63
West South Central	28	23	42	32	37	56	51	51	46	79
Mountain	19	0	0	0	19	38	29	0	0	0
Pacific	15	0	[2] 28	20	9	12	23	17	[2] 9	[5] 3

INFLUENZA DEATH RATES

105 cities	34	42	33	34	27	27	30	22	15	[4] 14
New England	35	30	30	35	32	27	30	20	10	7
Middle Atlantic	24	29	22	21	16	24	17	14	10	12
East North Central	33	49	40	38	27	24	33	23	16	11
West North Central	33	42	46	39	37	50	48	31	11	[4] 11
South Atlantic	33	53	12	28	26	12	43	26	24	10
EastSouth Central	91	120	86	69	74	80	86	51	51	80
West South Central	107	76	36	36	46	36	25	31	15	20
Mountain	48	48	38	181	86	38	76	48	19	57
Pacific	16	12	53	29	12	29	12	12	16	12

PNEUMONIA DEATH RATES

105 cities	222	217	206	204	201	192	203	167	151	[4] 127
New England	229	211	219	251	211	206	186	149	161	134
Middle Atlantic	214	217	199	215	190	204	223	206	185	143
East North Central	241	222	214	182	190	190	211	148	130	125
West North Central	175	173	166	193	228	171	136	72	77	[4] 58
South Atlantic	246	290	252	234	238	232	191	195	156	136
East South Atlantic	366	286	269	269	343	206	286	194	160	166
West South Central	178	178	168	168	168	173	158	127	138	112
Mountain	210	172	200	162	267	210	219	124	124	162
Pacific	155	131	159	159	119	98	147	127	123	78

[2] Spokane, Wash., not included. Report not received at time of going to press.
[3] Sioux Falls, S. Dak., and Tacoma, Wash., not included.
[4] Sioux Falls, S. Dak., not included.
[5] Tacoma, Wash., not included.

Number of cities included in summary of weekly reports and aggregate population of cities in each group, estimated as of July 1, 1923

Group of cities	Number of cities reporting cases	Number of cities reporting deaths	Aggregate population of cities reporting cases	Aggregate population of cities reporting deaths
Total	105	97	28,898,350	28,140,934
New England	12	12	2,098,746	2,098,746
Middle Atlantic	10	10	10,304,114	10,304,114
East North Central	17	17	7,032,535	7,032,535
West North Central	14	11	2,515,330	2,381,454
South Atlantic	22	22	2,566,901	2,566,901
East South Central	7	7	911,885	911,885
West South Central	8	6	1,124,564	1,023,013
Mountain	9	9	546,445	546,445
Pacific	6	3	1,797,830	1,275,841

FOREIGN AND INSULAR

THE FAR EAST

Wireless health news messages.—The following data were sent by wireless from the far eastern bureau of the health section of the League of Nations located at Singapore, to headquarters at Geneva, Switzerland:

Week ended Saturday, May 9, 1925

Port	Plague		Cholera		Smallpox	
	Cases	Deaths	Cases	Deaths	Cases	Deaths
Calcutta	-------	0	-------	49	107	100
Bombay	-------	4	-------	0	14	18
Madras	-------	0	-------	0	41	18
Rangoon	-------	24	-------	5	63	24
Karachi	-------	3	-------	0	7	3
Negapatam	-------	0	-------	0	0	0
Singapore [1]	6	6	0	0	0	0
Penang	0	0	0	0	0	0
Batavia	0	0	0	0	0	0
Soerabaya	0	0	0	0	1	-------
Samarang	0	0	0	0	0	0
Belawan Deli	0	0	0	0	0	0
Macassar	0	0	0	0	0	0
British North Borneo	0	0	0	0	0	0
Bangkok	1	1	0	0	1	-------
Saigon and Cholon	0	0	0	1	-------	-------
Hongkong [1]	-------	-------	-------	-------	-------	-------
Shanghai [2]	-------	-------	-------	-------	-------	-------
Nagasaki	0	0	0	0	3	-------
Manila	0	0	0	0	0	0
Kobe	0	0	0	0	0	0
Shimonoseki	0	0	0	0	0	0
Yokohama	0	0	0	0	0	0

[1] Infected rats found.
[2] Report not received for week ended May 9, 1925.

CANADA

Mosquito destruction—Fredericton, Nova Scotia.—Information received under date of April 30, 1925, shows that measures for the destruction of mosquitoes have been put into effect at Fredericton, Nova Scotia, Canada. The ponds and marshes in the vicinity of the city have been sprayed with oil.

CZECHOSLOVAKIA

Communicable diseases—January–March, 1925.—During the period January 1 to March 31, 1925, communicable diseases were notified in Czechoslovakia as follows:

Disease	Cases	Deaths	Province showing greatest number of cases and deaths
Anthrax	8	1	Bohemia, cases, 4; Russinia, 1 death.
Cerebrospinal meningitis	62	18	Slovakia, cases, 22; deaths, 2.
Diphtheria	1,101	83	Bohemia, cases, 544; deaths, 52.
Dysentery	72	2	Slovakia, cases, 23; Bohemia, deaths, 2.
Malaria	8	----------	Bohemia, cases, 4.
Paratyphoid fever A	2	----------	Bohemia.
Paratyphoid fever B	21	----------	Do.
Scarlet fever	2,683	84	Bohemia, cases, 1,311; deaths, 45.
Trachoma	651	----------	Moravia, cases, 211.
Typhoid fever	1,280	126	Slovakia, cases, 474; deaths, 38.
Typhus fever	54	2	Russinia, cases, 53; deaths, 2.

Typhus fever outbreak.—The occurrence during the period under report of 54 cases of typhus fever with 2 deaths, indicates unusual conditions in the prevalence of this disease, only 8 cases having been reported during the preceding 6-month period. From December 31, 1924, to the latter part of March, 1925, 28 cases of typhus fever were reported from the small town of Smerekov and its immediate vicinity. As the town is situated 8 miles from the main lines of travel it was quickly isolated by the health authorities and placed in charge of a divisional unit operating in the section of the Republic. No workers were permitted to leave the town.

JAVA

Further relative to epidemic malaria—Soerabaya.[1]—Reports of the prevalence of epidemic malaria among natives at Kedamean, Soerabaya Residency, Java, have been received as follows: Week ended February 2, 1925, 1,752 cases with 19 deaths; week ended March 2, 1925, 449 cases with 8 deaths; week ended March 9, 1925, 72 cases with 9 deaths. For the week ended March 16, only 17 cases were reported, with 1 death. During a period of 4 months, 6,000 cases of malaria were reported at Kedamean.

MADAGASCAR

Plague—March 1–15, 1925.—During the period March 1 to 15, 1925, 104 cases of plague with 87 deaths were reported in the island of Madagascar, occurring in the Provinces of Itasy, Moramanga, and Tananarive. Of the cases, 65 were stated to be bubonic, 14 pneumonic, and 25 septicemic in type. For distribution according to Province, see page 1224.

MEXICO

Cerebrospinal meningitis—State of Morelos—Epidemic stated to have ceased.—Under date of May 16, 1925, epidemic prevalence of cerebrospinal meningitis in the State of Morelos, Mexico,[1] was stated to have ceased. A few sporadic cases were reported on the date quoted at Cuernavaca.

[1] Public Health Reports, May 1, 1925, p. 916.

UNION OF SOUTH AFRICA

Smallpox—Typhus fever—March, 1925.—During the month of March, 1925, 9 cases of smallpox, of which 3 cases were in the white and 6 in the native population, and 41 cases of typhus fever with 7 deaths, of which 5 cases were in the European population, were reported in the Union of South Africa. For distribution of occurrence of typhus fever according to locality, see page 1225.

VIRGIN ISLANDS

Communicable diseases—April, 1925.—During the month of April, 1925, communicable diseases were reported in the Virgin Islands of the United States as follows:

Island and disease	Cases	Remarks	Island and disease	Cases	Remarks
St. Thomas and St. John:			St. Croix:		
Chancroid	1		Chicken pox	1	
Dengue	20		Filariasis	13	Bancrofti.
Dysentery	2	Unclassified.	Leprosy	2	
Gonorrhea	5	1 St. John.	Malaria	2	Malignant tertian.
Malaria	1	St. John. Benign tertian.	Syphilis	1	Secondary.
Syphilis	1	Secondary.	Trachoma	1	
Tetanus	2		Tuberculosis	3	Chronic pulmonary.

[1] Public Health Reports, May 8, 1925, p. 972.

CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER AND YELLOW FEVER

The reports contained in the following tables must not be considered as complete or final as regards either the lists of countries included or the figures for the particular countries for which reports are given.

Reports Received During Week Ended June 5, 1925 [1]

CHOLERA

Place	Date	Cases	Deaths	Remarks
India:				
Calcutta	Apr. 5–11	52	48	
Madras	Apr. 19–25	1	1	

PLAGUE

Egypt				Jan. 1–Apr. 29, 1925: Cases, 24; deaths, 14.
City—				
Suez	Apr. 2–22	2	2	
Province—				
Beni-Souef	Jan. 18	1	1	
Dakhalia	Jan. 7	1	1	
Fayoum	Apr. 5–14	3	2	
Girgeh	Jan. 9–Apr. 5	2	2	
Kalioubiah	Jan. 5–Apr. 22	5	2	
Menoufieh	Jan. 1–Apr. 9	8	4	
Minia	Apr. 1–5	2		
India:				
Karachi	Apr. 19–25		1	
Madras Presidency	do	27	16	
Madagascar				Mar. 1–15, 1925: Cases, 104; deaths, 87. Bubonic, 65; pneumonic, 14; septicemic, 25.
Itasy Province	Mar. 1–15	3	3	
Moramanga Province	do	2	2	
Tananarive Province	do	99	82	
Tananarive town	do	3	3	Pneumonic.
Other localities	do	96	79	
Straits Settlements:				
Singapore	Apr. 5–11	2	1	

[1] From medical officers of the Public Health Service, American consuls, and other sources

CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER—Continued

Reports Received During Week Ended June 5, 1925—Continued

SMALLPOX

Place	Date	Cases	Deaths	Remarks
Algeria:				
Algiers	Apr. 1-30	6		
Brazil:				
Porto Alegre	Apr. 12-18		1	
British South Africa:				
Northern Rhodesia	Mar. 17-Apr. 14	9		
Canada:				
British Columbia—				
Vancouver	May 4-17	5		
Ceylon:				
Colombo	Apr. 12-18	1		Port case.
China:				
Amoy	Apr. 5-18		8	Prevalent in surrounding district.
Antung	Apr. 12-26	5		
Canton	Apr. 12-18			Present.
Chungking	Apr. 5-11			Prevalent.
Foochow	Apr. 5-18			Present.
Manchuria—				
Dairen	Mar. 16-Apr. 5	14	3	
Harbin	Apr. 15-21	1		
Nanking	Mar. 29-Apr. 18			Prevalent.
France:				
Boulogne-sur-Mer	Apr. 1-30	1	1	
Gibraltar	May 4-10	2		
Great Britain:				
Newcastle-on-Tyne	May 3-9	3		
India:				
Calcutta	Apr. 5-11	404	313	
Karachi	Apr. 19-25	5	2	
Madras	do	64	27	
Indo-China:				
Saigon	Mar. 29-Apr. 4	8	2	Including 100 square kilometers of surrounding country.
Japan:				
Taihoku	Apr. 4-10	1		
Mexico:				
Guadalajara	May 12-18		3	
Mexico City	Apr. 26-May 2	7		Including municipalities in Federal district.
Persia:				
Teheran	Feb. 19-Mar. 19		9	
Spain:				
Malaga	May 3-9		4	
Switzerland:				
Berne	Apr. 12-18	1		
Turkey:				
Constantinople	Apr. 16-30	3		
Union of South Africa				Mar. 1-31, 1925: Cases, 9; white, 3; native, 6.

TYPHUS FEVER

Chile:				
Concepcion	Apr. 14-20		1	
Valparaiso	Apr. 5-25		3	
China:				
Manchuria—				
Harbin	Apr. 8-14	1		
Czechoslovakia				January-March, 1925: Cases, 54; deaths, 2.
Egypt:				
Alexandria	Apr. 2-8	1		
Greece:				
Saloniki	Mar. 31-Apr. 20	2		
Mexico:				
Mexico City	Apr. 26-May 2	9		Including municipalities in Federal district.
Turkey:				
Constantinople	Apr. 24-30	1		
Union of South Africa				Mar. 1-31, 1925: Cases, 41; deaths, 7. Native—cases, 36; deaths, 7. White or European—cases, 5.
Cape Province				Mar. 1-31, 1925: Cases, 17; deaths, 3.
Natal				Mar. 1-31, 1925: Cases, 6; deaths, 2.
Orange Free State				Mar. 1-31, 1925; Cases, 9; deaths, 2.
Transvaal				Mar. 1-31, 1925: Cases, 4.
Yugoslavia:				
Belgrade	Apr. 24-30	2		

CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER—Continued

Reports Received from December 27, 1924, to May 29, 1925 [1]

CHOLERA

Place	Date	Cases	Deaths	Remarks
Ceylon				June 29–Dec. 27, 1924: Cases, 14; deaths, 13. Dec. 28, 1924–Jan. 24, 1925: Cases, 24; deaths, 17. Oct. 19, 1924, to Jan. 3, 1925: Cases, 27,164; deaths, 16,228. Jan. 4–Mar. 29, 1925: Cases 26,127; deaths, 15,462.
Colombo	Nov. 16–22	1		
Do	Jan. 11–24	2	2	
India				
Bombay	Nov. 23–Dec. 20	4	4	
Do	Jan. 18–24	1	1	
Calcutta	Oct. 26–Jan. 3	59	51	
Do	Jan. 4–Mar. 21	205	164	
Do	Mar. 29–Apr. 4	49	46	Reported to be epidemic May 9, 1925.
Madras	Nov. 16–Jan. 3	69	40	
Do	Jan. 4–Mar. 7	139	99	
Do	Apr. 5–18	3	1	
Rangoon	Nov. 9–Dec. 20	9	2	
Do	Jan. 4–Apr. 11	20	13	
Indo-China				Aug. 1–Sept. 30, 1924: Cases, 14; deaths, 10. Dec. 1–31, 1924: Cases, 5; deaths, 2.
Province—				
Anam	Aug. 1–31	1	1	
Cambodia	Aug. 1–Sept. 30	6	5	
Do	Dec. 1–31	1		
Cochin-China	Aug. 1–Dec. 31	10	5	
Saigon	Nov. 30–Dec. 6	1		
Do	Mar. 15–21	1	1	
Tonkin	Dec. 1–31	1	1	
Siam:				
Bangkok	Nov. 9–29	4	2	
Do	Jan. 18–Mar. 21	8	5	

PLAGUE

Azores:				
Fayal Island—				
Castelo Branco	Nov. 25			Present with several cases.
Feteira	do	1		
St. Michael Island	Nov. 2–Jan. 3	30	13	
Do	Jan. 18–24	3	1	
Brazil:				
Bahia	Jan. 4–Apr. 18	11	7	
Santos	Year, 1924	2		Bubonic.
British East Africa:				
Tanganyika Territory	Nov. 23–Dec. 27	17	10	
Do	Jan. 18–Mar. 14	18	12	
Uganda	Aug.–Dec., 1924	279	243	
Do	Jan. 1–31	29	28	
Canary Islands:				
Las Palmas	Jan. 21–23	2		Stated to be endemic.
Do	Feb. 4	1		Stated to have been infected with plague Sept. 30, 1924.
Do	Mar. 26	1	1	
Realejo Alto	Dec. 19	3	1	Vicinity of Santa Cruz de Teneriffe.
Teneriffe—				
Santa Cruz	Jan. 3	1		In vicinity.
Celebes:				
Macassar	Oct. 29			Epidemic.
Ceylon:				
Colombo	Nov. 9–Jan. 3	12	9	
Do	Jan. 4–Apr. 14	21	21	
China:				
Foochow	Dec. 28–Jan. 3			Present.
Nanking	Nov. 23–Mar. 7			Do.
Shing Hsien	October, 1924		790	
Ecuador				Mar. 16–Apr. 15, 1925: Cases, 10; deaths, 4.
Chimborazo Province—				
Alausi District	Jan. 14		14	At 2 localities on Guayaquil & Quito Ry.
Daule	Mar. 16–31	1		
Guayaquil	Nov. 16–Dec. 31	9	3	Rats taken, 27,004; found infected, 92.
Do	Jan. 1–Apr. 15	68	29	Rats taken, 67,317; found infected, 294.
Naranjito	Feb. 16–Mar. 15	1		
Yaguachi	Feb. 1–Mar. 15	2	1	
Egypt				Year 1924: Cases, 373. Jan. 1–Apr. 22, 1925: Cases, 24; deaths, 14.

[1] From medical officers of the Public Health Service, American consuls, and other sources.

CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER—Continued

Reports Received from December 27, 1924, to May 29, 1925—Continued

PLAGUE—Continued

Place	Date	Cases	Deaths	Remarks
Gold Coast				September - December, 1924: Deaths, 52.
Greece:				
Patras	Apr. 5	1		
Hawaii:				
Honokaa	Nov. 4	1		Plague-infected rodents found Dec. 9, 1924, Jan. 15—Apr. 28 and 30, 1925. Vicinity Pacific Sugar Mill, Island of Hawaii.
India				Oct. 19, 1924, to Jan. 3, 1925: Cases, 28,154; deaths, 21,505. Jan. 4–Mar. 28, 1925: Cases, 57,672; deaths, 48,562.
Bombay	Nov. 22–Jan. 3	4	3	
Do	Jan. 4–17	2	2	
Do	Feb. 8–Apr. 4	56	47	
Calcutta	Jan. 18–24	1	1	
Karachi	Nov. 30–Dec. 6	2	1	
Do	Jan. 4–Feb. 21	12	11	
Do	Mar. 29–Apr. 18	6	6	
Madras Presidency	Nov. 23–Jan. 3	685	487	
Do	Jan. 4–24	658	511	
Do	Mar. 8–14	80	48	
Rangoon	Oct. 26–Jan. 3	26	25	
Do	Jan. 4–Apr. 11	187	164	
Indo-China				Aug. 1–Sept. 30, 1924: Cases, 25; deaths, 20. Dec. 1–31, 1924: Cases, 11; deaths, 11. Corresponding month, 1923: Cases, 15; deaths, 5.
Province—				
Anam	Aug. 1–Sept. 30	4	4	
Do	Dec. 1–31	5	5	
Cambodia	Aug. 1–Sept. 30	18	15	
Do	Dec. 1–31	6	6	
Cochin-China	do	3	1	
Saigon	Dec. 25–31	1	1	Including 100 square kilometers of surrounding territory.
Do	Jan. 11–17	2	1	Do.
Iraq	June 29–Jan. 3	20	14	
Bagdad	Mar. 22–28	1	1	
Japan	Aug. 10–Dec. 6	19		
Java:				
East Java—				
Blitar	Nov. 11–22			Province of Kediri; epidemic.
Pare	Nov. 29			Do.
Samarang	Mar. 22–28	2	2	
Sidoardja	Jan. 2			Declared epidemic, Province of Soerabaya.
Soerabaya	Nov. 16–Dec. 31	71	72	
Do	Jan. 15–Mar. 25	25	22	Mar. 29–Apr. 4, 1925: 2 plague rats found.
Soerakarta	Feb. 20			Epidemic plague in one locality.
West Java—				
Cheribon	Oct. 14–Nov. 3		14	
Do	Nov. 18–Dec. 22		80	
Do	Jan. 1–14		44	
Do	Feb. 5–11		13	
Do	Feb. 19–25		13	
Do	Mar. 5–11		14	
Pasoeroean	Dec. 27			Province. Epidemic in one locality.
Pekalongan	Oct. 14–Nov. 3		29	
Do	Nov. 18–Dec. 31		177	Pekalongan Province.
Do	Jan. 1–14		81	
Do	Feb. 5–11		36	
Do	Feb. 19–25		38	
Do	Mar. 5–11		28	
Probalingga	Dec. 27			Province. Epidemic.
Tegal	Oct. 14–Dec. 31		26	
Do	Jan. 1–14		37	Pekalongan Province.
Do	Feb. 5–11		7	
Do	Feb. 19–25		10	
Do	Mar. 5–11		3	
Madagascar:				
Fort Dauphin (port)	Nov. 1–Dec. 15	12	5	
Do	Feb. 1–15	1	1	Bubonic.
Itasy Province	Nov. 1–Dec. 15	4	2	
Do	Feb. 1–28	3	3	
Majunga (port)	Nov. 1–30	1	1	
Moramanga Province				Nov. 1–Dec. 15, 1924: Cases, 49; deaths, 34. Jan. 16–Feb. 28, 1925: Cases, 6; deaths, 6.
Tamatave (port)	Nov. 1–30	1	1	
Tananarive Province				Oct. 16–Dec. 31, 1924: Cases, 298; deaths, 274.
Do				Jan. 1–Mar. 15: Cases, 456; deaths, 387.
Tananarive (town)	Mar. 1–15	3		

CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER—Continued

Reports Received from December 27, 1924, to May 29, 1925—Continued

PLAGUE—Continued

Place	Date	Cases	Deaths	Remarks
Mauritius Island				Year 1924: Cases, 161; deaths, 144.
District—				
Flacq	Dec. 1-31	5	4	
Pamplemousses	do	1	1	
Plaines Wilhems	January - December, 1924.	54	47	Not present March, April, May.
Port Louis	February - December, 1924.	101	92	
Mexico:				
Tampico	Apr. 6, 1925			Plague rat found in vicinity of Government wharves.
Morocco:				
Marrakech				Feb. 9, 1925: Present in native quarter of town. Stated to be pneumonic in form and of high mortality.
Nigeria				August-November, 1924: Cases, 387; deaths, 317.
Palestine:				
Jerusalem	Mar. 3-9	1		
Peru:				
Callao	February, 1925	6	6	
Siam:				
Bangkok	Dec. 28-Jan. 3	1	1	
Do	Jan. 25-Mar. 21	7	6	
Siberia:				
Transbaikalia—				
Turga	October, 1924		3	On Chita Railroad.
Straits Settlements:				
Singapore	Nov. 9-15	1	1	
Do	Jan. 4-Apr. 4	27	8	
Syria:				
Beirut	Jan. 11-Apr. 10	2		
Turkey:				
Constantinople	Jan. 9-15	5	5	
Union of South Africa	Nov. 22-Jan. 3	28	15	In Cape Province, Orange Free State, and Transvaal.
Do	Jan. 4-Apr. 4	55	23	Do.
On vessels:				
S. S. Conde				At Marseille, France, Nov. 8, 1924. Plague rat found. Vessel left for Tamatave, Madagascar, Nov. 12, 1924.
Steamship	November, 1924	1	1	At Majunga, Madagascar, from Djibuti, Red Sea port.

SMALLPOX

Algeria				July 1-Dec. 31, 1924: Cases, 409.
Algiers	Jan. 1-Mar. 31	10		Jan. 1-20, 1925: Cases, 107.
Arabia:				
Aden	Jan. 25-Apr. 18	14	1	
Argentina:				
Buenos Aires	Mar. 15-21	1		
Belgium	Jan. 1-Feb. 10	4		
Bolivia:				
La Paz	Nov. 1-Dec. 21	20	11	
Do	Jan. 1-Mar. 31		12	
Brazil:				
Pernambuco	Nov. 9-Jan. 3	100	27	
Do	Jan. 4-Mar. 28	111	56	
British East Africa:				
Kenya—				
Mombasa	Jan. 18-Feb. 28	66	14	
Do	Mar. 8-28	29	7	
Uganda—				
Entebbe	Oct. 1-31	4		
Tanganyika Territory	Feb. 15-21	1		
British South Africa:				
Northern Rhodesia	Oct. 28-Dec. 15	57	2	
Do	Jan. 27-Feb. 2	3		Natives.
Southern Rhodesia	Jan. 29-Mar. 25	4	1	
Bulgaria:				
Sofia	Mar. 12-18	1		Varioloid.

CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER—Continued

Reports Received from December 27, 1924, to May 29, 1925—Continued

SMALLPOX—Continued

Place	Date	Cases	Deaths	Remarks
Canada:				
Alberta—				
Calgary	Mar. 15-21	1		
British Columbia—				
Ocean Falls	Mar. 7-27	6		Very mild.
Vancouver	Dec. 14-Jan. 3	32		
Do	Jan. 4-Apr. 12	305		
Do	Apr. 19-May 3	11		
Victoria	Jan. 18-Apr. 25	11		
Manitoba—				
Winnipeg	Dec. 7-Jan. 3	14		
Do	Jan. 4-Feb. 27	30		
Do	Apr. 5-11	1		
New Brunswick—				
Northumberland	Feb. 8-14	1		County.
Ontario				Nov. 30-Dec. 27, 1924: Cases, 33. Dec. 28, 1924, to Apr. 25, 1925: Cases, 69; deaths, 1.
Hamilton	Jan. 24-30	1		
Kingston	Apr. 12-18	1		
Ottawa	Mar. 29-Apr. 4	1		
Do	May 3-9	2		
Welland	Mar. 22-Apr. 25	7		
Ceylon				July 27-Nov. 29, 1924; Cases, 27; deaths, 1.
Colombo	Jan. 18-Feb. 7	4		
Do	Mar. 8-Apr. 11	16		
China:				
Amoy	Nov. 9-Feb. 21			Present.
Do	Feb. 22-Mar. 28		11	
Antung	Nov. 17-Dec. 28	5		
Do	Jan. 5-Feb. 14	15	1	
Do	Mar. 2-Apr. 5	9	1	
Canton	Mar. 15-Apr. 11			Prevalent.
Chefoo	Mar. 15-21			Prevalent. No foreign cases.
Chungking	Mar. 22-Apr. 18			Stated to be widely prevalent; less than in period in year 1924.
Foochow	Nov. 2-Mar. 28			Present.
Hongkong	Nov. 9-Jan. 3	6	2	
Do	Jan. 4-Feb. 7	9	7	
Do	Feb. 15-Apr. 4	27	13	
Manchuria—				
Dairen	Jan. 19-Mar. 15	4		
Harbin	Jan. 15-Feb. 11	5		
Nanking	Jan. 4-Mar. 28			Do.
Shanghai	Dec. 7-27	1	2	
Do	Jan. 18-Mar. 7		8	
Do	Apr. 12-25	2	1	
Chosen:				
Seoul	Dec. 1-31	1		
Do	Mar. 1-31	2		
Colombia:				
Buenaventura	Feb. 15-Apr. 4	3		
Santa Marta	Mar. 15-28			Present in mild form in localities in vicinity.
Cuba:				
Santiago	Apr. 12-18	3	1	
Czechoslovakia				Apr.-June, 1924: Cases, 1; occurring in Province of Moravia.
Dominican Republic:				
Puerta Plata	Mar. 8-21	3		
Dutch Guiana:				
Paramaribo	Apr. 20	1		
Ecuador:				
Guayaquil	Nov. 16-Dec. 15	4		
Egypt:				
Alexandria	Nov. 12-Dec. 31	10		
Do	Jan. 8-28	8		
Do	Feb. 26-Mar. 4	1		
Cairo	Jan. 29-Feb. 4	1	1	
Esthonia				Dec. 1-31, 1924: Cases, 2.
France				July-December, 1924: Cases, 81.
Do	January, 1925	10		
Dunkirk	Mar. 2-8	1		From vessel. In quarantine.
St. Malo	Feb. 2-8	7	1	Believed to have been imported on steamship Ruyth from Sfax, Tunis.
Germany				June 29-Nov. 8, 1924: Cases, 7.
Frankfort-on-Main	Jan. 1-10	1		
Gibraltar	Dec. 8-14	1		
Gold Coast				July-December, 1924: Cases, 106; deaths, 1.

CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER—Continued

Reports Received from December 27, 1924, to May 29, 1925—Continued

SMALLPOX—Continued

Place	Date	Cases	Deaths	Remarks
Great Britain:				
England and Wales	Nov. 23–Jan. 3	472		
Do	Jan. 4–Apr. 18	2,047		
Newcastle-on-Tyne	Jan. 18–Feb. 21	9		
Do	Mar. 1–May 2	2		
Greece				January–June, 1924: Cases, 170; deaths, 27.
Do				July–December, 1924: Cases, 38; deaths, 26.
Saloniki	Nov. 11–Dec. 22	3		
Do	Feb. 17–Mar. 2	4		
Haiti:				
Cape Haitien	Mar. 22–Apr. 2	6		
India				Oct. 19, 1924, to Jan. 3, 1925: Cases, 12,564; deaths, 2,857. Jan. 4–Mar. 28, 1925: Cases, 54,626; deaths, 12,494.
Bombay	Nov. 2–Jan. 3	30	18	
Do	Jan. 4–Apr. 4	601	307	
Calcutta	Oct. 26–Jan. 8	307	170	
Do	Jan. 4–Mar. 21	2,669	1,875	
Do	Mar. 29–Apr. 4	392	260	
Karachi	Nov. 16–Jan. 3	16	2	
Do	Jan. 4–Feb. 14	52	6	
Do	Feb. 22–Apr. 18	85	23	
Madras	Nov. 16–Jan. 3	122	48	
Do	Jan. 4–Mar. 7	552	212	
Do	Mar. 15–Apr. 18	489	197	
Rangoon	Oct. 26–Jan. 3	86	28	
Do	Jan. 4–Feb. 7	287	49	
Do	Feb. 15–Apr. 11	1,121	225	
Indo-China				Aug. 1–Sept. 30, 1924: Cases, 223, deaths, 76. Dec. 1–31, 1924; Cases, 485; deaths, 114.
Province—				
Anam	Aug. 1–Sept. 30	49	11	
Do	Dec. 1–31	167	26	
Cambodia	Aug. 1–Sept. 30	40	9	
Do	Dec. 1–31	30	13	
Cochin-China				Aug. 1–Sept. 30, 1924: Cases, 115; deaths, 49. Dec. 1–31, 1924: Cases, 50; deaths, 13.
Saigon	Nov. 16–Jan. 3	17	5	Including 100 square kilometers of surrounding country.
Do	Jan. 4–Feb. 21	32	8	
Do	Mar. 1–28	39	6	Do.
Tonkin	Aug. 1–Sept. 30	19	7	
Do	Dec. 1–31	238	62	
Iraq	June 29–Jan. 10	138	67	
Do	Jan. 11–20	4	2	
Bagdad	Nov. 9–Dec. 27	2	1	
Do	Mar. 1–28	2		
Italy				June 29–Dec. 27, 1924: Cases, 63.
Jamaica				Nov. 30, 1924–Jan. 3, 1925: Cases, 50. Reported as alastrim.
Do				Jan. 4–Apr. 25, 1925: Cases, 275. Reported as alastrim.
Kingston	Nov. 30–Dec. 27	4		Reported as alastrim.
Japan				Aug. 1–Nov. 15, 1924: Cases, 4.
Nagasaki	Feb. 9–Apr. 26	31	9	
Taiwan	Jan. 1–31	1		
Java:				
East Java—				
Pasoerocan	Oct. 26–Nov. 1	9	1	
Do	Nov. 12–19			Epidemic in 2 native villages.
Soerabaya	Oct. 19–Dec. 31	685	212	
Do	Jan. 15–Mar. 25	559	78	
West Java—				
Batam	Oct. 14–20	2		
Batavia	Oct. 21–Nov. 14	2		
Do	Dec. 20–Jan. 2	19	4	
Buitenzorg	Dec. 25–31	1		Batavia Residency.
Cheribon	Oct. 14–Nov. 24	15		
Do	Jan. 1–28	3		
Krawang	Jan. 15–21	1		
Pekalongan	Oct. 14–Nov. 24	22		
Do	Dec. 25–31	3		Province.
Pemalang	Jan. 8–14	1		Pekalongan Residency.
Preanger	Nov. 18–24	1		
Latvia				Oct. 1–Nov. 30, 1924: Cases, 5. Jan. 1–Feb. 28, 1925: Cases, 6.

CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER—Continued

Reports Received from December 27, 1924, to May 29, 1925—Continued

SMALLPOX—Continued

Place	Date	Cases	Deaths	Remarks
Lithuania				Jan. 1-31, 1925: Cases, 2.
Malta				Apr. 1-15, 1925: Cases, 3.
Mexico:				
Chiapas (State)	Mar. 1			Reported severely prevalent.
Durango	Dec. 1-31		5	
Do	Jan. 1-Apr. 30		29	
Guadalajara	Dec. 23-29		1	
Do	Jan. 6-Mar. 23		4	
Do	Apr. 21-May 11		11	
Mexico City	Nov. 23-Dec. 27	5		
Do	Jan. 11-Apr. 25	62		
Monterey				Jan. 24, 1925: Outbreak. Mar. 14, 1925, present.
Oaxaca (State)	Mar. 1			Reported severely prevalent.
Salina Cruz	Dec. 1-31	1	1	
Do	Feb. 22-Mar. 31	7	1	
Saltillo	Feb. 22-Apr. 11		2	
San Luis Potosi	Mar. 29-May 9		4	
Tampico	Dec. 11-31	5	4	
Do	Jan. 1-Apr. 30	66	20	
Torreon	Apr. 1-30	1	1	
Tuxpam district	Apr. 17-May 7	20	3	
Vera Cruz	Dec. 1-Jan. 3		10	
Do	Jan. 5-Apr. 19		39	
Villa Hermosa	Dec. 28-Jan. 10			Present. Locality, capital, State of Tabasco.
Yucatan (State)	Apr. 5-11			In country towns.
Nigeria				January-June, 1924: Cases, 357; deaths, 87.
Do				July-November, 1924: Cases, 87; deaths, 25.
Paraguay:				
Asuncion	Jan. 4-10		1	
Persia:				
Teheran	Sept. 23-Dec. 31		12	
Do	Jan. 1-Feb. 18		10	
Peru:				
Arequipa	Nov. 24-30		1	
Do	Jan. 1-Feb. 28		4	
Philippine Islands:				
Manila	Mar. 29-Apr. 4	3		
Poland				Sept. 21-Dec. 28, 1924: Cases, 30; deaths, 2. Jan. 4-Feb. 14, 1925: Cases, 15; deaths, 1.
Portugal:				
Lisbon	Dec. 7-Jan. 3	17		
Do	Jan. 4-Apr. 25	140		Jan. 4-Apr. 18, 1925: Deaths, 35.
Oporto	Nov. 30-Dec. 27	3	2	
Do	Jan. 11-Mar. 14	3		
Do	Apr. 12-25	2		
Russia				January-June, 1924: Cases, 18,229. July-November, 1924: Cases, 3,665.
Senegal:				
Dakar	Mar. 16-22	4		
Siam:				
Bangkok	Dec. 28-Jan. 3	1	1	
Do	Jan. 18-Feb. 21		19	
Do	Mar. 1-21	11	4	
Sierra Leone:				
Freetown	Feb. 7-Mar. 15	3		
Kaiyima	Mar. 9-15	1		
Spain:				
Barcelona	Nov. 27-Dec. 31		5	
Do	Mar. 19-25		1	
Cadiz	Nov. 1-Dec. 31		51	
Do	Jan. 1-Feb. 28		10	
Madrid	Year 1924		40	
Do	January-February		13	
Malaga	Nov. 23-Jan. 3		97	
Do	Jan. 4-May 2		98	
Valencia	Nov. 30-Dec. 6	2		
Do	Feb. 15-May 2	6		
Straits Settlements:				
Singapore	Feb. 22-Apr. 4	4	1	

CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER—Continued

Reports Received from December 27, 1924, to May 29, 1925—Continued

SMALLPOX—Continued

Place	Date	Cases	Deaths	Remarks
Switzerland:				
Berne	Mar. 15-Apr. 11	4		
Lucerne	Nov. 1-Dec. 31	19		
Do	Jan. 1-31	24		
Syria:				
Aleppo	Nov. 23-Dec. 27	13		
Do	Jan. 4-Feb. 28	71	18	
Beirut	Feb. 11-20	1		
Do	Apr. 1-10	1		
Damascus	Jan. 6-13	2		
Do	Feb. 11-20	22		
Tripoli:				
Tripoli	July 14-Jan. 2	53		
Tunis:				
Tunis	Nov. 25-Dec. 29	42	35	
Do	Jan. 1-Apr. 22		325	
Do	Apr. 30-May 6		13	
Turkey:				
Constantinople	Dec. 13-19	5		
Do	Mar. 16-Apr. 15	5	1	
Union of South Africa				Nov. 1-Dec. 31, 1924: Cases, 14. Jan. 1-31, 1925: Cases, 4—natives.
Cape Province	Feb. 1-21			Outbreaks.
De Aar District	Jan. 25-31			Outbreak at railway camp.
Do	Nov. 9-Jan. 17			Outbreaks.
Natal	Mar. 1-7			Do.
Orange Free State	Nov. 2-8			Do.
Ladybrand District	Jan. 15-31			Outbreak on farm.
Transvaal	Nov. 9-Jan. 10			Do.
Do	Feb. 1-21			Outbreaks.
Uruguay				January-June, 1924: Cases, 101; deaths, 2.
Do				July-November, 1924: Cases, 53; deaths, 2.
Yugoslavia	Year 1924	330	64	
Do	Jan. 1-Feb. 28	6	1	
Belgrade	Mar. 1-Apr. 7	6		
On vessel:				
S. S. Eldridge	Mar. 23	1		At Port Townsend, from Yokohama and ports.
S. S. Habana	Feb. 18	1		At Santiago de Cuba, from Kingston, Jamaica.
S. S. Ruyth				At St. Malo, France, January, 1924, from Sfax, Tunis; believed to have imported smallpox infection.

TYPHUS FEVER

Algeria				July 1-Dec. 20, 1924: Cases, 101; deaths, 14.
Algiers	Nov. 1-Dec. 31	5	1	
Do	Jan. 1-Apr. 20	14	7	In villages, department of Algiers: Cases, natives, 24; Europeans, 3.
Argentina:				
Rosario	Jan. 1-31		1	
Bolivia:				
La Paz	Nov. 1-Dec. 31	3		
Do	Jan. 1-31	2		
Do	Mar. 1-31	1		
Bulgaria				January-June, 1924: Cases, 191; deaths, 28.
Do				July-October, 1924: Cases, 5.
Chile:				
Concepcion	Nov. 25-Dec. 1		1	
Do	Jan. 6-12		2	
Do	Jan. 27-Feb. 2		1	
Iquique	Nov. 25-Dec. 1		2	
Do	Feb. 1-Mar. 28		2	
Talcahuano	Nov. 16-Dec. 20		5	
Do	Jan. 4-10		1	
Valparaiso	Nov. 25-Dec. 7		4	
Do	Jan. 11-Mar. 28		17	

CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER—Continued

Reports Received from December 27, 1924, to May 29, 1925—Continued

TYPHUS FEVER—Continued

Place	Date	Cases	Deaths	Remarks
China:				
Antung	Mar. 16–22	1		
Chosen:				
Chemulpo	Feb. 1–28	1		
Seoul	Nov. 1–30	1	1	
Do	Feb. 1–Mar. 31	6	2	
Czechoslovakia				December, 1924: Cases, 5.
Do	Jan. 1–31	14		
Egypt:				
Alexandria	Dec. 3–9	1	1	
Do	Mar. 12–18	1		
Cairo	Oct. 1–Dec. 23	13	8	
Do	Jan. 22–28	1		
Esthonia				Dec. 1–31, 1924: Cases, 5.
Do	Jan. 1–31	4		
France				July–October, 1924: Cases, 7.
Gold Coast				Oct. 1–31, 1924: 1 case.
Greece				May–June, 1924: Cases, 116; deaths, 8.
Do				July–December, 1924: Cases, 40; deaths, 4.
Athens	Feb. 1–Apr. 10		10	
Saloniki	Nov. 17–Dec. 15	3	2	
Do	Jan. 25–31	1		
Japan				Aug. 1–Nov. 15, 1924: Cases, 2.
Latvia				October–December, 1924: Cases, 30. Feb. 1–28, 1925; Cases, 11.
Lithuania				August–October, 1924: Cases, 15; deaths, 1.
Do				Jan. 1–31, 1925: Cases, 27; deaths, 2.
Mexico:				
Durango	Dec. 1–31		1	
Do	Mar. 15–Apr. 30	1	2	
Guadalajara	Dec. 23–29		1	
Mexico City	Nov. 9–Jan. 3	80		Including municipalities in Federal District.
Do	Jan. 11–Apr. 25	96		
San Luis Potosi	Mar. 8–14		1	
Do	Apr. 26–May 2		1	
Morocco				November, 1924: Cases, 5.
Palestine				Nov. 12–Dec. 29, 1924: Cases, 10.
Ekron	Dec. 23–29	1		
Jerusalem	do	2		
Do	Jan. 20–26	1		
Mikveh Israel	do	1		
Petach-Tikvah	Mar. 24–30	1		
Ramleh	Feb. 10–Mar. 23	2		
Tiberias	Feb. 24–Mar. 2	2		
Peru:				
Arequipa	Nov. 24–Dec. 31		3	
Do	Mar. 1–31		1	
Poland				Sept. 28, 1924–Jan. 3, 1925: Cases, 751; deaths, 57. Jan. 4–Feb. 11, 1925: Cases, 827; deaths, 68.
Portugal:				
Lisbon	Dec. 29–Jan. 4		2	
Do	Apr. 6–12		1	
Oporto	Jan. 4–Feb. 7	2		
Rumania				January–June, 1924: Cases, 2,906; deaths, 328.
Do				July–December 1924: Cases, 288; deaths, 38.
Constanza	Dec. 1–20	1		
Do	Feb. 1–28	2		
Russia				Jan. 1–June 30, 1924: Cases, 95,682.
Leningrad	June 29–Nov. 22	12		July–November, 1924: Cases, 34,729.
Spain:				
Madrid	Year 1924		3	
Malaga	Dec. 21–27		1	
Sweden:				
Goteborg	Jan. 18–Feb. 28	2		
Tunis				July 1–Dec. 20, 1924: Cases, 40.
Tunis	Mar. 5–25	9	1	
Do	Apr. 2–May 6	25	5	
Turkey:				
Constantinople	Nov. 15–Dec. 19	6	1	
Do	Jan. 2–Mar. 7	9	1	

CHOLERA, PLAGUE, SMALLPOX, TYPHUS FEVER, AND YELLOW FEVER—Continued

Reports Received from December 27, 1924, to May 29, 1925—Continued

TYPHUS FEVER—Continued

Place	Date	Cases	Deaths	Remarks
Union of South Africa				Nov. 1-Dec. 31, 1924: Cases, 345; deaths, 87. Jan. 1-Feb. 28, 1925: Cases, 159; deaths, 17; native. In white population cases, 12.
Cape Province	Nov. 1-Dec. 31	126	24	
Do	Jan. 1-Mar. 15	74	9	
Do	Mar. 22-Apr. 4			Outbreaks.
East London	Nov. 16-22	1		
Do	Jan. 18-Apr. 4	3	2	
Port Elizabeth	Feb. 22-Mar. 7	1	1	
Natal	Nov. 1-Dec. 31	130	50	
Do	Jan. 1-Feb. 28	43	5	
Do	Mar. 1-Apr. 4			Do.
Durban	Feb. 15-Mar. 28	4		
Orange Free State	Nov. 1-Dec. 31	59	8	
Do	Jan. 1-Feb. 28	32	3	Native.
Transvaal	Nov. 1-Dec. 31	30	5	
Do	Jan. 1-Feb. 28	10		Do.
Yugoslavia				Year 1924: Cases, 319; deaths, 22. Jan. 1-Feb. 28, 1925: Cases, 87; deaths, 8.
Belgrade	Nov. 24-Dec. 28	5		
Do	Apr. 8-14	2		

YELLOW FEVER

Gold Coast	October-November, 1924.	4	4	
Salvador:				
San Salvador	June-October, 1924.	77	28	Last case, Oct. 22, 1924.