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Received February 17, 1966. P.S.E.B.M., 1966, v122.

Studies of the Effect of Dimethylsulfoxide on Permeability of Dermal Connective Tissue.* (31115)

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Dimethylsulfoxide (DMSO) was found to be an effective histological preservative of mammalian cells when used at low concentrations(1-5). Several other chemical, physiological and therapeutic properties of this compound have also been reported(6,7). The ability of DMSO to translocate drugs through animal tissues has been ascribed to its high solvating power and membrane-permeability enhancing effect(7). The present study was undertaken to investigate the effect of this compound on the permeability of connective tissue in which the hyaluronic acid of the ground substance plays a major role(8).

The experiments were performed on albino male rats of CFN strain 9 to 10 months old. For measuring permeability our previously described method(8,9) based on the rate of dermal diffusion of dye alone or in combination with test substance was used. In this method 0.05 ml of a 0.4% solution of Evans blue in saline (pH 7.3) with or without the test substance was injected intradermally. Three injections on each animal were made for the control (generally dye alone) and 3 for the test compound. The contours of the blue spots thus produced were traced onto semi-transparent paper at 30, 60, 120 and 180 minute intervals after injection. The spots traced on paper were cut out, weighed and their areas calculated as mm^2.

Since 3 spots were obtained on each animal for the control and 3 others for test substance, the results reported in Table I are thrice the number of animals used for the test. Thus each animal served as its own control. The area of each spot was entered as an individual result in the calculation of the average value and standard deviation.

Four concentrations of DMSO were tested: 0.2, 2, 5 and 50%. Since it was previously observed that an association of a factor enhancing tissue permeability with an inhibitor potentiated the effect of the inhibitor(8,9), DMSO at a concentration of 0.2% was tested for its permeability effect in combination with diethyldithiocarbamate of sodium (DEDTC) at 2% concentration. DEDTC was previously found markedly to inhibit connective tissue permeability(9,10). Hence, it was of interest to test the effect of DMSO on permeability in the presence of this strong inhibitor.

The results show (Table I) that at concentrations of 0.2, 2 and 5% DMSO did not enhance the permeability as defined by this method. In fact, a slight inhibition of skin "permeability" (about 6 to 7%) was observed at a concentration of 0.2% DMSO. It behaved like other substances which affect permeability at low concentrations but which were inactive at higher concentrations(8,9). However, at a concentration of 50%, DMSO enhanced markedly the permeability when tested by the diffusion method. When DMSO was used with DEDTC, the inhibitory effect was slightly enhanced as compared to the effect obtained with DMSO alone (Table I).

A striking parallelism was earlier observed between the *in vitro* oxido-reductive depolymerization of hyaluronic acid (tested by rate of reduction in viscosity) and the *in vivo*

* This work was supported by the U.S.P.H.S. Nat. Inst. for Arthritis and Metab. Dis., Grant A-4619. The authors wish to thank Mrs. Anne O'Brien-Botticelli for valuable technical assistance.

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TABLE I. Effects of Dimethylsulfoxide (DMSO) on Dermal Diffusion of Evans Blue in Living Rats.

Conc of DMSO	Average spread area (mm²) for:						Permeability change (%) at:			
	Control* 30 min	DMSO†	Control 60 min	DMSO	Control 120 min	DMSO	Control 180 min	DMSO	30 min	60 min	120 min	180 min
0.2‡	120 ± 20	108 ± 20	131 ± 21	127 ± 25	151 ± 19	143 ± 25	161 ± 22	150 ± 26	−10	− 3	− 5	− 7
2	124 ± 13	125 ± 14	144 ± 14	144 ± 12	177 ± 19	171 ± 16	199 ± 23	194 ± 27	+ 1	± 0	− 3	− 3
5	125 ± 19	124 ± 20	142 ± 16	141 ± 19	160 ± 19	163 ± 17	182 ± 18	181 ± 20	− 1	− 1	+ 2	− 1
50	123 ± 17	156 ± 14	129 ± 12	163 ± 15	147 ± 18	185 ± 16	157 ± 14	209 ± 15	+27§	+26§	+26§	+33§
	DC + DMSO†		DC + DMSO		DC + DMSO		DC + DMSO					
0.2	124 ± 12	127 ± 16	130 ± 13	134 ± 11	145 ± 12	151 ± 14	151 ± 11	164 ± 23	+ 2	+ 3	+ 4	+ 9

* 400 mg Evans blue dissolved in 100 ml saline and adjusted to pH 7.3.
† Dissolved in saline containing Evans blue (4 mg/ml) and adjusted to pH 7.3.
‡ Each experiment was performed on 6 rats, 9 to 10 mo old, weighing 380 to 400 g. Three injections (0.05 ml) were made for control and 3 for test substance on each animal.
§ $P < 0.01$.
DC = Diethyldithiocarbamate of sodium at 2%.

changes of connective tissue permeability. Usually substances which depolymerized hyaluronic acid *in vitro* enhanced skin "permeability" *in vivo,* but compounds which acted as inhibitors of depolymerization inhibited "permeability"(8,9). Hence, the "permeability" enhancing effect of agents active at physiological concentrations and their depolymerizing effect on hyaluronic acid, appear to be two aspects of the same phenomenon(8,11). It was postulated that the inhibitory effect might be due to the scavenging of free radicals involved in such reactions(12). Such a mechanism could be the basis of the anti-inflammatory action of most of the substances which inhibit connective tissue permeability (9).

DMSO is also known to act *in vitro* as an inhibitor of the oxido-reductive depolymerization of hyaluronic acid(13). This effect of DMSO appears to be parallel with its slight inhibition of permeability observed at 0.2% concentration in the present study. The marked diffusion effect of this compound at the unphysiological concentration of 50% (Table I) was presumably due to tissue damage. Such an inflammatory reaction involving the circulatory system seems to occur when DMSO is used to promote the absorption of topically applied pharmaceutical compounds. It is unlikely, on the basis of the *in vitro* mentioned studies(13), that the dermal barrier is affected. A similar effect of tissue damage was previously observed for hydrogen peroxide at the concentration of 60%(10). At the concentration of 50%, DMSO was reported to act as an acute toxic agent when administered by oral route in combination with various pharmacological compounds(14).

Summary. Dimethylsulfoxide (DMSO) did not enhance tissue permeability at concentrations of 0.2, 2 and 5% when tested by the spreading method. In contrast, a slight inhibition of permeability (about 6%) was observed at a concentration of 0.2%. Its enhanced effect on skin "permeability" at a concentration of 50% was apparently due to tissue damage.

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Received February 10, 1966. P.S.E.B.M., 1966, v122.

Effects of d-Amphetamine on Plasma and Tissue Electrolyte Concentrations of Aggregated and of Hyperthyroid Mice.* (31116)

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Many actions of amphetamine can be influenced by environmental stresses such as forced exercise(1), aggregation or crowding (2), and nonaversive electric grid shock(3). The potentiating effects of thyroid hormones on certain of the actions of amphetamine have also been described(4). The toxicity of amphetamine is markedly enhanced in both aggregated and hyperthyroid mice; similar biochemical changes precede or accompany the death of mice in both groups. For example, in aggregated and in hyperthyroid mice, but not in control mice, amphetamine produces a pronounced depletion of tissue glycogen, hypoglycemia and depletion of tissue norepinephrine stores. The importance of these chemical changes in relation to the enhanced toxicity of amphetamine has been discussed previously(5-8).

Alterations in carbohydrate metabolism are accompanied by electrolyte shifts(9). Catecholamines are also known to affect changes in electrolyte metabolism(10). Since alterations in the tissue contents of both glycogen and catecholamines accompany stress-enhanced amphetamine toxicity, it was realized that certain manifestations of this toxicity might result in part from tissue compartmental shifts of electrolytes. Accordingly, the effects of amphetamine on tissue electrolyte and water content were examined in control, aggregated and hyperthyroid mice.

Methods. Male albino mice (Charles River Mouse Farms) weighing 24-30 g were used throughout this study. Mice used in the aggregation studies were housed in groups of 24-30 until the day of the experiment. The experiments consisted of injecting mice with saline or d-amphetamine sulfate (10 mg/kg) and placing them, 4 per cage, into small wire mesh cages measuring 9 × 9 × 9 cm. Mice used in the 'hyperthyroid' study were injected with 1-triiodothyronine (0.5 mg/kg) on three consecutive days. During this time they were housed in community cages in groups of 24-30. On the fourth day hyperthyroid mice were injected with saline or d-amphetamine sulfate (10 mg/kg) and placed individually into cages similar to those described for the aggregation studies. Four hours before the start of each experiment food but not water was removed from the animal cages. The intra-

* Supported by Grant AM 06275 from Nat. Inst. of Arthritis and Metab. Dis.