requires 40–50 minutes baking, and often a little extra time is given it, in an attempt to prevent the subsequent development of 'rope'— a not infrequent occurrence in our climate. The oven temperatures are, if anything, a little lower for 'Standard' bread than for white bread which, however, requires only about thirty minutes baking. One is therefore led to the conclusion that it is the longer period of heating which brings about the greater degree of destruction of thiamin. Published data support this contention[7,8].

It follows from our findings that, while 'Standard' bread has some advantages over an 85 per cent extraction loaf, increased thiamin content is not one of them.

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[1] *Proc. Nutr. Soc.*, **4**, 1 (1946).
[2] Holman, W. I. M., *Nutr. Abstr. Rev.*, **15**, 387 (1946).
[3] Golberg, L., Thorp, J. M., and Sussman, S., *S. Afric. J. Med. Sci.*, **10**, 87 (1945).
[4] Medical Research Council. War Memorandum No. 14. "Nutritive Values of Wartime Foods." (Tables compiled for the Accessory Food Factors Committee.) (H.M. Stationery Office, 1945.)
[5] Ministry of Food. Scientific Adviser's Division, *Nature*, **153**, 154 (1944).
[6] Golberg, L., Thorp, J. M., and Sussman, S., *S. Afric. J. Med. Sci.*, in the press.
[7] Schultz, A. S., Atkin, L., and Frey, C. N., *Cereal Chem.*, **19**, 532 (1942).
[8] Dawson, E. R., and Martin, G. W., *J. Soc. Chem. Ind.*, **61**, 13 (1942).

Polarographic Behaviour of Adenine

WORK in this laboratory on differences in mechanism between normal and malignant cells has shown the great need for improved methods of analysis and characterization of nucleic acids. Investigations which I began before the War indicated to me the value of the polarograph as an analytical tool in the study of inorganic cell constituents, and there was reason to believe that it had great possibilities in the organic field. Excellent accounts of general polarographic methods are now available[1,2].

Accordingly the behaviour of certain purine and pyrimidine bodies at the dropping mercury electrode was studied. Pech[3] had stated that compounds belonging to the purine group were not reducible at this electrode, with the exception of uric acid, which was reducible only after prolonged exposure to air in solution with lithium carbonate.

The present investigations show quite definitely that adenine, adenosine, adenylic acid and an adenine-cytosine dinucleotide are all reducible at the dropping mercury cathode and give well-defined diffusion currents or 'polarographic waves' when in solution in $0\cdot1$ N perchloric acid, and also in buffer solutions of perchloric acid and potassium perchlorate of similar concentration. Under the same conditions, no reduction occurs and no waves are obtained for guanine, guanosine, guanylic acid, or for cytidine or cytidylic acid, or for uracil. Pure cytosine was not available and was not tested, but for reasons given later in connexion with adenine, it seems unlikely that
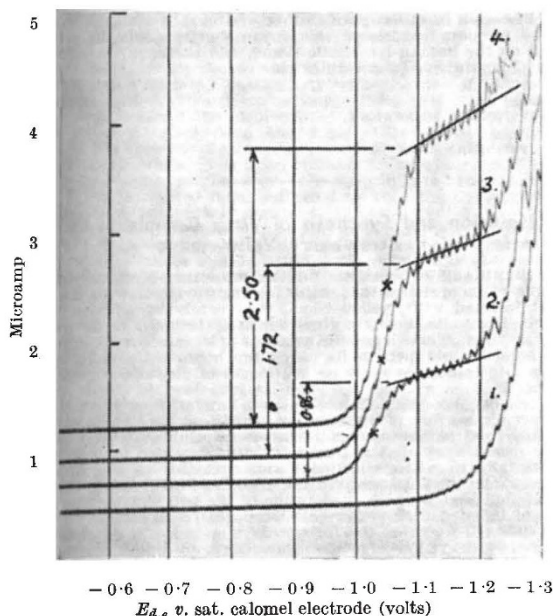
cytosine will be reducible if cytidine and cytidylic acid are not. Thymine was not available for test, but as 5-methyl uracil it is unlikely to be reduced when uracil itself is not.

An extensive study was made of the reduction at the dropping mercury cathode of adenine, adenosine and adenylic acid. In all three cases the diffusion current (i_d) was proportional to concentration (C) over the ranges investigated, C being throughout of the order of 10^{-4} M. For adenine in concentrations between $0\cdot50 \times 10^{-4}$ M and $1\cdot96 \times 10^{-4}$ M the value of i_d/C was $17\cdot0$ μA./millimol. per litre for the particular capillary used and at $25°$ C. The value of i_d/C for the other compounds decreases with increasing complexity, that is, in the order adenine, adenosine, adenylic acid, and the adenine-cytosine dinucleotide. There is no systematic variation of i_d/C with pH for any of these compounds.

The half-wave potentials ($E_{1/2}$) are not constant but become more negative with increasing concentration. For adenine in perchlorate buffer of pH $1\cdot30$ at $25°$ C., $E_{1/2}$ varies from $-1\cdot046$ to $-1\cdot073$ volts $\pm$ 3 mV. v. the saturated calomel electrode over the concentration range $0\cdot50 \times 10^{-4}$ M to $1\cdot96 \times 10^{-4}$ M. Similar values are obtained for the other compounds.

The half-wave potentials vary in a linear fashion with hydrogen ion concentration, becoming increasingly negative as the pH increases. For adenine at a concentration of $0\cdot50 \times 10^{-4}$ M in perchlorate buffers of pH varying from $1\cdot30$ to $2\cdot24$, at $25°$ C., the value of $E_{1/2}$ changes from $-1\cdot046$ to $-1\cdot129$ volts $\pm$ 3 mV. v. the saturated calomel electrode. The other compounds behave similarly.

Temperature coefficients of both $E_{1/2}$ and i_d have been determined, and the values of the respective coefficients are similar for all three compounds.

The logarithmic plots of the waves of the three substances are very similar, log $i/(i_d - i)$ against $E_{d.e}$ being linear for the greater part of the wave. Calculations based on the slopes of these lines give fractional values for the number of electrons involved. For adenine $n = 1\cdot33$. This, together with the fact that $E_{1/2}$ varies linearly with pH, suggests that hydrogen ions are also involved. For a reversible reaction involving two hydrogen ions and two electrons, the plot of $E_{d.e}$ against log $(i_d - i)/i^2$ should be linear with a slope of $0\cdot0295$ at $25°$ C. All three substances give reasonably linear plots of $E_{d.e}$ against log $(i_d - i)/i^2$ over the whole wave, and the values of the slopes of these lines range from $0\cdot026$ for adenine to $0\cdot029$ for adenylic acid, in fair agreement with the theoretical value for such a reaction. The observed variation of $E_{1/2}$ with concentration is also to be expected from these linear plots. It must be pointed out, however, that the value of i_d/C for adenine is more than twice as large as the corresponding value for a divalent metal ion such as zinc, so that a larger number of electrons may be involved. Various experimental considerations point to the possibility of the reduction occurring at the double-bond linking nitrogen at position (1) with carbon at position (6) in the purine ring. It is thought that this reduction process may have considerable biological significance.

The evidence obtained shows that in all three substances, and presumably also in the adenine-cytosine dinucleotide, it is the adenine moiety which enters into the electro-reduction, and that the other adenine-containing compounds are so constituted that neither the sugar nor the phosphoric acid groups can prevent the reduction.

In conclusion, it is pointed out that the polarographic reduction of adenine enables it to be estimated quantitatively in the presence of any one or all of the other purine or pyrimidine bodies mentioned here. For quantities of the order of 1 microgram the accuracy of estimation is about $\pm$ 2 per cent, but it is possible to detect much smaller quantities than this. The method is of direct and simple application to the estimation of adenine in the hydrolysates of either ribose or desoxyribose nucleic acids. Work on these lines is being continued, and further details will be published later.

These investigations are being undertaken for the British Empire Cancer Campaign (Birmingham Branch).

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[1] Kolthoff, I. M., and Lingane, J. J., *Chem. Rev.*, **24**, 1 (1939).
[2] Kolthoff, I. M., and Lingane, J. J., "Polarography" (Interscience Publ., 1941).
[3] Pech, J., *Coll. Czechoslov. Chem. Commun.*, **6**, 126 (1934).

POLAROGRAMS OF ADENINE IN $0\cdot1$ N PERCHLORIC ACID AT $25°$ C., SHOWING CONSTANCY OF i_d/C AND VARIATION OF $E_{1/2}$ WITH CONCENTRATION. CONCENTRATIONS OF ADENINE: (1) ZERO, (2) $0\cdot50 \times 10^{-4}$ M, (3) $0\cdot99 \times 10^{-4}$ M, (4) $1\cdot48 \times 10^{-4}$ M. THE POINTS MARKED WITH A CROSS INDICATE HALF-WAVE POTENTIALS. THE CURRENT ZERO HAS BEEN SHIFTED FOR EACH TRACE

Utilization of Groundnut-Cake Hydrolysate as Medium for Production of Streptomycin

SINCE the announcement of the discovery of streptomycin[1] rapid progress has been made in the treatment of infectious diseases which are resistant to penicillin[2-9]. The studies of Schatz et al.[2], on the influence of the composition of the media on the production of the active agent by *A. griseus*, revealed that the formation of streptomycin requires the presence of a certain organic substance supplied by meat extract or corn steep liquor. The formation of streptomycin was not affected by the nature of the peptone used, though the addition of glucose increased the yield of the antibiotic. Recently, Le Page and Elizabeth Campbell[10] have found that yeast extract media fortified with minerals gives the most satisfactory production of the antibiotic with *A. griseus*.

For some time past we have been interested in the utilization of enzyme hydrolysate of groundnut-cake as a medium for the production of antibiotics from members of the Penicillium and Aspergilli groups. The encouraging results obtained with these led us to the study of the formation of the antibiotic from *A. griseus*.

Enzyme digest of groundnut-cake prepared under specific conditions was distributed in 5 ml. aliquots into sterile tubes 15 cm. long and $1\cdot7$ cm. in diameter and autoclaved at 15 lb. pressure for half an hour. The tubes were then inoculated with a spore suspension of *A. griseus*