Effects of Intravenous Dimethylsulfoxide (DMSO) and Tween 80 on Atherosclerosis in Autosexing Pigeons[1] (36810)

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(Introduced by E. S. Vesell)

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In 1951, Kellner, Correll and Lodd (1) showed that the severity of atherosclerosis in rabbits on a high-cholesterol diet could be reduced by the intravenous administration of surface-active agents such as Tween 80 (20%). In 1967, Herzmann (2) demonstrated, in young cockerels being fed a diet high in cholesterol, that the addition of a 1% solution of dimethylsulfoxide (DMSO) to the drinking water could cause a 50% reduction in serum cholesterol levels without having any effect on aortic cholesterol. DMSO has some properties of surface-active agents (3), and increases the permeability of biologic membranes (4) to many constituents of the blood, including steroids (5). It seemed logical, therefore, to follow up Herzmann's experiments by determining the actual effects of DMSO on atheromatous plaques in the aorta and coronary arteries, as well as on serum levels of cholesterol and triglycerides. Pigeons were chosen as the experimental animal because much work has been done with atherosclerosis in this species (6–9). Tween 80 was employed as a control for the surface-active effects of DMSO.

Materials and Methods. Forty-eight autosexing pigeons (44 males, 4 females) 6 to 7 yr old were obtained from the Palmetto Pigeon Farm in Sumter, SC. Dimethylsulfoxide (DMSO, 90%) was obtained from E. R. Squibb & Sons in New Brunswick, NJ; Tween 80, from the Rugar Chemical Co., Inc., of Irvington, NJ. The high-cholesterol diet consisted of Purina Pigeon Pellets coated with 2% cholesterol and 4% lard; for the normal diet, the coating was omitted.

The experimental design is outlined in Table I. The birds were separated into groups of 6, each group being given injections of the indicated substance (2.5 ml/kg) every Tuesday and Friday in weeks 4 through 10. All blood samples were taken from the wing veins after 20 hr of fasting. In the cholesterol-fed pigeons, sample 1 was taken at the beginning of the experiment; sample 2, after 3 wk of the high-cholesterol diets; samples 3, 4, and 5, after 3, 5 and 7 wk of diet and injections. In the control pigeons (fed a normal diet), sample 1 was omitted and sample 2 represents the base line value. The entire experiment lasted 10 wk.

At the end of week 10, the birds were killed. The aortas were removed and quick-frozen to —70°, and the coronary arteries and hearts were placed in a 10% solution of neutral buffered formalin. The aortic content of total cholesterol, cholesterol esters, and free cholesterol was determined and three standard blocks, each containing tissue from the left ventricle and septum, were removed starting near the base of the heart. Frozen sections made from each of these blocks were stained with Sudan IV and hematoxylin.

The serum cholesterol and triglyceride determinations were made on a Technicon Auto Analyzer; the method of Levine and Zak (10) was used for cholesterol and the method of Kessler and Lederer (11) for triglycerides.

The aortas were homogenized individually in a mixture of chloroform and methanol (2:1) by means of a Thomas tissue grinder,

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TABLE I. Design of Experiment.

Treatment:	Normal saline		Tween 80		DMSO		DMSO/Tween 80	
Dietary cholesterol:	Normal	High	Normal	High	Normal	High	Normal	High
No. of birds:	6	6	6	6	6	6	6	6
Concn of drug (%)								
Weeks 4, 5, 6	—		20		1.8		1.8 + 20	
Weeks 7 & 8	—		30		3.6		3.6 + 30	
Weeks 9 & 10	—		30		7.2		7.2 + 30	

the homogenates being allowed to stand in the chloroform–methanol mixture for 4 hr in order to extract the lipids. The mixture was then centrifuged and the supernatant was collected and divided into 2 equal parts. The total cholesterol in one part was determined by the method of Schonheimer and Sperry and quantitated with Liebermann-Burchard reaction.

Cholesterol esters in the other part were determined by adding 2 ml of digitonin (0.5%) and evaporating the supernatant to dryness, then adding 2 ml of acetone and again evaporating the fluid. The esters were then quantitated by the Liebermann-Burchard reaction. Free cholesterol was determined by subtracting the value for cholesterol esters from that for the total cholesterol.

Results. Serum cholesterol levels (Table II) for birds on normal diets varied only slightly, the means ranging from 217 mg/100 ml (after 3 wk of DMSO and Tween 80) to 292 mg/100 ml (after 3 wk of Tween 80 alone). Those birds fed a high-cholesterol diet had a significant rise ($p < .05$) in all four treatment groups, the means of blood sample 2 ranging from about 1150 to 1250 mg/100 ml. No significant changes were observed following specific drug treatment in any group.

Serum triglyceride levels (Table III) did not appear to be significantly affected by diet or treatment, although three samples in the groups being fed a normal diet (blood sample 4 in the Tween 80 group and samples 4 and 5 in the DMSO/Tween 80 group) were significantly lower than those of saline-treated birds on the same diet. The levels in birds fed a normal diet ranged between 39 and 100

TABLE II. Serum Cholesterol Levels (mg/100 ml).[e]

Treatment	Dietary cholesterol	Blood sample 1	2	3	4	5
Normal saline	Normal		250 ± 7	264 ± 22	252 ± 9	245 ± 21
	High	299 ± 8	1165 ± 185[b]	1117 ± 192[b]	1073 ± 227[c]	846 ± 167[c]
Tween 80	Normal		248 ± 14[a]	292 ± 21[a]	288 ± 18[a]	270 ± 16[a]
	High	278 ± 7	1152 ± 123[ba]	1103 ± 140[ba]	1017 ± 108[ba]	1078 ± 140[ba]
DMSO	Normal		263 ± 15[a]	267 ± 13[a]	276 ± 10[a]	253 ± 8[a]
	High	277 ± 15	1240 ± 259[ca]	1534 ± 469[da]	1384 ± 451[da]	1150 ± 443[da]
DMSO and Tween 80	Normal		234 ± 8[a]	217 ± 9[a]	249 ± 11[a]	227 ± 13[a]
	High	307 ± 12	1244 ± 242[ca]	1276 ± 249[ba]	1240 ± 227[ba]	1306 ± 358[ca]

[a] No significance compared to groups on the same diet which were treated with saline ($p > .05$).

[b] Significance of differences when compared to groups on a normal diet which were given the same treatment: $p < .001$; [c] $p < .01$; [d] $p < .05$.

[e] Mean ± standard error.

TABLE III. Serum Triglyceride Levels (mg/100 ml).[e]

Treatment	Dietary cholesterol	Blood sample 1	2	3	4	5
Normal saline	Normal		159 ± 13	72 ± 10	86 ± 9	66 ± 9
	High	92 ± 5	144 ± 14[cd]	91 ± 16[c]	80 ± 16[c]	53 ± 6[c]
Tween 80	Normal		330 ± 173	49 ± 12	49 ± 9[b]	50 ± 7
	High	79 ± 6	142 ± 16[cd]	59 ± 11[c]	53 ± 11[c]	47 ± 8[c]
DMSO	Normal		285 ± 128	110 ± 17	89 ± 7	90 ± 10
	High	99 ± 3	154 ± 27[cd]	121 ± 27[c]	122 ± 43[c]	87 ± 23[c]
DMSO and Tween 80	Normal		205 ± 41	55 ± 12	45 ± 5[a]	39 ± 4[b]
	High	110 ± 18	138 ± 15[cd]	51 ± 9[c]	51 ± 9[c]	47 ± 10[c]

[a] Significance of differences when compared to groups on the same diet which were treated with saline: $p < .01$; [b] $p < .05$.

[c] No significance compared to groups on a normal diet which were given the same treatment ($p > .05$).

[d] See footnote 2 in text.

[e] Mean ± standard error.

mg/100 ml (excluding sample 2);[2] those on high-cholesterol diets ranged from 47 to 154 mg/100 ml.

The high aortic cholesterol levels observed in all groups (Table IV) are probably attributable to the age of the pigeons. Only in the DMSO group did the aorta show significantly higher levels of total cholesterol and cholesterol esters in the pigeons on a high-cholesterol diet than in those on a normal diet. No drug treatment had a significant effect in any group when the values are compared to those for saline controls.

The different treatments and dietary regimens appeared to have no significant effect on the size of the plaques in the coronary arteries. All hearts appeared grossly normal, and microscopic sections of the coronary arteries showed only mild to moderate atherosclerosis. The largest plaque observed among all the pigeons (Fig. 1) was found in a bird which had been fed a high-cholesterol diet and treated with DMSO.

[2] Each group of pigeons fed a normal diet included one female; and for reasons that we cannot explain the triglyceride level in sample 2 was abnormally high in each female. Since this sample was obtained prior to any drug therapy, it was disregarded. In subsequent samples the female pigeons had a triglyceride level in the same range as the males.

Discussion. This experiment using 6- to 7-yr-old autosexing pigeons failed to show that DMSO and Tween 80, alone or in combination, in the dosages employed had any consistent significant effect on either the prevention or the reduction of atherosclerosis, as judged by serum levels of triglyceride and cholesterol, aortic cholesterol content, and histological findings in the coronary arteries. It is apparent, however, that a high cholesterol diet does raise serum cholesterol levels significantly within a short period of time. Thereafter a plateau is reached—possibly because the lipoprotein carriers become saturated. The high-cholesterol diet did not have any consistent effect on the serum triglyceride levels.

The data from this experiment suggest that intravenous DMSO in the dosage employed has no effect on serum cholesterol levels. The apparent contradiction between this finding and the results of Herzmann (2) has two possible explanations: (a) DMSO does not lower serum cholesterol levels in this breed of pigeon as it does in young cockerels, or (b) serum levels of DMSO were not sufficiently high and/or sustained to achieve a reduction in cholesterol levels. Herzmann placed the DMSO in the drinking water, so that the young cockerel had a more constant level of the drug; it is possible that giving DMSO

TABLE IV. Aortic Cholesterol (mg/g Aorta).[d]

Treatment	Dietary cholesterol	Total cholesterol	Cholesterol esters	Free cholesterol
Normal saline	Normal	21 ± 2	9 ± 1	12 ± 1
	High	33 ± 6	15 ± 3	19 ± 2
Tween 80	Normal	37 ± 10[a]	20 ± 7[a]	17 ± 4[a]
	High	35 ± 3[a]	20 ± 4[a]	15 ± 5[a]
DMSO	Normal	23 ± 2[a]	9 ± 1[a]	14 ± 2[a]
	High	33 ± 2[ba]	18 ± 2[ca]	14 ± 3[a]
DMSO and Tween 80	Normal	26 ± 3[a]	10 ± 2[a]	16 ± 3[a]
	High	29 ± 3[a]	20 ± 3[a]	9 ± 3[a]

[a] No significance compared to groups on the same diet which were treated with saline ($p > .05$).

[b] Significance of differences when compared to groups on a normal diet which were given the same treatment: $p < .01$; [c] $p < .05$.

[d] Mean ± standard error.

intravenously twice a week, as in the present study, does not maintain an effective level in the blood and tissues. DMSO is metabolized rather rapidly, 50% of an oral or intravenous dose being recovered in the urine of guinea pigs and beagles within 24 to 36 hr (12); the peak levels in tissues seem to be reached about 2 to 6 hr after oral or intravenous administration.

Although this study confirmed Kellner, Correll and Lodd's findings (1) that Tween 80 has no statistically significant effect on serum cholesterol and triglyceride levels, there was a trend for the Tween 80 and the DMSO/Tween 80 groups on both diets to have lower serum triglyceride levels than ei ther the saline-treated groups or the DMSO groups. The fact that there was no major difference between blood samples 3, 4, and 5 in the Tween 80 groups indicates that, if Tween 80 does have an effect on serum triglycerides, this effect quickly reaches its peak and is not altered by changes in concentration of the drug. The combination of DMSO and Tween 80 seemed to be no more effective than Tween 80 alone.

Random blood samples from each group were tested for serum lipoprotein levels, but no consistent results were obtained. Total lipoproteins and the ratio of beta to alpha lipoproteins tended to rise with the high-cholesterol diets, but no treatment had a sig-

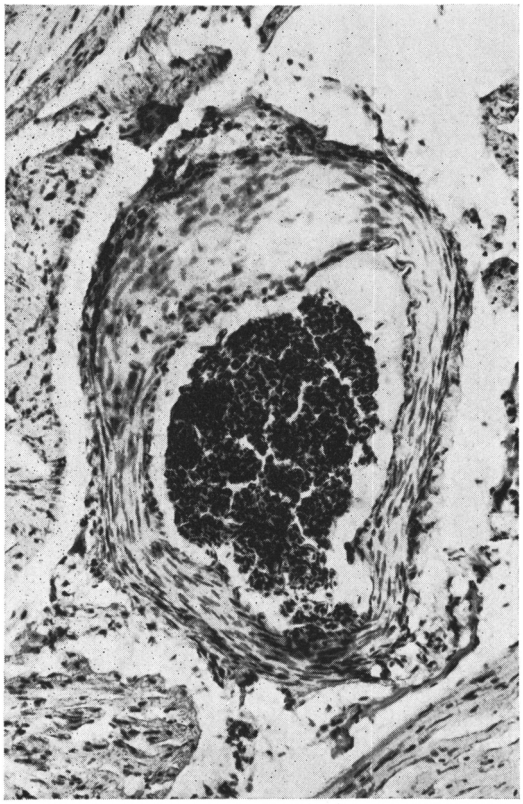

FIG. 1. Cross section through coronary artery showing the most extensive plaque observed in this study (Sudan IV and hematoxylin, 150×). The internal elastic lamina has been disrupted by the atheromatous plaque.

nificant effect on these levels.

Since no one has proven the absolute relationship between atherosclerosis and serum levels of cholesterol or triylgcerides, the more interpretable data relevant to atherosclerosis were the aortic cholesterol levels and the plaques in the coronary arteries. Mansfield and Howard (13) demonstrated at autopsy that isolated perfusion of the aortas and iliac arteries of human beings with a 15 to 25% solution of DMSO removed some total lipids, of which cholesterol was a minor component. If fibrosis of vessels is nothing but an inflammatory response, as McGill, Geer and Strong (14) have suggested, the anti-inflammatory effects of DMSO (15) should reduce or prevent fibroblastic proliferation (16). In our experiment, however, DMSO and Tween 80, alone and in combination, failed to reduce aortic cholesterol levels or the incidence of coronary artery plaques. Since all the groups of pigeons in our study had high levels of cholesterol in the aorta, regardless of diet, it is possible that experiments using youger birds might exhibit some prophylactic effect which this experiment could not.

Although this experiment failed to demonstrate that DMSO or Tween 80 is effective in the reduction or prevention of atherosclerotic plaques in autosexing pigeons, it does not rule out this possibility. Many of the apparent differences were not statistically significant because of the small number of animals in each group. DMSO, in particular, has many properties that could make it useful in the treatment of atherosclerosis. Before the true potential of either of these agents can be accurately evaluated, it will be necessary to carry out additional experiments over longer periods of time, using other experimental models, a greater number of animals in each group, different methods of administration, and increased concentrations of the drugs.

Summary. The effects of intravenously administered DMSO and Tween 80 were studied in autosexing pigeons on normal and high-cholesterol diets. These drugs, alone or in combination, did not have a significant effect in the dosage employed on aortic cholesterol content, serum cholesterol and triglyceride levels, or incidence of coronary artery atherosclerosis. Although previous reports have shown that these surface-active drugs can reduce the severity of atherosclerosis (Tween 80) or serum cholesterol levels (DMSO) in other species, these conclusions in autosexing pigeons were not supported under the conditions used in this study.

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