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The Toxicity of Nikethamide at Different Times of the Day.

By

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In an earlier paper (CARLSSON and SERIN 1950) it was reported that the subcutaneous toxicity of nikethamide to white mice (N, N-Diethylpyridine-3-carboxamide), varied according to the time of day of the injection. The mortality among those injected between 12 a.m. and 6 p.m. was distinctly higher than among those injected between 6 p.m. and 12 p.m.

The purpose of the present study was to make a systematic examination of the toxicity of a single constant dose of nikethamide given to mice at different hours of the day.

Experimental conditions.

The animals were divided into two experimental series. In the first series the animals consisted of adult white male mice from the same stock (Stock A), as was used in the previous study. The animals weighed 26.7 ± 0.2 g (s = 2.2 g). The experiments were carried out on the same day in July. The room temperature was practically constant throughout the duration of the experiments.

Five groups, each of 25 animals, were injected with an interval of 6 hours between groups i.e., at 2 a.m. 8 a.m. 2 p.m. 8 p.m. and 2 a.m.

Before the animals were used for the experiments they had been kept under natural alternations of light and darkness for at least one week in the room in which the tests were carried out. They were fed on "mouse-bread" and water: food and water were always available until the time of the injection. Reference should be made to the previous paper (CARLSSON and SERIN, 1950) for further details.

The mean weights of the animals in each group and the temperature at which the tests were performed are seen in table 1.

The experimental conditions for the second series differed but slightly from those for the first. The experiments were performed on the same day in June one year later. The material was heterogeneous and consisted of male mice of stock A with an average weight of 28.6 $\pm$ 0.4 g (s = 2.9 g) and of both male and female mice of another stock (Stock B) with an average weight of 22.9 $\pm$ 0.6 g (s = 3.3 g) and 19.7 $\pm$ 0.4 g (s = 3.2 g).

The animals' food consisted of a mixture of crushed cereals (oats and barley) and water. They were fed every day at approximately 12 a.m. Food and water were always available. The temperature of the room in which they were kept was practically constant throughout the duration of the experiments.

Four groups, 40 animals in each, were given injections of nikethamide with an interval of 6 hours between each group as in the previous series. The injections were given at 2 p.m. 8 p.m. 2 a.m. and 8 a.m. All the groups were equal as regards sex and stock.

In all other respects the experimental conditions were the same as in the previous series. For further details see table 2.

In both series each animal was given 0.30 g nikethamide in the form of a 1 per cent aqueous solution of Coramine "Ciba". Nikethamide was deposited subcutaneously with the aid of a syringe graduated in 0.01 ml.

In other respects the experimental conditions were the same as in the previous experiments (CARLSSON and SERIN, 1950).

Results.

The results in the first experimental series (table 1) show that the mortality in the two groups injected at 2 a.m. was only 20 and 24 per cent, whilst that of the other groups was 52—68 per cent. The difference was 37 $\pm$ 8.2 per cent and thus statistically significant.

Table 2 shows the results in the second experimental series. Here the mortality reached a maximum at 2 p.m. and a minimum at 2 a.m. The difference in mortality between these two times in this experimental series was 35 $\pm$ 10.5 per cent and is again statistically significant. A comparison between the different groups of animals in series II shows that the mortality of the mice injected varied in approximately the same manner irrespective of sex and stock.

Table 1.

Toxicity of 0.30 g nikethamide per kg body weight injected subcutaneously at different times of the day into white male mice of the same stock (Stock A). The tests were carried out in July. Each group consisted o 25 animals.

Injection at	Mean weight g	Temperature	Mortality per cent
2 a.m.	26	20°.1	20
8 a.m.	25	20°.1	52
2 p.m.	27	20°.2	56
8 p.m.	27	20°.1	68
2 a.m.	26	19°.8	24

Neither was there any notable difference between series I and series II. In both there was a distinct minimum death rate at 2 a.m. Also the average mortality was practically equal in both series, *viz*, 50 per cent and 53 per cent respectively.

Table 2.

Toxicity of 0.30 g nikethamide per kg body weight injected subcutaneously at different times of the day into white mice. The tests were carried out in June. Each group consisted of 40 mice.

	Stock	Sex	Mean weight g	Nr. of animals	Mortality per cent
Group I.	A	male	27	15	67
Injected at 2 p.m.	B	male	24	9	67
Temperature 22°.8.	B	female	20	16	75
Group II.	A	male	28	15	40
Injected at 8 p.m.	B	male	22	9	44
Temperature 22°.5	B	female	20	16	56
Group III.	A	male	30	15	33
Injected at 2 a.m.	B	male	23	9	44
Temperature 22°.2.	B	female	19	16	31
Group IV.	A	male	30	15	47
Injected at 8 a.m.	B	male	23	9	56
Temperature 22°.5.	B	female	20	16	75

As there was no marked difference between the results obtained in the two experimental series it seemed justified to combine them. The pooled material is illustrated in fig. 1.

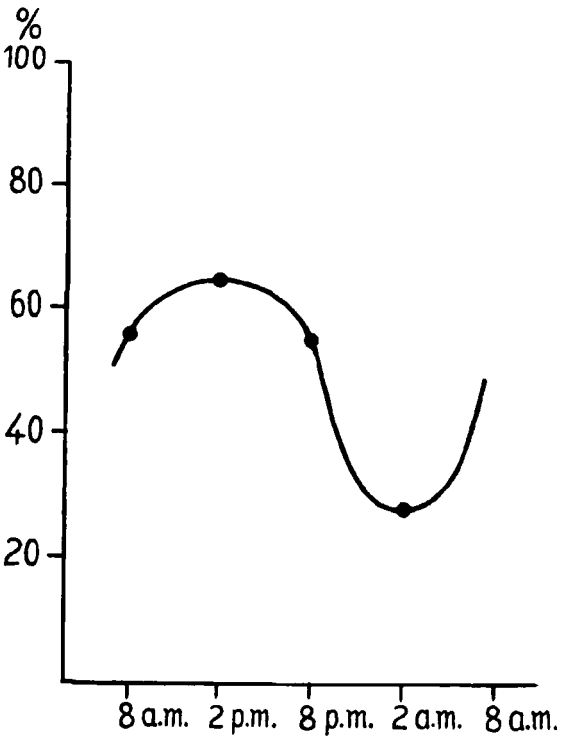

Fig. 1. Toxicity of nikethamide, 0.30 g per kg body weight given subcutaneously to white mice at different times of the day. Abscissa: time of day. Ordinate: mortality per cent.

In the first series we studied the voluntary activity of the animals during the 24-hour day by means of a simple mechanical arrangement. Twenty-five animals were placed in a box suspended by elastic rubber bands. Movement of the box was recorded on a kymograph. Judged by the curves thus traced the voluntary activity of the animals was greatest at about 2 a.m. and least at about 2 p.m. (See fig. 2).

Discussion.

In an earlier study (CARLSSON and SERIN 1950) it was observed that the toxicity of nikethamide in white mice varied at different times of the day. The tests were carried out during three months. Because of small differences in weight, which seemed to have a diminishing effect on the diurnal variation in the toxicity of nikethamide, it was not possible to get a clear idea of the extent of this variation. Neither was it possible to draw any definite conclusions as to the time of maximum and minimum effects.

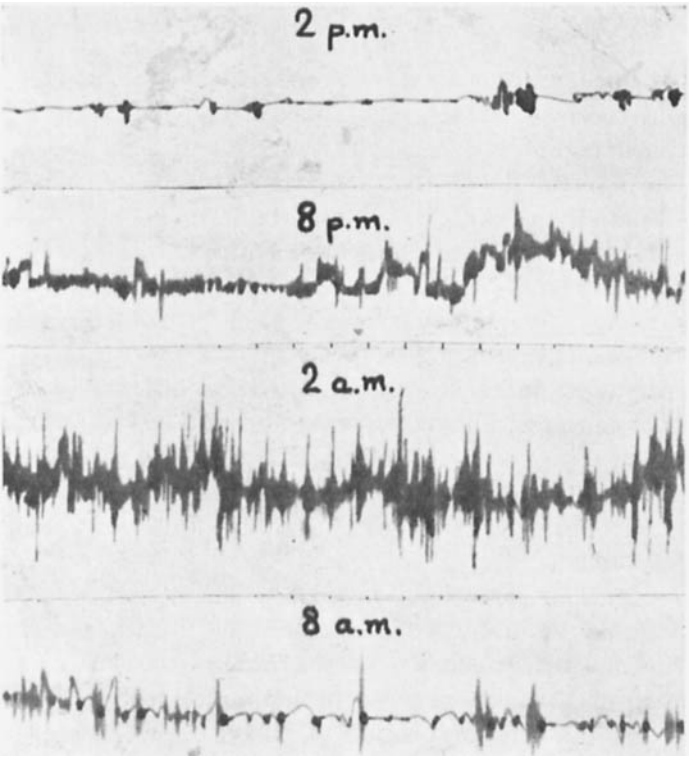

Fig. 2. Voluntary activity in white mice at different times of the day. Each record represents the activity during half an hour.

The present experiments were performed to study systematically the toxicity of nikethamide at various times of the day.

The experimental conditions were standardized as far as possible. A uniform dose of 0.30 g/kg of nikethamide ("Coramine", Ciba) was given subcutaneously to each animal. Each experimental series was performed in the course of a 24-hour day. In each experimental series the different groups injected at various times of the day were substantially identical in body weight, stock and sex. The significance of the results is emphasized by the fact that animals of both sexes and of different stocks were used. Room temperature throughout all the experiments was practically constant, although it was different for the two series (approximately 20°.0 and 22°.5 C. respectively), but this did not seem to effect the average daily mortality or variation in toxicity during a 24-hour day. Moreover, the animals had been previously kept for at least one week in the room in which the experiments were performed. The animals of each series were kept on different dietaries so that the phenomenon can hardly be associated with any special diet.

It will be apparent that the experimental conditions were well-controlled and that the variation in toxicity can hardly be attributed to any other factor than the time of day at which the injection was made.

As far as we know, no diurnal variation in the lethal effect of a substance has hitherto been recorded. Fluctuations in the convulsive effect of insulin during the day have however been described, but opinions differ about the times of maximum and minimum effect (ÅGREN, WILANDER and JORPES 1931; HEMMINGSEN 1933).

Investigations are at present being made to find an explanation for this variation in toxicity. Judging by the preliminary experiments described above, the maximum spontaneous activity of the animals coincided with the minimum degree of toxicity of the nikethamide and *vice versa*. In view of the apparently stimulating effect of nikethamide on the central nervous system this correlation is remarkable.

These findings open a new approach to the study of diurnal rhythm, a problem that has recently aroused increasing interest (for a recent review see KLEITMAN 1949).

Until convincing evidence of the wider occurence or non-occurence of such variations in the toxicity has been produced, the possibility of similar behaviour by other substances should be taken into account in toxicity tests.

Summary.

Toxicity tests of nikethamide injected subcutaneously into white mice at different hours of the day showed that the mortality following the same dose varied considerably according to the time of the day when the injection was given. A maximum mortality was recorded among the mice injected at 2 p.m., and a minimum among those injected at 2 a.m. There seems to exist an inverse relationship between the toxicity of nikethamide and the voluntary activity of the animals.

REFERENCES

Carlsson, A. and F. Serin: *Acta pharmacol.* 1950, **6**, 181.
Hemmingsen, A. M.: *Quart. J. Pharm. Pharmacol.* 1933, **6**, 39.
Kleitman, N.: *Physiol. Reviews* 1949, **29**, 1.
Ågren, G., O. Wilander and E. Jorpes: *Biochem. J.* 1931, **25**, 777.