of significant enzymatic activity from the cytoplasm-free nuclei also favors the interpretation that the locus is exclusively mitochondrial, but it must be conceded that these nuclei could have suffered extraction of enzyme in the course of purification.

It is of interest to note that the mitochondrial localization of monoamine oxidase might have been expected from the precedent of other systems which transfer electrons to oxygen: d-amino acid oxidase(10), uricase (11), and processes dependent upon cytocrome oxidase(12). This convergence of oxidative systems suggests that the mitochondrion is a favorable location for the reduction of oxygen; whether or not there is a more intimate functional association between these processes and an enzyme that deaminates potent pharmacological agents is a question that awaits future investigation.

Summary. (1) The monoamine oxidase activity of rat liver cells is localized predominantly, and perhaps exclusively, in the mitochondria. (2) In this respect it conforms to a precedent since other aerobic processes have been shown to have a mitochondrial localization.

10. Claude, A., Harvey lectures, 1947, v43, 121.

11. Schein, A. H., Podber, E., and Novikoff, A. B., *Fed. Proc.*, 1950, v9, 224.

12. Schneider, W. C., Claude, A., and Hogeboom, G. H., *J. Biol. Chem.*, 1948, v172, 451; Hogeboom, G. H., Claude, A., and Hotchkiss, R., *J. Biol. Chem.*, 1946, v165, 615.

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Streptococcal Bacteriostatic Antibody in Patients Treated with Penicillin.* (19007)

GEORGE DAIKOS AND LOUIS WEINSTEIN.

From the Haynes Memorial and Evans Memorial, Massachusetts Memorial Hospitals and the Department of Medicine, Boston University School of Medicine, Boston.

The antibacterial activity of the serum of patients infected with the beta-hemolytic *Streptococcus* has been studied by a number of investigators. Bacteriotropins for this organism were first demonstrated by Hare(1). Kuttner and Lenert(2) noted that the bacteriostatic antibody was type-specific, persisted for many months, and was unrelated to the development of antistreptolysin O. From a detailed study of 3 patients, Rothbard(3) concluded that this immune substance first appeared in the third to fifth week following infection, was present for at least 37 weeks and was type specific. A higher incidence of bacteriostatic antibody in rheumatic fever patients than in those with uncomplicated streptococcal infections was observed by Rothbard, Watson, Swift, and Wilson(4). The close association between the presence of type-specific nucleoprotein (M) in the infecting *Streptococcus* and the later development of antibacterial immunity has been recognized(5-7).

The administration of penicillin to patients with acute streptococcal pharyngitis suppresses the formation of antistreptolysin O and anti-streptokinase(8-12). The work re-

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1. Hare, R., *J. Path. and Bact.*, 1932, v35, 701.

2. Kuttner, A. G., and Lenert, T. F., *J. Clin. Invest.*, 1944, v23, 151.

3. Rothbard, S., *J. Exp. Med.*, 1945, v82, 93.

4. Rothbard, S., Watson, R. F., Swift, H. F., and Wilson, A. T., *Arch. Int. Med.*, 1948, v82, 229.

5. Lyons, C., and Ward, H. K., *J. Exp. Med.*, 1935, v61, 531.

6. Rothbard, S., and Watson, R. F., *J. Exp. Med.*, 1948, v87, 521.

7. Baily, M. D., *J. Immunol.*, 1949, v64, 245.

8. Weinstein, L., and Tsao, C. L., PROC. SOC. EXP. BIOL. AND MED., 1946, v63, 449.

9. Rantz, L. A., Boisvert, P. J., and Spink, W. W., *Science*, 1946, v103, 352.

ported in this paper was carried out for the purpose of determining whether antibacterial immunity was affected in the same manner as the antibodies for the soluble products of the organism, by therapy with penicillin. The results indicate that depression of formation of bacteriostatic antibody may follow the exhibition of antibiotic and that the effect is related in degree to dose and route of administration.

Methods. The streptococci used in the bacteriostatic tests were obtained from 2 sources: (1) direct isolation from the respiratory tract of patients with scarlet fever, and (2) stock strains of known type. The latter were employed only when the initially isolated organism was lost; in these instances, the serological type of the original isolation had been determined and a stock culture of the same type was substituted. Most of the stock strains maintained the matt colonial form. Those isolated from patients tended to become smooth and were passed through mice periodically to reestablish the virulent type. Only matt-mouse virulent strains were studied. The bacterial suspensions used in the determination of antibacterial antibody were prepared in the following manner: An actively-growing 12-15-hour-old culture in yeast-tryptose-heart infusion broth was centrifuged and the sediment resuspended in the same medium. Dilutions of this suspension—10^{-2} to 10^{-6}, varying with the individual organism—were made in broth and fresh human blood added. The indirect bacteriostatic test was carried out according to the method of Todd(13) as modified by Ward(14) and Rothbard(3). The reaction was considered positive if a 2+ difference in growth was observed in at least 2 dilutions. The blood was obtained from individuals who did not have streptococcal disease and was not used unless examination revealed the absence of bacteriostatic antibody against the type of *Streptococcus* employed in the test. The direct determination of bacteriostatic antibody was made according to the procedure of Kuttner and Lenert(2). Controls consisted of bacterial suspensions without blood and plasma plus bacterial suspension. The serums studied were obtained from cases of scarlet fever at the time of admission to the hospital and at periodic intervals thereafter and were stored at —20°C until used. All of the patients were treated with penicillin. Blood was drawn, therefore, just prior to an injection—at least 12 hours after a dose—and examined for antibiotic activity before use in the test; detectable quantities of drug were never present. Only those persons from whom the beta-hemolytic *Streptococcus* was isolated at the time or within 24 hours of admission were studied. They were treated in the following manner: Twelve were given 250,000 units of the drug intramuscularly every 12 hours for 10 days; 19 received the same quantity of antibiotic at the same interval orally. In 12 cases 150,000 units was administered parenterally twice daily for 10 days; 11 were treated with a similar quantity by mouth for one week.

Results. All of the strains of *Streptococcus* studied belonged to group A and included, in order of frequency, types 3, 1, 4, 5, 12, 19, 23 and 30. Twenty-three per cent of the entire group of patients revealed demonstrable bacteriostatic antibody. In 3 instances this was detected only by the direct method; in 15 both the indirect and direct tests were used. In the remainder only the indirect procedure was employed.

Examination of Table I shows that the appearance of bacteriostatic antibody was related to the quantity and route of administration of penicillin. Of those who received 250,000 units of antibiotic intramuscularly, none developed type-specific antibacterial immunity. The administration of the same dose of drug by mouth, however, permitted the appearance of antibody in 21%. A moderate difference in the occurrence of antibacterial

10. Kilbourne, E. D., and Loge, J. P., *J. Clin. Invest.*, 1948, v27, 418.

11. Denny, F. W., Wanamaker, L. W., Brink, W. R., Rammelkamp, C. H., Jr., and Custer, E. A., *J.A.M.A.*, 1950, v143, 151.

12. Wanamaker, L. W., Rammelkamp, C. H., Jr., Denny, F. W., Brink, W. R., Houser, H. B., Hahn, E. O., and Dingle, J. H., *Am. J. Med.*, 1951, v10, 673.

13. Todd, E. W., *Brit. J. Exp. Path.*, 1927, v8, 1.

14. Ward, H. K., *J. Exp. Med.*, 1930, v51, 675.

TABLE I. Relation of Dose and Route of Administration of Penicillin to Development of Bacteriostatic Antibody.

Penicillin units, ×1000	Route of admin.—duration of treatment	No. of patients	% of patients developing bacteriostatic antibody
250	Intramuscular—every 12 hr for 10 days	12	0
250	Oral—every 12 hr for 10 days	19	21.1
150	Intramuscular—every 12 hr for 7 days	12	33.3
150	Oral—every 12 hr for 7 days	12	41.5

activity in the serum was also detected when 150,000 units of penicillin was administered by different routes. The influence of the quantity of drug used is illustrated by the fact that of all of the cases receiving 250,000 units, regardless of route of administration, only 12.9% developed bacteriostatic antibody while the serums of 37.5% of those given 150,000 units were bacteriostatic. In practically all instances in which an antibacterial effect of the serum was demonstrable, the level of activity was considerably less than that observed by Rothbard(3) in untreated patients. In only one case in the present study was the titer high; this was a youngster with scarlet fever due to a type 12 *Strep. pyogenes* in whom the organisms persisted in the pharynx for 8 days.

Bacteriostatic antibody was first detected 3 weeks after the onset of infection in 6 cases; in 3 it appeared only after 9-15 weeks. In 5 patients antibacterial activity of the blood was demonstrable in the first or second week of illness and disappeared at the time of convalescence. This probably represents the nonspecific immune response reported by Tillet (15) in the acute phase of streptococcosis. In the remainder of the group bacteriostasis became evident initially between the 4th and 8th weeks. Bacteriostatic antibody persisted for at least 15 weeks in all of the patients; in 2 it was still present after 42 weeks. In 4 cases it was detectable after 15 but not after 24 weeks. These findings suggest that penicillin not only may inhibit completely the development of antibacterial immunity but may also decrease the quantity and shorten the duration if the antibody appears.

The serological varieties of beta-hemolytic *Streptococcus* present in those with positive bacteriostatic tests were type 3 in 4 cases, type 12 in 4, type 5 in 2, and types 1 and 3 in the remainder. Persistence or recurrence of streptococci in the pharynx did not influence the development of antibody, with the possible exception of the one patient cited above.

Serial determinations of antistreptolysin O and anti-hyaluronidase titers revealed no relation between their development and that of the antibacterial antibody since they were found just as frequently in the absence as in the presence of bacteriostatic activity in the serum.

Discussion. The results obtained in this study indicate that the administration of penicillin to patients with streptococcal infection suppressed the appearance of bacteriostatic activity. An indirect relationship between the quantity of drug used and the level of antibacterial immunity was apparent. When antibody was present, the titers were usually lower than those reported in patients receiving no specific therapy. The development of antibacterial activity was inhibited to a greater degree by the intramuscular than the oral route of administration of antibiotic. No relation between anti-hyaluronidase, antistreptolysin O and bacteriostatic antibody was noted. These results confirm the findings of Jawetz(16) in mice. The depression of antistreptococcal antibody production by penicillin is probably related to the rapid elimination of the infecting organisms(8). The higher incidence of antibacterial antibody in patients given smaller doses of drug or treated by mouth tends to support this possibility since both of these result in slower eradication of the bacteria and longer persistence of the antigenic stimulus than when large quantities or the parenteral route are used. The import-

15. Tillet, W. S., *J. Exp. Med.*, 1937, v65, 147.

16. Jawetz, E., *Arch. Int. Med.*, 1946, v77, 1.

ance of the time of contact between the host and organism prior to treatment in the development of immunity has been pointed out by Harrison(17) and Curnen and MacLeod (18). Although the production of serum precipitins and mouse protective antibodies in pneumococcal infections in man is not affected by the exhibition of sulfonamide or penicillin, there is some depression of antibody formation if animals are treated early(19). With delay in therapy or the use of a very large infecting inoculum immunity develops to a satisfactory level(19).

Although only a very small number of patients developed secondary streptococcal complications, there was no correlation between the appearance of clinical or bacteriologic relapse and the development of bacteriostatic antibody. In one case only was there an indication that a suppurative sequela may have influenced the degree of antibacterial immunity.

The inhibition of the development of bacteriostatic antibody in patients ill with streptococcal infections by penicillin therapy probably is of greater clinical importance than a similar depression of anti-streptolysin O, anti-streptokinase and anti-hyaluronidase. The antibacterial antibody protects against reinvasion by a homologous serological type; the other antibodies probably play little or no role in the defense against invasion by the organism. Certain schedules of penicillin treatment, therefore, actually depress the development of effective immunity and permit reinfection by the same serologic type of *Streptococcus* which caused the initial disease.

Conclusions. (1) The administration of penicillin to patients with streptococcal pharyngitis depresses the formation of type-specific antibacterial antibody. (2) There is a relation between the dose and route of administration of penicillin and the degree to which antibacterial activity develops. (3) Suppression of antibody formation by penicillin is probably due to rapid removal of the antigenic focus. (4) Failure to develop type-specific antibacterial antibody for the *Streptococcus* following penicillin therapy increases the risk of subsequent infection by homologous serological types.

17. Harrison, P. E., *J. Inf. Dis.*, 1946, v79, 101.
18. Curnen, E. C., and MacLeod, C. M., *J. Exp. Med.*, 1942, v75.
19. Skinsnes, O. K., and Wooldridge, R. L., *J. Inf. Dis.*, 1948, v83, 78.

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Microwave Diathermy Treatment of Frostbite. (19008)

ROBERT B. LEWIS. (Introduced by Herman I. Chinn.)
From the Department of Pathology, USAF School of Aviation Medicine, Randolph Air Force Base, Randolph Field, Texas.

Several investigators(1-5) have demonstrated the beneficial effect of rapid thawing with warm water on frostbitten tissue with respect to necrosis.

The purpose of this investigation was to determine the effect of immediate rewarming of frostbitten tissue with microwave diathermy on the incidence and extent of necrosis.

Materials and methods. The experiments were performed on 78 male albino rabbits of more than 2,500 g body weight, of which 39 were treated with diathermy and the remainder served as controls. Two degrees of cold

1. Arev, T. Y., *Sovet. vrach. Zhur.*, 1939, v43, 391; Translated by E. Hope.
2. Fuhrman, F. A., and Crismon, J. M., *J. Clin. Invest.*, 1947, v26, 476.
3. Lempke, R. E., and Shumacker, H. B., Jr., *Yale J. Biol. and Med.*, 1949, v21, 321.
4. Finneran, J. C., and Shumacker, H. B., Jr., *Surg., Gynec. and Obst.*, 1950, v90, 430.
5. Pichotka, J., and Lewis, R. B., *U. S. Armed Forces Med. J.*, in press.