blood. In several of the experiments this depression persisted for as long as an hour after penicillin had been completely excreted from the blood, on others there was a more rapid return to the control value for that particular patient.

The bleeding time, which in the control group also remained constant, fell when penicillin was given (Graph III). The curve of descent was not quite so marked, and from these early results seemed more transient.

The prothrombin times have so far not shown a characteristic uni-directional change.

DISCUSSION

This consistent, penicillin-evoked alteration of the blood possesses two components. The first one, the hastening of the coagulation of the blood, has already been described. The second is an even more startling change in the nature of the clot itself. There is produced, as a result of the increased penicillin level in the blood, a non-retractile clot; the blood is dark and exceedingly viscous in its flow, and when coagulation is complete it appears solidified. The appearance of the coagulated blood is that of an artificially produced solid thrombus. The mechanism of this peculiar clotting response is deserving of complete analysis, and such studies are planned.

There is a twofold clinical significance to be attached to these findings. First, they serve to emphasize the danger of thrombus formation with penicillin administration. This becomes more important with the newer tendency to increase dosages employed to augment clinical effect. Second, they suggest investigative studies to test the value of the drug as a coagulant in hemorrhagic disorders.

The authors wish to acknowledge their appreciation for the cooperation and encouragement given to them by Col. G. V. Emerson, Commanding Officer of the Harmon General Hospital, and Lt. Col. William O. Johnson, Chief of the Surgical Service.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

STANDARDIZATION OF STREPTOMYCIN[1]

IN the isolation and purification of antibiotic substances, the bacteriologist has to depend upon biological assays. Until the chemical nature of the active agent has been established and until a suitable chemical test has been developed, the chemist, as well, has to depend upon such methods. This is true also of the pharmacologist and of the clinician, who study the effectiveness of such materials *in vivo*. The unit of measurement adopted for a certain purpose, however, may not be suitable or may prove too small when applied to a totally different problem or use. As a result of such different applications, new units of measurement of the antibiotic substance may have to be introduced.

In the case of penicillin, for example, the dilution unit originally employed for measuring the activity of this material using a standard test organism, namely, *Staphylococcus aureus,* was later replaced by the Oxford unit.[2] This unit represented "that amount of penicillin which, when dissolved in 50 ml of meat extract broth, just inhibits the growth of the test strain of *Staphylococcus aureus.* Thus material containing one unit of penicillin per mg just inhibits the growth of *S. aureus* at a dilution of 1 to 50,000." When crystalline penicillin was finally obtained, the value of the Oxford unit was adjusted to the weight of the product, 1 unit corresponding to 0.6 micrograms of the crystalline material.[3]

A similar situation has now arisen in establishing standard units for another antibiotic substance, streptomycin. The previous method for measuring the antibacterial activity of this material was based upon its bacteriostatic effect against a given strain of *Escherichia coli.*[4] A unit of streptomycin was thus defined as that amount of material which will inhibit the growth of the particular strain of *E. coli* in 1 ml of nutrient broth or other suitable medium. This unit proved to be satisfactory for production and isolation studies of streptomycin;[5] it appeared to be satisfactory also for pharmacological investigations, especially when small animals were used.[6] For clinical purposes,

[1] Journal Series Paper, New Jersey Agricultural Experiment Station, Rutgers University, Department of Microbiology.

[2] H. W. Florey and M. A. Jennings, *Brit. Jour. Exp. Path.,* 23: 120–123, 1942.

[3] M. V. Veldee, R. P. Herwick and R. D. Coghill, *chairman,* SCIENCE, 101: 42–43, 1945; P. Hartley, *ibid.,* 101: 637–638, 1945.

[4] A. Schatz, E. Bugie and S. A. Waksman, *Proc. Soc. Exp. Biol. and Med.,* 55: 66–69, 1944.

[5] S. A. Waksman, E. Bugie and A. Schatz, *Proc. Staff Meet. Mayo Clinic,* 19: 537–548, 1944.

[6] H. J. Robinson, D. G. Smith and O. E. Graessle, *Proc. Soc. Exp. Biol. and Med.,* 57: 226–231, 1944.

however, too many units are required,[7] thus giving the impression that large doses of the material are needed for effective chemotherapeutic purposes.

The problem of defining a streptomycin unit becomes especially complicated when attempts are made to compare the *in vivo* activity of streptomycin with that of penicillin, especially when the measurement of the latter is based on the Oxford unit.[8] The fact that the basis for the standardization of the two materials is different, and, further, the use of two different test organisms can only lead to much confusion. This becomes clear when one compares the above figures given for the Oxford unit with the unit of streptomycin which is based upon inhibition of growth in 1 ml of medium; the Oxford unit is thus found to represent a far greater *in vitro* activity than the streptomycin unit.

Because of these considerations, it is proposed here to establish the following three units for designating streptomycin:

(1) An S unit, or that amount of material which will inhibit the growth of a standard strain of *E. coli* in 1 ml of nutrient broth or other suitable medium. This unit will thus correspond to the original *E. coli* unit.

(2) An L unit, or that amount of material which will inhibit the growth of a standard strain of *E. coli* in 1 liter of medium. An L unit is thus equivalent to 1,000 S units.

(3) When crystalline material becomes available, a weight unit will become possible. One can even now prepare for it by recognizing a G unit, comparable to one gram of the crystalline material. Should this material show an activity of 1,000 *E. coli* units per 1 mg, it will be equivalent to 1,000,000 S units, to 1,000 L units and to 1 G unit, per gram of material.

For the purpose of studying the production of streptomycin and its concentration in the medium as well as for the isolation of the material, and especially for measuring its concentration in blood, urine and other body fluids, the *E. coli* or the new S unit may still be used. For the purpose of utilizing the material for clinical treatments, however, the new L or even the G unit would no doubt prove to be far preferable. Thus, a good culture broth will have 100 to 200 S units of streptomycin per 1 ml. If a patient is treated with streptomycin, instead of using 1,000,000 to 5,000,000 units daily, on the basis of the old unit, the equivalent in terms of the new units will be 1,000 to 5,000 L units of streptomycin or 1 to 5 G units. The latter will thus be roughly equivalent to about 5 gm of the purified material. The concentration of streptomycin in the blood of the patient may be 0.5 to 50 S units, depending on dosage used and rate of excretion.

The units of measurement of streptomycin are thus based upon the inhibition of growth of a standard strain of *E. coli,* as determined by the dilution method, using either a series of dilutions in liquid media or the agar plate streak method. The actual determinations are carried out by the agar diffusion or so-called cup method, using either a standard strain of *Bacillus subtilis* or *S. aureus.* Other convenient procedures, such as the turbidimetric method, can also be employed. A given preparation of streptomycin, preferably the crystalline product when it becomes available, is used as a standard for determining the potency of unknown lots of broth or of the isolated product.

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[7] H. A. Reimann, W. F. Elias and A. H. Price, *Jour. Am. Med. Asn.,* 128: 175–180, 1945.

[8] F. R. Heilman, *Proc. Staff Meet. Mayo Clinic,* 20: 169–176, 1945.

DARKFIELD ILLUMINATORS IN MICROSCOPY

IT is usually recommended that a microscope be equipped with a special darkfield condenser and a funnel stop solely for darkfield work. An improvised darkfield stop has been found to be a satisfactory substitute for the darkfield condenser in the experience of the writer.

A special stop is generally supplied with the microscope by the manufacturer. An improvised darkfield stop can be made by having a piece of thin metal cut in the form shown in the illustration (Fig. 1), and of such a size that the outer narrowing ring fits snugly into the slot provided in most substage Abbe condensers.

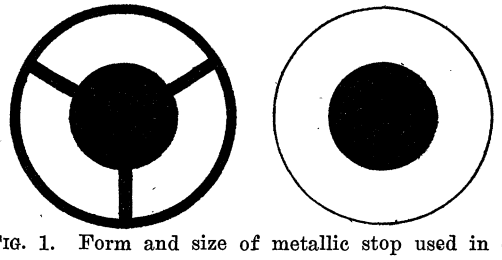

FIG. 1. Form and size of metallic stop used in conjunction with the Abbe condenser to produce darkfield illumination. Fig. 2. Form and size of stop made by pasting a circular disc of black paper on a glass disc for use in conjunction with the Abbe condenser to produce darkfield illumination.

Another type of stop can be made by pasting a circular disc of black paper in the center of a large