fered with 0.1% sodium acetate,[12] directly into the pars distalis.

In 12 of the 20 experimental animals which survived this operation for 48 hours the copper did not reach the hypophysis; the hypophyseal capsule remained intact. None of these 12 revealed ruptured or hemorrhagic follicles at autopsy. This group included 5 injected with copper acetate in an India ink suspension, and the ink was found in the retropharyngeal region below the sella and in the cavernous sinuses. Of the 8 whose hypophyses actually received copper, 6 ovulated. Two of the 6 had hypophyses penetrated to the top in such a way that some of the copper could possibly have reached the hypothalamus, but in the other 4 no such condition existed. Three of the 8 injected hypophyses were entered with the copper-ink medium and of these, 2 ovulated; but in all 3 most of the ink was located inferior to the sellar region, none above the diaphragma sellae. The effects of mechanical trauma were controlled by a series of 7 animals into each of whose hypophyses a bipolar electrode was inserted,[10] and a second series of 23 rabbits whose hypophyses were injected with acetylcholine-eserine solution.[11] None of the former and only one of the latter ovulated.

At first glance our results, ovulation in 75% of 8 rabbits, on intrapituitary injection of copper, might seem unconvincing compared with Harris' 80% of 13 animals on injection of copper into the third ventricle, especially since Harris used a dosage only a third as great as ours. However, most of our injected material backed out of the sella, away from the hypophysis and hypothalamus, while Harris' copper could have reached and stimulated the hypophysis directly rather than the hypothalamus as he assumed. His copper, deposited in the third ventricle, may well have been picked up by the proximal capillary plexus of the hypophyseal portal system and carried by the portal veins directly to the adenohypophysis. Brooks' failure to induce ovulation with copper in stalk-sectioned animals is difficult to explain in terms of a direct-action hypothesis, since presumably an adequate arterial blood supply to the adenohypophysis remained intact after his operation. The possibility does exist, however, since he could not test them by the mating response and since he supplied no estrogen, that his copper-treated stalk-sectioned animals were anestrous.

To summarize, the intrahypophyseal injection of 1/100th of the systemic dose of copper acetate, an agent whose effect on stimulating the release of hypophyseal LH is not blocked by anti-adrenergic or anti-cholinergic drugs, has led to ovulation in 6 out of 8 rabbits. It is, therefore, concluded that at least part of the copper effect is a direct stimulation of adenohypophyseal cells.

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Reduction of Potassium Tellurite by Living Tissues.* (17369)

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The property of soluble colorless tetrazolium salts to form insoluble red to purplish formazans as reduction products can be used for the demonstration and microscopic localization of reducing activity in living tissues.[1-4]

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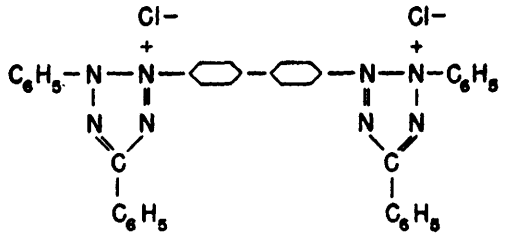
1 Mattson, A. M., Jensen, C. O., and Dutcher, R. A., *Science*, 1947, **106**, 294.

2 Pratt, R., and Dufrenoy, J., *Stain Technology*, 1948, **23**, 137.

Potassium tellurite introduced into bacteriology by Klett[5] and later utilized by Conradi and Troch[6] for the isolation of diphtheria bacilli, was found to be a suitable reagent for this purpose since reduced insoluble tellurium imparts a distinct black color. A similar reagent had been previously employed by Hasegawa[7] for the determination of the germinability of cereal seeds.

Method. A 0.1% solution of potassium tellurite C.P. in M/10 phosphate buffer pH-7.4 gave reproducible and consistent results. For comparison, a saturated solution of "neotetrazolium chloride" obtained from Pannone Chemical Company, Farmington, Conn. in physiological saline solution or phosphate buffer was likewise used. The formula of this compound is:



Results and discussion. 24 hour cultures of various bacteria (*E. coli, S. typhimurium, S. oranienburg, S. montivideo, S. typhosa, Shigella alkalescens, Proteus morganii, Proteus vulgaris, Pseudomonas aerugenosa, B. subtilis, Staphylococcus, Streptococcus viridans, C. diphtheriae*) grown in 2% peptone water or nutrient broth reduced both "neotetrazolium chloride" and potassium tellurite. 0.2 cc of the reagents were added to 3 cc of the culture medium. A strong reduction was also seen by several strictly anaerobic bacteria, (*C. tetani, C. botulinum, C. perfringens*) grown in tryptose broth, regardless of whether the cultures after the reagent had been added were returned to strictly anaerobic condition or left outside in the air. In general, the reduction of potassium tellurite required several hours while that of "neotetrazolium" was apparent within 30-60 minutes. Under the microscope, many of the bacteria as well as the cocci showed similar inclusions with both reagents. The ones following treatment with "neotetrazolium"[4] were larger and coarser, but their general shape and distribution was alike with both preparations.

Suspensions of fresh[8] and of air-dried yeast[9] reduced tetrazolium salts as well as potassium tellurite. Under the microscope, many yeast cells showed fine and coarse granules of similar morphology with both preparations.

Material from various kinds of exudates from human patients were suspended in potassium tellurite and "neotetrazolium chloride" solutions at 37°C. In a considerable amount of polymorphonuclear leucocytes, reducing activity was revealed by the occurrence of purplish red and black granules respectively, of similar appearance and distribution. Thin tissue pieces from rat and rabbit organs as well as from operatively removed human material were suspended in potassium tellurite and "neotetrazolium chloride" solutions and kept at 37°C. Within 15 to 30 minutes, a discoloration took place increasing in intensity within the next hours. The extent of discoloration was similar with both reagents. Kidney tissue, *e.g.*, showed the strongest reaction while intestines reacted only moderately and fat tissue very weakly. A more consistent and deeper penetration of the tissues was however noted with potassium tellurite.

The distribution of reduced tellurium can be studied in frozen sections prepared from formalin fixed tissues cut at 15 to 25 μ that had been immersed for 8 to 12 hours in the potassium tellurite solution previous to fixation. Paraffin embedding may also be employed but has so far given inferior results. Particularly satisfactory preparations were obtained with kidney tissue of animal as well

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[4] Antopol, W., Glaubach, S., and Goldman, L., *Public Health Reports,* 1948, **63**, 1231.

[5] Klett, A., *Zeitschr. Hyg.,* 1900, **33**, 137.

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[7] Hasegawa, K., *Japanese J. of Botany,* 1936, **8**, 1.

[8] Kuhn, R., and Jerchel, D., *Ber der Deutchen Chem. Ges.,* 1941, **74B**, 949.

[9] Gunz, F. W., *Nature,* 1949, **163**, 98.

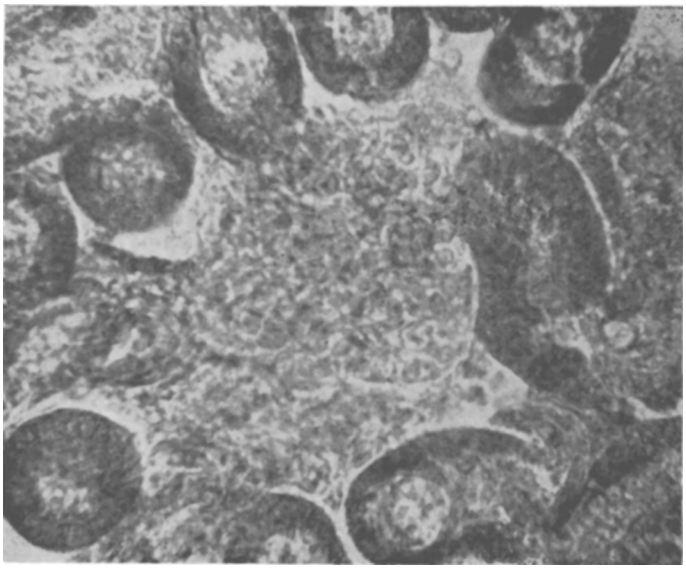

FIG. 1.
A frozen section from a rabbit kidney showing tellurium deposition in the proximal convoluted tubules. × 450.

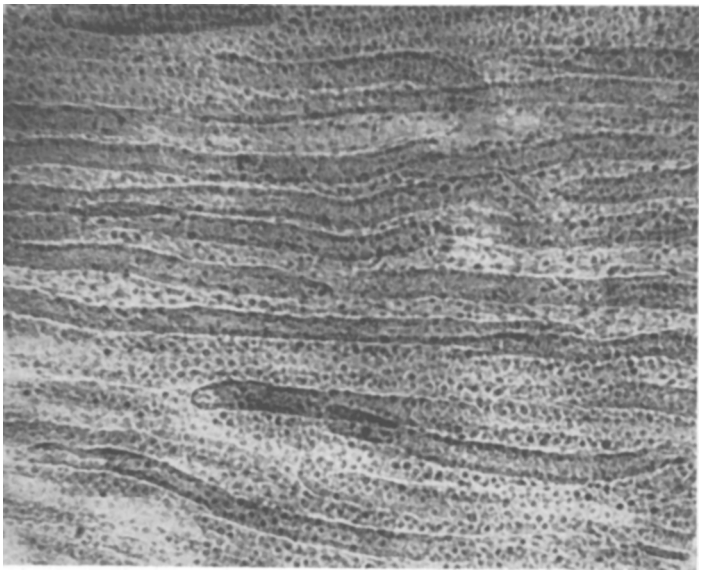

FIG. 2.
A frozen section from rabbit kidney showing tellurium deposition mainly in ascending limbs of Henle's loops in the outer zone of the medulla. × 150.

as human origin. In the cortex, the proximal and distal convoluted tubules, as well as ascending limbs of Henle's loops, showed deposition of tellurium (Fig. 1). Within the outer zone of the medulla, ascending and descending limbs of Henle stained selectively (Fig. 2) while in the innermost portion of the medulla, occasional collecting tubules revealed reducing activity. The intensity of the staining reaction varied considerably from field to

field. In general, however, proximal convoluted tubules and ascending limbs of Henle's loop reacted strongest.

Intravital reduction of tellurium in the kidney could also be demonstrated with potassium tellurite, although the reaction was less extensive. Two groups of 6 adult rats received a daily intraperitoneal injection of 0.8 cc of "neotetrazolium chloride" and potassium tellurite solution, respectively, for 16 days. On gross examination, the cortical portion of the kidney was stained. Microscopically, reduction took place only in the proximal convoluted tubules. The depositions of tellurium as well as of the formazan were considerably less intense than in supravitally stained kidney tissue. With potassium tellurite there was however very little reduction in the liver, while considerable dye deposition took place in the parenchymal cells with "neotetrazolium chloride".[4] The deposition of tellurium in the kidney took place in such a manner as to suggest a localization within large droplets and rods closely simulating the microscopic appearance of kidney sections that had been specifically stained for mitochondria. On chemical examination, Schneider found various enzymes including succinic dehydrogenase to be associated with the mitochondria or large granule fraction from rat liver and kidney homogenates.[10]

The conditions under which potassium tellurite is reduced are closely similar to those under which "neotetrazolium chloride" is acted upon. Boiling, alcohol, acetone, and formalin fixation destroy the reducing ability of animal tissue for both reagents. Antopol and coworkers[4] have noted that in the test tube, cysteine and glutathione at pH-7.0 reduce "neotetrazolium chloride" in contrast to cysteine and methionine, thus indicating the importance of active SH groups. An identical behavior was found with potassium tellurite. Correspondingly, treatment with sulfhydryl reagents prevented the reduction of tetrazolium salts by fresh tissue. Similarly, pieces of fresh kidney immersed for one hour in a 0.1% solution of iodocetamide[11] and then incubated up to 20 hours in potassium tellurite or "neotetrazolium" remained completely unstained. Control tissue immersed first in physiological saline solution and then treated with the reagents showed a very strong reaction. There is however considerable lack of specificity for inhibitors of the reduction of tetrazolium salts by living tissues.[12] This would indicate that a number of reducing enzymes, acting possibly on various materials within the cell could be involved in the reducing process.

Summary. Reducing activity in living tissue can be demonstrated with the aid of potassium tellurite. Under the microscope, the sites of reduction are indicated by insoluble dark tellurium. Various bacteria, including strictly anaerobic ones as well as yeast and leucocytes from human material, reduced posassium tellurite. Among different mammalian tissue examined, kidney gave the most constant result, permitting histochemical localization in certain portions of the nephron. Active SH groups apparently are essential for the reduction of potassium tellurite by living tissues.

The writer appreciates the technical assistance of Miss Esther Levenkron.

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