The Use of Potassium Gluconate in Hypopotassemia

Adolph Bernhard

Achelis Laboratory, Lenox Hill Hospital, New York

The approach to the problem of hypopotassemia has been greatly simplified by the development of the flame photometer. Hypopotassemia has been found in diabetic acidosis, infantile diarrhea, and after major surgical operations. The prompt administration of potassium has been found to be extremely beneficial when such insufficiencies have been shown to exist.

The ideal substance for the administration of potassium would be a salt in which the anion of the compound exerts slight or no pharmacological action. Compounds of this nature would be the metallic salts of gluconic acid. The parent radical of this group of salts, gluconic acid, is chemically a penta-hydroxy caproic acid, which is derived by oxidation of the aldehyde group in the sugar D-glucose. Gluconic acid does not display any marked physiological action, and it has been shown that gluconic acid is tolerated without injury or disturbance to the digestive system. The radical is also of relatively low toxicity upon parenteral injection. One of these compounds, calcium gluconate, has been successfully employed during the past 20 years in the treatment of tetany and other calcium deficiencies.

This communication reports the use of another gluconate, the potassium salt, in hypopotassemia, and its method of preparation. Potassium gluconate[1] is a white crystalline powder having a mol wt of 234.24, and containing 16.7% potassium. It has a mild saline taste, is quite soluble in water, and is stable in air. A 30% solution of potassium gluconate is prepared with pyrogen-free triple-distilled water, filtered through a fritted Pyrex glass disk, transferred to 20-ml size ampules, and autoclaved.[2] Twenty ml of this solution contains 1 g of potassium (25.5 mEq). The solution has a pH of 7.0 and is colorless and stable. It may be added to the usual parenteral infusion mixtures. Although the usual source of potassium for both oral and parenteral administration has been potassium chloride, the gluconate is the solution of choice when the chloride ion is undesirable.

Potassium gluconate may be given orally in orange juice, Coca-Cola, tea, etc. One g of potassium gluconate is equivalent to 167 mg or 4.2 mEq of potassium. Parker (*1*) has shown that the gluconate when given orally seems to be less irritating than the chloride, and is well tolerated.

Potassium gluconate has been used as a source of potassium in hypopotassemia in the surgical and medical wards of the Lenox Hill Hospital for the past 6 months with excellent results. A more detailed report will be submitted at a later date.

[1] Potassium gluconate through the courtesy of Chas. Pfizer & Co., Brooklyn, N. Y.

[2] We are indebted to Samuel Gordon, Endo Products, Richmond Hill, N. Y., for supplying ampules of potassium gluconate.

Reference

1. PARKER, F. P. *Southern Med. J.*, **33**, 1301 (1940).

Tetrazolium Salt

F. E. Smith

Pal Chemicals Limited, London, England

2,3,5-Triphenyl tetrazolium chloride, commonly known as tetrazolium salt, or TTC, was first prepared by von Pechmann and Runge (*1*) in 1894. It occurs as a white to pale-yellow crystalline powder that darkens on exposure to light. The reasons for this color change have been investigated by Weygand and Frank (*2*), and further by Hausser, Jerchel, and Kuhn (*3*). It is readily soluble in water, and melts with decomposition at about 245° C.

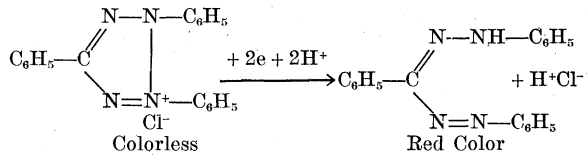
In 1941, Kuhn and Jerchel (*4*) synthesized a number of substituted tetrazolium salts by an improved procedure and called attention to the fact that their dilute solutions stained yeast, garden cress, and bacteria. They found that the reduction of the colorless salt solutions to red compounds that dyed the plant tissues was not due to the presence of glutathione, ascorbic acid, or cysteine, for the latter substances did not reduce these salts below a pH of 9.0, whereas the characteristic reductions observed on yeast, garden cress, and bacteria took place in neutral solutions.

As a result of various studies, Lakon (*5*) substituted triphenyl tetrazolium chloride for the toxic compound sodium selenite in his "topographic method" for testing the germinating ability of seeds. By a comprehensive series of comparative staining and germination tests, he was able to show that it was possible to predict the germinability of corn, oats, rye, wheat, and barley by observation of the embryo parts that were stained red by the insoluble formazan deposited in viable tissues.

Nadvornik (*6*), using Lakon's method, found that results concordant with those obtained by germination and superior to those given with selenite could be obtained with seeds of shrubs and fruit trees. Porter, Durrell, and Romm (*7*) used the method and found a close agreement between the percentage of stained embryos and the percentage of normal sprouts obtained in standard germination tests with corn, wheat, rice, buckwheat, popcorn, soybeans, and Bahia grass. Less satisfactory agreement was found in a comparison of the two methods when applied to vetch and to some lots of oats, peas, and barley. Cottrell (*8*) found good agreement between results obtained with the tetrazolium salt test and those obtained by the standard germination tests; the results were within the legal requirements for accuracy for cereal seed testing. Bishop (*9*) has discussed its use in the evaluation of malting barley. Shuel (*10*) reported that new barley, oats, and wheat incubated in a 1% solution at

48° C gave a good color reaction, which appeared to be a quick, reliable index of germinability, but for old seeds with a viability of less than 60% the test was much less accurate. Goodsell (*11*) introduced into the staining technique drying of the seed at 43° C to 12% or less moisture. In a second paper, Cottrell (*12*) reported that wheat, barley, oats, peas, and vetches gave results as reliable as those obtained in standard germination tests, but found small seeds difficult to examine. However, Flemion and Poole (*13*), in another paper published at about the same time, stated that they found great difficulty in interpreting the staining in testing the seed viability of many species, but their trial did not include any cereals. Bennett and Loomis (*14*) found that freezing injury in seed corn could be determined with fair accuracy by staining with a 0.05% solution, provided viability was fairly high and the corn had been stored for a period after freezing. Further reports on seed viability tests have been given by Lakon (*15*) and Crocioni (*16*). In the first of a promised series of papers on methods of adapting the tetrazolium method to the determination of the viability of small seeds, Hyde (*17*) reported work with Chewing's fescue (*Festuca rubra*). Holmes (*18*) found that with the seeds of conifers the tetrazolium method gave a fairly constant overestimate of the actual figure found by germination tests. Raggio and de Raggio (*19*) obtained satisfactory results using a 0.1% solution with seeds of the following species: *Pinus, Quercus, Laurus, Ricinus, Phaseolus, Vigna, Sesbania, Gossypium, Hibiscus, Lycopersicum, Solanum, Citrullus, Cucumis,* and *Helianthus*. In three further papers, Lakon (*20*) has given the detailed technique to be followed in determining the viability of peas and conifers. In a comparison of the dinitrobenzene, selenite, and tetrazolium methods, which are described and compared with physiological methods, Fink and Schwieger (*21*) concluded that, although more rapid, the new methods did not differentiate between germinating energy and germinating power. Brewer (*22*) reported its use in estimating damage in artificially cured peanuts.

Mattson, Jensen, and Dutcher (*23*) confirmed the work of Lakon with seed corn and the observations of Kuhn and Jerchel with yeast. They found that many other viable materials, in addition to seeds and yeast, reduce neutral solutions of tetrazolium salt: the fleshy parts of apples, oranges, and grapes; the gill area of mushrooms; carrot roots; white and sweet potatoes; young leaves; the stigmas and ovaries of certain pollinated flowers; bull spermatozoa; and the blastoderm of hen's eggs. The serum of bull spermatozoa and the chalazae of egg white gave a positive reaction. The reduction of the tetrazolium salt was not due to sugars, for reducing sugars formed the red formazan only above pH 11.0, whereas the above-mentioned materials would reduce the compound at acidities below pH 7.0. They stated that the use of tetrazolium salt should have a distinct advantage over many indicators as a viability test, since it was one of the comparatively few organic compounds that was colored in the reduced state. In the presence of viable tissue the colorless solution of triphenyl tetrazolium chloride formed the insoluble red triphenyl formazan by the following reaction:



Colorless — Red Color

It was quite evident that enzyme systems were responsible when this reduction took place in plant and animal materials, since tissues heated at 82° C or higher lost their ability to reduce this salt. Furthermore, it was probable that the reduction was caused by dehydrogenase systems requiring coenzymes I or II, for Jerchel and Mohle (*24*) had shown that the apparent redox potential of tetrazolium salt was about −0.08 v. Thus it was possible for the compound to act as an electron acceptor for many pyridine nucleotide dehydrogenases. They found that one of these holoenzymes, glucose dehydrogenase-coenzyme I, in the presence of its substrate, reduced the salt at pH 6.6, and other experiments had indicated that the enzyme systems responsible for the reduction of the tetrazolium salts were present in a wide variety of living tissues. They suggested that in all probability the reduction of these compounds by enzymes of living cells could not be considered a general test for life, but nevertheless the unusual properties of these reagents suggested that they might be utilized in many types of biological research involving differences in tissue viability. Their suggestions have now been followed up with some good results. Lederberg (*25*) reported some further experiments on its use with enzymatic reducing systems. Waugh (*26*) observed that sections of living trees and shrubs became stained in the cambium layer, whereas dead sections were unaffected; more recently, Roberts (*27*) has made a detailed survey of reducing tissues in vascular plants. Morse (*28*), in studies on the bleaching of vegetables, found that the system responsible for the reduction to formazan was one more easily inactivated by heat than either the catalase or peroxidase systems of vegetables.

Straus, Cheronis, and Straus (*29*) obtained successful results in the detection of malignant tumors by differential staining, the disadvantage being the fact that the red stain was difficult to distinguish, and Schuermann (*30*) found that, following application to ulcerated tissues *in vivo,* reduction took place relatively more rapidly with malignant tumors than with benign lesions. But when Seligman, Gofstein, and Rutenburg (*31*) examined the distribution of radioiodine-labeled tetrazolium in mice, they found there were no great differences in the radioactivity of tumors and that of other tissues, and concluded that neoplasms did not reduce the salt; this was confirmed by Masouredis, Shimkin, McMillan, and Fox (*32*), who found that following intravenous injection in mice radioactivity rapidly disappeared from the circu-

lating blood and was rapidly excreted by the liver and kidneys; transplantable mammary carcinoma and lymphoma were found to contain less radioactivity than most normal tissues. These conflicting results are difficult to interpret, but as may be seen from a paper by Wrenn, Good, and Handler (*33*), certain dyes are known to penetrate altered tissues, and it may become practice to detect neoplasms preoperatively by means of isotope-labeled dyes.

Gall (*34*) used tetrazolium salt to estimate the reducing activity of bean tissue cultured in the plant-growth substance 2,4-dichlorophenoxyacetic acid. Pratt, Dufrenoy, and Pickering (*35*) found it a valuable reagent in studies of cellular physiology, which could also be used in pencillin assays with *Staph. aureus,* and in another paper, Dufrenoy and Pratt (*36*) reported its use in testing the reducing activity of sugar-cane stalks. Later, Tynen and Underhill (*37*) reported its utility in the histological demonstration of subcutaneous fat, and Gunz (*38*) reported on its rapid reduction by fresh brewers yeast; he also obtained reduction with a cell-free yeast extract, but heating the yeast or the extract to 60° C inhibited the reaction. Fred and Knight (*39*) reported on its reduction by *Penicillium chrysogen,* Black and Kleiner (*40*) on its use in the measurement of respiration of tissue slices, and Kun and Abood (*41*) on its use in the colorimetric estimation of succinic dehydrogenase. Mixner (*42*) showed that its use as a test for the viability of bull spermatozoa was precluded by its toxicity to the spermatozoa and its inability to stain adequately. Stein and Gerarde (*43*) later reported that it failed to indicate cell viability of chick-heart fibroblasts *in vitro,* presumably owing to the failure of the compound to penetrate living cellular membranes. Fults, Schaal, and Michaelson (*44*) reported a study of the tetrazolium salt reactions of 42 physiologic races of the common potato scab organism *Actinomyces scabies*; although there was no absolute correlation between parasitism on Bliss Triumph potatoes and the tetrazolium reaction, the parasitic races gave a significantly greater mean reaction than the non-parasitic types. Differentiation of bacterial species and variations within species were reported by Huddleson and Baltzer (*45*); 0.01% or less of tetrazolium salt was incorporated in agar and plated in Petri dishes, and differences were seen in the size, shape, and shade of the red central areas, with pronounced differences in the delicate pastel tints in the borders. Bielig, Kausche, and Haardick (*46*) investigated formazan formation at reducing loci in bacteria with varying conditions of pH and temperature, and at the same time Bielig and Querner (*47*) showed that it could be used in growth studies with marine animals, a 0.1% solution in sea water being nontoxic to a large number of species. Sonnenblick, Antopol, and Goldman (*48*) have reported some interesting findings on its influence on the growth and cytology of root tips of onions. An excellent review in Spanish of the uses and action of tetrazolium salts has recently been given by Raggio and de Raggio (*49*).

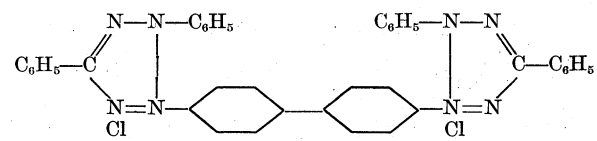
Weiner (*50*) has reported it to be a qualitative test for chemicals which are reducing compounds, the method employed being as follows:

To 2 drops or a few crystals of the unknown compound, 1 ml of water was added (or if insoluble in water, isopropyl alcohol was used). Four drops of normal sodium hydroxide followed by 4 drops of a 0.1% aqueous solution of tetrazolium salt was added, and the mixture allowed to stand for several minutes after shaking. A reddish precipitate (or solution if isopropyl alcohol was used) formed if a reducing substance was present.

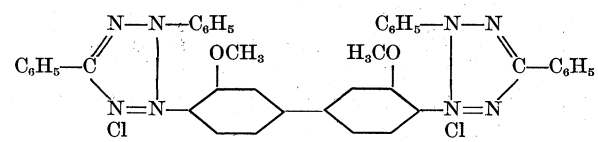
Recently, Mattson and Jensen (*51*) reported its use in the quantitative colorimetric determination of reducing sugars at 4,900 A, and Trevelyan, Procter, and Harrison (*52*) have since reported its use in the detection of these sugars on paper chromatograms. The dried, developed paper chromatogram strip was treated with a 0.5% solution in chloroform, and after it had been allowed to dry it was sprayed with alcoholic sodium hydroxide. Red spots developed on standing overnight or on heating for 5 min in a moist atmosphere, in the presence of reducing sugars; non-reducing disaccharides such as glucose and trehalose did not react.

It will be seen that tetrazolium salt is a valuable compound for workers in many fields, but, as it has certain limitations, some work has been directed toward the preparation of new compounds with similar but possibly more desirable properties.

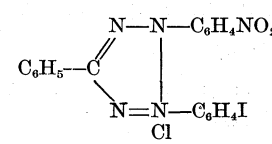
From these, the chief to emerge so far are neotetrazolium salt, 2,2-(*p*-diphenylene)bis(3,5-diphenyltetrazolium chloride) (I), its dimethoxy derivative (II) known as blue tetrazolium, and 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride (III):



(I)



(II)



(III)

Neotetrazolium salt gives a deep-purple to black color on reduction, as opposed to the red color given by tetrazolium salt itself, and is thus more easily distinguished in tissues. Antopol, Glaubach, and Goldman (*53*) reported that with fresh tissue slices, fibrous tissue stained little if at all, whereas muscle and

epithelial and parenchymal tissues such as liver and kidney stained well; malignant tumors stained rapidly and intensely. Its action on various bacteria and on onion root tips was also discussed. In another paper, the same authors (*54*) reported its use as a tool in the study of active cell processes, and Narahara, Quittner, Goldman, and Antopol (*55*) recorded its use in the study of *Escherichia coli* metabolism. For the test used on milk for the presence of brucellosis in cattle herds, Wood (*56*) has prepared a stable antigen for the *Brucella* ring test by the reduction of neotetrazolium salt.

Blue tetrazolium, which forms a deep-blue pigment on reduction, has been used by Rutenberg, Gofstein, and Seligman (*57*) to demonstrate enzymes in normal and neoplastic tissues, but it is ten times more toxic *in vivo* (mice) than tetrazolium salt itself; it has also been used in the histochemical demonstration of succinic dehydrogenase in tissue sections by Seligman and Rutenburg (*58*).

2-(*p*-Iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl tetrazolium chloride recently reported by Atkinson, Melvin, and Fox (*59*), along with some other iododerivatives, is far less photosensitive than tetrazolium salt itself and has the additional advantage of giving very rapid staining with less diffusion into unstained tissue.

Further reports on these and possibly on new, improved members of this useful type of compound will be awaited with interest.

References

1. VON PECHMANN, H., and RUNGE, P. *Ber.* **27**, 2920 (1894).
2. WEYGAND, F., and FRANK, I. *Z. Naturforsch.*, **3b**, 377 (1948).
3. HAUSSER, I., JERCHEL, D., and KUHN, R. *Chem. Ber.*, **82**, 195 (1949).
4. KUHN, R., and JERCHEL, D. *Ber.*, **74B**, 941, 949 (1941).
5. LAKON, G. *Mitt. intern. Ver. Samenkontrolle*, **12**, 1 (1940); *Ber. deut. botan. Ges.*, **57**, 191 (1939); **60**, 299, 434 (1942).
6. NADVORNIK, J. *Věstník Ceské Akad. Zemědělské*, **20**, 160 (1946).
7. PORTER, R. H., DURRELL, M., and ROMM, H. J. *Plant Physiol.*, **22**, 149 (1947).
8. COTTRELL, H. J. *Nature*, **159**, 748 (1947).
9. BISHOP, L. R. *European Brewery Convention Congress (Scheveningen)*, 8 (1947); *Chemistry & Industry*, 779 (1947).
10. SHUEL, R. W. *Sci. Agr.*, **28**, 34 (1948).
11. GOODSELL, S. F. *J. Am. Soc. Agron.*, **40**, 432 (1948).
12. COTTRELL, H. J. *Ann. Applied Biol.*, **35**, 123 (1948).
13. FLEMION, F., and POOLE, H. *Contribs. Boyce Thompson Inst.*, **15**, 243 (1948).
14. BENNETT, N., and LOOMIS, W. E. *Plant Physiol.*, **24**, 162 (1949).
15. LAKON, G. *Ibid.*, 389.
16. CROCIONI, A. *Humus*, **3**, 15 (1946).
17. HYDE, E. O. C. *New Zealand J. Sci. Technol.*, **31**, 13 (1949).
18. HOLMES, G. D. *Rept. Forest Research*, 34 (1949). (Forestry Commission, London, 1950.)
19. RAGGIO, M., and DE RAGGIO, N. M. *Bol. lab. botán. fac. agron. (La Plata)*, No. 3 (1950).
20. LAKON, G. *Saatgutwirtsch*, **2**, 37, 60, 83 (1950).
21. FINK, H., and SCHWIEGER, E. *Brauwissenschaft*, **2**, 39; **3**, 68 (1950).
22. BREWER, H. E. *Science*, **110**, 451 (1949).
23. MATTSON, A. M., JENSEN, C. O., and DUTCHER, R. A. *Ibid.*, **106**, 294 (1947).
24. JERCHEL, D., and MOHLE, W. *Ber.*, **77B**, 591 (1944).
25. LEDERBERG, J. *J. Bact.*, **56**, 695 (1948).
26. WAUGH, T. D. *Science*, **107**, 275 (1948).
27. ROBERTS, L. W. *Bull. Torrey Botan. Club*, **77**, 372 (1950).
28. MORSE, R. E. *Fruit Products J.*, **29**, 13, 25 (1949).
29. STRAUS, F. H., CHERONIS, N. D., and STRAUS, E. *Science*, **108**, 113 (1948).
30. SCHUERMANN, H. *Klin. Wo-chschr.*, **28**, 464 (1950).
31. SELIGMAN, A. M., GOFSTEIN, R., and RUTENBURG, A. M. *Cancer Research*, **9**, 366 (1949).
32. MASOUREDIS, S. P., *et al. J. Natl. Cancer Inst.*, **11**, 91 (1950).
33. WRENN, F. R., JR., GOOD, M. L., and HANDLER, P. *Science*, **113**, 525 (1951).
34. GALL, H. J. F. *Botan. Gaz.*, **110**, 319 (1948).
35. PRATT, R., DUFRENOY, J., and PICKERING, V. L. *Stain Technol.*, **23**, 137 (1948).
36. DUFRENOY, J., and PRATT, R. *Am. J. Botany*, **35**, 333 (1948).
37. TYNEN, T., and UNDERHILL, S. W. F. *Nature*, **164**, 236 (1949).
38. GUNZ, F. W. *Ibid.*, **163**, 98 (1949).
39. FRED, R. B., and KNIGHT, S. G. *Science*, **109**, 169 (1949).
40. BLACK, M. M., and KLEINER, I. S. *Ibid.*, **110**, 660 (1949).
41. KUN, E., and ABOOD, L. G. *Ibid.*, **109**, 144 (1949).
42. MIXNER, J. P. *J. Dairy Sci.*, **32**, 1013 (1949).
43. STEIN, R. J., and GERARDE, H. W. *Science*, **111**, 691 (1950).
44. FULTS, J. L., SCHAAL, L. A., and MICHAELSON, M. E. *Soil Sci. Soc. Am., Proc.*, **13**, 287 (1948).
45. HUDDLESON, I. F., and BALTZER, B. *Science*, **112**, 651 (1950).
46. BIELIG, H. J., KAUSCHE, G. A., and HAARDICK, H. *Z. Naturforsch.*, **4b**, 80 (1949).
47. BIELIG, H. J., and QUERNER, H. *Ibid.*, 21.
48. SONNENBLICK, B. P., ANTOPOL, W., and GOLDMAN, L. *Trans. N. Y. Acad. Sci.*, (II), **12**, 161 (1950).
49. RAGGIO, M., and DE RAGGIO, N. M. *Ciencia e invest. (Buenos Aires)*, **7**, 35 (1951).
50. WEINER, S. *Chemist-Analyst*, **37**, 56 (1948).
51. MATTSON, A. M., and JENSEN, C. O. *Anal. Chem.*, **22**, 182 (1950).
52. TREVELYAN, W. E., PROCTER, D. P., and HARRISON, J. S. *Nature*, **166**, 444 (1950).
53. ANTOPOL, W., GLAUBACH, S., and GOLDMAN, L. *U. S. Pub. Health Service. Pub. Health Repts.*, **63**, 1231 (1948).
54. ———. *Trans. N. Y. Acad. Sci.*, (II), **12**, 156 (1950).
55. NARAHARA, H. T., *et al. Ibid.*, 160.
56. WOOD, R. M. *Science*, **112**, 86 (1950).
57. RUTENBURG, A. M., GOFSTEIN, R., and SELIGMAN, A. M. *Cancer Research*, **10**, 113 (1950).
58. SELIGMAN, A. M., and RUTENBURG, A. M. *Science*, **113**, 317 (1951).
59. ATKINSON, E., MELVIN, S., and FOX, S. W. *Ibid.*, **111**, 385 (1950).

Radioactive Tracers in Solid Solution Investigations

William T. Foley and Paul A. Giguere

Department of Chemistry,
Laval University, Quebec, Canada

During recent work on the exact freezing point of hydrogen peroxide we had to ascertain definitely whether this compound forms solid solutions with water. The experimental evidence reported previously in that connection (*1*) was considered doubtful, as it was based on an inadequate method, namely, direct analysis of phases. Indeed, the high viscosity of the solutions and the tendency of hydrogen peroxide to decompose on melting made it practically impossible to isolate completely the crystals from the mother liquor. On the other hand the so-called wet residue method of Schreinemakers (*2*) required addition of a third substance fairly soluble in both water and hydrogen peroxide, which did not catalyze decomposition of the latter or interfere with its chemical determination. It was further desirable that this substance