mice subjected to a thermal stimulus. Both are ineffective in those administered phenylquinone. This difference is possibly related to differences between visceral and somatic pain.

Both isomers antagonize the effects of reserpine as measured by the reversal of the reserpine induced prolongation of hexobarbital hypnosis and the reserpine induced prolongation of the reaction time of mice exposed to a thermal stimulus.

This unusual "hyperalgesia" or "hyperacuity" property of dexoxadrol suggests that it may be an agent which will increase awareness.

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1. Hardie, W. R., Halverstadt, I. F., Allen, R. E., Abst. of Papers 141st Meeting, Am. Chem. Soc., March, 1962, p. 3N.

2. Hidalgo, J., Gallin, J., Williams, B., Thompson, C. R., *Pharmacologist,* 1961, v3, 69, Fall.

3. Garcia Leme, J., Rocha e Silva, M., *J. Pharm. Pharmacol.,* 1961, v13, 734.

4. Eddy, N. B., Leimbach, D. J., *J. Pharmacol.,* 1953, v107, 385.

5. Hendershot, L. C., Forsaith, J., *ibid.,* 1959, v125, 237.

6. Snedecor, G. W., *Statistical Methods,* 5th Ed., Iowa State Coll. Press, Ames, 1956.

7. Miller, L. C., Tainter, M. L., PROC. SOC. EXP. BIOL. MED., 1944, v57, 216.

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Effect of Environmental Temperature on Tetanus Intoxication in Mice.* (28596)

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While studying acute tetanus poisoning in mice we observed that body temperature began to fall at an early stage in intoxication. Attempts to maintain body temperature by keeping the poisoned animals at an ambient temperature of 35°C resulted in a considerable decrease in survival time. More complete studies were therefore undertaken to determine the effect of environmental temperature and dosage on survival time.

Method. The toxin used was a sterile filtrate from cultures of *Cl. tetani* prepared by Wyeth Labs., Radnor, Pa. The effective toxin content, estimated by nitrogen determinations and electrophoretic analysis, was 180 μg/ml.

The animals used were white female Swiss mice weighing from 23 to 30 g. They had been kept in an animal house at temperatures of 15° to 22°C, and were thus acclimated to one of the experimental temperatures (21°C).

Groups of 21 mice were injected via the intraperitoneal route with 1 of 6 graded doses (0.01 to 0.8 ml of the toxin filtrate, equivalent to 1.8 to 144 μg of toxin). These groups were then divided into 3 sub-groups of 7 animals each. Each sub-group was then kept at either 5°C, 21°C or 35°C and closely observed until the animals died. Survival times of the individual animals were recorded in minutes. For the 72 μg dose, 2 sets of 21 animals were used. Groups of control animals were also kept at these 3 temperatures. None of these died during the experiments.

To quantitate the original observation on the fall of body temperature noticed in the animals kept at room temperature (21°C) after poisoning, the internal and surface temperatures of control and poisoned animals were measured with thermistor probes. Yellow Springs Instruments probe #402 was used for measurements of rectal temperature and YSI probe #409 was used for measuring the surface temperature. This latter probe was taped to the unshaven abdomen of the animal. The probes were kept in place by a small harness

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which did not restrict the freedom of movement of the animals. After an initial period of familiarization (30-40 minutes) to accustom them to the probes, one of each pair of animals was injected with 0.4 ml of the crude toxin (72 μg). The control animal received either no injection or 0.4 ml of saline (2 experiments). The internal and surface temperatures of both animals were measured on a YSI telethermometer at 5 minute intervals until the poisoned animal died. Apart from a transient fall in temperature immediately after injection, saline had no effect. All these experiments (5) were made at 21°C. Measurements were not made at the extreme temperatures.

Results. The effects of varying doses and environmental temperatures on the survival times are shown in Fig. 1. The doses have been arranged logarithmically along the abscissa in order to accommodate the 80-fold range. The height of each bar represents mean survival time for each sub-group at a given temperature and dose. The central narrow line represents standard error of survival time for the sub-group. At the 3 highest doses, there is a marked optimum of survival at 21°C. Although the survival time at 5°C closely approximated that at 21°C for the lowest doses, minimum survival times were always found for animals kept at 35°C. Statistical analysis of the double set of animals given the 72 μg dose showed that the differences in survival time produced by the different temperatures were statistically significant (between 35°C and 21°C, $P < 0.01$; between 21°C and 5°C, $P < 0.05$; and between 5°C and 35°C, $P < 0.02$).

The effect of changing the dose is similar for all 3 environmental temperatures. For doses between 1.8 and 9 μg of toxin, survival time was approximately proportionate to the logarithm of the dose, but at high dose levels survival time is apparently independent of the dose and constant for any given temperature.

Fig. 2 illustrates results obtained in one of the 5 experiments in which the body temperature was measured during the course of intoxication. The results of all 5 of these experiments were consistent. Both the surface

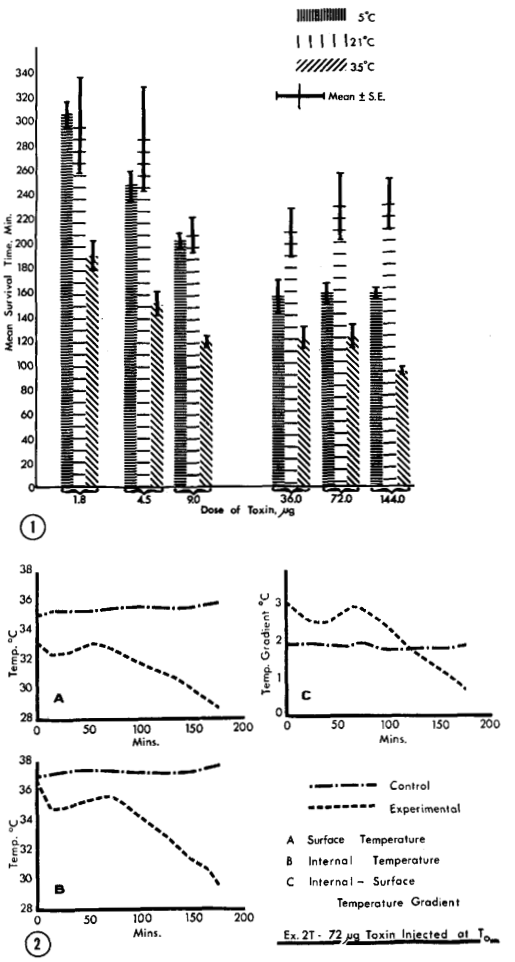

FIG. 1. Effect of varying doses of tetanus toxin on survival times of mice kept at 3 environmental temperatures.

FIG. 2. Internal and surface temperatures recorded from poisoned and non-poisoned animals.

(A) and the internal (B) temperatures of the control animals remained constant during the experiment. In the poisoned animals there was an initial fall in temperature, more marked internally than externally, followed by a period of relative temperature stability. After 50 to 60 minutes, both internal and external surface temperatures began to fall steadily with the difference between them also diminishing steadily. Surface temperature began to fall slightly before internal temperature.

Discussion. The most important feature of these results is the relationship between the apparent toxicity of the toxin, as reflected in

survival times, and environmental temperature. The relationship between dose and survival time follows a common pharmacologic pattern. That is, a saturation of the animal with the largest doses and a progressive increase in survival time as the dose is reduced.

Wright has reviewed(1) the relationship between environmental temperature and tetanus intoxication in poikilothermic animals. The effectiveness of the toxin increases with temperature and shows no optimum. Wright considers this to be due to the effect of temperature on rate of binding of the toxin at its active sites.

This explanation cannot be applied to our observations. The data presented in Fig. 2 support the hypothesis that the toxin depresses, either primarily or secondarily, the rate of heat production in the poisoned animals. Under conditions where the control animal remained in heat balance there was slow cooling of the poisoned animal. Increased heat loss would have been reflected in an initial increase in surface temperature due to vasodilatation and a sharp decrease in the internal-surface temperature gradient at the beginning of cooling. In our experiments this did not occur and blanching of the paws, ears and tail indicated vasoconstriction. We therefore consider that our results show reduced heat production in the poisoned animal.

This concept also provides an explanation of the effect of temperature on survival times of the poisoned animals. The physiological response to either high or low temperature stress is marked by a considerable increase in the metabolic rate. If the action of the toxin is upon heat production, then it is to be expected that its maximal effect should be observed under circumstances where the demands for increased metabolism are greatest; that is, at extreme temperatures. This effect will also be most clearly observed at the highest dose levels where the animals are saturated with toxin and factors affecting its rate of dispersion in the body will be minimized.

Our results do not provide direct evidence as to whether the reduction in heat production is a primary or secondary effect of the toxin. Morax and Marie(2) and Meyer and Ransom(3) proposed in 1903 that tetanus toxin acted on the central nervous system. Later work by Brooks, Curtis and Eccles(4) demonstrated inhibition of polysynaptic reflexes. Harvey(5) showed that toxin interfered with neuromuscular junctional transmission. Action of the toxin on central neurones might well produce a decrease in heat production through interference with muscular function. However, evidence is now beginning to accumulate that more fundamental biochemical lesions may result from tetanus intoxication. Michelazzi *et al*(6) showed that ATP metabolism in skeletal muscle was affected in tetanus poisoning, and Wensinck and Cohen(7) found abnormalities in glycogen metabolism. Balough(8) demonstrated decreased cellular respiration in brain cells poisoned with tetanus toxin.

As a morphologic correlate of these data Price and Nishi(9) observed degeneration of renal tubular mitochondria in chronic tetanus intoxication and we(10) have observed changes in the mitochondria of brain and muscle cells in mice poisoned under the same conditions as those used in this study.

The possibility thus arises that the neurophysiologic abnormalities in tetanus intoxication are only the most easily observed result of more widespread subcellular damage.

Summary. Observations were made on survival times of mice poisoned with relatively large doses of tetanus toxin and kept at either 5°C, 21°C or 35°C. The results showed that survival times were markedly influenced by the temperature over a wide range of doses. Animals kept at 35°C after poisoning died very much more quickly than those kept at 21°C (the temperature of acclimatization) and in general animals kept at 5°C also showed markedly reduced survival times. When measurements of the body temperatures of mice were made during intoxication it was found that body temperature decreased steadily from an early stage of intoxication. The results are interpreted as showing that tetanus toxin interferes either primarily or secondarily with the processes of heat production and the possibility that the mode of action of tetanus toxin is via a widespread

injury at the subcellular level is discussed.

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1. Wright, G. P., *Pharmacol. Rev.,* 1955, v7, 413.
2. Morax, V., Marie, A., *Anal. de l'Inst. Pasteur,* 1903, v17, 335.
3. Meyer, H., Ransom, F., *Arch. f. Exp. Path. u. Pharmacol.,* 1903, v39, 369.
4. Brooks, V. D., Curtis, D. R., Eccles, J. C., *J. Physiol.,* 1957, v135, 655.
5. Harvey, A. M., *ibid.,* 1939, v96, 348.
6. Michelazzi, M., Mor, M. A., Dianzani, M. U., *Experientia,* 1955, v11, 73.
7. Wensinck, F., Cohen, J. A., *Biochem. et Biophys. Acta,* 1953, v10, 184.
8. Balough, E., cit. in G. Kassay and M. Valeria, *Iserl. Orvost.,* 1951, v3, 401.
9. Price, Z., Nishi, Y., Travelling Exhibit, Electron Microscope Soc. Am., 1961.
10. Zacks, S. I., Sheff, M. F., *J. Neuropath. and Exp. Neurol.,* in press.

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Morphology and Hatching Power of the Developing Chick Embryo Following X-Irradiation.* (28597)

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Gilman and Baetjar(1) published some of the earliest observations on the effects of irradiation upon the chick embryo. These workers described various conditions following irradiation, such as deformities of growth, patchy distribution of feathers, and hemorrhages in the occipital region. Wilson(2), using radium rays determined the amount necessary to cause the death of the chicken embryo at various stages of development. It was found that a diminishing radiosensitivity from the first to the sixth day of incubation occurred. Results of extensive studies were reported by Stearner *et al*(3), who determined that the periods of radiosensitivity following irradiation treatment fall roughly into 3 intervals: 0 to 1 day, 2 to 5 days and 6 to 12 days. Particular types of responses accompanied each of these divisions.

Karnofsky *et al*(4) determined that roentgen rays exerted both an acute and a delayed toxic effect upon the chick embryo. Acute death occurred within 20 hours post-irradiation while delayed death occurred approximately 10 days after irradiation. It was further determined that young embryos are not highly susceptible to the acute effects, but that they develop their maximum sensitivity during the 8 to 10 days of incubation. Developmental abnormalities were common, especially those of the beak, limbs, head and eyes. Result of studies on many kinds of organisms, by Landauer(5), revealed that there are definite and well-defined stages of embryonic development during which certain environmental agencies will change the fate of particular parts. Muller *et al*(6) in a study of $LD_{50}/22$ days of incubation dose curves for the chick embryo reported that sensitivity to the combined injury mechanisms of X-rays increases through the first 3 days of development, then decreases through the 12th day where it plateaus at approximately 750 roentgens. Irradiation through the 12th day of development also evoked a progressive depression in growth rates of the surviving chicks.

The present studies were designed to demonstrate the effects of X-irradiation upon the developing chick embryo, the resulting abnormalities and their degree of occurrence. The chicken embryo was chosen for these studies as it offers a number of advantages which enhance its application to X-irradiation experiments. Since the chick is a vertebrate, it might be expected to respond in a manner similar to other vertebrates. The size

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