TABLE I.
Effect of Iodide on the Thyroid Glands of Rats at Room Temperature or at 4°C.

Group	Treatment	No. of animals*	Wt gain	Thyroid weight, mg/100 g	Epithelial height, microns	Colloid diameter, microns§
A	Room temp. stock diet	5	10.8	10.4	9.1	41
B	Room temp. stock diet 0.05% KI	4	—26.3	12.7	3.5	60
C	4°C stock diet	11†	—18	12.8	12.9	34
D	4°C stock diet 0.05% KI	5‡	—43	13.4	8.3	47

* No. of experimental animals varies due to deaths during the course of the experiment. Figures represent animals surviving at end of 21 days.
† Including 4 animals killed at end of 2 weeks.
‡ Including 2 animals killed at end of 2 weeks.
§ Mean of long and short diameters.

develops like a salivary gland which later loses its excretory pathways, leaving the thyroglossal duct as a remnant. Colloid may, therefore, be considered as a secretory product of the thyroid gland trapped in the alveoli. Iodide is known to cause hypersecretion and hypersalivation and may therefore also be considered to induce secretion of colloid into the thyroid follicles. Such colloid would, from the results of Wolff and Chaikoff, be expected to be low in organic iodine.

Summary. The hyperplasia of the thyroid epithelium produced in rats by exposure to cold-room conditions for 3 weeks can be prevented by increasing the iodide level of the diet. The mechanism of this effect would appear to correspond to the involuting action of iodide in the thyroids of patients with hyperthyroidism. It is difficult to explain these effects on the basis of the inhibition of hormone synthesis, and it is submitted that iodide may stimulate the secretion of colloid into the lumen of thyroid follicles by a mechanism related to its stimulating influence on salivation.

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Tetanus Prophylaxis with Penicillin-Procaine G.

MILAN NOVAK, MILTON GOLDIN, AND WELTON I. TAYLOR.
From the Department of Bacteriology, College of Medicine, University of Illinois, Chicago.

Clostridium tetani is sensitive to penicillin *in vitro*[1] and *in vivo*.[2,3] The development of penicillin-procaine has resulted in prolonged therapeutic blood levels following a single injection.[4,5] Claims have been made for inhibitory levels which are maintained for as

1 Abraham, E. P., Chain, E., Fletcher, C. M., Gardner, E. D., Heatley, N. G., Jennings, M. A., Florey, H. W., *Lancet*, 1941, **2**, 177.

2 Weinstein, L., and Wesselhoeft, C., *New England J. Med.*, 1946, **233**, 681.

3 Diaz-Rivera, R. S., Deliz, L. R., Berio-Suarez, J., *J.A.M.A.*, 1948, **138**, 191.

4 Herrell, W. E., Nichols, D. R., and Heilman, F. R., *Proc. Staff Meet., Mayo Clinic*, 1947, **22**, 567.

5 Sullivan, N. P., Symmes, A. T., Miller, H. C., Rhodehamel, H. W., Jr., *Science*, 1948, **107**, 169.

TABLE I.
Mortality in Penicillin-Treated Mice Infected with *Cl. tetani.*

Series	Units of penicillin	Hours before penicillin	Total animals	Survivors	Time to death (days)	Mortality, %
A	0	0	32	1	<4	96.8
B	150	0	25	20	7-10	20.0
C	150	0	25	5	<5	80.0*
D	300	0	25	21	4- 8	16.0
E	150	3	25	21	7-10	16.0
F	150	6	18	14	6-10	22.2
G	150	24	19	9	<5	52.6

* Penicillin injected IM in opposite leg rather than infected leg.

long as 4 or 5 days following injection of 300,000 or 600,000 units.

The possibility of penicillin action against spores of tetanus in tissue during the incubation period of the disease posed the probability of its use as a prophylactic agent. Its value in this instance would be especially desirable in minor injuries where there is hesitancy in the use of antitoxin because of the dangers from foreign protein sensitization. Its use to prevent toxin formation in tissue would seem more rational than use of antitoxin to neutralize toxin after it is formed.

The results obtained with mice as shown in data presented demonstrate a definite prophylactic effect. More detailed evidence will be presented later.

*Methods. Clostridium tetani** was grown in Trypticase Soy Broth with added fresh calf brain at 37°C for about 12 days, then filtered through sterile cheesecloth to remove meat particles, centrifuged and resuspended in sterile distilled water in 8 oz. screw-capped bottles. Toxin and vegetative cells were destroyed by heating in a water bath for 30 minutes at 80°C. Spore suspensions were then shaken vigorously 30 times to suspend spores evenly, and counted by adding serial dilutions in duplicate to melted and cooled Trypticase Soy Broth (1.5% agar added) deeps. After solidification a one-half inch layer of 2% stratifying agar was poured on top to insure adequate anaerobiosis. The appearance of macroscopic colonies upon incubation yielded a count only of viable spores. Spore suspensions were then adjusted to 1,000,000/ml. and stored at 4°C.

* Courtesy of Parke Davis and Company, Detroit, Mich.

Since it has been adequately demonstrated that the spores of *Cl. tetani* are not infective in healthy tissue, $CaCl_2$ in 5% solution was used as a tissue debilitant.[6-8]

The number of spores necessary to produce approximately 100% mortality in White Swiss mice was determined by injecting mice with 0.1 ml 5% $CaCl_2$ immediately followed by 10, 100, 1,000, 10,000, and 100,000 spores of *Cl. tetani* intramuscularly in the inner surface of the left hind leg. Mice receiving 10 and 100 spores survived; those receiving 1,000 spores died with symptoms of tetanus in 3-5 days, and with 10,000 and 100,000 spores, 2-3 days. Thus 1,000 spores was chosen as an LD_{100} infective dose.

To determine the possible prophylactic value of penicillin in tetanus, Penicillin G Procaine in oil with 2% aluminum monostearate,† containing 300,000 units/ml was used. The contents of one disposable cartridge containing 300,000 units were added to 99 ml sterile sesame oil and shaken 30 times before each usage to procure a homogenous suspension. With this dilution 0.1 ml contained 300 units penicillin, 0.05 ml contained 150 units. (The latter is comparable to 600,000 units for an adult human being.) Injections of penicillin were made in the same leg used for the infective dose of spores and $CaCl_2$, except in one series (Table I, Series C) where the opposite leg was used.

The time lapse between the injections of

6 Bullock, W. E., and Cramer, W., *Proc. Royal Soc. London*, 1919, B, **90**, 513.

7 Russell, D. S., *Brit. J. Exp. Path.*, 1927, **8**, 377.

8 Fildes, P., *Brit. J. Exp. Path.*, 1927, **8**, 387.

† Supplied by Abbott Laboratories, North Chicago, Ill.

TABLE II.
Statistical Analysis of Data on Mortality and Time to Death.

Series	Mortality		Time to death (Days)		
	%	P value*†	Mean	S.D.	P value*‡
A	96.8	—	3.31	1.42	—
B	20.0	<.01	8.40	3.47	<.01
C	80.0	>.10	3.90	1.69	>.05
D	16.0	<.01	6.25	2.51	<.01
E	16.0	<.01	7.75	2.94	<.01
F	22.2	<.01	6.75	3.07	<.01
G	52.6	<.01	4.10	2.11	>.05

* Series compared with Series A.
† P values taken from Chi-square 4-fold table including Yates correction; value less than 0.05 indicates significant difference.
‡ P values taken from Fisher's "t" table; value less than 0.05 significant.
S.D., Standard Deviation.

the infective dose and penicillin was varied, since other reports on the clostridia[9-15] indicate that elapsed time is an important factor in protection.

White Swiss mice of 15 to 35 g weight were used with a holding period of 10 days after the initial infective dose. Results after this interval are shown in Table I.

A statistical analysis of data[16] on mortality and time to death of mice receiving penicillin prophylaxis and untreated controls is presented in Table II.

The P value (likelihood of difference arising through chance alone) of the mortality of each series as compared with controls (Table II, Series A) was obtained from a Chi-square test of independence 4-fold table including Yates correction. P values of series B, D, E, F, and G (Table II) are significant.

The statistical analysis of the difference in time to death in days between each series and series A includes the mean time to death in days and the standard deviation with the P value obtained from Fisher's table indicating the significance of the data. Series B, D, E, and F have significant values.

Discussion. Both the reduction in mortality and prolongation of life of treated mice indicate the prophylactic effect of the penicillin-procaine compound. Administration of penicillin within 24 hours after the injection of spores of *Cl. tetani* serves to reduce the mortality as compared with the untreated controls. The lowest mortalities resulted when 150 and 300 units of penicillin were given in the infected leg immediately, and when 150 units were given after 3 and 6 hours' delay (Table I, Series B, D, E, and F). These series having low mortalities were also observed to have the greatest time lapse before symptoms and deaths. When compared to the rapid completion of the disease as observed in the controls (Table I, Series A), the noticeably longer time required before symptoms and deaths occur in the low mortality series receiving penicillin (Table I, Series B, D, E, and F) may indicate that the number of organisms capable of producing toxin is considerably reduced and that only when there are organisms surviving in remaining necrotic areas, which multiply and produce a lethal dose of toxin, does a fatality result. The similarity between the mortality rates

[9] Chain, E., Florey, H. W., Gardner, A. D., Heatley, N. G., Jennings, M. A., Orr-Ewing, J., and Sanders, A. G., *Lancet*, 1940, **2**, 226.

[10] McIntosh, J., and Selbie, F. R., *Lancet*, 1942, **2**, 750.

[11] Dawson, M. H., Hobby, G. L., Meyer, K., and Chaffee, E., *Ann. Int. Med.*, 1943, **19**, 707.

[12] McKee, C. M., Hamre, D. M., and Rake, G., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 211.

[13] McIntosh, J., and Selbie, F. R., *Lancet*, 1943, **2**, 224.

[14] Hac, L. R., and Hubert A. C., PROC. SOC. EXP. BIOL. AND MED., 1943, **53**, 61.

[15] Hac, L. R., *J. Inf. Dis.*, 1944, **74**, 164.

[16] Snedecor, G. W., *Statistical Methods*, Ames, Iowa, Iowa State College Press, 1946, 485 pp.

and time lapse before death in series B, where an immediate injection of penicillin was given, and in series E and F where administration of penicillin was delayed for 3 and 6 hours respectively, may be explained by the fact that it is generally acknowledged that penicillin is most effective against sensitive microorganisms during the period of active growth and multiplication; therefore the efficacy of penicillin administered after 3- or 6-hour lapses may indicate that those times allow germination of spores[8] and the vegetative cells are then inhibited by the concentration of penicillin available. Since Penicillin G Procaine in oil is reported to maintain concentrations at effective therapeutic levels for 96 hours, the longer time lapse before symptoms appear may be accounted for if it is only after this level has dropped that a lethal toxin is produced by survivors.

Higher mortalities were observed in series C and G. When 150 units of penicillin was administered with no delay but in the leg opposite the necrosis, 80% of the mice died (Table I, Series C). When the penicillin was injected into the necrotic leg after a 24-hour delay, 52.6% of the mice died (Table I, series G). The indication in series C is that (1) a sufficient amount of penicillin does not reach the necrotic area to inhibit growth and production of toxin by *Cl. tetani*, with resultant death of the animal, or (2) that organisms produce a lethal dose of toxin before being inhibited, with this latter possibility being especially applicable to series G. The rate at which penicillin is released and the rate and extent of penetration into necrotic areas are probably the significant factors. The similarity of series C and G to series A (Table I) in the short time lapse before death indicates that toxin production had taken place rapidly, and if series G is compared with series F this observation is further verified since the additional 18 hours time lapse before penicillin was administered caused a 30% increase in mortality.

Conclusions. Results indicate that penicillin-procaine G in sesame oil and 2% aluminum monostearate is of significant value prophylactically in lowering the mortality of mice experimentally infected with a lethal dose of detoxified spores of *Clostridium tetani*. In addition to decreasing the mortality, it retards the development of symptoms and resulting deaths. Injected into the necrotic areas, it is more effective than the same unitage injected at an uninfected site. Whether this is caused by insufficient penetration of the drug due to the presence of necrotic tissue or to interference by the calcium chloride has not been determined.

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Further Observations on Isosensitization to the Rh Factor.

ALEXANDER S. WIENER.

From the Serological Laboratory, Office of the Chief Medical Examiner, New York City.

The purpose of this report is to describe the results of experiments on Rh sensitization in man. The findings are of significance in relation to the pathogenesis of intragroup hemolytic transfusion reactions,[1,2] and the pathogenesis of erythroblastosis fetalis,[3] as well as the practical problem of producing Rh testing sera.[4]

The subjects used in these experiments were 47 adult male individuals. Each individual was subjected to a series of injections of group

1 Wiener, A. S., and Peters, H. R., *Ann. Int. Med.*, 1940, **13**, 2306.

2 Unger, L. J., and Wiener, A. S., *Am. J. Clin. Path.*, 1945, **15**, 280.

3 Wiener, A. S., *Am. J. Dis. Child.*, 1946, **71**, 14.

4 Wiener, A. S., and Gordon, E. B. S., *Am. J. Clin. Path.*, 1947, **17**, 67.