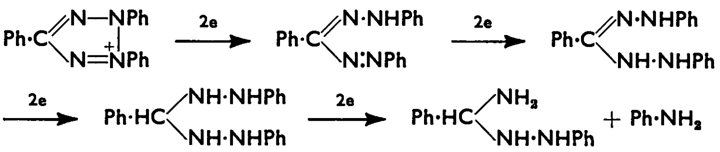
603. *Tetrazolium Compounds. Part V.* Polarography of Triphenyltetrazolium Bromide and Some of its Substituted Derivatives.*

By H. Campbell and P. O. Kane.

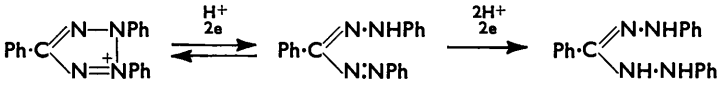
Triphenyltetrazolium bromide gives anomalous polarographic waves in aqueous solution, believed to be due to adsorption effects. Use of 50% methanol–water solutions gives more regular polarograms, and up to 4 waves are obtained involving a total of 8 electrons per molecule. The postulated reduction reactions, ionisation changes being neglected, are as follows, at high pH values :



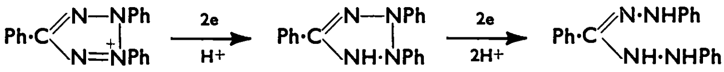
At low pH values the first two reactions occur at the same potential. From the dependence of half-wave potential on pH, probable mechanisms of reduction are discussed. Substituted triphenyltetrazolium salts behave similarly to the parent compound. The relations of half-wave potential to structure and bacteriostatic activity are discussed.

Although considerable interest has been shown in the reduction of substituted triphenyltetrazolium ions in living organisms (see Part I), it is only recently that physicochemical studies of the oxidation–reduction properties of these compounds have been made under experimental conditions which allow significant conclusions to be drawn. Jambor,[1] who has reviewed the earlier literature, made a polarographic study of aqueous solutions of triphenyltetrazolium chloride over the potential range 0 to about $-1{\cdot}0$ v *versus* the normal calomel electrode and has concluded that the mechanism is :

(i) At pH >6, two separate reduction waves, corresponding to two successive reactions, the first being thermodynamically reversible :



(ii) At pH <6, one reduction wave corresponding to two successive reactions, the half-wave potentials of which are not sufficiently different to allow separation into two waves (an adsorption forewave is present in the pH range 5·5—8·5) :



Although there is considerable agreement between the experimental results and conclusions of the present authors and those of Jambor, there are important points of disagreement which are :

(*a*) The absence of a flat residual current curve at high pH values from Jambor's polarograms (*e.g.*, his Fig. 8, curve 1, and Fig. 9, curves 8—11) suggests that there were impurities in his solutions which were oxidised at potentials close to the half-wave potential of the triphenyltetrazolium ion. Such impurities may well affect the shape of the reduction waves. They may also account for the oxidation wave of triphenylformazan in alkaline ethanol, reported by Jambor as evidence for the thermodynamic reversibility of the reduction of triphenyltetrazolium chloride to triphenylformazan. We have found no evidence for such an oxidation wave (see our Fig. 3).

---

* Part IV, *J.*, 1954, 2968.

[1] Jambor, *Acta Chim. Acad. Sci. Hung.*, 1954, **4**, 55.

(*b*) We cannot agree with Jambor's interpretation of the effect of pH on half-wave potential. In our opinion, there is no evidence from either our results or Jambor's for postulating the formation of 1 : 2-dihydro-2 : 3 : 5-triphenyltetrazole in the reduction of the triphenyltetrazolium ion at low pH values.

Since there is this disagreement between Jambor and the present authors and since our studies cover a wider range of reduction potentials and include a number of substituted triphenyltetrazolium ions, we are presenting our experimental results in detail in this paper.

EXPERIMENTAL METHODS

Triphenyltetrazolium bromide and the substituted compounds listed in Table 5 (p. 3138) have been examined. The latter were bromides (in most experiments), chlorides, and sulphates and the results obtained were independent of the anion. The synthesis of these compounds is described in Part I of this series (see Table 5).

A manually operated polarograph, built in this laboratory, was used. Half-wave potentials were measured in volts relative to the saturated calomel electrode and were corrected for ohmic potential differences across the polarographic cell. Currents were measured in microamperes (μA). Limiting currents are measured as the difference between the value of the current plateau or inflexion point and the residual current of the supporting electrolyte. Measurements were made in buffered solutions at 25° ± 0·2°. 0·002% Carbowax 4000 (a polyethylene glycol of molecular weight *ca.* 4000) was used as maximum suppressor.[2] pH values were measured with a Doran glass electrode and Cambridge pH meter. Calibration of the electrode with standard buffers showed that in the pH range 4—9·2 the measurements were accurate within ±0·02 pH. No corrections have been applied to the measured pH values of 50% methanol–water solutions. The apparatus for controlled potential electrolysis consisted of a potentiostat,[3] electrolysis cell,[4] and hydrogen–oxygen coulometer.[5]

The electrode characteristics were: mercury flow rate, 2·60 mg./sec.; drop time in 0·1N-potassium chloride on open circuit, 4·51 sec.

The parent compound, triphenyltetrazolium bromide, was investigated over the pH range 2—12 at one concentration and at pH 6·7 over a range of concentrations. Anomalous waves, believed to be due to adsorption, were obtained in aqueous solutions and were especially noticeable above pH 7. They were largely eliminated by the use of 50% methanol–water buffer solutions. On exposure of solutions to light, an additional wave was slowly produced which was shown to be due to the photochemical oxidation product of triphenyltetrazolium bromide which has been reported[6] as being 5-phenyl-2 : 3-(2 : 2′-diphenylene)tetrazolium bromide. Substituted triphenyltetrazolium ions were studied only at pH 6·7 in aqueous 10^{-4}M-solution.

The controlled-potential electrolysis apparatus was used for preparing reduction products and for the determination of the value, *n*, of the number of electrons per molecule involved in the electrode reaction. Lingane[5] claims that by this method he can distinguish between $n = 17$ and $n = 18$ for picric acid, an error of less than 5% being implied. In our experience, however, when the reduction product is readily oxidised by oxygen contained in the nitrogen bubbling through the solution, as in the present case, a positive error of as much as 30% can arise if " nitrogen free from oxygen " (British Oxygen Company; <10 p.p.m. of oxygen) is used.

RESULTS AND DISCUSSION

Triphenyltetrazolium Bromide.—Polarograms. Fig. 1*a* shows polarograms of triphenyltetrazolium bromide at a constant $0{\cdot}229 \times 10^{-3}$M-concentration in water over the pH range 1·83—8·34, which are in limited agreement with those of Jambor. At pH values <4·07, two waves (β and γ) are present, and at pH values >4·91 a third wave (δ) is revealed by the displacement of the hydrogen-wave to more negative potentials. With increasing pH, the β-wave splits, giving a small fore-wave (α) at pH 6·22 and again at pH 7·08 (β' and β''). At a constant pH of 6·7 a similar splitting of the β-wave occurs on increasing the concentration, as is shown in Fig. 1*b*. At pH 8·34, a maximum which cannot easily be

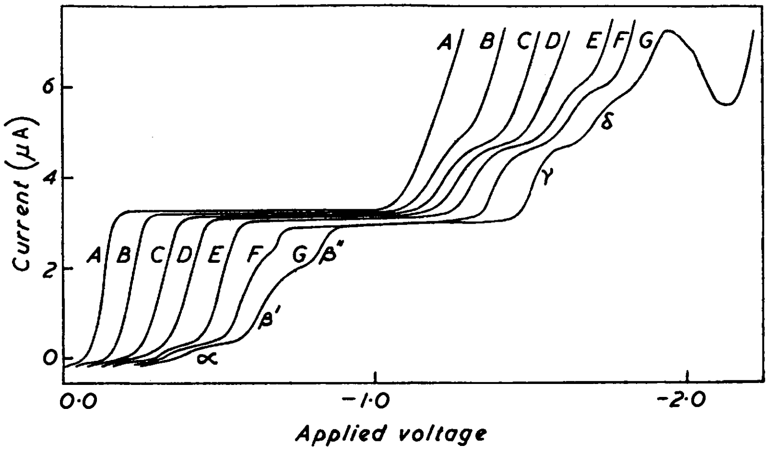
---

[2] Kane, Ph.D. Thesis, London, 1955.
[3] Hickling, *Trans. Faraday Soc.*, 1942, **38**, 27.
[4] Lingane, Swain, and Field, *J. Amer. Chem. Soc.*, 1943, **65**, 1348.
[5] Lingane, *ibid.*, 1945, **67**, 1916.
[6] Hausser, Jerchel, and Kuhn, *Chem. Ber.*, 1949, **82**, 195.

suppressed appears in the δ-wave. Polarograms cannot be determined in more alkaline solutions owing to large, irregular fluctuations of the galvanometer.

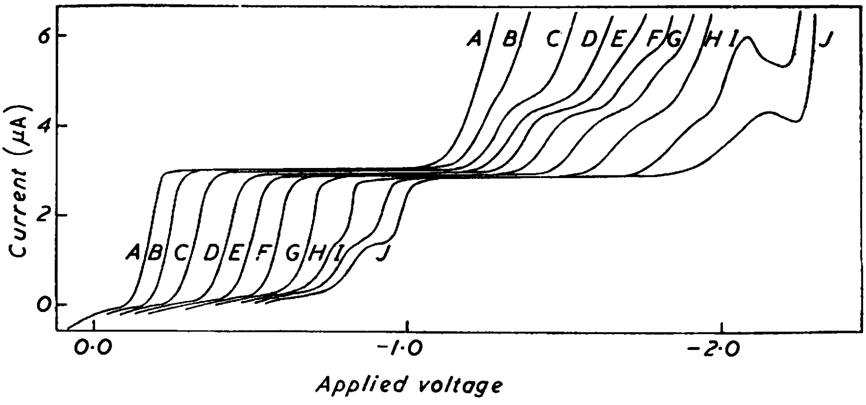
Further information about this anomalous splitting of the waves is obtained from the variation of the polarograms with concentration. The height of the α-wave is independent of concentration over the range of concentrations studied and is directly proportional to the mercury head on the dropping electrode. Brdicka [7] attributed a similar wave in the polarographic reduction of methylene-blue to adsorption of the reduction products on the mercury surface. If a similar explanation holds here, triphenyltetrazolium bromide is reduced to the same product in both the α- and the β-wave, the half-wave potential of the α-wave being less negative because the free energy of adsorption of the reduction product changes the overall free energy of the reduction process. The constant height of the α-wave is due to saturation of the mercury surface with the adsorbed reduction product, and the triphenyltetrazolium ions reduced in the α-wave cannot contribute to the β-wave. Consequently, as found experimentally (Fig. 2*a*), the limiting current β-wave should be

FIG. 1*a*. *Polarograms of triphenyltetrazolium bromide in aqueous solution.*



Triphenyltetrazolium bromide, 0·229 × 10^{-3}M. pH values : *A*, 1·83; *B*, 2·96; *C*, 4·07; *D*, 4·91; *E*, 6·22; *F*, 7·08; *G*, 8·34.

FIG. 1*b*. *Polarograms of triphenyltetrazolium bromide in 50% v/v aqueous methanol solution.*



Triphenyltetrazolium bromide, 0·243 × 10^{-3}M. pH values : *A*, 2·31; *B*, 2·94; *C*, 4·04; *D*, 5·41; *E*, 6·18; *F*, 7·17; *G*, 8·22; *H*, 9·14; *I*, 10·46; *J*, 11·45.

Voltage scale, correct for *A*, is displaced —0·04 v for each succeeding polarogram in Figs. 1*a* and *b*.

---

[7] Brdicka, *Coll. Czech. Chem. Comm.*, 1947, **19**, 522.

proportional to the concentration of the triphenyltetrazolium ion in the concentration range in which the β-wave does not split into multiple waves.

In Fig. 2*a*, the limiting currents of all the waves are plotted against the concentration of triphenyltetrazolium ion in aqueous solution at pH 6·7. Half-wave potentials are also given. The small limiting current of the α-wave has not been plotted. The γ- and the δ-waves are normal in that the limiting currents are proportional to concentration, and the half-wave potentials are very nearly independent of concentration. The half-wave potential of the β-wave at low, and of the β′-wave at high, concentrations is very nearly independent of concentration, indicating that they are due to the same electrode reaction. The limiting current of the β-wave at low, and of the β″-wave at high, concentrations is approximately proportional to concentration, indicating that the number of electrons per molecule involved in the electrode reaction and, hence, probably, in formation of the reduction product is the same for the β- and the β″-wave. At high concentrations, it appears that some of the triphenyltetrazolium ions are prevented from taking part in the normal electrode reaction causing the β- and the β′-wave and are then reduced at the more negative potentials of the β″-wave. Unlike the α-wave, where it has been postulated that a free energy of adsorption of the reduction product makes reduction easier than normally, the β″-wave must involve an additional reaction stage, the free energy of which makes the reduction more difficult than normally. This extra stage may be the adsorption, at high concentrations, of triphenyltetrazolium ions. At >1·0 × 10^{-3}M-concentration the polarograms again change in nature, the β′-, the γ-, and the δ-wave becoming merged into a single wave whose half-wave potential is between those of the γ- and the δ-wave and whose height is equal to the combined heights of the β′-, γ-, and δ-wave. No explanation can, at present, be advanced for this phenomenon.

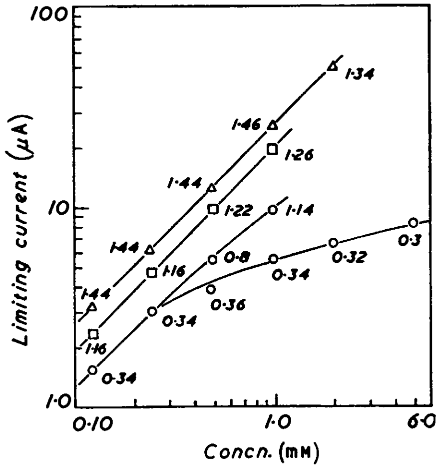


FIG. 2*a*. *Relations between limiting current and concentration for triphenyltetrazolium bromide in aqueous solution at* pH 6·7.

○ β-wave (β′ and β″ at high concn.). □ γ-wave. △ δ-wave.

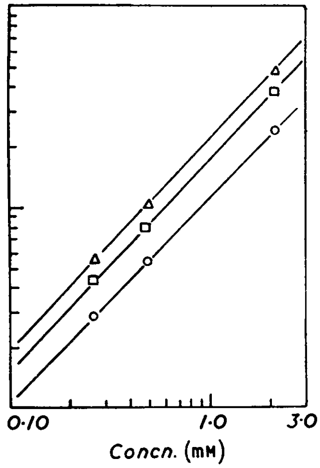


FIG. 2*b*. *Relations between limiting current and concentration for triphenyltetrazolium bromide in* 50% *v/v aqueous methanol at* pH ~7.

○ β-wave. □ γ-wave. △ δ-wave.

Numerals in Fig. 2*a* are negative values of half-wave potential.

Adsorption effects in the polarography of acridine derivatives [8] were eliminated by the use of a supporting electrolyte containing large concentrations of methanol. By using buffered 50% v/v methanol–water solutions of triphenyltetrazolium bromide, anomalous behaviour is considerably reduced (Figs. 1*b* and 2*b*). The α-wave is absent and the behaviour of the β-wave is normal to higher values than in the absence of methanol. Fig.

---

[8] Kaye and Stonehill, *J.*, 1951, 27, 2638.

2*b* shows that, over a wide range of concentration at about pH 7, the limiting currents for the β-, γ-, and δ-waves are proportional to concentration. In solutions more alkaline than about pH 8, the β-wave splits into two equal waves, β_1 and β_2, the electrode reactions of which do not involve adsorption as postulated for the β′- and β″-waves in the absence of methanol. The mechanism of the electrode reaction associated with β_1- and β_2-waves is discussed in greater detail below.

Number of electrons involved in electrode reactions. The number, *n*, of electrons involved in each electrode reaction is of value in establishing the nature of the reduction product associated with each polarographic wave. Two methods have been used to evaluate *n*, *viz.*, application of the Ilkovic equation, and coulometry.

The experimentally determined equivalent conductance at infinite dilution of aqueous solutions of triphenyltetrazolium bromide at 25° is 98·0. From the limiting conductance [9] at 25° of the bromide ion, 78·2, the ion conductance of triphenyltetrazolium is calculated as 19·8, and D_0 as $5{\cdot}29 \times 10^{-6}$ cm.^{2}/sec. This value is in good agreement with that obtained by Jambor [1] (5×10^{-6} cm.^{2}/sec.) from direct diffusion measurements in a Lamm diffusion apparatus. The diffusion coefficient for the triphenyltetrazolium ion in 50% v/v aqueous methanol was calculated as $3{\cdot}50 \times 10^{-6}$ cm.^{2}/sec. by using the Ilkovic equation and assuming that, at equal concentrations of triphenyltetrazolium ion below that at which anomalous effects appear, the difference in wave heights of the β-wave in aqueous and in methanolic solution is entirely due to differences in the diffusion coefficient. The diffusion current constant *D′*, where, with the usual symbolism,

$$D' = \bar{i}_d/cm^{2/3}t^{1/6} = 605nD^{\frac{1}{2}}$$

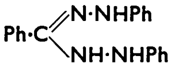
showed less than 2% variation with pH. The values of *n* in Table 1 were calculated. The value *n* = 4 for the β-wave was in agreement with Jambor's results.

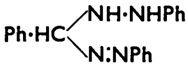
TABLE 1. *Diffusion current data and values of* n *for triphenyltetrazolium bromide.*

Wave	Solvent	pH range	*D′*	*n*
β	Water	1·8—6·2	5·50	3·95
γ	Water	4·0—8·3	8·35	6·00
δ	Water	6·2—7·1	11·1	7·98
β_1	50% Methanol	9·1—11·5	2·18	1·94
$\beta(\equiv\beta_2$ above pH 9)	50% Methanol	2·33—11·5	4·50	4·00
γ	50% Methanol	5·4—8·2	7·05	6·25
δ	50% Methanol	8·2—9·1	9·15	8·15

A value for *n* of 4·72 for the β-wave was obtained from the quantity of electricity required to reduce a given weight of triphenyltetrazolium bromide in aqueous 0·5N-sulphuric acid, with the cathode potential controlled to allow only the electrode reaction of the β-wave to occur and corrections for the small residual current due to electrolysis of the supporting electrolyte. Since the diffusion current of the β-wave does not vary appreciably with pH, this value of *n* which, in the present case, is a maximum value owing to errors discussed above, is not in disagreement with the value *n* = 4 given in Table 1.

Nature of the reduction processes. The reduction product of the β-wave, which unlike triphenylformazan, is colourless and soluble in 0·5N-sulphuric acid, has been prepared by controlled potential electrolysis. Its rapid oxidation in air to triphenylformazan made it difficult to determine its chemical properties but proves that the molecule is not split into two or more parts during the reduction associated with the α- and the β-wave, and that

(I) 

 (II)

2 of the 4 electrons are probably used in opening the tetrazolium ring. Since it is known that the phenyl group cannot be reduced at the dropping mercury electrode in the potential range 0 to —2·0 v, two structures (I) and (II) are possible for the full reduction product. These may be tautomeric forms; both contain the readily oxidised hydrazo-group. Apart

---

[9] International Critical Tables, 1926, Vol. VI, p. 230.

from the opening of the tetrazolium ring, they are formed by the reduction of N=N and C=N bonds and a comparison of the polarographic data for the triphenyltetrazolium ion, azobenzene, and benzylideneaniline (Table 2) favours (I) as the structure of the reduction

TABLE 2. *Half-wave potentials of azobenzene, benzylideneaniline, and triphenyltetrazolium ion at 25° in aqueous methyl alcohol (50% v/v) at ca.* pH 7.

Buffer, KH_2PO_4–Na_2HPO_4 (each 0·05M). Concn. of compound *ca.* 10^{-3} mole/l.

Compound	$-E_{\frac{1}{2}}$ (v)	Compound	$-E_{\frac{1}{2}}$ (v)
Ph·N=N·Ph	0·46	Triphenyltetrazolium ion (0·5 × 10^{-3}M)	0·44 (β wave)
Ph·N=CH·Ph	1·35		1·33 (γ wave)

product of the β-wave. The high concentration of methyl alcohol in solution probably affects the reproducibility of the solution junction potential, but the half-wave potential of the β-wave of the triphenyltetrazolium ion is very much more nearly in agreement with that of azobenzene than with that of benzylideneaniline.

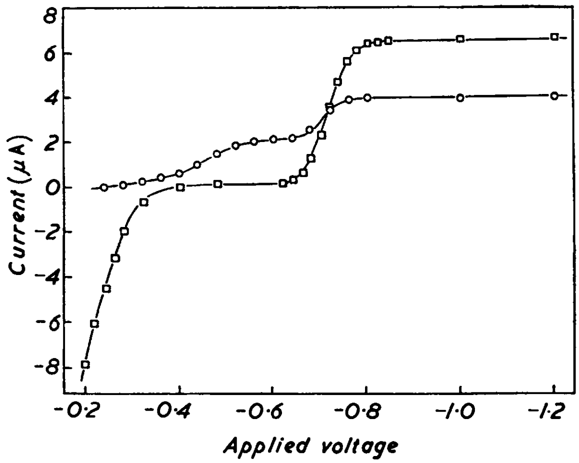
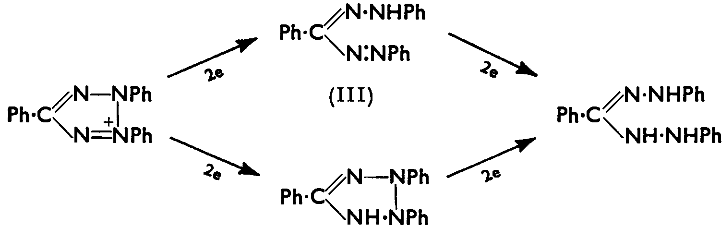


FIG. 3. *Polarograms of* 2·43 × 10^{-4}M-*triphenyltetrazolium bromide* (○) *and* 8·32 × 10^{-4}M-*triphenylformazan* (□) *in* 95% *methanol–water at high* pH.

Two reaction routes are possible for the two separate waves, each of 2 electrons, into which the β-wave splits in solutions more alkaline than about pH 8, both routes yielding structure (I) for the final reduction product. One route involves the reduction of triphenylformazan (III) so that a choice between these possible routes can be made from a comparison of the half-wave potentials of triphenylformazan with those of the β_1 and the β_2-wave. Owing to its extremely low solubility in solvents containing high proportions of water, triphenylformazan was studied in 95% v/v methanol. Since pH values in 95% methanol



are uncertain and solution junction potentials may be appreciable, polarograms (Fig. 3) of both triphenylformazan and the triphenyltetrazolium ion were determined in this solvent, with 0·2N-lithium hydroxide as supporting electrolyte. The concentrations were adjusted so that the currents flowing at the half-wave potentials of the β_2-wave of triphenyltetrazolium bromide and the single wave of triphenylformazan were approximately equal.

This minimised errors due to ohmic potential drop. Half-wave potential data from these polarograms are summarised in Table 3.

TABLE 3. *Polarographic data for the triphenyltetrazolium ion and triphenylformazan in 95% methanol at high* pH *values.*

Compound	$-E_{\frac{1}{2}}$ (v)	D'
Triphenyltetrazolium ion, β_1 wave	0·44	3·1
β_2 wave	0·70	6·2
Triphenylformazan	0·67	3·1

The single reduction wave of triphenylformazan corresponds reasonably closely with the β_2-wave, suggesting that the reduction mechanism is the same and that triphenylformazan is the reduction product of the β_1-wave. The absence of an oxidation wave of half-wave potential —0·44 v indicates that the reduction of the triphenyltetrazolium ion to triphenylformazan is thermodynamically irreversible. This is in complete disagreement with Jambor's results [1] for ethanol–water solutions. It is considered very unlikely that this change of solvent would alter a reversible to an irreversible reduction.

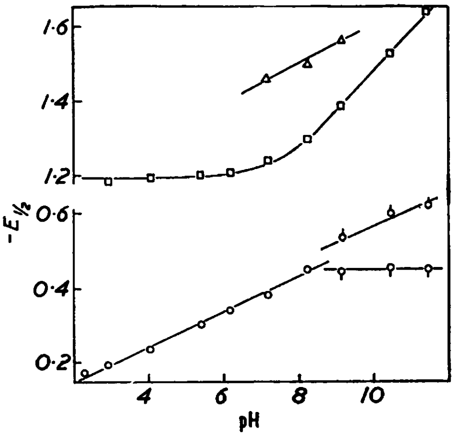


FIG. 4. *Relation between half-wave potential and* pH *for triphenyltetrazolium bromide in* 50% *v/v methanol.*

○ β-wave. ♀ β_1-wave. ♂ β_2-wave. □ γ-wave. △ δ-wave.

No attempt was made to isolate the reduction products of the γ- and the δ-waves. The agreement between the half-wave potentials of the γ-wave and of benzylideneaniline (Table 2) suggests that the C=N bond of structure (I) is reduced, giving $Ph{\cdot}CH(NH{\cdot}NHPh)_2$. The δ-wave must represent rupture of the molecule, and one of the hydrazo-bonds may be reduced since these are probably the weakest in the molecule:

$$Ph{\cdot}CH(NH{\cdot}NHPh)_2 \xrightarrow{2e} NH_2{\cdot}CHPh{:}NH{\cdot}NHPh + Ph{\cdot}NH_2$$

Fig. 4 shows the effect of pH on the half-wave potentials of the triphenyltetrazolium ion. The results were obtained from Fig. 1*b*, and were in substantial agreement with Jambor's although showing much less scatter. Kaye and Stonehill [8] have developed somewhat cumbersome equations to express the pH-dependence of half-wave potential for acridine derivatives. Kane [2] has simplified these equations. Considering a reversible electrode reaction of the type,

$$A + ne + qH^+ \rightleftharpoons B$$

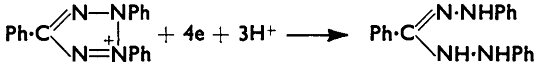
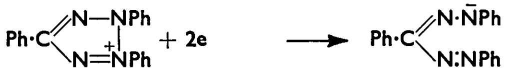
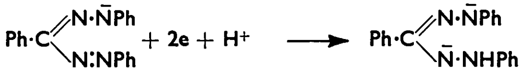
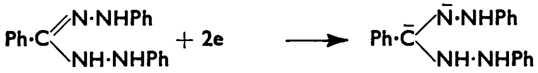
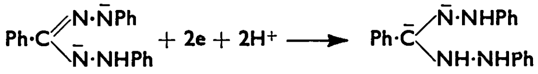
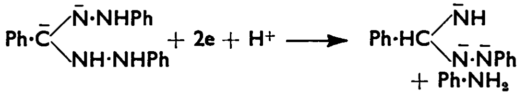
we then have:

$$E_{\frac{1}{2}} = E_0 - 0{\cdot}059q/n \text{ pH at } 25° \quad . \quad . \quad . \quad . \quad . \quad . \quad (3)$$

This equation is a generalisation of those derived by Kaye and Stonehill.[8] The half-wave potential varies rectilinearly with pH when q is constant. This occurs when both oxidant and reductant show either no change or an identical change of degree of ionisation with pH. From the slope of this straight line (referred to below as the pH slope of the wave) and the known value of n, the value of q can be calculated. If the ionic state of one level of oxidation is known, that of the other may be calculated. Although equation (3) is strictly valid only for reversible reactions, it is frequently found that electrode reactions which are, overall, irreversible obey this equation or its less generalised forms approximately.[2, 8] Occasionally, large discrepancies between theory and experiment are found. It is, therefore, of interest to apply equation (3) to the reduction steps of the triphenyltetrazolium ion and on this basis the reductions given in Table 4 are considered most probable. There is only one serious divergence between theory and experiment, *viz.*, the γ-wave at pH 9—12. Although the pH slope of the wave (—0·100 v) suggested values of q/n of $\frac{3}{2}$ (—0·089 v) or $\frac{4}{2}$ (—0·118 v) rather than $\frac{2}{2}$ (—0·059 v), the following argument shows that the last value is probably correct. Over the pH range 9—12, the compound being reduced in the γ-wave must be the same compound, in the same state of ionisation, as the reduction product of the β_2-wave. This compound is known from the pH-dependence of the β_1- and the β_2-wave to possess two negative charges. In the pH range 2—8, the reduction product of the γ-wave has been shown to possess two negative charges and cannot, therefore, be less negative at higher pH values. Consequently, not more than two hydrogen ions can be added over the pH range 9—12.

The formulation of reduction products with as many as three negative charges is not to be taken as indicating that these structures are necessarily stable ions which have a free existence, but, more probably, that the rate of addition of hydrogen ions is much slower than the electron addition which is the potential-determining step.[2] Kaye and Stonehill [8] have postulated equally improbable ions as reduction products of acridines.

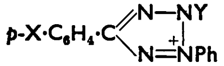
TABLE 4. *Probable reduction reactions of triphenyltetrazolium bromide.*

Wave	pH range	Probable reaction	pH slope (volts) Theoretical	pH slope (volts) Experimental
β	2—8	 $\mathrm{Ph{\cdot}C}\Big\langle\begin{matrix}\mathrm{N{-}NPh}\\ \mathrm{N{=}\overset{+}{N}Ph}\end{matrix} + 4e + 3H^+ \longrightarrow \mathrm{Ph{\cdot}C}\Big\langle\begin{matrix}\mathrm{N{\cdot}NHPh}\\ \mathrm{NH{\cdot}NHPh}\end{matrix}$	—0·045	—0·045
β_1	9—12	 $\mathrm{Ph{\cdot}C}\Big\langle\begin{matrix}\mathrm{N{-}NPh}\\ \mathrm{N{=}\overset{+}{N}Ph}\end{matrix} + 2e \longrightarrow \mathrm{Ph{\cdot}C}\Big\langle\begin{matrix}\mathrm{N{\cdot}\bar{N}Ph}\\ \mathrm{N{:}NPh}\end{matrix}$	0	0
β_2	9—12	 $\mathrm{Ph{\cdot}C}\Big\langle\begin{matrix}\mathrm{N{\cdot}\bar{N}Ph}\\ \mathrm{N{:}NPh}\end{matrix} + 2e + H^+ \longrightarrow \mathrm{Ph{\cdot}C}\Big\langle\begin{matrix}\mathrm{N{\cdot}\bar{N}Ph}\\ \mathrm{\bar{N}{\cdot}NHPh}\end{matrix}$	—0·030	—0·037
γ	2—8	 $\mathrm{Ph{\cdot}C}\Big\langle\begin{matrix}\mathrm{N{\cdot}NHPh}\\ \mathrm{NH{\cdot}NHPh}\end{matrix} + 2e \longrightarrow \mathrm{Ph{\cdot}\bar{C}}\Big\langle\begin{matrix}\mathrm{\bar{N}{\cdot}NHPh}\\ \mathrm{NH{\cdot}NHPh}\end{matrix}$	0	0
γ	9—12	 $\mathrm{Ph{\cdot}C}\Big\langle\begin{matrix}\mathrm{N{\cdot}\bar{N}Ph}\\ \mathrm{\bar{N}{\cdot}NHPh}\end{matrix} + 2e + 2H^+ \longrightarrow \mathrm{Ph{\cdot}\bar{C}}\Big\langle\begin{matrix}\mathrm{\bar{N}{\cdot}NHPh}\\ \mathrm{NH{\cdot}NHPh}\end{matrix}$	—0·059	—0·100
δ	7—9	 $\mathrm{Ph{\cdot}\bar{C}}\Big\langle\begin{matrix}\mathrm{\bar{N}{\cdot}NHPh}\\ \mathrm{NH{\cdot}NHPh}\end{matrix} + 2e + H^+ \longrightarrow \mathrm{Ph{\cdot}HC}\Big\langle\begin{matrix}\mathrm{\bar{N}H}\\ \mathrm{\bar{N}{\cdot}\bar{N}Ph}\end{matrix} + \mathrm{Ph{\cdot}NH_2}$	—0·030	—0·03

Substituted Triphenyltetrazolium Salts.—Results. The half-wave potentials and data on bacteriostatic activity are given in Table 5.

(a) *Effect of structure on half-wave potential.* For any reversible four-electron/molecule reduction, a change in redox potential of 0·100 v is equivalent to a change in free energy of reduction of 10 kcal./mole. If it is assumed that a similar relation holds for thermodynamically irreversible reductions, it follows that the effect of *para*-substituents on the free energy of reduction of the triphenyltetrazolium ion was less than ±3 kcal./mole. If these changes of free energy are wholly associated with changes in potential energy of the tetrazolium molecule (whether by inductive or mesomeric effect of substituents) then such potential energy changes are quite small. The only *ortho*-substituted compound investigated (the penultimate one of Table 5) possessed the most negative half-wave potential for the β-wave and is in broad agreement with the results of Jerchel and Mohle [10] who found that in a series of 5-alkyl-2 : 3-diaryltetrazolium compounds *ortho*-substituents caused much greater changes of reduction potential than *para*-substituents. Although, there are no data on the resonance energy of tetrazolium compounds, the value for 1 : 3 : 5-triphenylbenzene is 25 kcal./mole, excluding Kekulé resonance.[11] The value for the triphenyltetrazolium ion excluding Kekulé resonance, and that of the tetrazolium ring might be expected to be less than this since steric factors prohibit coplanarity of the molecule. This is borne out by the results of Jerchel and Mohle [10] who found that replacing the 5-phenyl group of triphenyltetrazolium chloride by methyl or carboxyl changed the reduction potential by less than 0·01 v in either case. It is clear, then, that no profound changes of half-wave potential with simple substitution in the benzene rings are to be expected, which is borne out by Table 5.

TABLE 5. *Half-wave potential, structure, and bacteriostatic activity of substituted triphenyltetrazolium derivatives.**



		$-E_{\frac{1}{2}}$ (v *vs.* S.C.E.) at pH 6·7				Max. diln.† for bacteriostatic activity	
X	Y	α	β	γ	δ	*S. aureus*	*B. coli*
H	Ph	0·15	0·35	1·15	1·5[a]	1 : 8,000	<1 : 2,000
NH_2	Ph	0·20	0·35	1·09	1·5[a]	1 : 16,000	<1 : 500
NHAc	Ph	0·13	0·34	1·10	[b]	1 : 2,000	<1 : 2,000
NO_2	Ph	0·08	0·38	1·17	1·6[a]	1 : 32,000	1 : 1,000
p-NHAc·C_6H_4·SO_2·NH	Ph	0·18	0·36	1·22	[b]	N.T.	N.T.
p-NH_2·C_6H_4·SO_2·NH	Ph	0·17	0·35	1·12	[b]	1 : 8,000	1 : 8,000
NC	Ph	[b]	0·36	[b]	[b]	N.T.	N.T.
Br	*p*-C_6H_4Br	[b]	0·34	1·10	[b]	N.T.	N.T.
H	*p*-NHAc·C_6H_4	0·12	0·36	1·12	1·4[a]	1 : 8,000	<1 : 2,000
H	*p*-C_6H_4Cl	0·12	0·32	1·14	1·4[a]	1 : 16,000	1 : 125
H	*p*-$C_6H_4Pr^i$	0·16	0·35	1·12	1·4[a]	N.T.	N.T.
H	*p*-C_6H_4Ph	[c]	0·33	1·11	1·4[a]	1 : 256,000	1 : 4,000
H	4 : 3-NH_2·C_6H_3Cl	0·12	0·38	1·14	1·40	1 : 128,000	1 : 1,000
H	4 : 2-NH_2·C_6H_3Cl	0·14	0·42	1·13	1·41	1 : 32,000	1 : 1,000
H	4 : 3-NH_2·C_6H_3(OH)	[c]	0·41	1·17	1·40	1 : 32,000	1 : 1,000

[a] Maximum. [b] Wave overlapped by other waves. [c] Wave absent or too small for accurate measurement.

* Ref. for the 1st compound : von Pechmann and Runge, *Ber.*, 1894, **27**, 2920. Ref. for the others : Ashley, Davis, Nineham, and Slack, *J.*, 1953, 3881.

† Freeman, Pattinson, Alexander, and Grose, personal communication; N.T. = not tested.

Relation between half-wave potential and bacteriostatic activity. Bacteriostatic and biological activity is always associated with the formation of triphenylformazan, which has no separate existence during polarographic reduction of triphenyltetrazoliun salts at pH 7. The α- and the β-wave are believed to include, as part of the electrode reaction, the opening of the tetrazolium ring such as occurs in reduction to triphenylformazan. The α-wave is characteristic of the reduction under the special conditions of adsorption of the reduction product on a mercury surface. This condition is, of course, not present in biological systems and must, therefore, be ignored. Although the β-wave involves reduction beyond triphenylformazan, its half-wave potential can probably be regarded as a measure of the ease of reduction to triphenylformazan. If the half-wave potential of the ring-opening and the other stage of the β-wave electrode reaction were markedly different, it would be expected that the single β-wave should be replaced by two separate waves, as is found in more alkaline solution. The thermodynamic irreversibility of the reduction does not allow complete identity between half-wave and redox potentials to be assumed. The results in Table 5 show that, whereas there is a wide variation in bacteriostatic activity, there is little variation in the half-wave potential of the β-wave and there is no correlation between bacteriostatic activity and half-wave potential. If the bacteriostatic activity

---

[10] Jerchel and Mohle, *Ber.*, 1944, **77**, 591.

[11] Pauling and Sherman, *J. Chem. Phys.*, 1933, **1**, 606.

of triphenyltetrazolium salts is due to their acting as electron-acceptors for an enzyme system, then some factor such as availability at the enzyme " site of action," rather than reduction potential, must be influencing the bacteriostatic activity. Alternatively, the the active species may be triphenylformazan, the soluble triphenyltetrazolium ion being a method of transport to the enzyme. Data on the reduction potentials of substituted triphenylformazans corresponding to the tetrazolium salts of Table 5 are not, as yet, available.

The authors thank the Directors of May & Baker Ltd. for permission to publish these results.

MESSRS. MAY & BAKER LTD., DAGENHAM, ESSEX. [*Received, November* 18*th*, 1955.]