μg/ml thiamine HCl, 1.0 μg/ml calcium pantothenate and inorganic salts.[8] All media were adjusted to pH 7.6. Cultures were incubated at 37°C for 3 days. Strains 161 R, 147 N, 196 N and 43 R were grown in 1000, 200, 50 and 5 units/ml of penicillin respectively. Toxins were produced and assays of enterotoxin and hemolysin were carried out by previously described methods.[9-11] Lethal factor was detected by the intravenous injection of mice; dermonecrotoxin by intracutaneous injection of rabbits with 0.1 ml of a 1:20 dilution of toxin in saline.

Counts of viable bacteria revealed no difference in the amount of growth in media containing penicillin compared to the same media without penicillin.

Positive tests for enterotoxin[9,10] were obtained in monkeys or cats with centrifugates of enterotoxic cultures grown in all of the above media with and without penicillin.

Results of assays for hemolysins, lethal factor and dermonecrotoxin are summarized in Table I. The potency of the hemolysins, lethal factor and dermonecrotoxin produced by strains naturally or artificially resistant to penicillin was markedly reduced in cultures grown in Amigen liquid containing penicillin. However, in semisolid Amigen and veal infusion agar cultures, questionable effect of penicillin was noted. Penicillin in the same concentration failed to neutralize preformed hemolysins, lethal factor and dermonecrotoxin.

Summary. The natural resistance of 15 enterotoxic strains of staphylococci to penicillin was found to range from 0.05 to 500 units per ml. Production of staphylococcal hemolysins, lethal factor and dermonecrotoxin, but not of enterotoxin, was inhibited by growing selected naturally and artificially resistant strains in Amigen liquid medium containing sublethal concentrations of penicillin. Inhibition was not observed in similar experiments using semisolid Amigen and veal infusion media.

[8] Surgalla, M. J., and Hite, K. E., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 244.

[9] Davison, E., Dack, G. M., and Cary, W. E., *J. Infect. Dis.*, 1938, **62**, 219.

[10] Dack, G. M., *Food Poisoning*, University of Chicago Press, 1943.

[11] Surgalla, M. J., and Hite, K. E., *J. Infect. Dis.*, 1945, **76**, 78.

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Studies on Thermal Sedation in Suppression of the Symptoms of Tetanus Toxin

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The following experiments on the physiological effects of temperature suggest that cold narcosis, as seen in reptiles and some warm-blooded vertebrates, will modify the development of symptoms resulting from tetanus toxin. It is possible that cold narcosis might be used as a method of sedation and as an adjunct to therapy in such diseases as rabies and tetanus.

Because of the wide range in temperature tolerance exhibited by the diurnal reptiles and because of their similarity to the mammals with respect to optimum temperature, which in many lizards range from 36°C to 41°C, and because of their thermal simplicity and the greater facility with which these animals can be utilized in temperature experiments, it was agreed that any common diurnal species of reptiles would be suitable for experimentation, and the northern crested lizard *Dipsosaurus dorsalis dorsalis* was selected as being most convenient as to size,

thermal requirements, responses, and availability.

Although it is stated in the literature[1] that reptiles do not respond to tetanus toxin, experience in the field and laboratory suggested that there was great possibility of error in the previous experiment due to lack of appreciation of the vital necessity of maintaining optimum thermal condition. In most experimental work with reptiles, the full importance of the temperature factor has not been recognized.

In the pilot experiment, because reptiles had been reported as not susceptible to tetanus toxin, the arbitrary enormous dose of 20,000 guinea pig M.L.D.'s (minimal lethal dose) was employed in testing a lizard weighing 65 g. It was planned that, in case the symptoms of tetanus did not appear within a few days' time, the animals were to be exposed to optimum temperatures of 34°C to 40°C in the hope that during the approach towards or into this normal temperature range, the onset of symptoms might reveal a critical threshold at which neural and muscular effects might develop and below which partial cold narcosis might prevent their appearance.

Contrary to expectation, the original and all subsequent experiments demonstrated the fact that these animals, when held at temperatures ranging from 10°C through to the optimum of 38°C, are by no means immune to the effects of tetanus toxin although at all low temperatures there is a marked retardation in the appearance of symptoms.

Exp. I. Three specimens of *Dipsosaurus dorsalis dorsalis* were given the following doses: 20,000 guinea pig M.L.D.'s in one instance, for the other 2, 10,000 guinea pig M.L.D.'s each. The toxin was injected intramuscularly in the femoral regions in these and all subsequent experiments. The animals were then maintained at temperatures of 24°C to 26°C until the onset of symptoms. In all instances first symptoms appeared as slight tremors in the head and tail regions. These tremors appeared first in the individual receiving 20,000 guinea pig M.L.D.'s. The first perceptible tremors occurred 36½ hours after inoculation. Two hours after onset of primary symptoms, action of the hind legs was impaired and the tail was arched strongly in a lateral position.

This specimen which had received 20,000 guinea pig M.L.D.'s was heated to 32°C at which time opisthotonic contractions arched the tail and back to a dorsolateral position. Sound or sudden movements taking place in close proximity to the animal caused convulsions, although fairly periodic spasms were observed even without extra stimulation. Three hours after the appearance of symptoms this individual was placed in an incubator refrigerator at a temperature of 10°C-11°C and was maintained under these conditions until death occurred 212 hours later.

The remaining 2 subjects which had been given 10,000 guinea pig M.L.D.'s, only half the dosage administered to the first lizard, showed these initial tremors 40 hours after injection. One of these was immediately placed in a temperature of 10°C-11°C while the other was maintained at room temperature. At room temperature (27°C-28°C) death took place between 72 and 80 hours after inoculation. Prior to death, violent symptoms of tetanic convulsions and opisthotonic contraction threw the animal into a strong sigmoid curve with the head inclined upward at a sharp angle. This posture is retained with only slight relaxation even after death. (Fig. 2).

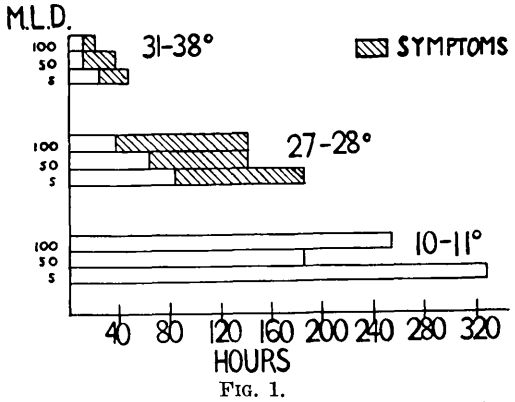

FIG. 1.
Duration of life in desert iguana, injected with tetanus toxin, with respect to maintenance temperature.

[1] Metchnikoff, E., *Ann. Inst. Past.*, 1897, **11**, 801.

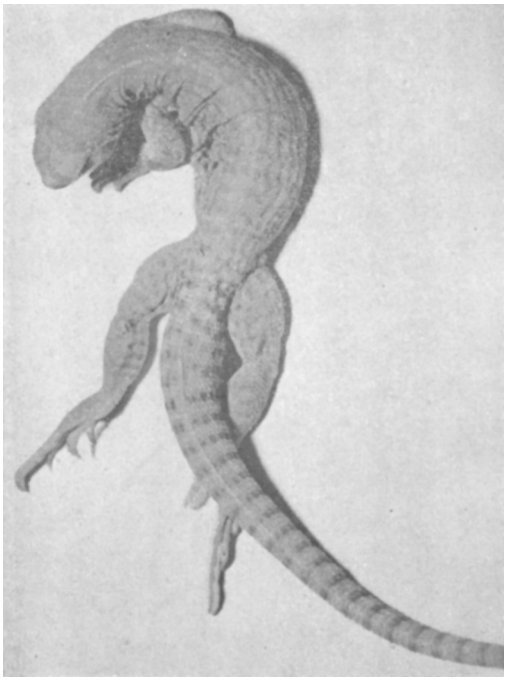

FIG. 2.
Dead *Dipsosaurus* showing characteristic posture attendant on death from tetanus.

Eighty hours after inoculation, the third specimen, which had been maintained at 10°C, was removed from the cold chamber and allowed to warm to room temperature, in this case 25.5 to 27°C. On warming, locomotory movements were limited to forelimbs but movement was poorly coordinated. The hind legs were immobilized and were strongly adpressed to the tail, although locomotion could be carried on with the front legs alone.

As temperatures rose from 25.5-27°C, convulsive, tetanic contractions became progressively more violent and, as reported above, it was noticeable that auditory and visual stimuli, as well as vibrations and movements taking place in close proximity to the animal precipitated these convulsive reactions.

At 28°C the body became still more violently flexed, remaining in this position almost continuously. The flexure was so violent that the animal was forced to lie on one side. At 30°C the head was flexed strongly to the left and the forelimbs showed limited mobility and were flexed backwards under the body. After being exposed to warming temperatures for 23 minutes, the animal defecated for the first time and the body temperature was noted as being 31°C. Twenty-five minutes after removal from the cold chamber and while still with body temperature of 31°C, it was first noted that respiratory movement had ceased, but it is possible that respiration may have stopped shortly before.

Concomitant with cessation of respiration, the general reactions became fewer and less severe and the animal remained in a contorted, prostrate position. Only 52 minutes after being removed from the control chamber, and with body temperature having risen to only 31.5°C, the animal died.

Animal No. I, which received double the dosage given the other 2 individuals, was maintained continuously at a temperature of 10°C thus acting as a control for No. II and III. In spite of such a heavy dosage, this animal outlived the others by a total of 212 hours, whereas, the individual chilled for 80 hours then warmed to 32°C died after only 52 minutes at a moderate temperature for these animals. Since the optimum temperature for this species approximates 37°C and may rise to 43°C with no discomfort to the animal, it is clearly apparent that death in so short a time cannot be attributed to overheating or overstimulation. At least one factor causing death from tetanus toxin seems to be the violence of contraction, thus with approach toward normal neuromuscular reactions, the toxin-produced reaction may cause traumatic injury and thus death. It seems improbable that either the amount or the degree of diffusion of the neurotoxic element can have caused death where no symptoms were evoked; on the contrary, this would appear to be a case of death induced by some other toxic effect or by too prolonged chilling, which seems rather improbable, or some other unknown factor.

In these experiments the most clearly discernible effect of temperature is the very marked lowering of the intensity of all tetanic reactions and their gradual intensification attendant on rising temperatures. This cor-

relation of violence of reaction with temperature change is in harmony with the anticipated results and is also in accord with the generally known effect of temperature changes with respect to metabolic rates in general.

Exp. II. The extreme sensitivity of these reptiles to tetanus toxin suggested that a second series of lizards be tested with lighter dosages in order that some approximation to their M.L.D.'s might be obtained prior to any additional expenditure of living material. For this reason, similar treatment was utilized but dosages of 100, 800 and 4,000 guinea pig M.L.D.'s were administered to the animals.

Following inoculation, the lizards were first maintained at 26-28°C; that is, 10° below their optimum. Thus they were held under conditions which seemingly would retard slightly the onset of symptoms.

Although all 3 lizards appeared normal for the first 48 hours, that with 4,000 units showed extreme symptoms 75 hours after inoculation. The 800 guinea pig M.L.D. individual displayed only the primary evidence of involvement, tremors of the head and cervical regions. All 3 animals were placed in the refrigerator incubator at 10°C as soon as symptoms were detected. The 100-unit individual showed no symptoms and was continued at room temperatures pending development of evidence of toxic effect.

After 90 hours at 26-28°C, the individual with 100 guinea pig M.L.D.'s was found to be suffering from partial paralysis of the hind legs and was then placed with the other 2 individuals at a temperature of 10°C.

Death of the 4,000-unit individual occurred sometime between 196 to 214 hours after inoculation, the time lapse probably being somewhat curtailed owing to a 2-hour period of warming to 31°C while photographs were being taken to illustrate symptoms.

The remaining 2 individuals, those at 800 and 100 units, while still refrigerated, showed marked opisthotonic contractions up to 244 hours, these symptoms gradually diminishing in intensity until immobility, possibly from prolonged exposure to abnormally low temperature, concealed the reaction. These conditions led imperceptibly to death some 270 hours ($\pm$ 12 hours) after inoculation.

Exp. III. In this investigation, an attempt was made to accomplish the following:

1. Test more fully the retarding effects of low temperature.

2. Test the accelerating effect of optimum temperature.

3. Determine the effect of small dosages of the toxin on:

   a. Animals continuously cooled to torpidity (10°C) from the time of inoculation to the natural termination of the experiment.

   b. A series maintained at temperatures of 27-28°C, and

   c. A series at temperatures slightly above the optimum but within voluntary tolerance range.

4. Determine the approximate lizard M.L.D. for this species under 3 conditions: namely,

   a. When cooled to torpidity.

   b. Maintained at approximately minimum-activity level, and

   c. At and slightly above the optimum.

Procedure. Nine lizards were divided into 3 groups, one of which received 100 M.L.D.'s, another 50 guinea pig M.L.D.'s, and the third 5 guinea pig M.L.D.'s, thus reducing the dosage from that of the maximum administered in the first experiment to 1/200, 1/400, and 1/4,000 of that massive inoculation. From each of these dosage groups, one individual was placed in the incubator-refrigerator at 10°C, one in a cage at room temperature 26-28°C, and a third was placed in a heating cabinet at 38°C, the approximate optimum temperature, although still 5°C below temperatures selected by these animals under natural conditions and some 7-9° below the lethal temperature for the species. Throughout the first 17 hours in a heating cabinet, temperatures were raised slowly from 31-38°C and thereafter were maintained at the latter level.

In order to postulate the exact relationship between different thermal levels and the progress of the intoxication, larger series of animals as well as greater standardization of

procedures would have been required, conditions not compatible with objectives in these preliminary experiments.

In spite of the exploratory nature of these tentative trials, it is apparent that the life expectancy of these lizards, when under normal thermal conditions—that is, 31-38°C—is less than 2 days; whereas with moderate cooling to 27-28°C or chilling to 10-11°C, there is a material prolongation of life, in the former case by 7 to 8 days, and in the latter as much as 14 days. Unfortunately, controls were not run to determine how long a normal lizard would survive at this temperature (ice box).

Although these results might have been predicted by the use of Van't Hoffs Law, the ameliorating effects are greater than the Q_{10} of 2, and approach those of 3 to 4. This larger factor may be due to inherent differences of tissues or to physiological functions, but at present it seems more probable that the explanation may lie in the realm of normal differences of the Q_{10} for different thermal levels.

Conclusions. 1. Contrary to reports indicating that "cold blooded" creatures are not susceptible to tetanus toxin, the desert iguana is susceptible and for temperature studies is an excellent experimental animal. 2. Lowering of temperature markedly prolongs life in the desert iguana inoculated with tetanus toxin. From Van't Hoffs Law one would expect a slowing down of the effects of the toxin at lower temperatures. 3. There is a tremendous field awaiting study on the effects of altering bodily temperature on the course of disease, especially where toxic factors are concerned.

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Use of Thiosulfate Clearance As a Measure of Glomerular Filtration Rate in Acidotic Dogs.*

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The renal clearance of thiosulfate has recently been proposed as a measure of glomerular filtration rate in the normal dog[1] and in man.[2] Since the majority of the ionic constituents of the glomerular filtrate are more or less completely reabsorbed during their passage through the renal tubules, the apparent tubular rejection of this anion constitutes an interesting anomaly. The present study of the excretion of thiosulfate in the acidotic dog was undertaken with the following 2 ends in view. If thiosulfate were to undergo no reabsorption in the tubules of animals with disturbed acid base relationships, as is apparently true in normal animals, that fact would strengthen the concept that its clearance constitutes an adequate and broadly applicable measure of filtration rate. If the thiosulfate clearance could be used as a measure of filtration rate in acidotic animals, it would have the following advantage over the creatinine clearance. Thiosulfate, unlike creatinine, is devoid of buffer properties. Hence, it can be predicted that the administration of this substance would not affect the rate of excretion of titratable acid, as does the administration of creatinine.[3]

In this study it has been found: (1) that the thiosulfate clearance provides an accurate means of quantitating glomerular function in

* Aided by grants from the John and Mary R. Markle Foundation and from the United States Public Health Service.

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2 Newman, E. V., Gilman, A., and Philips, F. S., *Bull. Johns Hopkins Hosp.*, 1946, **79**, 229.

3 Pitts, R. F., and Alexander, R. S., *Am. J. Physiol.*, 1945, **144**, 239.