EFFECT OF DOISYNOLIC ACID ON EXPERIMENTAL ATHEROMA IN THE COCKEREL

BY

R. S. F. CAMPBELL, T. D. V. LAWRIE, J. C. MACLAURIN, AND R. PIRRIE

From the Department of Veterinary Pathology, University of Glasgow, and the Department of Cardiology, Glasgow Royal Infirmary

Received October 14, 1958

The striking difference in the incidence of atherosclerosis in men and women has drawn attention to the possible protective value of oestrogens (Katz and Stamler, 1953). A good degree of protection against coronary atheroma in cholesterol-fed chickens can be given by endogenous oestrogen in physiological proportions (Pick *et al.*, 1953) as well as by administered oestradiol benzoate (Pick *et al.*, 1952 *a* and *b*). In contrast, oestradiol benzoate is ineffectual against aortic lesions. Mixed conjugated oestrogens (premarin) and parenteral diethyl-stilboestrol resemble oestradiol in their ability to inhibit coronary lesions (Stamler *et al.*, 1953).

The feminizing effect of oestrogens is an obvious deterrent to their use in the treatment of human atherosclerosis and attempts have been made to obviate this difficulty by using structurally-related substances of low potency. Stamler *et al.* reported a lack of success. Recently, Cook *et al.* (1958) described lipid-depressing effects with a newer steroid of weak oestrogenicity. The work described here concerns methoxy bisdehydro-doisylnolic acid-7-benzoate (MDDA).

MATERIALS AND METHODS

The experiment was similar to that devised by Pick *et al.* (1952 *b*). Seventy-two Golden Legbar $\times$ Light Sussex cockerels, a breed previously tested for susceptibility to experimental atheroma, were obtained at the age of eight weeks from the West of Scotland Agricultural College Poultry School. After placing in individual cages, the birds were fed *ad libitum* on the particular diet for eight weeks, with the exception of one group killed at five weeks to prove the establishment of lesions prior to treatment. The daily food intake was estimated once a week from the fifth week onwards (Table I) and the birds were weighed on the first, fifth, and eighth weeks (Fig. 1).

The normal and treated control groups (1 and 2) received commercial chick mash, others being given chick mash into which was incorporated 2 per cent cholesterol and 5 per cent cottonseed oil by means of a food mixer. The experiment included the following groups of twelve birds.

- (1) Normal diet only.
- (2) Normal diet with 5 mg. MDDA intramuscularly daily, during 6th to 8th weeks inclusive.
- (3) Cholesterol diet only.
- (4) Cholesterol diet with 1 mg. MDDA intramuscularly daily during 6th to 8th weeks inclusive.
- (5) Cholesterol diet with 5 mg. MDDA intramuscularly daily, during 6th to 8th weeks inclusive.
- (6) Cholesterol diet: killed after five weeks.

At autopsy the heart, brachiocephalic arteries, aorta, and first part of the iliac arteries were examined microscopically for atheroma, and any lesions classified in slight, moderate, and severe grades. *Slight* implies focal lesions up to about 2 mm. in diameter, white or cream-coloured, accompanied by minimal thickening of the intima. *Moderate* implies plaques up to about 5 mm. in diameter, cream to ivory-yellow in colour, with distinct intimal thickening and linear ("tree-trunk") ridging. *Severe* implies larger plaques of cream to yellow colour merged into diffuse atheroma, with distinct thickening and ridging or distortion of the wall.

After fixation in 10 per cent formol saline, three transverse slices were taken from the broadest part of the heart. One frozen section was prepared from each slice, stained by Sudan IV and hæmalum, and a microscopical count of atheromatous coronary arteries carried out. Criteria for the presence of atheroma were intimal thickening or plaque formation. Sudanophilia of intima or media without structural change was regarded as a negative finding.

TABLE I
MEAN FOOD INTAKE OF EACH GROUP

Weeks	Food intake g. per day			
	5th	6th	7th	8th
Group 1 ..	99	118	139	135
Group 2 ..	94	120	121	136
Group 3 ..	98	106	115	126
Group 4 ..	90	116	132	127
Group 5 ..	98	108	122	130

FIG. 1.—Mean body-weight of each group, at the start, after five weeks, and after eight weeks.

RESULTS

Sexual Inhibition. Birds receiving MDDA showed a varying degree of inhibition of sexual development, as judged by gross examination. It was seen that in those on a regime of 5 mg. the testes were small and the comb and wattles undersized as compared with the normal group. Of the birds receiving 1 mg. MDDA daily, inhibition occurred in eight; the remaining four showed testes, comb, and wattles of normal size.

Atheroma. (a) *Aorta.* The incidence of aortic lesions is presented in Table II. The cholesterol-induced lesions were more severe in the thoracic than in the abdominal aorta as noted by Dauber and Katz (1942). Indeed the abdominal lesions were absent from a majority of birds. The incidence of aortic lesions, however, was not affected by MDDA but was similar in the three groups receiving cholesterol.

TABLE II
THE INCIDENCE OF AORTIC ATHEROMA

Vessel examined	Degree of atheroma	Group				
		1	2	3	4	5
Thoracic aorta	Severe	0	0	6	3	6
	Moderate	0	0	6	7	6
	Slight	0	0	0	2	0
	Absent	12	12	0	0	0
Abdominal aorta	Severe	0	0	0	0	0
	Moderate	0	0	2	2	1
	Slight	1	0	2	3	2
	Absent	11	12	8	7	9

(b) *Coronary arteries.* Analysis of variance shows no significant difference in the incidence of atheroma in the birds fed with cholesterol. The results of the arterial counts are shown in Table III.

TABLE III
PERCENTAGE INCIDENCE OF CORONARY ARTERY LESIONS

	Mean	Range
Group 3 ..	34.7	8.3-48.9
Group 4 ..	22.8	4.0-36.2
Group 5 ..	34.2	18.0-52.7

DISCUSSION

It appears that MDDA in daily doses of 1 or 5 mg. for three weeks has no effect on the incidence of aortic or coronary atheroma in chickens produced by a 2 per cent cholesterol diet over an eight-week period. Further, there was clear evidence that the drug, especially in the higher dosage, exerted considerable oestrogenic activity.

Pick *et al.* (1952 *b*) demonstrated that in the cockerel oestradiol benzoate caused regression of established coronary lesions when given over the last five weeks of a thirteen-week period of cholesterol feeding. If this effect of oestradiol benzoate is a parallel function of its oestrogenicity, MDDA might have been expected to produce a similar trend, in view of the oestrogenic activity as judged by sexual inhibition in the present experiment. Its failure to do so suggests that oestrogenicity is not necessarily a measure of the effect against atheroma. A similar tentative conclusion on lipid effects and oestrogenic properties was reached in respect of the steroid tested by Cook *et al.* (1958).

SUMMARY

The effects of methoxy-bisdehydro-doisylnolic acid-7-benzoate on atheroma induced by cholesterol in cockerels is described. When given in daily doses of 1 mg. and 5 mg. over the latter three weeks of an eight-week feeding experiment it had no significant effect on the incidence of aortic or coronary artery atheroma. The significance of these findings is briefly discussed.

This research programme has been supported by a grant from the Scottish Hospital Endowments Research Trust. We are indebted to Dr. J. H. Wright and Professor J. W. Emslie for their guidance and helpful criticism, to Dr. Hewett of Organon Laboratories Ltd. for his kindness in supplying the drug, and to Dr. R. A. Robb for his aid with the statistical analysis of results.

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