scientific personnel. Continued military research as well as the great peace-time industrial undertakings on the horizon will together demand more technologists than have ever been available, and this at a time when we have allowed our scientific personnel resources to dwindle seriously. This promises to become one of our large post-war problems. The promotion of the general technological welfare is at stake.

Anything that can be done, no matter how small, to accelerate the reaccumulation of this important human resource is worth doing and should be done.

Several efforts are now under way to encourage the return of young scientists and others to school. The encouragement being given veterans to return to school has been given wide publicity. The American Council on Education has facilitated the academic recognition of military in-service training through their evaluation[2] of large numbers of military training programs. Although the effect of these efforts should be great, they do not particularly stress the training needs in science and technology.

Recently, the National Research Council announced[3] a plan for granting fellowships for competent young scientists to study toward the doctorate. This will be an important step toward replenishing the supply of scientific personnel.

Another step would be to enlarge the opportunities for young scientists to continue their training on a part-time basis. This is something that can be done at once without waiting for the cessation of hostilities and the release of scientific workers from their present jobs. At the present time, a graduate student wishing to carry on his studies must look to local part-time educational facilities. Although the Office of Education's E.S.M.W.T. courses have been of considerable service, this student must usually look to evening classes at the nearest university. These facilities are frequently meager and inconveniently situated. Although there may be a wide choice of evening classes from a liberal arts standpoint, the choice in technological subjects may be slight and primarily at an elementary level. Furthermore, the campus on which these courses are given may be far from the place of employment of the student, and with long working hours and with overcrowded or slow transportation facilities the obstacles to this student taking the course he needs become very great.

In order to facilitate the attendance of these scientific personnel at the courses they need, it is proposed that universities and institutions employing scientific personnel together make a concerted effort to provide the courses needed at a convenient place. It would be necessary to find out what courses are needed by the employees of particular institutions and then to provide these courses at a convenient time and place—say, within the institution immediately after working hours. The students would then register with the university just as if the course were given on the campus.

Students who are able to pursue their studies on a part-time basis now are more likely to return to university campuses to complete their graduate requirements when the time comes for them to leave their present jobs. Not only would the students and universities benefit, but in the long run the institution would also benefit through the increased competence and value of their employees.

PHILIP N. POWERS,
Training Director

NAVAL ORDNANCE LABORATORY,
WASHINGTON, D. C.

SPECIAL ARTICLES

CRYSTALLINE REINECKATES OF STREPTOTHRICIN AND STREPTOMYCIN

STREPTOTHRICIN, the antibiotic agent produced by *Actinomyces lavendulae*,[1,2,3,4,5] and streptomycin, a closely related substance from *Actinomyces griseus*,[5,6,7,8,9] have been intensely studied recently in view of their possible utility for the control of infections resistant to penicillin. As a result, both substances have been characterized with regard to their antibacterial spectra, protective and toxic effects and other biological properties. However, published information concerning their chemical nature is scant and is in essence confined to the statement that they are both water-soluble bases; as to purification methods, only the preparation of crude concentrates has been described.[1,6]

[2] American Council on Education, "Guide to the Evaluation of Educational Experiences in the Armed Services."

[3] SCIENCE, March 30, 1945, p. 322.

[1] S. A. Waksman and H. B. Woodruff, *Proc. Soc. Exp. Biol. and Med.*, 49: 207, 1942.

[2] J. W. Foster and H. B. Woodruff, *Arch. Biochem.*, 3: 241, 1943.

[3] G. Rake, D. M. Hamre, F. Kavanagh, W. L. Koerber and R. Donovick, *Jour. Med. Sci.*, 1945.

[4] H. J. Robinson and D. G. Smith, *Jour. Pharm. Exp. Therap.*, 81: 390, 1944.

[5] A. Schatz and S. A. Waksman, *Proc. Soc. Exp. Biol. and Med.*, 57: 244, 1944.

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

[7] D. Jones, H. J. Metzger, A. Schatz and S. A. Waksman, SCIENCE, 100: 103, 1944.

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

[9] R. Donovick, W. L. Koerber and G. Rake, to be published.

We have succeeded in isolating from submerged culture filtrates of the respective *Actinomycetes* both streptothricin and streptomycin in form of their crystalline salts with Reinecke acid, which are antibiotically active in proportion to their content of the base component.

STREPTOTHRICIN

A mere outline of the procedure employed by us for the purification of streptothricin will have to suffice here. In brief, it involves absorption of the active principle on charcoal followed by elution with dilute mineral acid essentially as described by Waksman and Woodruff,[1] precipitation with phosphotungstic acid, conversion of the bases liberated from the phosphotungstate into a crude picrate and fractionation of the latter by chromatographic methods. One or more of the resulting fractions, after removal of the picric acid, yielded water-soluble amorphous products possessing a potency of 400–500 units[10] per mg. Addition of Reinecke salt, $NH_4[Cr(SCN)_4(NH_3)_2]$, to an aqueous solution of such highly active fractions resulted in the formation of crystalline precipitates, from which pure streptothricin reineckate was secured by fractional crystallization.

Streptothricin reineckate crystallizes in form of clusters of fine platelets which decompose at 192–194° (corr.) after sintering at 184°. Its potency was determined in repeated tests as 256 units per mg. The antibiotic activity of the salt is entirely due to its base component, as the ammonium salt of Reinecke acid in comparable concentrations is completely devoid of bacteriostatic properties.

The analytical composition of preparations isolated from two different fermentation batches, after drying *in vacuo* at 70°, was as follows: C, 25.0, 24.9; H, 3.94, 4.3; N, 23.8, 24.2; Cr, 10.54, 10.1; S, 25.8, –; H_2O loss: 4.1, 4.1. The analyses of two other independently prepared specimens fell into the same range.

These data suggest an empirical formula, $C_{21}H_{39}O_7N_{17}S_8Cr_2$ (calc: C, 25.19; H, 3.93; N, 23.76; S, 25.56; Cr, 10.38), which corresponds to the di-reineckate of a base $C_{13}H_{25}O_7N_5$.[11] We wish to make it clear that the formula for the free base, though it is supported by confirmatory evidence given below, should be regarded as provisional, since the molecular weight is not known.

[10] The streptothricin unit referred to has been defined in previous publications (see references 2 and 3). The same unit has been adopted for the assay of streptomycin. Exchange of samples of streptomycin with Dr. S. A. Waksman showed that this unit corresponds very closely to his *E. coli* streptomycin unit.

[11] The absence of sulfur in streptothricin and streptomycin has been demonstrated qualitatively on partially purified preparations of the respective hydrochlorides.

Decomposition of several specimens of the reineckate with silver sulfate yielded streptothricin sulfate in form of an amorphous white powder which consistently assayed 500–530 units per mg. (calc. for streptothricin sulfate from the reineckate: 650 units per mg). The biological assay and elementary analysis of such preparations are rendered uncertain by their high water content (about 7 per cent.), pronounced hygroscopicity after drying, the presence of some free base and contamination by small amounts of break-down products of Reinecke acid, namely, chromium sulfate (about 2.5 per cent.) and ammonium sulfate (up to 7 per cent.). However, the analyses of three independently prepared specimens showed a C:N ratio of 13:5 (after correction for ammonia nitrogen), in good agreement with the above tentative formulation. Furthermore, they leave no doubt as to the high oxygen content of the base.

The α-amino nitrogen, determined by the manometric method of Van Slyke, was 20–22 per cent. of the total nitrogen (4 and 30 minutes reaction, both corrected for ammonium ion present). This finding supports the analytical evidence that streptothricin contains at least five nitrogen atoms, two of which are present as salt-forming basic groups. Streptothricin is free of O-methyl, N-methyl and hydrolyzable acetyl groups.

The ultraviolet absorption spectrum shows only end absorption, which rules out the presence of aromatic groups or other chromophores giving rise to specific absorption above 220 mμ.

Streptothricin is stable between pH 1 and 8.5, but is destroyed more or less rapidly by higher degrees of alkalinity. It gradually reduces Tollens' reagent and neutral permanganate solution in the cold, and Fehling solution immediately on boiling. It gives positive biuret and ninhydrin reactions, whereas the ferric chloride, nitroprusside, Molisch, Sakaguchi and Schiff tests are negative.

Attempts to secure other crystalline salts have been unsuccessful, nor has acylation of the basic group(s) with various aliphatic and aromatic acyl chlorides so far yielded any crystalline derivatives. All the acylated products are neutral, which indicates that there are no free acidic groups present in streptothricin. Some significant facts emerged from experiments in which benzoyl chloride was used as the acylating agent. When the reaction was carried out in aqueous bicarbonate solution, that is, under conditions favoring benzoylation of the basic groups only, a product was obtained which analyzes well for a dibenzoyl derivative of the base $C_{13}H_{25}O_7N_5$. (Found: C, 56.56; H, 5.82; N, 12.11. Calc. for $C_{27}H_{33}O_9N_5$: C, 56.74; H, 5.82; N, 12.25.) On the other hand, benzoylation in pyridine yielded what appears to be a penta- or

hexabenzoyl derivative of a base of this composition. It may be inferred from these results that streptothricin contains 3 or 4 hydroxyl groups in addition to 2 acylatable basic groups.

Hydrolysis of streptothricin with hydrochloric acid has yielded two water-soluble bases in form of their crystalline picrates, on which we shall report in a separate communication.

STREPTOMYCIN REINECKATE

This compound was obtained from submerged culture filtrates[9] by a procedure similar in many respects to that employed in the isolation of streptothricin. It crystallizes from water in thin plates which decompose at 162–164° (corr.). Two preparations derived from different fermentation batches were analyzed after drying at 70° *in vacuo* (H_2O–loss: 6.1 per cent., 5.8 per cent.): C, 27.1, 26.9; H, 4.65, 4.31; N, 21.4, 20.8; S, 20.8, 21.2; Cr, 8.53, 8.27. The inconsistencies in some of the analytical figures preclude the assignment of a definite empirical formula at the present time. However, formulae $(C_{14}H_{26}O_7N_9S_4Cr)_n$ or $(C_{14}H_{26}O_8N_9S_4Cr)_n$, corresponding to $(C_{10}H_{19}O_{7-8}N_3)_n$[11] for the basic component, are in reasonable agreement with the above data and provide a basis for calculating the potency of free streptomycin. Since the two analyzed preparations of the crystalline reineckate assayed 370 and 410 units (10) per mg, respectively, it follows that the potency of the pure streptomycin base should lie between 800 and 910 units per mg. Decomposition of one of these preparations with silver sulfate yielded streptomycin sulfate assaying 850 units per mg.

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J. FRIED
O. WINTERSTEINER
DIVISION OF ORGANIC CHEMISTRY,
SQUIBB INSTITUTE FOR MEDICAL RESEARCH
NEW BRUNSWICK, N. J.

CHRONIC INTERMITTENT ANOXIA AND IMPAIRMENT OF PERIPHERAL VISION[1]

IN general, the methods which have been employed in testing visual functions under conditions of acute anoxia have failed to reveal reliable signs of impairment below altitude pressures of 12,000 feet above sea level.[2] Comparable studies for possible effects of chronic intermittent anoxia have been lacking. With the great expansion of aviation in the present war, it became imperative to establish a sound basis for Service regulations governing the use of oxygen equipment on repeated missions. Twenty male subjects, selected to meet the age, physical and mental standards of the Service Air Corps, were exposed in a low pressure chamber to a simulated altitude of 10,000 feet above sea level for five or six hours per day, six days per week, for a period of four to six weeks. Before, during and after the period at altitude, peripheral vision was systematically studied in each subject by means of a newly developed, quantitative campimeter.[3]

During the third or fourth week of exposure at 10,000 feet, thirteen of the twenty subjects developed a marked and progressive impairment of peripheral vision. This impairment was reflected by failure to see targets briefly exposed in the peripheral field that had previously been readily detected. It occurred in some subjects without any associated alteration of central vision, although in others both functions were impaired. It developed insidiously in the sense that the subjects were unaware of it. Once established, it could not be relieved immediately by the inhalation of 100 per cent. oxygen at altitude and it could still be detected on the same day at ground level, although to a less marked extent. The effect was not immediately reversible in some subjects at the end of the four- or six-week period; in some instances days or weeks elapsed before return to preexposed levels of visual efficiency. One subject, who developed a very marked impairment of peripheral vision with delayed recovery, was reexposed nine months later. During the second exposure, however, he breathed 100 per cent. oxygen during the middle hour of each daily five-hour period at altitude. Under these conditions no impairment of peripheral vision occurred.

Four additional subjects were likewise chronically exposed to a particular altitude ranging from 11,500 to 18,000 feet. In these individuals the impairment of peripheral vision appeared earlier, was more marked and was recovered more slowly as the altitude, or the degree of anoxia, was increased. These subjects were studied by Bryan and Ricketts[4] in their search for possible effects upon the adrenal cortex of chronic intermittent anoxia. They were maintained

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Chicago.

[2] H. G. Armstrong, "Principles and Practice of Aviation Medicine," second ed. Chapter xviii. Baltimore: Williams and Wilkins Co. 1943.

[3] W. C. Halstead, *Arch. Neurol. and Psychiat.*, 52: 252, 1944.

[4] A. H. Bryan and H. T. Ricketts, *Jour. Clin. Endocrinol.*, 4: 450, 1944.