DOES THE INGESTION OF ALCOHOL INFLUENCE THE DEVELOPMENT OF ARTERIOSCLEROSIS IN FOWLS?*

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Since Aschoff stated that a lifelong indulgence in Rhenish wine and severe atheroma are not found together (1), the view has been widely held that chronic alcoholics show less severe arteriosclerosis than do moderate drinkers of alcohol (2, 3). It has been suggested that alcohol may act to increase the solubility of cholesterol in plasma (3), but the low lethal concentration of blood alcohol would argue against such a view (4). It has also been pointed out that the less severe arteriosclerosis presumably occurring among alcoholics may result from a changed food consumption (4). So far it has not been possible to resolve the question as to whether ingested alcohol *per se* can protect against the development of arteriosclerosis.

It seemed to us that a solution to this problem might be obtained by studying the effects of alcohol in animals in which all caloric factors, including alcohol intake, were controlled. A single attempt has thus far been made in this direction (5), but unfortunately the important variable of food intake was not controlled, and consequently the conclusions drawn are open to question. In view of this paucity of experimental information about a subject of such importance, it was decided to initiate a long term experiment in fowls that would include not only the naturally occurring type of arteriosclerosis but also the type induced experimentally.

EXPERIMENTAL

A total of 224 white Leghorn cockerels, 3 months of age, obtained from the Poultry Division, was used in three separate experiments. The first two experiments were designed to study the effects of orally administered ethyl alcohol on naturally occurring and on estrogen-induced (stilbestrol) arterial disease. Various concentrations of ethyl alcohol were fed to chickens, and it was found that a 15 per cent solution was well tolerated. The administration of a 20 per cent solution resulted in a sharp reduction in food and alcohol intake. In the third experiment, white wine was substituted for ethyl alcohol, and the same experimental regimen was followed. The design of each experiment is shown in Table I.

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Experiment 1.—104 cockerels were divided into 4 groups (A to D) of 26 each, and each bird was assigned a number, as follows: 1 A to 26 A, 1 B to 26 B, 1 C to 26 C, and 1 D to 26 D.

Each bird of group A was injected subcutaneously, every 2 months, with two 15 mg. pellets containing, in all, 24 mg. of diethylstilbestrol. The birds of this group were fed Purina broiler chow *ad libitum*, and allowed access, also *ad libitum*, to a 15 per cent ethyl alcohol solution for drinking purposes. The food and fluid intake of each bird was measured at 48 hour intervals.

TABLE I

Design of Experiments

Experiment	Group	No. of birds per group	Stilbestrol injected	Broiler chow	Alcohol administration		Glucose administration	
					Concentration	Amount	Concentration	Amount
1	A	26	+	Fed *ad libitum*	15 per cent ethyl alcohol	*Ad libitum*		
	B	26	None	Pair-fed with A	15 per cent ethyl alcohol	Paired with A		
	C	26	"	" " "	None	None	24.4 per cent solution	Isocaloric with alcohol intake of A
	D	26	+	" " "	"	"	24.4 per cent solution	Isocaloric with alcohol intake of A
2	A	12	+	Fed *ad libitum*	15 per cent ethyl alcohol	*Ad libitum*		
	B	12	None	" " "	15 per cent ethyl alcohol	" "		
	C	12	"	Pair-fed with B	None	None	24.4 per cent solution	Isocaloric with alcohol intake of B
	D	12	+	" " A	"	"	24.4 per cent solution	Isocaloric with alcohol intake of A
3	WA	18	+	Fed *ad libitum*	White wine*	*Ad libitum*		
	WB	18	None	" " "	" "	" "		
	WC	18	"	Pair-fed with B	None	None	19.1 per cent solution	Isocaloric with wine intake of B
	WD	18	+	" " A	"	"	19.1 per cent solution	Isocaloric with wine intake of A

* 12.1 per cent alcohol by volume.

The birds of group B were fed the Purina broiler chow and received the 15 per cent ethyl alcohol solution for drinking purposes. Each bird of this group was pair-treated with a corresponding bird of group A. Thus, the amounts of food and alcohol allowed bird 1 B during any given 48 hour period were determined by the amounts ingested by bird 1 A during the preceding 48 hours. In this way, the caloric intake of each bird of group B was made equal to that of a bird with a corresponding number in group A.

The 26 birds of group C served as controls, and each bird of this group received, during any given 48 hour period, (*a*) an amount of Purina broiler chow equal to that ingested by a corresponding bird of group A during the preceding 48 hours; and (*b*) a volume of a 24.4 per cent glucose solution equal to that of the 15 per cent alcohol solution ingested by the corresponding bird of group A. (1 cc. of this glucose solution has the same calorific value as 1 cc. of the 15 per cent alcohol solution.) In this way the fluid as well as the caloric intake of each bird of group C was made equal to that of the bird with a corresponding number in groups A and B.

The birds of group D were treated with diethystilbestrol exactly as described for group A. Their food intake was determined by the food intake of the birds of group A. Each bird of group D received, for drinking purposes, an amount of 24.4 per cent glucose solution isocaloric with the alcohol ingested by a corresponding bird of group A.

Experiment 2.—In this experiment the birds of two groups, A and B, were allowed access, *ad libitum*, to the broiler chow and the 15 per cent alcohol solution. Each bird of group C was pair-fed with a corresponding bird of group B, and each bird of group D was pair-fed with a corresponding bird of group A. All birds of groups C and D received the 24.4 per cent glucose solution for drinking purposes. Each bird of group C was allowed an amount of this glucose solution isocaloric with that of the alcohol ingested during the previous 48 hours by a corresponding bird of group B. In the case of group D, the amount of glucose solution ingested by a given bird was made isocaloric with that of the alcohol ingested by a corresponding member of group A.

Experiment 3.—The design of this experiment was similar to that of Experiment 2 except that (*a*) wine,[1] which contained 12.1 per cent ethyl alcohol by volume, was substituted for the 15 per cent ethyl alcohol solution, and (*b*) the concentration of the glucose solution was reduced to 19.1 per cent.

Plasma Cholesterol.—Plasma cholesterol was determined twice on each bird before the start of the experimental regimen and then at monthly or bimonthly intervals during the remainder of the experimental period. Total plasma cholesterol was determined by a modification of the method of Sackett (6).

Postmortem Examination of Birds.—When a bird of Experiment 1 died or was sacrificed, the remaining three birds in its group were sacrificed at the same time. In experiments 2 and 3, if a bird died, only its pair-fed partner was sacrificed at that time. At postmortem examination, all major viscera were weighed, and sections of them were fixed in neutral formalin. The thoracic and abdominal aortas were excised, and the gross degree of arteriosclerosis was graded as follows:—

0, no visible changes in the gross.
0.5, extensive intimal yellow coloring lacking elevation.
1, plaques less than 3 mm.^{2} in total area, white to cream colored.
2, plaques with a total area greater than 3 mm.^{2} and cream to light yellow in color.
3, numerous plaques with a total area greater than 3 mm.^{2}, with a definitely yellow color.
4, severe arteriosclerosis; elevated lesions yellow and calcified.

Frozen sections were prepared from the midportion of the arch of the thoracic aorta and from the abdominal aorta approximately 1 cm. above the bifurcation. They were stained with Sudan IV and hematoxylin, and examined microscopically. The degree of intimal thickening (arteriosclerosis) and lipide infiltration of each vessel was graded separately from 0 to 4+.

RESULTS

The average daily food and alcohol intake per bird is recorded in Table II. An analysis of these data is given in the section dealing with statistical treatment.

The average values for the concentration of total plasma cholesterol for monthly or bimonthly intervals are given in Table III. Initially, the birds in Experiment 2 had the highest average total cholesterol values, while those

[1] The white table wine was kindly furnished by the Wine Advisory Board. It had the following composition (gm. per 100 cc.): total acid, 0.6; volatile acids, 0.06; and reducing sugars, 0.16.

TABLE II

Average Food and Alcohol Intake per Day per Bird

Experiment	Group	Intake and No. of remaining birds	Period (each 32 days) from start of experiment										
			1	2	3	4	5	6	7	8	9	10	11
1	A*	15 per cent alcohol, cc. per bird	110	93	81	87	97	84	103	108	102	124	92
		Diet, gm. per bird	70	60	47	49	52	47	55	68	70	75	66
		Remaining birds	26	24	21	15	12	10	6	4	3	3	3
2	A	15 per cent alcohol, cc. per bird	108	130	108	97	98	102	118	146	138	173	136
		Diet, gm. per bird	59	72	62	56	55	52	60	71	67	88	53
		Remaining birds	12	11	11	11	9	7	4	2	1	1	1
	B	15 per cent alcohol, cc. per bird	89	110	134	130	120	133	136	95	156	157	143
		Diet, gm. per bird	54	65	77	71	70	70	75	70	94	102	100
		Remaining birds	12	12	11	10	9	8	6	4	4	4	4
3	A	12.1 per cent wine, cc. per bird	93	107	113	109	104	97	110	140	137	134	121
		Diet, gm. per bird	51	56	52	47	48	43	45	60	63	69	71
		Remaining birds	18	18	17	16	12	9	8	5	5	5	4
	B	12.1 per cent wine, cc. per bird	93	103	107	116	121	122	126	114	125	121	128
		Diet, gm. per bird	54	64	64	59	66	62	60	59	66	66	65
		Remaining birds	18	17	17	17	17	17	15	14	13	12	9

* Birds of groups B, C, and D in Experiment 1 were pair-fed with A birds (see text).

TABLE III

Average Total Plasma Cholesterol of Birds

All values are expressed as mg. per 100 cc. of plasma

Experiment	Group	Treatment	Interval of experiment in mos.												
			0	1	2	3	4	5	6	7	8	9	10	11	12
1	C (birds 1–26)	Spontaneous, no alcohol	102	138	139	148	177	211	170	141	160	131	105	82	105
		No. of plasma determinations	34	20	23	18	9	10	4	7	1	3	2	1	3
	B (birds 1–26)	Spontaneous + alcohol	96	131	133	155	139	162	169	158	167	148	82	74	86
		No. of plasma determinations	39	19	22	19	8	12	7	7	1	3	2	2	3
	D (birds 1–26)	Stilbestrol, no alcohol	103	248	406	942	775	843	943	551	1100	449	941	345	720
		No. of plasma determinations	42	20	24	13	6	11	7	7	1	3	1	1	3
	A (birds 1–26)	Stilbestrol + alcohol	103	289	495	900	824	1005	733	691	431	219	1029	467	1029
		No. of plasma determinations	39	19	23	12	8	12	6	9	1	4	2	2	2
2	C (birds 27–38)	Spontaneous, no alcohol	132	—	131	177	105	103	127	89	111	—	162	—	176
		No. of plasma determinations	10		10	1	9	3	4	3	5		4		5
	B (birds 27–38)	Spontaneous + alcohol	134	—	154	109	100	156	118	112	103	—	112	—	124
		No. of plasma determinations	12		11	2	10	1	6	2	5		4		5
	D (birds 27–38)	Stilbestrol, no alcohol	147	—	184	—	705	—	367	631	157	—	216	—	569
		No. of plasma determinations	12		12		12		8	5	2		1		1
	A (birds 27–38)	Stilbesterol + alcohol	138	—	277	—	971	1105	382	1068	116	—	252	—	949
		No. of plasma determinations	11		11		12	2	5	2	2		1		1
3	C (birds W1–18)	Spontaneous, no wine	119	—	121	—	113	—	123	133	132	—	134	—	146
		No. of plasma determinations	18		17		16		14	1	9		12		8
	B (birds W1–18)	Spontaneous + wine	118	—	103	—	106	—	105	124	123	—	123	112	139
		No. of plasma determinations	18		17		17		12	2	12		11	3	7
	D (birds W1–18)	Stilbestrol, no wine	106	—	247	937	474	1085	704	1119	687	—	687	—	545
		No. of plasma determinations	18		18	1	15	4	8	1	3		5		4
	A (birds W1–18)	Stilbestrol + wine	107	—	241	—	728	—	880	1262	532	—	384	—	368
		No. of plasma determinations	18		17		14		6	1	4		5		3

TABLE IV

Gross Grading of Aortic Lesions in Experiments 1 and 2 (Alcohol Series)

Bird No.	Thoracic lesions								Abdominal lesions							
	Group A	Group D	A–D	Ordered differences	Group B	Group C	B–C	Ordered differences	Group A	Group D	A–D	Ordered differences	Group B	Group C	B–C	Ordered differences
1	0	2	−2	−2	0	0	0	0	2	3	−1	−3	1	0	1	−2
2	0.5	0.5	0	−2	0	0	0	0	0	0.5	−0.5	−3	0.5	0	0.5	−1
3	1	3	−2	−1	0.5	0	0.5	0	4	1	3	−2	0.5	1	−0.5	−1
4	3	0	3	−1	0	0	0	0	0	0	0	−2	1	0	1	−1
5	1	2	−1	−1	0	0	0	0	0	1	−1	−2	0	0	0	−0.5
6	2	2	0	−1	0	0	0	0	4	4	0	−1	0	0	0	0
7	0.5	0.5	0	0	0	0	0	0	0	3	−3	−1	0	1	−1	0
8	0.5	0.5	0	0	0	0	0	0	0	2	−2	−1	0	1	−1	0
9	0	0	0	0	0	0	0	0	0.5	1	−0.5	−1	0.5	0.5	0	0
10	2	2	0	0	0	0	0	0	3	4	−1	−0.5	0	0	0	0
11	0.5	0	0.5	0	0	0	0	0←	2	0	2	−0.5	2	0	2	0←
12	0.5	0.5	0	0←	0	0	0	0	0	2	−2	0←	0	0	0	0
13	0.5	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0
14	0.5	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0	0
15	0	0	0	0	0	0	0	0	0	2	−2	0	0	1	−1	0
16	0.5	0.5	0	0	0	0	0	0	0	3	−3	0	0	0	0	0
17	0	0	0	0	0.5	No autopsy	—	0	2	0	2	0	0	No autopsy	—	0
18	3	0	3	0	0	0	0	0	1	0	1	0	2	1	1	0
19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
20	0	1	−1	0	0	0	0	0	0	0	0	0	0	0	0	0
21	0	0	0	0	0	0	0	0	3	0	3	0	0	0	0	0
22	1	0	1	0	0	0	0	0	0	0	0	0	2	0	2	0
23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
24	1	0	1	0	0	0	0	0←	0	0	0	0	0	0	0	0←
25	4	0	4	0.5←	0	0	0	0	4	0	4	0←	0	0	0	0
26	0	0	0	0.5	0	0	0	0	0	0	0	0	0	0	0	0
27	3	0	3	0.5	0	0	0	0	0	1	−1	0	0	0	0	0
28	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
29	0	1	−1	1	0	0	0	0	0	0	0	0	0	0	0	0
30	1	0	1	1	0	0	0	0	4	0	4	0	0	0	0	0
31	1	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0.5
32	1	0	1	1	0	0	0	0	0	0	0	2	3	0	3	1
33	0	0	0	1	0	0	0	0	0	0	0	2	0	0	0	1
34	0	1	−1	2	0	0	0	0	3	0	3	3	0	2	−2	1
35	1	0	1	3	0	0	0	0	0	0	0	3	0	0	0	2
36	4	2	2	3	0	0	0	0	3	3	0	3	0	0	0	2
37	0	0	0	3	0	0	0	0.5	0	0	0	4	0	0	0	3
38	1	1	0	4	0	0	0		0	0	0	4	0	0	0	

in Experiment 1 had the lowest. However, between corresponding groups in a given experiment (B *vs.* C in Experiment 1, for example), the initial values were almost identical, and did not differ significantly from each other throughout the experimental period. In most of the groups there was a suggestion of a downward trend toward the end of the experiment, but this may have been a chance result, occurring because of the small number of determinations in the latter periods.

Two types of lesions were observed in the aortas of the birds. In the thoracic portion, lipide infiltration of the intima was followed by proliferation of

TABLE V

Gross Grading of Aortic Lesions in Experiment 3 (Wine Series)

Bird No.	Thoracic lesions								Abdominal lesions							
	Group A	Group D	A–D	Ordered differences	Group B	Group C	B–C	Ordered differences	Group A	Group D	A–D	Ordered differences	Group B	Group C	B–C	Ordered differences
W 1	2	2	0	−2	0	0	0	0	0	4	−4	−4	0	0	0	−2
2	2	0	2	−2	0	0	0	0	0	0	0	−3	2	0	2	0
3	0	0.5	−0.5	−1	0	0	0	0	0	2	−2	−2	0	0	0	0
4	0.5	0.5	0	−1	0	0	0	0	0	0	0	0	0	0	0	0
5	0.5	0	0.5	−0.5←	0	0	0	0	0	0	0	0←	0	0	0	0←
6	0	0	0	−0.5	0	0	0	0	0	0	0	0	0	0	0	0
7	2	0.5	1.5	−0.5	0	0	0	0	0	0	0	0	0	0	0	0
8	0	2	−2	0	0	0	0	0	0	0	0	0	0	2	−2	0
9	0	0.5	−0.5	0	0	0	0	0	0	0	0	0	0	0	0	0
10	2	3	−1	0	0	0	0	0	4	1	3	0	0	0	0	0
11	0.5	0.5	0	0	0	0	0	0	1	0	1	0	3	0	3	0
12	0	2	−2	0	0	0	0	0	0	0	0	0	0	0	0	0
13	2	0.5	1.5	0	0	0	0	0	4	2	2	0	2	0	2	0
14	2	3	−1	0.5←	0	0	0	0	3	0.5	2.5	1←	2	0	2	0←
15	3	0	3	1.5	0	0	0	0	3	0	3	2	0	0	0	2
16	0	0.5	−0.5	1.5	0	0	0	0	0	0	0	2.5	0	0	0	2
17	3	3	0	2	0	0	0	0	1	4	−3	3	0	0	0	2
18	0	0	0	3	0	0	0	0	0	0	0	3	0	0	0	3

intimal connective tissue cells with formation of intimal plaques. The lesions in the abdominal portion were characterized by the development of fibrous intimal plaques in which lipide material was deposited secondarily. These lesions differed in no way from those previously observed in male birds, normal and stilbestrol-injected (7, 8). The gross grading of lesions found in the thoracic and abdominal portions of the aorta are recorded in Tables IV and V.

Statistical Treatment of Data

For the purpose of statistical analysis, experiments 1 and 2 (see Table I) were grouped together, so that there were four groups of 38 birds each. These will be called the "alcohol series." Experiment 3, consisting of four groups of 18 birds each, called the "wine series," was considered separately. This divi-

sion gave two parallel sets of experiments, differing only in the number of birds used and in the substitution of wine for ethyl alcohol.

Groups A and D (Table I) then constituted one comparison on the effect of alcohol (or wine) in the stilbestrol-injected birds, and groups B and C constituted a separate comparison on the effects of alcohol (or wine) in the absence of any other treatment. For each bird in each series we made use of numerical scores describing two variables: (*a*) a gross grading of the thoracic aorta; and (*b*) a gross grading of the abdominal aorta (Tables IV and V). The microscopic gradings (degree of intimal thickening and lipide infiltration) agreed closely enough with the gross gradings so that they were not analyzed statistically.

If a substantial difference were observed between A and D, or between B and C, with respect to any of the scores, it would suggest that alcohol (or wine) had some effect, *provided the groups were identical with respect to all other factors which might influence the development of arteriosclerosis.* The most important of these other factors appear to be age, diet, and weight. The age distribution was equalized by dividing birds from a given hatch equally among all four groups in each experiment. The caloric intake was equalized by pair-feeding as described above. An analysis of food consumption at the beginning of each experiment and at about the halfway point (Table II) brought out the following facts. Among the A birds, those in Experiment 1 consumed the most, and those in Experiment 3 consumed the least, in the initial period. At the halfway point there were no significant differences. Among the B birds, there were no significant differences in either period. In both Experiment 2 and Experiment 3, the A and B birds consumed about the same amount initially, but the B birds were consuming considerably more at the halfway point. It should be noted, however, that these facts do not invalidate the merging of results from Experiments 1 and 2, since corresponding A and D birds had the same caloric intake, and the corresponding B and C birds had the same caloric intake, in either experiment.

The distribution of the initial weight differences between corresponding pairs of birds in A and D of the alcohol series had a mean significantly different from 0 at the 5 per cent level,[2] but not at the 1 per cent level. The corresponding distributions for B and C in the alcohol series, and for all groups in the wine series, had means not significantly different from zero. It was therefore assumed that in each case the two groups to be compared consisted of pairwise identical birds at the beginning of the experiment.

[2] The expression "the difference is significant at the X per cent level" means that the observed results would occur by chance X per cent of the time or less, if there were really no difference between the two groups; *i.e.*, if there had been no selection for weight. More pronounced differences will be significant at lower levels, and differences significant at levels higher than 5 per cent are called "not significant." The significance of the above figures was measured by means of the statistic "t" described in reference 9.

On the other hand, within each group, the individual weights and caloric intakes of the birds varied considerably. For this reason the sums of the gross gradings for the groups fail to provide a valid measure of the relative degree of arteriosclerosis. For example, the total scores of groups A and D in Experiment 3 for thoracic lesions are almost identical, but this finding can not be taken to indicate that there is no effect of wine in arterial disease. Indeed, a hypothetical example can be constructed in which the total gross gradings are equal in two groups which nonetheless differ significantly in their degree of arterial disease. The need for a more precise analysis is thus clearly indicated.

At the suggestion of Professor Elizabeth Scott, of the Statistical Laboratory of this University (to whom we are indebted for some of the computations), we used Nair's method of confidence intervals for the median (10) described on page 259 of reference 9. As used here, it eliminates the "blurring" effect of variation within the groups, and has the additional advantage that no assumptions need be made about the distributions of the scores. In the present case, for example, the usual assumption of normality does not hold.

What is done is to take, for a given variable in a given series, the differences in the scores of two corresponding birds in the two groups being compared. The resulting collection of numbers can be considered as a sample from a population of numbers whose precise distribution is unknown. However, since the corresponding birds are identical except for the treatment with alcohol or wine, the differences are as likely to be positive as they are to be negative if alcohol had no effect. That is, the median of the population will be 0 if alcohol (or wine) had no effect. Therefore, one possible test of the hypothesis of "no effect" is to obtain from the data a confidence interval for the median, and to reject the hypothesis if the interval does not include 0. An "X per cent confidence interval" for the median may be interpreted as follows: If an experiment is repeated a large number of times, and if the X per cent confidence interval is computed for each experiment, then X per cent of the intervals so computed will include the true population median. In this paper we have chosen to compute 95 per cent confidence intervals. Then, if the median is really 0, only 5 per cent of all such possible intervals will fail to include 0. In other words, the test proposed above has five chances in a hundred of detecting a false effect.

An approximate 95 per cent confidence interval may be obtained by arranging the differences in order from the smallest to the largest, and taking the interval from the m-th to the n-th smallest, where m and n are chosen from Table XXV of reference 9. If there are 38 differences, for example, the interval extends from the 13th to the 26th smallest. If there are 18 differences, it extends from the 5th to the 14th smallest.

As an example, consider the variable "gross grading of thoracic lesions" in the comparison A *vs.* D of the wine series, shown in Table V. The scores of the 18 birds in each group, the 18 differences between corresponding birds, and the 18 differences, in order, are given. The arrows indicate the 5th and 14th observations. The confidence interval, from -0.5 to 0.5, therefore includes 0, and so for this variable under the stated conditions, the hypothesis of "no effect" is accepted. Table VI shows the results of similar computations on all the variables for both comparisons in the alcohol and wine series. On the basis of these results, there is no reason to reject the hypothesis that alcohol is ineffective under any of the experimental regimens used here.

The use of the approximate 95 per cent confidence interval implies that the probability of finding an effect when none in fact exists is less than five in a hundred. Since we have accepted the hypothesis of "no effect," a word should be said about the probability of our having failed to detect a real effect. For a given comparison with a given variable in a given

series, the differences computed here can be considered, as we said above, to be a sample from a hypothetical population of differences. Under the hypothesis of no effect, half of the differences in this population would be negative. If alcohol or wine had a real protective effect, more than half of these differences would be negative. The greater the protective effect, the greater the proportion of negative differences until, with complete protection, all the differences would be negative, that is, the severity of the lesions of a bird in the alcohol- or wine-treated group would always be less than that of the corresponding bird in the other group. Thus, the protective effect of alcohol, if it existed, could be described numer-

TABLE VI

95 per Cent Confidence Intervals for Median Score Differences, Both Series

Experiment	Comparison	No. of birds	Variable	Confidence interval
1 and 2	A *vs.* D	38	Thoracic aorta	0 to 0.5
			Abdominal aorta	0 to 0
	B *vs.* C	37	Thoracic aorta	0 to 0
			Abdominal aorta	0 to 0
3	A *vs.* D	18	Thoracic aorta	−0.5 to 0.5
			Abdominal aorta	0 to 1
	B *vs.* C	18	Thoracic aorta	0 to 0
			Abdominal aorta	0 to 0

TABLE VII

Probability of Failure to Detect Various Degrees of Protective Effect for Two Sample Sizes

Sample size	Degree of protective effect			
	0.6*	0.7*	0.8*	0.9*
18	0.90	0.67	0.28	0.03
38	0.81	0.34	0.03	0.00

* Expressed as proportion of negative differences in a hypothetical population. See text for explanation.

ically by giving the proportion of negative differences in our hypothetical population—0.50 corresponding to no protection, and 1.00 corresponding to complete protection. Table VII shows, for the two sample sizes used here, the probability that we failed to detect different degrees of protection, when the degree of protection is expressed in this way.

Taking account of both the experimental results and the "strength" of the statistical analysis as shown in Table VII, we may draw the following conclusions: First, neither alcohol nor a wine containing 12 per cent of it had any effect on the development of arteriosclerosis or lipide infiltration of the aorta in chickens, as measured by the two variables analyzed here. Second, the procedure leading to the above conclusion was such that, although we may

have missed a slight effect, it is extremely unlikely that there is a substantial effect that we failed to detect.

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SUMMARY

The effect of alcohol ingestion upon the development of naturally occurring and stilbestrol-induced arteriosclerosis was studied in the domestic fowl. In two experiments, a 15 per cent ethyl alcohol solution was used for drinking purposes, and in a third experiment wine containing 12 per cent of it was administered. The caloric intake of both food and alcohol was carefully controlled by pair-feeding, a glucose solution being used for drinking purposes to equalize the caloric intakes of the control birds with those of the alcohol-treated birds. A total of 224 cockrels was studied, and the period of observation for each experiment lasted 12 months.

The degree of arteriosclerosis in the thoracic and abdominal aortas was determined in the gross and microscopically, and the degree of lipide infiltration was determined microscopically. The gross grading of arteriosclerotic lesions agreed closely with the microscopic analysis of intimal thickening and lipide infiltration of the arterial wall.

The gross gradings were subjected to a critical statistical analysis which allowed precise statements to be made on the probability that a real effect would be overlooked. This analysis yielded no evidence that alcohol or the wine used had affected the degree of gross arteriosclerosis or lipide infiltration.

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