holder to be carried by the manipulator. A 50-ml. beaker is filled with 5 per cent aqueous silver nitrate in which a piece of silver wire is immersed. The latter is connected to the negative pole of a 6-volt d-c source (storage or dry batteries), while the leads from the electrodes are connected to the positive pole. The electrodes are rapidly immersed in the silver nitrate to a depth of about 2 mm. and immediately withdrawn. This is repeated four or five times, or until inspection under a microscope reveals tapered tips of the required shape and fineness. The angle of taper is determined by the speed of immersion and withdrawal, and can be varied accordingly. The silver remains soft and smooth and can be chlorided and insulated with shellac if desired. The great advantage of tapered silver electrodes is that the fine tips can be bent into any shape with fine forceps during the course of an experiment, and less than a minute is required to make new tapered tips, should the points break off.

The tendency to vibrate is slight since the electrodes can be made of relatively heavy silver wire, while the soft temper and fineness of the points greatly minimizes tissue damage which may occur when the electrodes are moved.

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Use of Sulfuric Acid-Dichromate Mixture in Cleaning Glassware

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Concentrated sulfuric acid saturated with potassium or sodium dichromate has been used for many years in cleaning glassware. In 1934 Laug (*3*) found that, although 10 rinsings of glassware with water removed all dichromate from the glass surface, there were still appreciable amounts within the glass, and that if the glass were allowed contact with water for several hours, it would yield the dichromate to solution. For example, in one experiment with a pyrex beaker 1.3 μg. of potassium dichromate was removed. Only by boiling it out with several successive changes of water could the dichromate be removed effectively. Richards (*4*) studied the effects of such small concentrations of dichromate on yeast and other microbial forms and found as little as 0.0001 μg./ml. to be toxic in some cases.

Because of the general use of this cleaning solution in most biological as well as chemical laboratories, it was considered of some importance to investigate further the retention of dichromate and the acid component by glassware and the effect of dichromate on certain representative laboratory procedures.

Sulfuric acid-dichromate cleaning solution was made up in the usual way (250–300 ml. saturated potassium dichromate plus approximately 3,500 ml. technical concentrated sulfuric acid) and was found to contain 28,100 μg. potassium dichromate/ml. (determined by iodimetry, using a solution of sodium thiosulfate standardized against potassium iodate). An extremely sensitive test for dichromate (*1*) entails the addition of diphenylcarbazide in an acid medium. The absorption curve of the resultant color product was determined on the Beckman spectrophotometer and a maximum extinction (E) observed at a wave length of 540 mμ. Working at this wave length, a curve relating concentration to E was established by which all subsequent analyses for dichromate were determined. By this method, as little as 0.01 μg./ml. can be detected. Determinations of pH were made using the Beckman pH meter.

Determinations of dichromate and pH were made on washings from a 5-ml. pyrex volumetric pipette, a 25-ml. pyrex volumetric flask, and a 250-ml. pyrex volumetric flask. The first 10 washes were done as rapidly as is usually done when washing glassware under a running faucet, and each washing was tested for pH and dichromate. In each case 6 to 10 rinses were required before the pH approximated that of the wash water. Four to 6 rinses removed most of the dichromate, although even after 10 rinses a small amount could be detected in some cases, e.g. the 10th washing from the 5-ml. pipette contained between 0.10 and 0.20 μg. As would be expected because of the relatively large glass surface to its contained volume, more rinses were required for a pipette than a flask. To demonstrate the quantity of absorbed dichromate not removed by such rapid washing, a 250-ml. pyrex volumetric flask was allowed contact with cleaning solution for 48 hours. The 11th rapid wash contained 0.1 μg. dichromate. The flask was then filled with water and allowed to stand for 22 hours at room temperature, after which the wash was concentrated to 10 ml. by heat evaporation. This wash contained 0.2 μg. dichromate.

The effect of dichromate on urease activity was determined by adding various amounts of dichromate solution to the reactants in a modified Karr method for determining urea nitrogen.[1] Dichromate in the range of 1–10 μg./ml. attained as much as 95 per cent inhibition of the enzyme urease. Since a direct relationship exists between enzyme concentration and concentration of inhibitor required for a specific amount

[1] A 1 per cent solution of Squibb's urease was used. The resulting ammonium carbonate solution (after reaction for 30 minutes at 55° C.) was nesslerized by Koch-McMeekin's reagent. The color intensity was measured at a wave length of 425 mμ on the Coleman spectrophotometer.

of inhibition, less dichromate would be needed for an inhibition in cases where the enzyme concentration is decreased from that used in these particular experiments.

The effect of dichromate on growth of two strains of *Staphylococcus aureus* was followed turbidimetrically (at 450 mμ. on the Coleman spectrophotometer) and checked by duplicate pour-plate dilution counts. In synthetic medium[2] as little as 1 μg./ml. was very toxic to growth, whereas in nutrient broth approximately 10 times as much dichromate was needed to obtain equivalent inhibition. This was undoubtedly due to binding of the heavy metal ion by constituents of the nutrient broth.

Because of the extreme difficulty in ridding glassware of dichromate after cleaning in "cleaning solution" and its great toxicity for living cells and enzymes, it is believed highly advisable in laboratories dealing with such material to clean all glassware by another method, such as 10 per cent nitric acid, a detergent, or 1–5 per cent trisodium phosphate.

[2] The medium used was that of Landy and Dicken (2) with the omission of sodium acetate, asparagine, guanine, xanthine, uracil, and folic acid.

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Letters to the Editor

The Effect of Urethane on Lymphatic Leukemia in Rats

In 1925 Hawkins and Murphy reported from this Laboratory that urethane anesthesia (ethyl carbamate) caused a rapid increase in the CO_2 content and pH of the whole blood of rabbits to a point where there was a marked uncompensated alkalosis. This reached its maximum in 24 hours and persisted for 48 hours. Accompanying this change was a marked fall in the circulating lymphocytes, similar in extent to that following a relatively large exposure to X-ray (*J. exp. Med.*, 1925, **42**, 609). Recently an investigation has been undertaken to test the effect of urethane on the development of transplanted lymphatic leukemia and lymphosarcoma in rats. Since starting this study, our attention has been called to two articles which have appeared in British journals. Haddow and Sexton, in testing urethanes on experimental animal tumors, noted that the most striking effect was upon leukemic cells (*Nature, Lond.*, 1946, **157**, 500). In the second paper, Paterson, Haddow, Thomas, and Watkinson compared the effect of urethane with deep X-ray on human leukemia (*Lancet*, 1946, 11 May, 677). They noted that the chemical agent produced a remarkably similar effect on the blood count and the enlarged lymph nodes to that resulting from the application of the standard method of deep X-ray therapy.

The material for our test was a transplanted disease of rats, which manifests itself as generalized lymphatic leukemia if the malignant cells are injected intraperitoneally or as a localized lymphosarcoma when the cells are inoculated into the subcutaneous tissue of the groin. The leukemic type of the disease develops rapidly, with marked increase in the circulating lymphocytes and extensive involvement of the thymus and lymph nodes. Death results in 8 to 12 days. The groin inoculations result in rapidly growing tumors which attain very large size and cause death of the rats in 16 to 21 days.

Among 50 inoculated rats, given from 50 to 100 mg. of urethane/100 grams of body weight, repeated 4 times a week, only 3 developed leukemia (6 per cent). Among 41 controls of the same strain, inoculated with the same material but receiving no treatment, 33 developed fatal leukemia (80.4 per cent). Among 30 rats inoculated in the groin with leukemia cells, and given the urethane treatment, only 9 (30 per cent) developed progressive tumors, while 26 of the 30 controls (86.6 per cent) died of lymphosarcoma.

We have previously demonstrated that adrenalectomy renders rats much more susceptible to our strain of leukemia (*Science*, 1943, **98**, 568). Furthermore, adrenal cortical and pituitary adenotropic hormones retard or prevent the development of the disease (*Science*, 1944, **99**, 303). In the light of these observations it may prove significant that the adrenals of rats given urethane in the dosage employed above show about 33 per cent increase in weight over those from normal, untreated animals. A similar increase in weight of the adrenals has been noted in rats which develop resistance to inoculated leukemic cells without treatment. We are attempting to evaluate the part played by the adrenals by treating inoculated, adrenalectomized rats with urethane.

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Wild Pineapples in Venezuela

Although pineapples, *Ananas ananas* (L.) Cockerell (*A. sativus* Schult. f.) were known to have originated in the American tropics and have been reported growing in the wild state in Brazil, Surinam, and Paraguay (*Pflanzenfam.* (2nd ed.), 1930, p. 154), not until recent observations by the author and V. Badillo in the Parguasa region of the Estado Bolívar, and simultaneously by others in neighboring regions, have they been definitely known to be in the wild state in Venezuela. They grow