both normal and leukemic spleens contained about 17% of the nitrogen of the whole chromosome fraction (Table I). The PNA contents, on the other hand, were strikingly different. Whereas the residual chromosome fraction from normal mouse spleen contained only 0.18-0.19 γ of PNA per γ of nitrogen, that from leukemic spleen contained 0.32 to 0.45 γ of PNA per γ of nitrogen, an increase of nearly 100%.

Discussion. It is of great importance to determine whether the excess PNA found in the residual chromosome fraction of leukemic spleen is an integral part of the chromosome structure or is contained in some other constituent of the preparation. The cytoplasm of the leukemic spleen cells contained large amounts of PNA.[2] That any significant amount of cytoplasm was left in these preparations seems unlikely, however, since careful microscopic observation failed to reveal it. The whole "nuclear fraction" of the leukemic spleens, however, was also extremely rich in PNA.[2] This is in agreement with the findings of Thorell,[6] who has shown, by ultraviolet spectrophotography, that the nucleus of the leukemic white cell contains 2 to 4 nucleoli rich in PNA, which are absent from the normal mature white cell. Since nucleoli sometimes remain attached to isolated chromosomes,[1] it is probable that some of the excess PNA found in the leukemic residual chromosome preparations is located in associated nucleoli; how much cannot be decided until preparations are obtained which do not clump so badly, and can be examined microscopically.

Summary. The residual chromosome fraction isolated from leukemic mouse spleen contained from 0.32 to 0.45 γ of pentose nucleic acid per γ of nitrogen, while that from normal spleen contained 0.18-0.19 γ per γ of nitrogen.

[6] Thorell, B., Studies on the formation of cellular substances during blood cell production. London, 1947.

16783

Isolation of a Streptomycin-Resistant Organism Capable of Utilizing Streptomycin for Growth.

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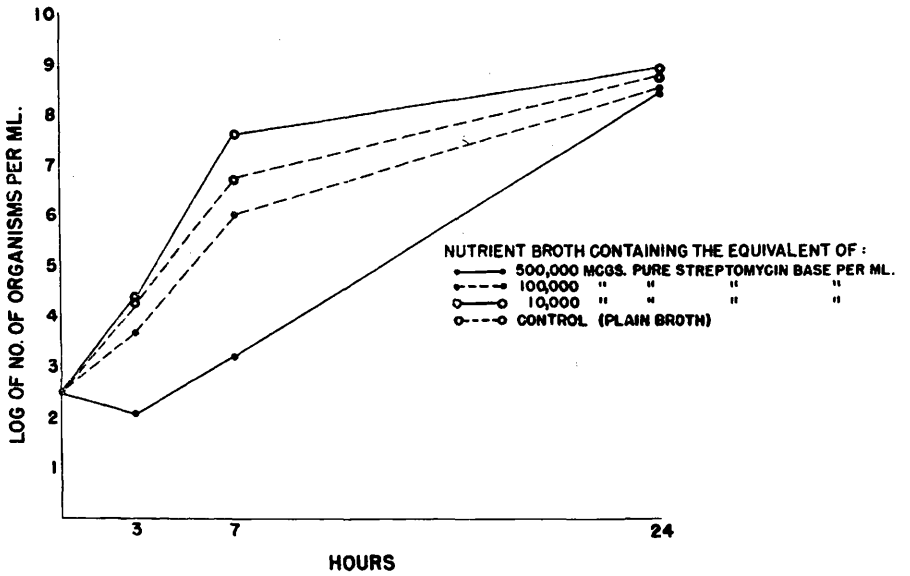
Since the introduction of streptomycin in 1944, the appearance of streptomycin-resistant variants in otherwise sensitive strains of microorganisms has been observed repeatedly. Miller and Bohnhoff[1] reported the isolation of variants of meningococci not only resistant to streptomycin but capable of growing only in its presence. These organisms were dependent upon streptomycin for multiplication both *in vitro* and *in vivo*. Paine and Finland,[2] described dependent variants of other bacterial species including *Staphylococcus aureus, E. coli, Ps. aeruginosa,* and *P. morgani.* More recently, 13 streptomycin-dependent strains of *M. tuberculosis* have been isolated in our own laboratory[3] from mice infected with the $H_{37}Rv$ strain and subsequently treated with streptomycin for a period of 31 days. The possibility that these organisms are capable of utilizing the nitrogen of streptomycin for growth has been suggested.

In the present communication, the isolation of a streptomycin-resistant organism capable of growing both in streptomycin as the sole source of nutrient and in broth containing no streptomycin is discussed.

[1] Miller, C. P., and Bohnhoff, M., *Science,* 1947, **105**, 620.

[2] Paine, T. F., and Finland, M., *Science,* 1948, **107**, 143.

[3] Lenert, T. F., and Hobby, G. L., *Am. Rev. Tuberculosis,* 1949, in press.



GRAPH 1.

Effect of streptomycin on the growth of a streptomycin-resistant gram negative rod (strain SMR).

Isolation. An aqueous solution of a highly purified form of streptomycin sulfate, prepared through an intermediate crystalline stage, in a concentration equivalent to 375,000 μg of pure streptomycin base per ml, was allowed to stand at room temperature for a period of several days. Subcultures were subsequently made from the solution (1) by streaking 0.05 ml of the undiluted solution onto beef infusion agar and onto Sabaraud's agar, and (2) by inoculating 0.1 ml of the undiluted solution and a 10^{-8} dilution of the solution into beef infusion broth and into fluid thioglycollate medium. Incubation was carried out at 5°C, 22°C, and 37°C. Luxuriant growth appeared in all cultures incubated at 22°C and at 37°C. No growth occurred at 5°C.

Cultural characteristics. Morphologically the organism is a short plump gram negative rod with rounded ends and varying slightly in size. At times the organism may appear almost coccoid in form. It is nonmotile and produces no spores.

The organism grows readily in diffuse form under aerobic or facultative anaerobic conditions in liquid media.* Growth occurs at pH ranging from 6.0 to 7.8 and at room temperature (22°C) and 37°C; no growth occurs at 5°C.

On plain or blood agar the organism grows in the form of a smooth opaque grayish-white colony, approximately 3-4 mm in size. It is nonhemolytic. On glucose or Sabaraud's agar, it grows in the form of a confluent viscous or mucoid slime.

Growth occurs rapidly in the presence of glucose, maltose, sucrose, levulose, arabinose, and d-galactose with the formation of acid and gas. In the presence of xylose, acid without gas is formed, while in the presence of lactose (0.1%), growth is slow and no acid is produced.

Growth occurs readily on sodium citrate agar. The organism is capable of reducing nitrates to nitrites. Acetyl methyl carbinol is not formed. In Voges-Proskauer-Methyl-Red broth, a powerful reducing substance is produced. In distilled water† and in distilled

* It is of interest to note that this organism is incapable of producing detectable amounts of penicillinase. It is sensitive to 80 units of penicillin per ml and to the equivalent of 40 μg of polymyxin B hydrochloride per ml.

† Baxter sterile distilled water was used in all experiments throughout this study. Analyses indicated a content of less than 10 p.p.m. total solids.

water† containing 3% methyl or ethyl alcohol, growth occurs rapidly within 24 hours.

Although morphologically suggestive of the Pseudomonas group of organisms, the data above are not sufficient to place this organism within this species.

Streptomycin Resistance. In view of the fact that this organism was originally isolated from a solution containing a high concentration of streptomycin, it is apparent that it is highly resistant to this antibacterial agent.

Graph I shows the rate of growth of the organism in broth containing varying concentrations of highly purified streptomycin. Highly purified streptomycin sulfate was diluted to 10,000, 100,000, and 200,000 μg of pure streptomycin base per ml. Plain broth was used as control. An 18-hour broth culture of the streptomycin-resistant organism was diluted in broth to a dilution of 10^{-5}, and 0.5 ml of this was inoculated into tubes containing 4.0 ml of each of the above solutions of streptomycin in broth. Incubation was carried out at 37°C. The number of organisms per cc was determined by colony counts at 0, 3, 7, 24 hours. No inhibition of growth was observed in the presence of streptomycin in concentrations equivalent to 10,000 or 100,000 μg of pure base. A concentration of streptomycin equivalent to 500,000 units of pure base produced a temporary lag in the growth of the organism, lasting for a period of 3 to 7 hours only. After 24 hours incubation, the number of organisms per ml was as great in the presence of the equivalent of 500,000 μg of pure streptomycin base per ml as in broth containing no streptomycin.

Utilization of streptomycin for growth. The fact that this organism was originally isolated from a solution of streptomycin in water suggested that the organism must be capable of utilizing streptomycin for growth.

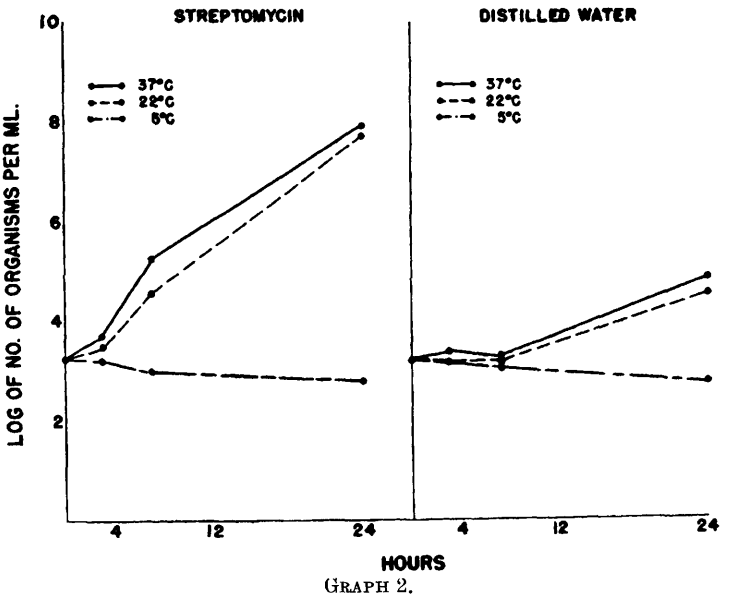
Highly purified streptomycin sulfate was diluted in sterile distilled water to a concentration equivalent to 333,000 μg of pure streptomycin base per cc. Four cc of this solution was pipetted into each of 3 tubes. As control, 4 ml of sterile distilled water was pipetted into each of 3 additional tubes. An 18-hour broth culture of the streptomycin-resistant strain was centrifuged and the sedimented organisms washed twice with sterile distilled water to remove traces of broth. The organisms were then resuspended in a volume of sterile distilled water equal to that of the original broth culture. The aqueous suspension of organisms was then diluted to 10^{-4}, and 0.1 ml of this dilution was inoculated into each of the 3 tubes of streptomycin solution and into each of the 3 tubes of sterile distilled water. Incubation was carried out at 37°C.

Two similar sets of tubes were prepared and incubated at 5°C and at 22°C, respectively.

The results are shown in Graph 2. No multiplication occurred at 5°C either in the aqueous solution of streptomycin or in distilled water. At both 22° C and 37°C however, heavy growth occurred in the aqueous solution of streptomycin. Whereas the rate of growth was somewhat slower than had previously been observed when the organism was grown in a broth solution of streptomycin (Graph 1), the number of organisms per ml after 24 hours incubation was as great in the aqueous solution of streptomycin as it previously had been in the broth solution of streptomycin. At 22°C and at 37°C, slight multiplication took place in the distilled water. The amount of growth occurring in the water alone was, however, far less than in the aqueous solution of streptomycin, and it is apparent, therefore, that this organism is capable of utilizing streptomycin for growth.

Pathogenicity. Mice of the Rockland regular strain inoculated intraperitoneally with an undiluted 4-hour plain broth culture died within less than 72 hours; mice similarly inoculated with dilutions of 10^{-1} - 10^{-7} all survived, indicating that the organism possesses little virulence for mice.

It has been observed by Miller and Bohnhoff that streptomycin-dependent strains of meningococci are virulent for mice only if the animals are treated simultaneously with streptomycin. The virulence of this strain, therefore, was tested in mice receiving 4 mg of streptomycin daily by the subcutaneous route. Again only those animals receiving undiluted culture failed to survive.



GRAPH 2.

The rate of growth of a washed suspension of a streptomycin-resistant gram negative rod in presence of streptomycin as source of nutrient.

When heat-killed suspensions of the organisms, grown on broth or agar, were injected intravenously into each of 3 rabbits, all of the animals showed marked pyrogenic responses consisting of temperature rises ranging from 1.2 to 2.2°C. These animals appeared ill soon after injection and 7 of the 9 died within 24 hours.

Seven-tenths of a ml of each of these suspensions, when administered intravenously to mice (Swiss strain), produced no reaction. It seems possible, therefore, that death of the rabbits receiving this material may have been due primarily to pyrogenic reactions rather than to the presence of other toxic substances.

All animals receiving the aqueous suspension of standard pyrogen prepared from *Ps. pyocyaneus*‡ survived with no untoward reaction other than rise in temperature.

Discussion. The isolation of an organism which is not only resistant to the antibacterial action of streptomycin but capable of multiplying in the presence of streptomycin as the sole source of nutrient has been described. Recently a second organism has been isolated which is also resistant to streptomycin and capable of growing in high concentrations of the drug. This organism is a slender gram-negative rod which grows on beef infusion agar in the form of an opaque creamy yellow colony, 2-3 mm in diameter. On blood agar it produces a grayish-red pigment after 1 to 2 weeks at room temperature. Sugars are not fermented readily.

The fact that such organisms may exist is significant. It is now apparent that microorganisms may be divided into 4 general categories, in relation to streptomycin: (1) the streptomycin-sensitive cells, including those that are capable of growing in culture media without streptomycin but are inhibited by the presence of streptomycin, (2) the streptomycin-resistant cells, which are capable of growing either in culture media alone or in media containing streptomycin, (3) streptomycin-resistant cells, which are capable of multiplying in culture media, in media containing streptomycin, or in aqueous solutions of streptomycin, and (4) streptomycin-dependent

‡ Prepared from a culture of *Ps. pyocyaneus* according to the method of Welch, Calvery, McClosky, and Price[4] and subsequently purified further by removal of protein.

[4] Welch, H., Calvery, H. O., McClosky, W. T., and Price, C. W., *J. Am. Pharm. Assn.*, 1943, **32**, 65.

cells, which multiply only in the presence of streptomycin. The frequency with which streptomycin-resistant variants and organisms capable of utilizing streptomycin for growth have been described has limited its usefulness as a chemotherapeutic agent.

Conclusions. The isolation and cultural characteristics of a streptomycin-resistant organism capable of multiplying in aqueous solutions of streptomycin has been described.

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Biological Studies with Arsenic[76]. II. Excretion and Tissue Localization.

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Arsenic[76] is characterized by a β radiation maximum of 3.04 MEV, a γ radiation maximum of 2.15 MEV, and a 26.8 hour half-life. The high energy beta and gamma emission simplify counting but necessitate shielding to protect personnel. The short half-life makes tracer experiments of long duration impossible; but the rapid decay is useful in therapeutic applications, as radiation dosage is easily controlled.

In order to calculate radiation dosage from any radioactive material to a particular tissue, it is essential to know not only the physical characteristics of the isotope employed, but also the concentration of the material in the tissue under consideration. For this reason, as well as for the more theoretical purposes discussed in an earlier paper,[1] we began a series of studies on the fate of radio-arsenic in laboratory animals, and, later, in man. Several phases of this research still are far from complete; however, because of the therapeutic potentialities of arsenic[76],[2] and because radioisotopes of arsenic are now offered for general distribution,[3] we feel that our studies on distribution and excretion may be of some interest and value to other experimenters.

Early work with radio-arsenic. Radioactive arsenic has been previously reported upon by Lawton and co-workers,[4] who administered 16-day arsenic (As^{74}) to a group of 6 cotton rats infected with *Litomosoides carinii,* sacrificing their animals 24 hours after intraperitoneal injection; by DuPont *et al.*,[5] who injected radio-arsenic intravenously in 37 rabbits; and by Hunter and co-workers,[6] who studied distribution in tissues, and in various chemical fractions of tissue, of radio-arsenic administered subcutaneously to rats, rabbits, guinea pigs, higher apes, and man. All of these experiments showed a remarkable degree of individual variation among animals of the same species receiving apparently identical treatment.

In general, all animals in all experiments exhibited greatest arsenic concentration in liver, kidney, spleen, and lung. Low arsenic uptake by the Brown-Pearce rabbit tumor was found by the DuPont group, who also reported no change in the tissue distribution pattern of tumor-bearing animals. Hunter *et al.* noted a remarkable difference between

1 Straube, R. L., Neal, W. B., Jr., Kelly, T., and Ducoff, H. S., Part I. PROC. SOC. EXP. BIOL. AND MED., in press.

2 Neal, W. B., Jr., Jacobson, L. O., Brues, A. M., Ducoff, H. S., Straube, R. L., and Kelly, T., *Am. Assn. for Cancer Research,* March 13, 1948, Atlantic City, N. J.

3 U. S. Atomic Energy Commission, Radioisotopes—Catalogue and Price List, No. 2, September, 1947.

4 Lawton, A. H., Ness, A. T., Brady, F. V., and Cowie, D. B., *Science,* 1945, **102**, 120.

5 DuPont, O., Ariel, I., and Warren, S. L., *Am. J. Syph. Gon. and Ven. Dis.*, 1942, **26**, 96.

6 Hunter, F. T., Kip, A. F., and Irvine, J. W., Jr., *J. Pharm. and Exp. Therap.*, 1942, **76**, 207; Lowry, O. H., Hunter, F. T., Kip, A. F., and Irvine, J. W., Jr., *ibid.*, 1942, **76**, 221.