Brit. J. Pharmacol. (1948), **3**, 116.

THE EFFECT OF ENVIRONMENTAL TEMPERATURE ON THE TOXICITY OF BAL

BY

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(Received July 21, 1947)

Peters, Stocken, and Thompson (1945) have already described the steps leading to the manufacture of BAL (2 : 3-dimercaptopropanol) the specification for which required that BAL should pass a toxicity test before being used for therapeutic purposes.

Groups of rats used as standard controls in assaying samples of BAL have shown a wide variation in response to intramuscular injections of 140 mg. per kg. of Oxford Standard BAL—the LD50 dose as found at Porton (Boyland and McDonald, 1943). Variations from 15 to 85 per cent mortalities occurred in different groups receiving this dose in assays made over several months. Such a wide difference in response was greater than would be expected by chance and some other external factor was suspected of contributing to the toxic effects of BAL.

The effects of toxic agents on rats are influenced by many factors (cp. Holck, 1942) among which the most notable is the room temperature at which the rats are kept during the experiment.

Temperature being the greatest variant to which Porton rats were subjected, experiments were conducted to see if this affected the mortality of rats to a standard dose of BAL.

PROCEDURE

Rats weighing between 120–180 g. were starved, but given water, for 24 hours prior to injection of BAL and kept in thermostatically controlled chambers at various temperatures for the whole of the starving period and until 72 hours after injection. Oxford Standard BAL was diluted with propylene glycol to give a concentration of 140 mg./c.c. Using a micrometer syringe, 1 c.c./kg. was injected into the muscles of the thigh of each rat and the mortality rate observed for 72 hours after injection. Most of the deaths occurred within 24 hours and only an occasional rat died after that period. For approximately 20 minutes, the average time taken to weigh and inject a group, the rats were exposed to ordinary room temperature.

RESULTS

The results obtained over the period between August, 1944, and May, 1945, are given in Table I.

TABLE I

Date	Temperature ° F.	Mortality rate	
		Actual	Per cent
29/8/44	39	10/10	100
16/10/44	39	10/10	100
19/10/44	42	8/10	80
1/11/44	43	7/10	70
1/5/45	45	12/20	60
27/4/45	49	13/20	65
1/5/45	50	9/20	45
9/10/44	54	10/20	50
2/10/44	55	8/20	40
24/4/45	55	4/20	20
19/9/44	60	5/20	25
25/9/44	60	7/20	35
17/4/45	60	9/20	45
29/8/44	65	3/10	30
31/8/44	65	7/20	35
24/4/45	69	5/20	25
27/4/45	70	8/20	40
2/10/44	71	8/20	40
25/9/44	75	10/20	50
17/4/45	75	10/20	50
31/8/44	80	14/20	70
19/9/44	80	12/20	60
1/5/45	80	15/20	75
9/10/44	81	16/20	80
29/8/44	84	9/10	90
1/11/44	84	18/20	90

Fig. 1 shows the same results plotted on probit scale with the best fitting curve, a parabola, symmetrical about the temperature 63° F. as calculated by Box (1945). This demonstrates quite a remarkable effect of temperature on toxicity. In view of the fact that variations from the curve are no more than could be expected to occur by chance, there is no evidence that seasonal variation occurred over the period that observations were made, other than that due to temperature difference. Fig. 2 shows the expected variation of the LD50 for BAL at different temperatures, calculated from Box's BAL–

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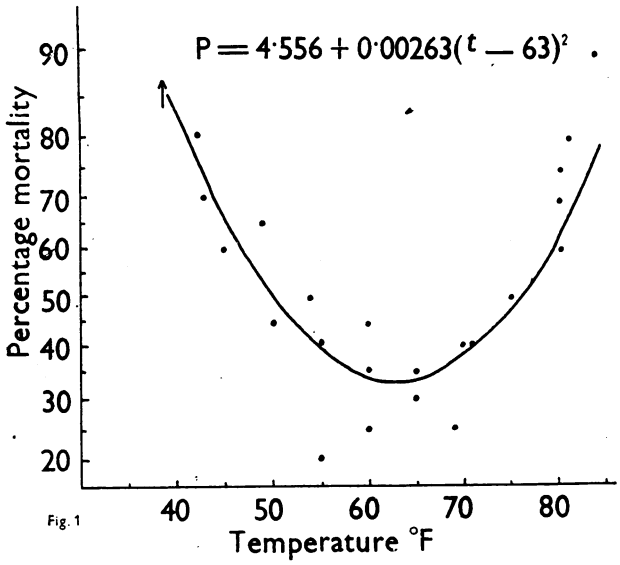

Fig. 1

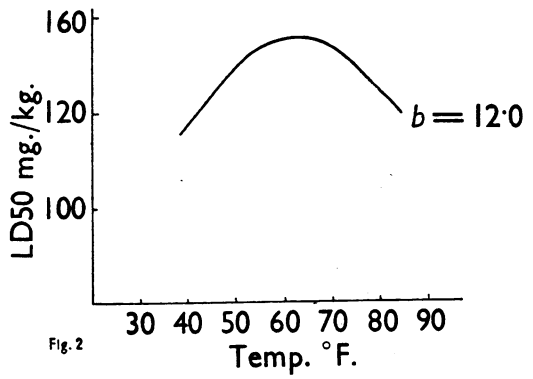

Fig. 2

emperature–toxicity formula:— LD50 = 152 − 0.07 t - 63)², where t = temp. in ° F. (Box, 1945).

Propylene Glycol

When propylene glycol is used for diluting BAL prior to injection, care should be taken to ensure that it is freshly distilled. We found, when using an old sample of propylene glycol in error, that we obtained much higher mortality rates than were expected in view of the previous results. However, when the sample was redistilled, the mortality rates were in keeping with the previous findings. These high results have therefore been discarded and our practice now is to distil off enough propylene glycol for an assay on the same day that the rats are injected.

Conclusions

The mortality rate of rats injected with BAL has been shown to be influenced by the temperature of their environment. The mortality was minimal at 63° F. (17.2° C.). Rats used for BAL assay should be kept at an even temperature or in a thermostatically controlled room if possible. No estimation of the relative toxicity of samples of BAL should be made without reference to the results obtained from a dose of a standard preparation given at the same time.

If propylene glycol is used as a vehicle for injection, it is preferable to distil off the required amount on the same day.

I am indebted to the Chief Scientist, Ministry of Supply, for permission to publish this paper, to Miss Cynthia Moore for technical assistance, and to Mr. G. E. P. Box for evolving a formula from data in Table I.

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