In 5 cases, the addition of epinephrine to samples of blood removed before injection was without effect.

*Summary.* Injections of epinephrine were found to produce the following reactions in patients with and without diseases of the blood-forming tissues, from whom samples of capillary or venous blood were withdrawn: 1. Tenderness over the spleen and long bones occurred in some of the patients who had diseases of the blood-forming tissues. 2. There was an increase in the erythrocyte and leukocyte counts regardless of whether or not the spleen was present. 3. The lymphocytes were the first blood-cells to increase in numbers following injection. 4. Immature cell-forms did not appear in numbers sufficient to indicate stimulation of the bone-marrow. 5. The hematocrit values were increased. 6. The mean corpuscular volume varied too greatly to be of significance. (The calculation of the mean corpuscular volume is at best only an approximation.) 7. The fragility of erythrocytes was not altered. 8. When added *in vitro* to whole blood, epinephrine did not produce significant changes. The results of these experiments are compatible with the hypothesis that the leukocytosis following injection of epinephrine is due to hemo-concentration.

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## Local Irritation from Sodium Bisulfite as Preservative in Epinephrine Solutions.*

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A large number of the epinephrine preparations on the market contain sodium bisulfite in a concentration of about 0.1%, to prevent the oxidation of the alkaloid. As far as we can determine, no studies have been made on the possible irritant properties of sodium bisulfite, so that its suitability for a therapeutic product does not seem to have been adequately established. We have, therefore, tested the material, to see whether irritant phenomena could be produced by this chemical, either in pure solution, or in the media in which it is used clinically in local anesthesia.

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Sodium bisulfite is an extremely acid salt, as shown by the measurements recorded in Table I, obtained with the DeEds glass electrode.[1]†

TABLE I.
Hydrogen Ion Concentrations of Sodium Bisulfite Solutions in Normal Saline and Local Anesthetic Solutions.

| Concentration Sodium Bisulfite % | pH of $NaHSO_3$ dissolved in | |
|---|---|---|
| | 0.9% NaCl | 2% Procaine HCl containing 1:25,000 Epinephrine HCl |
| 0.5 | 1.82 | 2.13 |
| 0.1 | 2.12 | 2.83 |
| 0.02 | 2.66 | 3.59 |
| 0.004 | 3.62 | 5.50 |
| 0.0008 | 6.95 | 5.58 |

A test for possible irritation of mucous membranes was made by instilling 3 times a few drops of the bisulfite solution in the conjunctival sacs of rabbits. A 0.5% concentration in saline solution caused immediate reddening and chemosis with profuse lacrimation; 0.1% concentration produced the same changes to a lesser degree, while a 0.02% concentration produced only moderate hyperemia. Since 0.1% is the concentration of bisulfite usually present in epinephrine solutions, there is a definite possibility that it would cause local irritation of mucous surfaces in the conjunctiva, and possibly other areas as well.

Irritation in subcutaneous areas may be tested for by means of the colloidal dyes.[2] For such a test, 0.5 cc. of each bisulfite solution in Table I, together with controls of saline and procaine-epinephrine solutions, were injected subcutaneously in the previously shaved abdominal skin of a rabbit. Then 10 mg. per kg. of trypan blue dissolved in 6% dextrose solution was injected intravenously. The colloidal particles of trypan blue are too large to pass through the endothelium of normal blood vessels. However, in an irritated or damaged skin-area, the large dye molecules cannot be held back by the endothelium and so escape into the tissues, thus causing locally a deep blue stain which sharply marks out the area of increased vascular permeability. According to this test, irritation was absent after the control solutions and those containing 0.004% bisulfite or less. The stronger solutions caused definite vascular damage in

[1] Kahler, H., and DeEds, F., *J. Am. Chem. Soc.*, 1931, **53**, 2998; DeEds, F., *Science*, 1933, **78**, 557.

† We are indebted to Mr. C. W. Eddy for making the pH measurements.

[2] Tainter, M. L., and Hanzlik, P. J., *J. Pharm. Exp. Therap.*, 1924, **24**, 179; Spagnol, G., *Arch. Exp. Path. Pharm.*, 1928, **137**, 250.

the injected areas as shown by the appearance of a deep blue color locally.

A final test of possible local toxicity from the bisulfite was made by injecting the solutions in Table I both intracutaneously and subcutaneously in the skin of the forearms of all 3 authors, using aseptic technic. For the intracutaneous injections, 0.05 cc. was used and for the subcutaneous, 0.1 cc. The subcutaneous injections of the bisulfite in saline solution caused intense hyperemia at time of injection, followed by some induration. Over 48 hours were required for the skin areas to return completely to normal. The intensity of the effects was proportional to the concentration of the bisulfite, and was definite even with the lowest concentration used. The intracutaneous injections of the saline-bisulfite solutions were painful and caused hyperemia lasting over 2 hours with concentrations of the bisulfite of 0.02% or higher, but were painless and blanched when the bisulfite was dissolved in the procaine-epinephrine solution. The presence of epinephrine stopped the flow of blood through the injected area, so that signs of inflammation did not become visible, even though tissue damage may have occurred. There was no necrosis or sloughing in any of the skin-areas, although some discoloration persisted for several days. The factor of individual sensitivity was present, since the reactions were not equally great in all 3 persons.

When a patient is given an injection of a 1:1000 epinephrine solution containing sodium bisulfite, as preservative, the tissues may be exposed locally to 0.1% concentration of the bisulfite. When the epinephrine solution is added to procaine, a common practice in making local anesthetic solutions, the resulting bisulfite concentration may fall between 0.001%, if the end concentration of epinephrine is 1:100,000, and 0.007%, with epinephrine concentrations of 1:16,000, as are sometimes used. The results obtained by us show that such concentrations of sodium bisulfite are not entirely innocuous, but may produce demonstrable, though variable, injury to the tissues at the site of application. The reason that irritation has not been recognized heretofore is that the strong vasoconstriction of epinephrine obscures or inhibits the inflammatory reddening and swelling,[3] although it cannot prevent damage to the cells. There is a definite possibility, therefore, that the local inflammatory reactions commonly seen, after epinephrine vasoconstriction has passed off, may be due, at least in part, to the irritant effects of bisulfite. This

[3] Tainter, M. L., *J. Pharm. Exp. Therap.*, 1926, **27**, 201; **33**, 129; Tainter, M. L., and Reichert, F. L., *Arch. Path.*, 1928, **6**, 872.

being the case, search should be made for substitute anti-oxidants which would be free from the comparatively minor, but nevertheless definite, disadvantages of sodium bisulfite.

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## Experimental Inoculations of Trichomonads from Man into the Prostate Gland of Rats.

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The purpose of these experiments was to determine the duration of experimental infections with the 3 human trichomonads in the prostate gland of the rat. According to certain recent workers *Trichomonas vaginalis* may occasionally be a causative agent of chronic prostatitis.

Although the incidence of trichomonad infection in the vagina of women is fairly high (10-69.9%),[1] positive reports of *T. vaginalis* in the prostate of men have been rare. Stuhler[2] found 16 positive cases out of a total of 32,000 prostate secretions; Riba and Perry[3] reported 2 cases. Nitschke[4] examined the urine and prostate fluid of 40 men suffering from non-specific urethritis and found 5 of them positive for *Trichomonas vaginalis*. Working in conjunction with Drs. A. M. Meads and T. I. Buckley of Oakland, California, we have been able to demonstrate one positive case of *T. vaginalis* out of a total of 100 patients examined. In all cases the expressed prostatic fluids were cultured in L.E.B. medium and were examined after each 2 to 4 transplants before being discarded as negative. This one positive case was a single man, age about 45 years, having a past history of gonococcal infection 15 years prior to this examination. There was no urethral discharge present, the gland was normal in size and quite tender on massage. Microscopic examination of the expressed prostatic fluid showed approximately 5% pus cells and numerous flagellates.

The combined effect of bacteria and protozoa probably accounts

[1] Bland, P. B., Goldstein, L., and Wenrich, D. H., *J. A. M. A.*, 1931, **96**, 157.
[2] Stuhler, L. G., *Proc. Staff Meeting Mayo Clinic*, 1933, **8**, 221.
[3] Riba, L., and Perry E., *J. Urol.*, 1929, **22**, 563.
[4] Nitschke, P. H., *J. A. M. A.*, 1936, **107**, 12.