The Effect of Dimethylsulfoxide on Behavioral Temperature Regulation* (32648)

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Dimethylsulfoxide (DMSO) has received much attention because of its possible, albeit controverted, therapeutic value. Relatively large doses can be safely given to experimental animals; e.g., the LD_{50} for ip administration in rats is 13.08 gm/kg (1). DMSO is an excellent solvent and has proved useful as a vehicle for the parenteral administration of antibiotics and other drugs. Biological actions of DMSO were recently reviewed (2).

DMSO has been widely used as a cryoprotective agent for single cells and tissues (3). Its effect on cold-exposed intact animals, however, has received little study. Intraperitoneal injection of DMSO (3–6 gm/kg) causes a slight fall in the body temperature of mice (4) and rats (5) maintained at ordinary room temperatures. The same doses can cause deep hypothermia and death within a few hours during exposure to an ambient temperature of 1°C (5). Oxygen consumption by cold-exposed rats and hamsters is significantly reduced following DMSO treatment (6), and this implies a reduction in heat production needed for the maintenance of thermal balance under cold stress. There is need for a fuller understanding of the action of DMSO with reference to thermal homeostasis. In an effort to help clarify this effect we studied thermoregulatory behavior, allowing the DMSO-treated subject to augment available heat externally by means of an operant heat reinforcement technique. This system was developed by others (7) and has been used for a variety of physiological evaluations (e.g., 8–10).

Methods. Subjects. The subjects were 58 unshaved, Long-Evans male rats, weighing an average of 281 (240–340) gm.

Apparatus. The behavioral chamber was a dimly illuminated plexiglas cylinder, 20 cm in diameter and 35 cm high, with an open top and a grid floor made of plastic rods. The chamber contained a single plastic response key which extended 3 cm into the apparatus and was 3 cm above the floor. A pressure of 8 gm was sufficient to depress the response key and cause the lighting of an overhead 250-W infrared heating lamp for 4 sec. The lamp was centrally placed 25 cm above the floor of the chamber. A continuous reinforcement schedule was used. The key had to be first released and pressed again before a second reinforcement was delivered. Responses made when the heat lamp was on had no effect. Programming was accomplished by a system of switching relays and timers. Data were recorded automatically on counters and cumulative recorders. The behavioral chamber was located in a room maintained at 1–3°C. Food and water were not available within the behavioral chamber.

Procedure. The experiment had three phases: *training, resting,* and *testing.* All individuals were treated identically until the final test phase. There was a 16-hour training session beginning at 5 p.m. at an ambient temperature of 2°C. Relative humidity was 60–80%. Following training, the subjects were rested for 7 hours with food and water at an ambient temperature of 24°C. They were then divided into four groups, injected ip with 5 ml of physiological saline or with a selected dose of DMSO in a 5-ml saline solution, and immediately placed once again in the behavioral chamber at an ambient temperature of 2°C. The four groups included: 1) 11 rats which received saline alone; 2) 17 rats which received 3 gm/kg DMSO; 3) 18 rats which received 6 gm/kg DMSO; and 4) 12 rats which received 6 gm/kg DMSO at the start of the experiment and a second dose of 6 gm/kg DMSO after 6 hours. For most of the experiments 'Baker Analyzed' (No. 9224) DMSO was used for preparing injections. In other experiments the DMSO was dried over CaH_2, distilled under vacuum, and then filtered through an 0.2 μ sterile membrane

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(The Nalge Co., No. 120). No response differences were noted for these preparations. Animals were weighed at the beginning and end of each phase of the experiment. Body temperatures were measured by rectal thermocouple, continuously when possible, during the three phases of the experiment.

Results. Training. Training patterns were similar for all groups. The mean response rates gradually increased until about 6 hours and then leveled. Such training responses are described in greater detail elsewhere (9). The mean 16-hour training response rate was 46.7 heat reinforcements/hour. Two rats which failed to train were not used in the subsequent tests. Subjects lost an average of 22 gm during the training session but rapidly regained more than half of this loss during the following rest period. Data on the body temperatures of rats during training procedures similar to those described here have been given elsewhere (9, 11).

Testing of saline controls. The mean response rate was 75.3 heat reinforcements/hour, 61.2% higher than during training, a typical second exposure result (Fig. 1, Table I). Figure 2 illustrates the response of a saline-treated subject which began to work steadily for heat after 3 hours of cold exposure. The rate at which this animal performed after 9 hours was about the same as it was at the start of its performance.

Testing of 3 gm/kg DMSO subjects. The mean response rate was 82.3 heat reinforcements/hour, 9.3% higher than saline controls (Fig. 1, Table I). An analysis of variance

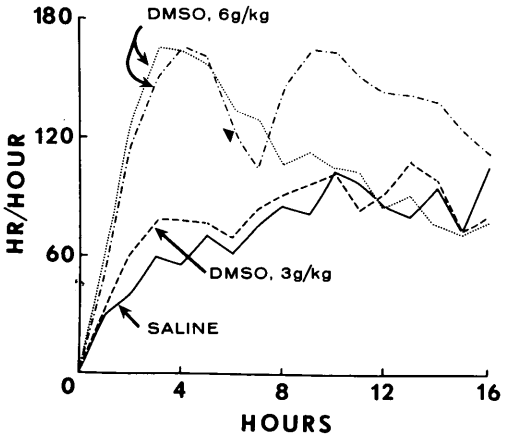

FIG. 1. Mean heat reinforcement (HR) rates during test sessions. The triangle indicates the time of the second DMSO injection for the two-dose series.

during the first 3 hours, a period during which there is a relatively large difference between the behavior of this group and the saline control group, indicates that the rate difference observed is not significant ($F = 0.8$).

Figure 2 illustrates a typical cumulative record obtained from these subjects. Body temperature records are incomplete because of difficulty in maintaining the continuity of thermocouple connections overnight. Available data indicate that body tempratures were not depressed below 30°C and that the greatest depression occurred about 1.5 hours following injection. This suggests a pattern which parallels that shown by the more complete 6 gm/kg DMSO series.

Testing of 6 gm/kg DMSO subjects. The

TABLE I. A Summary of Heat Reinforcement Response Rates at 3-Hour Intervals for Rats Treated with Saline or DMSO and Exposed to an Ambient Temperature of 2°C.

This was the second 16-hour session (the test session) in the heat reinforcement situation. Subjects were untreated during their initial training.

	Mean heat reinforcements/3 hour ± SE			
Time interval (hours)	Saline ($n = 12$)	3 gm/kg DMSO ($n = 18$)	6 gm/kg DMSO ($n = 16$)	Two doses 6 gm/kg DMSO ($n = 12$)
1–3	136 ± 25.1	179 ± 36.3	360 ± 27.3	318 ± 44.2
4–6	187 ± 26.4	229 ± 14.6	458 ± 28.6	453 ± 43.0
7–9	232 ± 34.6	271 ± 38.1	362 ± 22.1	392 ± 24.2
10–12	290 ± 40.1	276 ± 38.2	296 ± 27.0	468 ± 33.3
13–15	251 ± 33.0	305 ± 26.8	240 ± 28.0	408 ± 51.1

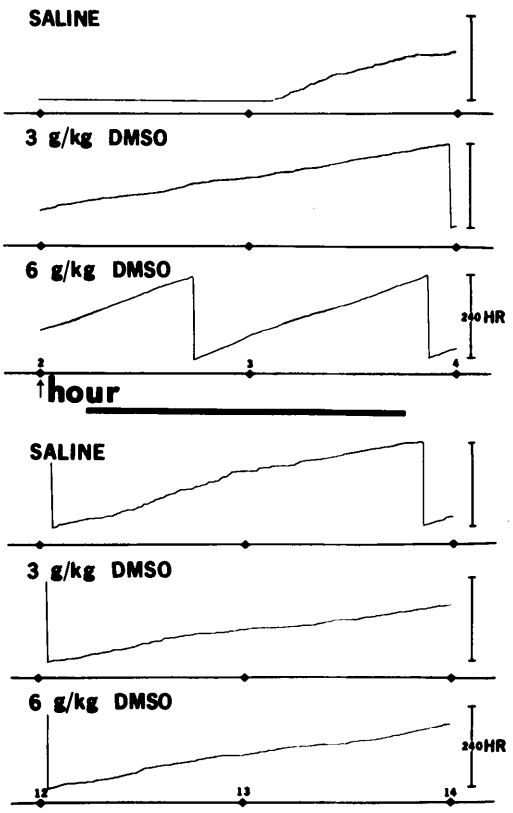

FIG. 2. Typical cumulative responses of rats on a continuous heat reinforcement schedule. Only hours 2–4 and 12–14 are shown. The ordinate represents cumulated responses, and the abscissa indicates time in hours. All records are from test sessions.

mean response rate was 111.7 heat reinforcements/hour, 48.3% higher than saline controls (Fig. 1, Table I). Performance peaked after 3 hours. A typical cumulative record is shown in Fig. 2. These animals began their DMSO test with an average body temperature of 36.8 (35.8–37.5)°C. The lowest body temperature reached in the course of the test averaged 31.6 (29.8–33.6)°C. This occurred 103 (67–148) min following injection. This precedes the point of maximum heat reinforcement by about 1.5 hours. Following the maximum body temperature depression, in each case there was a slow rise in body temperature. Earlier levels were not attained until after 4–7 hours.

An analysis of variance comparing the response rates of combined saline and 3 gm/kg DMSO subjects with the response rates of this 6 gm/kg DMSO group during the first 3 hours following treatment indicates a highly significant difference ($F = 32.9$). An analysis of variance between the saline group and the 6 gm/kg DMSO group also indicates a highly significant difference ($F = 28.0$).

Testing of subjects receiving two 6 gm/kg doses. The mean response rate was 135.2 heat reinforcements/hour, 79.5% higher than saline controls (Fig. 1, Table I). The first performance peak occurred after 3 hours and the second performance peak occurred 3 hours following the second injection.

Discussion. Treatment with the doses of DMSO used in these experiments reduces the ability of rats to withstand the stress of low environmental temperatures (5, 6, 12). The results of the present study clearly indicate a significant intensification of the thermoregulatory behavioral response by cold-exposed rats following treatment with 6 gm/kg DMSO. Similarly treated rats without the possibility of heat reinforcement have been reported to cool to body temperatures of 10°C within 6 hours (5). The availability of heat reinforcement allowed the subjects to avoid death due to hypothermia. The DMSO-induced vulnerability to cold stress was compensated for by their behavior. The responses of the two groups receiving 6 gm/kg DMSO (Fig. 1) were very similar early in the experiment. In each case there was a dramatic rise in response rate during the first 3 hours. The one-dose group then returned to control levels. The response of the two-dose group began to diminish, but then increased again in nearly identical fashion to the initial rise. The final decline was slower, possibly due to some accumulation of the drug.

It was judged from previous experiments that fatigue does not diminish the heat reinforcement response of a cold stressed animal during a 16-hour session. Nevertheless, it was decided to see if the decline in the performance curve for the 6 gm/kg DMSO subjects following the 3-hour peak was caused by fatigue. The two-dose response indicated that fatigue was not the determining factor. Rats remained physically capable of sustaining a high response rate. It may also be noted that lethargy or decreased motor activity which

may accompany DMSO treatment (13) failed to interfere crucially with the measured response.

The behavioral response peaked after about 3 hours. This is interesting if compared to a report by Ashwood-Smith (14) that the level of DMSO remaining in the brains of rats treated with 4.5 gm/kg DMSO also peaks at 3 hours. According to his data, our behavioral results parallel brain distribution of DMSO closer than any other tissue studied.

While there appears to be no doubt about the interference with thermal balance (4–6, 12), the specific stimulus for the observed behavioral effect remains unclear. Carlisle (11) states that, ". . . rates of responding and of obtaining reinforcement increase (a) when ambient temperature decreases, indicating that cold is drive-inducing, and (b) when magnitude of reinforcement increases . . ." In our experiments, ambient temperature, except as modified immediately surrounding the animal by heat reinforcement, and the magnitude of the reinforcement were kept constant. Satinoff (15) observed increased heat reinforcement when there was local cooling of the hypothalamus. Core cooling differences, however, probably do not explain the difference between the 3 and 6 gm/kg DMSO results. Although the present study did not yield complete body temperature information for the 3 gm/kg dose, it has been observed that both doses induced a mild hypothermia soon after administration (5, 6). Differences in the depth of hypothermia induced are slight, and it appears unlikely that this is the significant factor. Any increase in the sensitivity of the skin to cold should induce a greater effort at raising the ambient temperature. Possibly a change in sensory receptors is contributing to the behavioral effect. Carlisle (11) has speculated that the subject may work "to prevent a fall in body temperature" and he proposes an analogy between operant responding for heat and avoidance behavior. This speculation seems to apply well to our data. The 6 gm/kg DMSO dose may interfere more significantly with heat production and/or physical temperature regulation than either the 3 gm/kg DMSO dose, or, of course, saline for the control subjects. The result is that with less heat production and/or less control over heat loss, the 6 gm/kg subject must respond with greater frequency in order to maintain its body temperature within certain limits.

Food and water were withheld during behavioral training and testing because previous studies have shown that under these conditions rats not only show a more rapid onset of key-pressing for heat, but also maintain a more stable pattern of performance. The latter result particularly was desirable for our analysis. The utilization of a short rest period between training and testing sessions minimized complications arising from retention of training.

In summary, cold-exposed rats were trained to work for external heat by pressing a response key. Treatment with 6 gm/kg DMSO following training caused a significant increase in heat reinforcement performance; the highest response rates occurred after 3 hours, the post ip injection time at which the level of DMSO in the rat brain has been reported to reach its peak. DMSO-treated subjects are sensitive to the need for heat reinforcement and are able to respond vigorously in order to obtain the external heat required to supplement for impaired homeostatic mechanisms.

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1. Coujolle, F., Coujolle, D., Bouyssou, H., and Calvet, M., Compt. Rend. Acad. Sci. Paris **258**, 2224 (1964).

2. Leake, C. D., ed., Ann. N. Y. Acad. Sci. **141**, Art. 1 (1967).

3. Doebbler, G. F., Cryobiology **3**, 2 (1966).

4. Ashwood-Smith, M. J., Intern. J. Radiation Biol. **3**, 41 (1960).

5. Panuska, J. A., Malinin, T. I., and Mentz, R. J., Cryobiology **3**, 345 (1966).

6. Walker, T. M., Albrigo, J. L., and Panuska, J. A., Cryobiology **3**, 265 (1966).

7. Weiss, B. and Laties, V. G., Science **133**, 1338 (1961).

8. Yeh, S. D. J. and Weiss, B., Am. J. Physiol. **205**, 857 (1963).

9. Panuska, J. A., Cryobiology **1**, 334 (1965).

10. Baldwin, B. A. and Ingram, D. L., Physiol. and Behav. **2**, 15 (1967).

11. Carlisle, H. J., J. Comp. Physiol. Psychol. **61**, 388 (1966).

12. Altland, P. D., Highman, B., and Parker, M., Proc. Soc. Exptl. Biol. Med. 123, 853 (1966).

13. Willson, J. E., Brown, D. E., and Timmers, E. K., Toxicol. Appl. Pharmacol. 7, 104 (1965).

14. Ashwood-Smith, M. J., Ann. N. Y. Acad. Sci. 141, 45 (1967).

15. Satinoff, E., Am. J. Physiol. 206, 1389 (1964).

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Studies on the Etiology of Marek's Disease. I. Propagation of the Agent in Cell Culture (32649)

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Marek's disease (MD) is a highly contagious, lymphoproliferative disease causing severe mortality and economic loss in commercial chicken flocks. Affected birds often have lymphoid tumors in the nerves, skin, muscle, or visceral organs. The etiological agent is highly infectious but its nature is uncertain. Although the agent may be detected by inoculation of susceptible chicks, this technic is difficult and time consuming. A rapid and sensitive *in vitro* assay would facilitate research on many aspects of this disease.

Considerable effort has been directed toward the development of methods for *in vitro* propagation and assay. Biggs and Payne (1) failed to detect infectivity in chick embryo fibroblast cultures following inoculation with the B14 strain of MD. Vindel (2) isolated a filtrable, cytopathogenic agent from chickens with neurolymphomatosis but its relationship to the MD agent was not established. In recent studies (Witter *et al.* (3); B. W. Calnek, personal communication), monolayer cultures prepared from normal chick embryo bone marrow and other tissues were infectious for several weeks following treatment with cellular MD inocula. However, evidence for replication of agent in these *in vitro* systems was inconclusive and no morphological alterations were observed in infected cultures.

Kottaridis and Luginbuhl (personal communication) have observed a cytopathic effect (CPE) in chick embryo fibroblast cultures inoculated with bone marrow from birds with MD and these cultures reproduced MD when inoculated into chickens. We have just learned that Churchill and Biggs (4) have isolated a herpes-like virus from chick kidney cells inoculated with MD tumor cell suspensions that produced a CPE characterized by the presence of Cowdry Type A intranuclear inclusion bodies. Chicks inoculated with cultures showing typical CPE developed MD.

This report describes the propagation in duck embryo fibroblast (DEF) cultures of a cell associated, cytopathogenic agent derived from the JM strain of MD. Evidence presented here and in another report (5) suggests that this agent is the etiological agent of Marek's disease.

Materials and Methods. Marek's disease agent. Blood collected in heparin (20 units per ml) from chickens inoculated with the JM strain (6) of MD was used for inoculation of cell cultures. Propagation of the JM strain in chickens at this laboratory has been described previously (7).

Duck embryo fibroblast cultures. Fibroblasts were prepared by the trypsinization of decapitated 12 to 13-day-old duck embryos derived from flocks in Michigan and New York. Cells were diluted in growth medium, transferred to 100-mm petri dishes and incubated at 38–40°C in a humidified atmosphere containing 3–5% carbon dioxide. The growth medium consisted of medium 199[2] and nutrient medium F10[2] in a ratio of 4:5 plus 5% tryptose phosphate broth, 0.084% sodium bicarbonate and 4–6% calf or bovine fetal se-

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