Effect of Dimethylsulfoxide on Isolated-Innervated Skeletal, Smooth, and Cardiac Muscle. (31063)

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(Introduced by M. B. Sulzberger)
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Dimethylsulfoxide (DMSO) has been under active investigation for its solvent properties (1) and its ability to prevent freezing damage to tissues(2). Recently, it has been the subject of a number of investigations in laboratory animals and man. In general, DMSO has shown relatively little systemic toxicity (3,4). But daily intravenous administration to dogs has been reported by Willson *et al* (5) to cause a hemolytic anemia, hematuria, and cloudy swelling of the liver parenchyma, and in rats, large intravenous doses may cause death preceded by tremors, muscular weakness, and, occasionally, convulsions.

No detailed pharmacodynamic studies of the effects of this compound on isolated muscles and nerves have been reported thus far. The effects of DMSO were therefore studied by means of isolated, innervated guinea pig diaphragm, stomach, and cardiac atrium preparations.

Materials and methods. All studies employed organs from adult hybrid guinea pigs weighing 350-450 g, and all animals were anesthetized with pentobarbital sodium (60 mg/kg i.p.) prior to laparotomy for removal of the selected organ.

The pH of the organ bath was monitored continuously with a Beckman Zeromatic pH Meter during the course of the studies.

I. *Innervated guinea pig diaphragm.* After laparotomy, one hemidiaphragm was excised, along with its rib cage, and a segment of its motor innervation (phrenic nerve). The rib cage and hemidiaphragm were secured to a plastic holder which allowed electrical stimulation alternately to the muscle or nerve delivered at regular intervals by 2 stimulators (American Electronics Model 104-A). This preparation was placed into a 150 ml bath containing Krebs-Henseleit buffer(6), to which 5.4% glucose and 3.8% $MgSO_4$ had been added. The solution was saturated at 37° with 94% O_2 and 6% CO_2. The central tendon of the diaphragm was connected semi-isotonically by means of a light thread to a force displacement transducer (Statham Model UC2), coupled to a direct writing multiple channel polygraph (Gilson). After a 30-minute period of equilibration, baseline responses to muscle and nerve stimulation were recorded at 10-minute intervals for a 60-minute control period. At the end of this time, DMSO ranging from 0.6-6.0% final bath concentration was added. Supramaximal square wave stimuli (50 milliseconds duration, 50 cycles/sec and 1-5 volts) were used throughout. Four diaphragm-phrenic preparations were studied.

II. *Innervated guinea pig stomach.* The stomach and its vagal supply were removed surgically from anesthetized guinea pigs according to the technique of Greeff and Holtz (7). The preparation was inserted into a holder-stimulator which allows direct (muscle) and indirect (nerve) electrical stimulation. The square wave stimuli and organ bath used were the same as those described previously. The activity of the muscle was monitored by means of a light thread tied to the pyloric end of the preparation and recorded as with the diaphragm.

After a 30-minute equilibration period, baseline responses to electrical stimulation were obtained at 10-minute intervals for 60 minutes. DMSO of the desired concentration from 0.6-6.0% was added and the muscle responses to both direct and to nerve stimulation were recorded.

Five stomach-vagus preparations were studied.

III. *Innervated cardiac atrium.* The heart of an anesthetized guinea pig was rapidly removed and transferred into a shallow dish containing modified Krebs-Henseleit solution at 4°C. Both ventricles and all extraneous tissues were excised except for the atria and

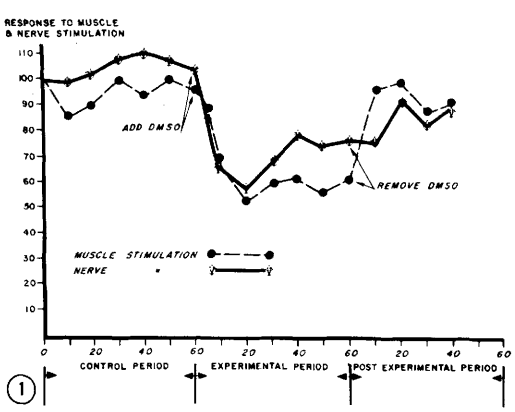

FIG. 1. Hemidiphragm—phrenic nerve preparation in a final organ bath concentration of 3% DMSO.

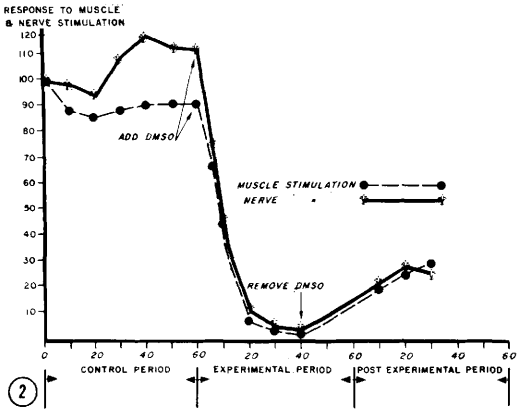

FIG. 2. Same as Fig. 1 except DMSO concentration 6%.

their vagal innervation. The isolated atrium-vagus preparation was inserted into a plastic muscle holder, a small metal clip was attached to the tip of the auricle, and the preparation transferred into a 150 ml constant temperature bath containing modified Krebs-Henseleit solution saturated at 37°C with 94% O_2 and 6% CO_2. A light silk thread was connected to the metal clip and semi-isotonically to a force-displacement transducer (Statham Model UC2 with a factor-of-ten lever) or inserted through the core of a linear displacement differential transformer (Sanborn Model 595 DT-005) acting on a low frequency spring. The output of the transformer was fed through a transducer converter (Sanborn Model 592-300) and recorded on a multichannel direct writing polygraph (Gilson) or through a pre-amplifier (Tektronix Type 122) to a storage oscilloscope (Tektronic Model 564). The vagus nerve was stimulated electrically at regular intervals with square wave pulses 50 milliseconds in duration, at a frequency of 50 cps, and 1-10 volts.

It was possible to record rate and amplitude of atrial contraction and establish accurately a vagal threshold which would produce complete arrest of the recordable contractions of the muscle.

The atria were allowed to equilibrate in the buffer for 30 minutes before recordings were initiated. After a suitable control period, concentrations of DMSO ranging from 0.6-6.0% were added to the bath. Eight atrial-vagus preparations were investigated.

In addition, ouabain (0.08 to 0.67 μg/ml) or digoxin (0.08 to 0.67 μg/ml) were added to the bath with and without 6.0% DMSO to ascertain whether potentiation or antagonism occurred.

Results. DMSO, in the concentrations used in this study, had no measurable effect on the pH of the buffered organ bath.

I. *Innervated diaphragm.* DMSO, at a final bath concentration of 0.6%, had no effect on the contractile response of the diaphragm to electrical stimulation of either the muscle or nerve.

With a 3% DMSO concentration, the amplitude of the response of both nerve and muscle was depressed to approximately 60% of the control level (Fig. 1). Four minutes after addition of the compound, fine muscle fasciculations could be observed during the resting intervals between stimuli.

A 6% DMSO concentration almost completely abolished, within 20 minutes, the response of the nerve-muscle preparation (Fig. 2). Fasciculations became noticeable after 4 minutes' exposure of the preparation to 6% DMSO (Fig. 3). These fasciculations diminished gradually in intensity and were no longer visible or recordable after 15 minutes. The response to muscle and nerve stimulation recovered only partially following the removal of DMSO and its replacement by fresh buffer. No signs of neuromuscular blockade were detected.

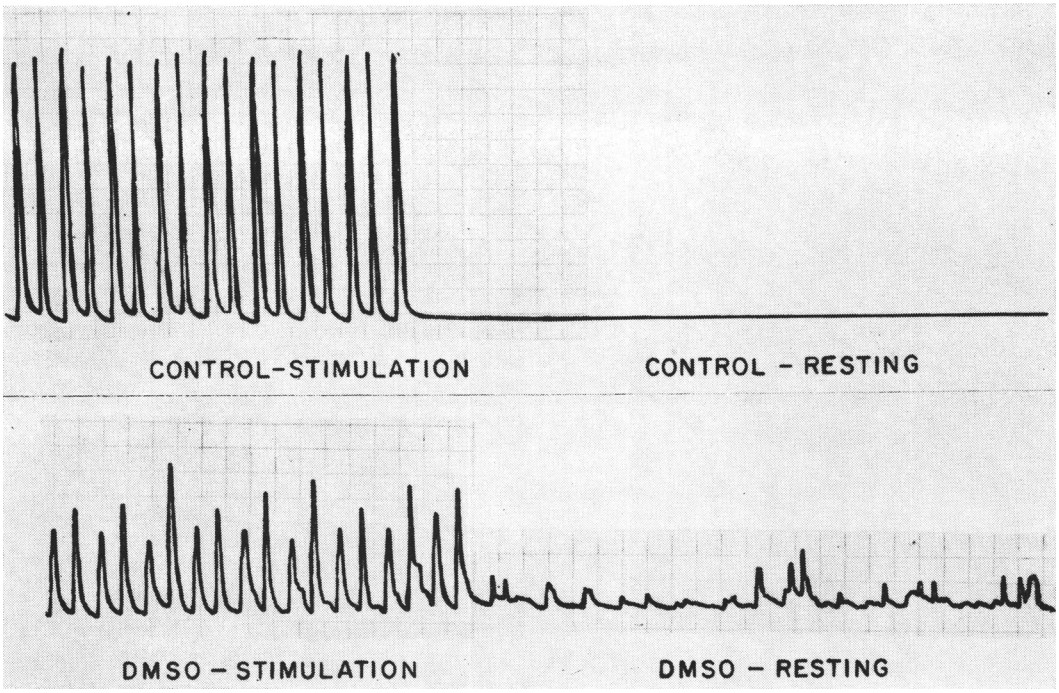

FIG. 3. Hemidiaphragm—phrenic nerve preparation showing polygraph recordings of alternate simulation of muscle and nerve on left and the resting state on the right. DMSO concentration 6%.

II. *Innervated stomach.* DMSO appeared to augment the response of the stomach to electrical stimulation of both nerve and muscle. However, there was no linear increase in response to stimulation as a function of increased concentration of DMSO.

At a bath concentration of 3%, DMSO produced the most marked enhancement of response to electrical stimulation. The contractility of the muscle to direct stimulation increased 15% within 10 minutes from the addition of DMSO to the bath while nerve stimulation elicited a 25-30% increase of contractile response.

A small increase in response to both nerve and muscle stimulation with 0.6% DMSO was also observed in one case.

The spontaneous activity of gastric muscle at rest was not increased.

III. *Innervated cardiac atrium.* DMSO in a concentration of 0.6% had no appreciable effect on either the rate or amplitude of atrial contraction though it did lower the vagal threshold approximately 20%.

At 3%, DMSO showed a negligible effect on rate and a moderate increase in the amplitude of contraction, though it consistently lowered the threshold to vagal stimulation.

A final bath concentration of 6.0% DMSO induced a slight increase in rate and a very marked (50-55%) increase in amplitude of muscle contraction within 5-10 minutes after addition to the bath (Fig. 5). The vagal threshold was lowered approximately 50% (Fig. 6).

This effect of DMSO on the atria was not influenced by prior addition of ouabain or digoxin to the bath.

Discussion. At concentrations of 3% and 6%, DMSO appears to decrease significantly the response of the diaphragm to direct and indirect electrical stimulation. It appears, therefore, that DMSO has direct skeletal muscle depressant properties, though there was no evidence of neuromuscular blockade. The spontaneous fasciculations which were regularly observed are of considerable interest. These could be due to an anticholinesterase action of DMSO, as neostigmine induces an analogous response *in vitro* and *in vivo*(8). To test whether DMSO did, indeed, inhibit cholinesterase, the activity of the en-

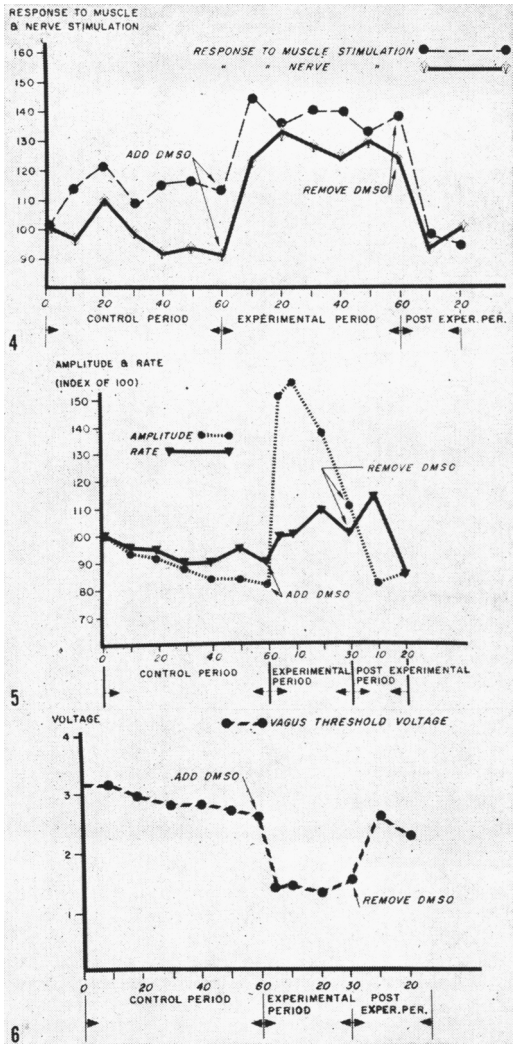

FIG. 4. Stomach—vagus nerve preparation in a final organ bath concentration of 3% DMSO.

FIG. 5. Cardiac atrium—vagus nerve preparation in a final organ bath concentration of 6% DMSO.

FIG. 6. Cardiac atrium—vagus nerve preparation in 6% DMSO showing the vagal threshold.

zyme was tested by the method of Hestrin (9). This involves incubation of cholinesterase with the suspected inhibitor, followed by incubation with acetylcholine. Free acetylcholine is determined after incubation. With this method, it was found that 0.78% (0.1 M) DMSO inhibits 16% of the reaction, 3.9% (0.5 M) DMSO inhibits 44% of the reaction, and 7.8% (1.0 M), inhibits 85% of the reaction. Thus, cholinesterase inhibition could reasonably account for the observed muscle fasciculation.

Cholinesterase inhibition could also explain the increase in tone of the smooth muscle of the stomach.

DMSO had no appreciable effect on atrial rate in any of the concentrations used, though 6% DMSO caused a marked increase in amplitude of atrial contraction. The lowering of vagal threshold was also very striking and two explanations seem reasonable. First, DMSO may increase the permeability of the cell membrane to such a degree that Na^+ is able to cross the membrane with a weaker stimulus. Second, the enhancement to vagal stimulation may be related to inactivation of cholinesterase at the cardiac-vagus endings. An analogous response has been observed with neostigmine.

Summary. Isolated, innervated guinea pig preparations have been used to study the effects of dimethylsulfoxide on skeletal, smooth, and cardiac muscles. DMSO depressed the response of the diaphragm to both muscle and nerve stimulation. In addition, it caused spontaneous skeletal muscle fasciculations. DMSO increased the response of the smooth muscle of the stomach to both muscle and nerve stimulation. The amplitude of atrial contraction is augmented considerably by DMSO, although the rate of contraction appears unaffected. Vagal threshold was lowered approximately 50% by a bath concentration of 6% DMSO. Although other explanations are possible, the observed fasciculations of the skeletal muscle, the increased tone of the stomach muscle, and the lowering of the vagal threshold by DMSO could all be due to cholinesterase inhibition.

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Influence of Adrenalectomy on Homologous Disease.* (31064)

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It is well established that the syndrome known as "homologous disease," "runt disease" or "secondary disease" is the result of an immune reaction elicited by the introduction of immunologically competent lymphoid cells into susceptible recipients incapable of rejecting the donor cells. This graft-*versus*-host reaction has been induced in fetal(1) newborn(2) and adult animals previously made tolerant of the homologous cells(3) and in adult animals pretreated with a lethal dose of whole body X-irradiation(4). Homologous disease can also be produced by injection of parental strain lymphoid cells into genetically tolerant F1 hybrid mice(5). Animals with the disease develop anemia, lethargy, and a hunched posture, and they frequently succumb as a result of the process.

We became interested in the influence of the adrenal glands on homologous disease because we had observed that animals suffering from homologous disease frequently had enlarged adrenal glands. In addition, it had been reported by Kaplan and Rosston(6) that adrenalectomy ameliorates homologous disease. We therefore considered the possibility that homologous disease might be in part the result of adrenal hyperactivity. Furthermore, there is good evidence that the adrenal steroid hormones exert an effect upon lymphoid tissue and upon immunological responsiveness. The injection of certain adrenal corticosteroids causes involution of lymphoid organs (7) and decreases immunological responsiveness to antigenic stimulation(8,9). Conversely, adrenalectomized animals frequently develop higher titers of circulating antibody than normal animals(10).

In the experiments to be reported here, we have studied the development and course of homologous disease produced by injection of spleen cells from parental strain mice into their F1 hybrids previously subjected to bilateral adrenalectomy. These experiments demonstrate that not only does removal of the adrenal gland fail to avert homologous disease produced in this way, but such manipulation increases both the incidence and the severity of this process. They also demonstrate that these effects of adrenalectomy can be prevented by administration of cortisone acetate.

Materials and methods. Inbred mice of the A and C3H strains and F1 hybrid mice resulting from the cross between A and C57-Bl/1 and between C3H and C57Bl/1 strains were used for these experiments. The A, C3H and C57Bl/1 strains of mice have been maintained in our colony by strict brother-sister mating since 1956, and are directly descended from the inbred colony of the late Dr. J. J. Bittner. All F1 hybrid mice were between 1½ and 3½ months of age at the time they received the spleen cell injection. Spleen cell suspensions were prepared by slicing spleens into several pieces and then expressing the splenic pulp gently from the capsule in a loose fitting Potter-Elvehjem glass homogenizer. All suspensions were prepared in lactate-Ringer's solution and were injected within one hour of preparation. The dosage of spleen cells injected in all experiments was 150 million per mouse, administered intravenously in a volume of approximately 0.25 cc into one of the lateral veins

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