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Hypothermia in the Mouse as a Bio-Assay of Endotoxin Protection Factor in Impure Penicillin.*

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In seeking an improved method of bio-assay of the endotoxin protection factor of impure penicillin,[1-4] it was found, as reported by Beck[5] and Zahl and Hutner,[6] that mice given bacterial endotoxins were unable to maintain their body temperature. In addition, we found that this hypothermia was less marked in animals treated with impure penicillin and that the degree of hypothermia was a function of the amount of impure penicillin administered.

Methods. Young adult (20 g) mice were kept in a constant temperature room at 24°C for at least 2 days before and during the entire time of each experiment. At the time the endotoxin was given the mice were placed in individual compartments which prevented them from huddling together and permitted the course of an individual mouse to be followed throughout the experiment.

0.5 ml of impure penicillin, containing 10,000 units of crystalline penicillin per ml, was given intraperitoneally at 20, 18 and 2 hours before the injection of the endotoxin.

The endotoxin, prepared from *Salmonella acrtrycke* as previously described[7] was administered intraperitoneally as an endotoxin-saline mixture, the total volume of fluid being 1.0 ml in all cases.

TABLE I.
Method of Median Calculation.

	Protected mice			
Hr after endotoxin	22 hr	26 hr	28 hr	30 hr
	37.1	37.6	38.0	38.2
	36.9	37.5	37.7	37.7
	36.7	37.4	37.5	37.7
	36.3	37.0	37.4	37.3
	35.9	36.8	37.2	37.2
	35.6	36.6	36.8	36.7
	35.3*	35.8*	36.0*	36.6*
	34.4*	35.7*	35.8*	36.3*
	34.3*	35.2*	35.7*	36.0*
	33.3*	35.0*	35.6*	35.8*
	30.9*	32.3*	34.1*	34.7*
	30.7*	31.3*	32.8*	32.1*
Median temp.	35.4	36.2	36.4	36.7
	Unprotected mice			
	32.8	33.3	34.6	35.0
	32.3	32.7	33.2	33.4
	29.4	31.3	30.6	26.6
	27.9	27.4	27.5	26.5
	27.9	27.1	26.9	26.4
	27.4	26.5	26.5	26.1
	26.9*	26.4*	26.4*	25.9*
	26.5*	26.3*	26.3*	25.9*
	26.0*	25.3*	26.1*	25.5*
	25.8*	25.2*	25.2*	Dead*
	25.7*	Dead*	Dead*	Dead*
	25.6*	Dead*	Dead*	Dead*
Median temp.	27.1	26.5	26.5	26.0

* Mice with body temperature below the median of the group.

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The impure penicillin was supplied through the courtesy of Dr. Robert D. Coghill of the Abbott Laboratories. The crystalline penicillin was supplied by Abbott Laboratories, Commercial Solvents Corporation, Lederle Laboratories, and Schenley Laboratories, Inc.

1 Boor, A. K., and Miller, C. P., *Science*, 1945, **102**, 427.

2 Miller, C. P., and Boor, A. K., PROC. SOC. EXP. BIOL. AND MED., 1946, **61**, 18.

3 Boor, A. K., Miller, C. P., and Hawk, W. D., *Fed. Proc.*, 1947, **6**, 240.

4 Miller, C. P., Hawk, W. D., and Boor, A. K., *Science*, 1948, **107**, 118.

5 Beck, L. V., A.A.A. S. Symposium on Approaches to Tumor Chemotherapy, 1947, p. 265.

6 Zahl, P. A., and Hutner, S. H., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 156.

7 Boor, A. K., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 168.

8 Nielsen, E. T., *Acta Medica Scand. Supplementum*, 1938, **90**, 168.

The rectal temperatures were determined by the use of a copper-constantan thermocouple and a galvanometer. One junction was inserted a distance of 3 cm into the mouse's rectum and the precautions advised by Nielsen[8] regarding its position were observed. The other junction was placed in a constant temperature water bath.

As a routine schedule, 4 temperature determinations were made at approximately 21, 24, 27 and 30 hours after the endotoxin was injected. Less than 4 determinations increased the standard error. Twelve animals in each group were found to be sufficient for a fairly quantitative result, giving a standard error of about 0.8°C for the average of 4 determinations. This meant that a difference of approximately 3°C between the two groups of mice was significant.

The median temperature of each group of mice was chosen for the final calculation rather than the mean temperature because the loss of a mouse by death raised the mean temperature

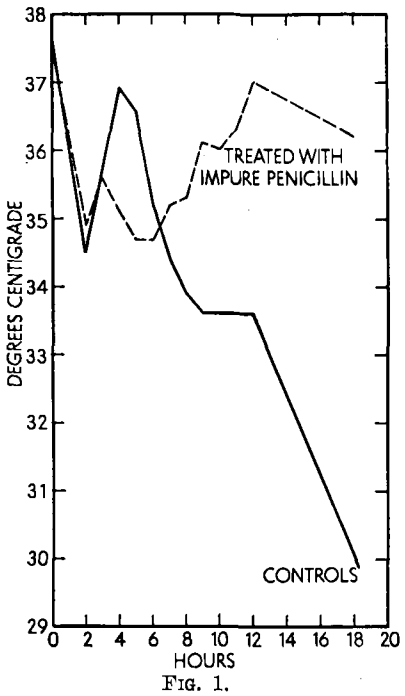

FIG. 1.

Median temperatures of mice injected with endotoxin.

Note the two phases of hypothermia after the injection of the endotoxin; the impure penicillin protects against the later more important phase of hypothermia.

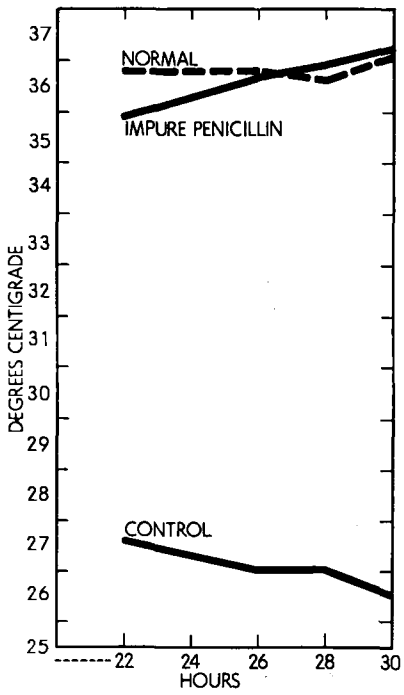

FIG. 2.

Median temperatures of mice injected with endotoxin.

Comparison of the median temperature of treated (impure penicillin) and control mice injected with endotoxin; and normals receiving no injection.

of the group. The dead mice were, therefore, listed at the bottom of their respective groups when the medians were determined and a more satisfactory computation resulted. This was felt to be a justifiable procedure since among 457 mice which died (of a total of about 1,500) 88% were animals with temperatures previously below and only 12% at or above the median. A sample experiment and calculation is given in Table I.

Experimental. Fig. 1 presents the results of a typical experiment. Two groups of mice were used: one protected by impure penicillin, the other untreated. Both groups were given 0.05 ml of the endotoxin intraperitoneally.

The results of an experiment using the routine procedure described under "methods" are shown in Fig. 2; the data appear in Table I. The temperatures of normal untreated mice kept under the same conditions and determined at the same time are also shown in this figure.

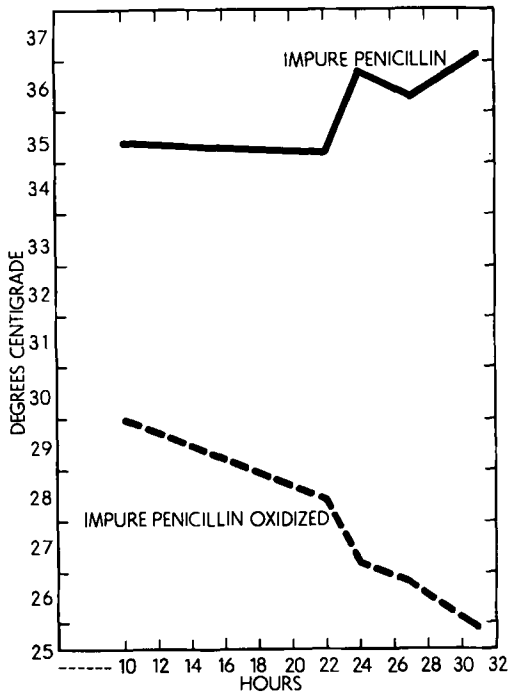

FIG. 3.
Median temperatures of mice injected with endotoxin.

Differences between protected and unprotected groups of mice as shown in Fig. 1 and 2 have been found in 25 separate experiments, using 1,140 mice in which temperatures were taken at least 4 times.

We have found a rough correlation between the degree of protection of a given substance computed by the LD_{50} and the anti-hypothermic effect. In order to investigate the correlation between the two types of experiments, the protective activity was destroyed by oxidation with hydrogen peroxide. The LD_{50} of the endotoxin in mice treated with oxidized impurity was 0.065 ml; in mice protected with unoxidized material the LD_{50} was 0.35 ml. The results of the two subsances in a temperature experiment are shown in Fig. 3.

The impure penicillin did not protect against the hypothermic effects of alpha naphthyl-thio-urea or crystalline insulin.

In order to obtain some quantitative results, an experiment consisting of 18 groups of 12 animals each was performed. The results can be seen in Fig. 4. This graph shows that the method can be used for a quantitative assay of the protective material; however, only a narrow range of the concentration of the protective material can be measured with one dose of endotoxin. In actual practice it was more economical of materials to use a constant dose of endotoxin and various dilutions of the protective factor. The curve shown in Fig. 5 plots the averages of 4 median temperatures of 4 groups of mice treated with varying concentrations of impure penicillin and injected with a standard dose of endotoxin.

Discussion. The greatest error in the method is the biological variation in the mice which necessitates the use of 12 animals in each group. The method also requires that the mice be kept in a constant temperature room and preferably in compartment cages to keep them separated from one another. It has the advantage of greater economy of mice and materials than a satisfactory determination of LD_{50} and is much more quantitative than the latter.

Autopsies, including microscopic examination, failed to disclose any significant trauma

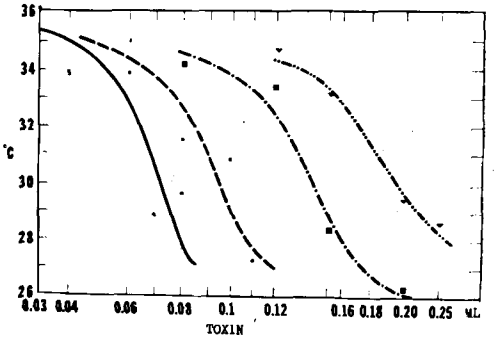

FIG. 4.
Median temperatures of mice injected with endotoxin.
This figure shows the effect of varying doses of a standard preparation of impure penicillin. Each point on the curves represents the average of 4 median temperature determinations between 20 and 30 hours after the administration of endotoxin.
——— Penicillin and saline treated controls.
– – – Treated with one part impure penicillin and 3 parts water.
—•— Impure penicillin diluted with an equal volume of water.
—••— Impure penicillin undiluted.

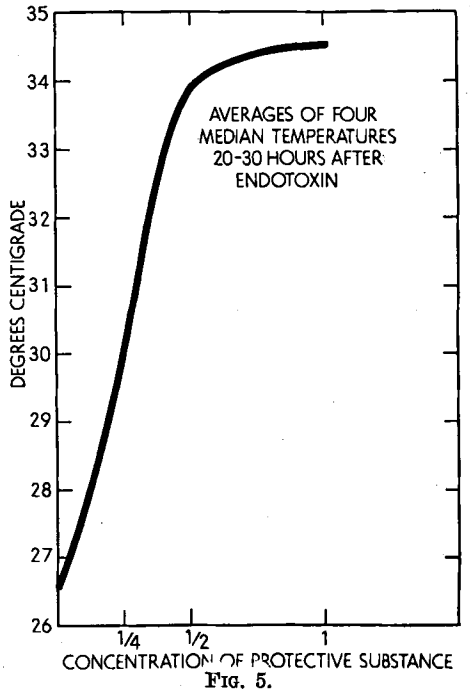

FIG. 5.

Effect of varying doses of impure penicillin. One can distinguish between the protective effect of penicillin and saline, impure penicillin diluted to ¼, and impure penicillin diluted to ½ by using 0.1 ml of endotoxin, as was done in this experiment. In order to distinguish half strength from undiluted material, one must dilute further.

from the insertion of the junction into the mouse.

Hypothermia may be regarded as the physiological response of the mouse to the endotoxin, comparable to fever in most other animals. That it is not merely an ante-mortem phenomenon is evidenced by the fact that less than one-third of the mice die in an experiment. In some instances mice have recovered after their temperatures have been as low as 26°C for 8 to 12 hours.

Summary and Conclusions. The endotoxin of *Salmonella aertrycke* injected intraperitoneally into mice caused a marked fall in body temperature. A certain phase of this hypothermia could be prevented to a significant degree by previous administration of impure penicillin. This phenomenon has been utilized for the quantitative assay of impure penicillin.

A correlation was demonstrated between the antilethal and the anti-hypothermic action of the impure penicillin against bacterial endotoxin in mice.

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Effect of Nicotinic Acid Monoethylamide on the Liver of the Young Rat.

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It has been reported by Brazda and Coulson[1] that a diet containing 1% coramine (niketh amide) produces a rapid and significant increase in the liver weight-body weight ratio of young rats. Nicotinamide, under similar conditions, produces no significant increase.[2] Inasmuch as coramine is disubstituted on the amide nitrogen whereas nicotinamide is unsubstituted it was decided to determine the effect produced by nicotinic acid monoethylamide in which the amide nitrogen is only monosubstituted. This communication reports the results obtained by use of this compound in the diet.

Experimental. The procedure employed was the same as that used in the study of the effect of coramine.[1] The nicotinic acid monoethylamide was mixed into the basic diet

[1] Brazda, Fred G., and Coulson, R. A., PROC. SOC. EXP. BIOL. AND MED., 1948, **67**, 37.

[2] Coulson, R. A., and Brazda, Fred G., PROC. SOC. EXP. BIOL. AND MED., 1947, **65**, 1.