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Hypocholesteremic Effect of Phenoxybenzamine (Dibenzylamine), An Adrenergic Blocking Agent

EXPERIMENTAL STUDIES WITH MONKEYS AND HUMAN VOLUNTEERS

By S. N. Jagannathan, M.Sc., S. G. Srikantia, B.Sc., M.B.B.S.,
and C. Gopalan, M.D., Ph.D.

■ The possible contribution of emotional stress and strain to the development of coronary artery disease has been frequently suggested, but precise proof of this is hard to obtain. In view of the difficulty inherent in properly defining what constitutes emotional stress, let alone measuring it, progress in this field of research has perforce been slow. There have been some studies, however, pointing to the influence of acute mental stress on serum cholesterol levels.¹⁻⁴ While the exact biochemical equivalent of mental stress has not been clearly established, it is generally agreed that acute emotional states are associated with an increased secretion of epinephrine. Bogdonoff et al.⁵ found that emotional episodes caused rapid mobilization of unesterified fatty acids from the body tissues into the circulation; similar results were found with infusions of epinephrine and norepinephrine. It has been reported⁶ that deposition of cholesterol in the intima is accelerated and intensified by epinephrine, and the catecholamine-phospholipid compounds seem to possess a particular affinity for arterial tissues.

The present study was originally undertaken with a view to elucidating the possible role of epinephrine in influencing serum cholesterol levels. While the experiments reported here have not been able to provide evidence for the role of emotional stress and epinephrine in atherosclerosis, they have yielded some new information regarding the effect of some substances on serum cholesterol levels.

Methods

The effect on serum cholesterol, in human sub-

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jects and in monkeys, of supplementing high-fat diets with Dibenzylamine,⁷ an orally effective adrenergic blocking agent, which acts by inhibiting the response of effector cells to the action of circulating epinephrine, was studied. In addition, the effect of the phenoxyethyl analogue of Dibenzylamine, G-D 131,⁸ which does not possess the adrenergic blocking property, but has been shown^{7, 8} to possess the other actions of Dibenzylamine, was also investigated. The following experiments were carried out:

EXPERIMENT 1: EFFECT OF SERUM CHOLESTEROL OF SUPPLEMENTATION OF DIBENZYLAMINE OR G-D 131 TO MONKEYS ON A HIGH-FAT DIET

Twenty-one adult male monkeys (*Macaca radiata*) maintained on an adequate stock diet containing 8 per cent fat for a period of not less than four weeks were divided into three groups of seven animals each, with respect to an even distribution of body weights and initial levels of serum cholesterol. The animals were fed a high-fat diet for a period of five weeks, at the end of which their serum cholesterol levels were determined. After this initial period, group 1 was continued on the same high-fat diet and served as control, whereas groups 2 and 3 were given, in addition to the high-fat diet, 5 mg. per day of Dibenzylamine and G-D 131, respectively. The diets were of "liquid-formula type" and contained skimmed-milk powder, 48 per cent; coconut oil, 25 per cent; vitamin mixture, 1 per cent;‡ salt mixture,§ 1.2 per cent;

*Phenoxybenzamine—Dibenzylamine capsules kindly donated by Smith, Kline and French Laboratories Ltd., England.

†N-(2-chloroethyl)-N-(cyclohexylmethyl)-ethylamine hydrochloride.

‡Provided per Kg. diet, the following vitamins in mg.: Thiamine HCl, 5; riboflavin, 8; niacin, 25; pyridoxine HCl, 5; calcium pantothenate, 25; folic acid, 2; menadione, 20; inositol, 100; and para-aminobenzoic acid, 100. Vitamin A, 7000 I.U., and vitamin C, 50 mg., were included in the daily diet of each monkey.

§Composed of, in Gm.: Potassium phosphate, 366; magnesium sulfate, 160; ferrous sulfate, 70; sodium chloride, 104; and potassium iodide, 10.

TABLE 1
*Effect of Administration of Dibenzylamine (Phenoxybenzamine) and Its Phenoxyethyl Analogue, G-D 131, on Serum Cholesterol Levels of Monkeys Fed a High-Fat Diet**

Group	Drug supplement	Number of monkeys	Serum cholesterol, mg./100 ml.			Significance of the change in serum cholesterol after drug supplementation
			Initial low-fat period level	Level 5 weeks after high-fat diet	Change in level after 25 days of drug supplementation	
1	Nil	7	149.0 ±9.13†	179.4 ±8.74	+ 0.86 ± 4.76	Not significant
2	Dibenzylamine	7	137.1 ±6.42	166.7 ±10.70	-13.71 ±13.76	<i>P</i> < 0.02
3	G-D 131	7	143.6 ±7.37	176.6 ±7.68	-21.0 ± 1.56	<i>P</i> < 0.001‡

*The diet contained coconut oil at a level of 25 per cent, supplying 45 per cent of total calories.

†Standard error of the mean.

‡The difference between the Dibenzylamine group and G-D 131 group was not significant.

choline-starch mixture, 1 per cent;* and sucrose, 23.8 per cent. The diets were administered to the animals by means of a stomach tube. Four animals in each group were given the daily feed in a single dose, while the other three animals received the same amount of diet in two equally divided doses, four hours apart. The details of the preparation and administration of the liquid diets have been described previously (Jagannathan⁹). Dibenzylamine and G-D 131 were added daily to the diet at the time of feeding. The three groups of animals were maintained on their respective diets for a period of 25 days, at the end of which their serum cholesterol levels were determined.

EXPERIMENT 2: EFFECT OF DIBENZYLAMINE ON THE INCREASE OF SERUM CHOLESTEROL IN MONKEYS MAINTAINED ON A HIGH-CHOLESTEROL HIGH-FAT DIET

Six adult male monkeys were divided into two groups of three animals each. Group 1 was fed a diet containing wheat flour, 52.3 per cent; casein, 12 per cent; salt mixture,¹⁰ 4 per cent; vitamin mixture, 1 per cent; choline chloride, 0.2 per cent; hydrogenated groundnut (peanut) fat, 30 per cent; and cholesterol, 0.5 per cent. Group 2 was fed the same high-cholesterol, high-fat diet plus a supplement of 5 mg. Dibenzylamine per animal per day. Serial determinations of serum cholesterol levels were done once in two weeks over a period of 15 weeks. Two monkeys, one from each group, were sacrificed at the end of 90 days and the other animals at the end of 105 days. The aortae were taken out and examined histologically for evidence of atheroma.

*Supplied 0.2 per cent of choline chloride in the diet.

EXPERIMENT 3: EFFECT OF DIBENZYLAMINE ON SERUM CHOLESTEROL LEVELS AND FECAL EXCRETION OF BILE ACIDS IN HUMAN SUBJECTS RECEIVING A HIGH-BUTTERFAT DIET

Three normal healthy human subjects, aged 25 years, were fed a diet providing about 3,000 calories, 90 Gm. protein, and 120 Gm. butterfat daily for a period of 32 days. Serum cholesterol levels were determined on two successive days before the experimental diet was started (initial values) and again at the end of 8 and 11 days on the experimental high-fat diet (end of period I). From the twelfth to the twenty-third day (period II), the subjects received an oral supplement of 10 mg. Dibenzylamine a day and serum cholesterol levels were estimated after 8 and 11 days in this period. The Dibenzylamine was then replaced by a placebo, but the fat diet was continued (period III). Serum cholesterol levels were again determined on the thirty-first and thirty-second days. Twenty-four hours' fecal output on the last three days of periods I and II was collected and duplicate samples were analyzed for the bile acids, cholic and dihydroxycholic (deoxycholic and chenodeoxycholic) acids, and Liebermann-Burchard chromogens.

The determination of serum cholesterol was carried out according to the method of Abell et al.¹¹ Cholic acid and dihydroxycholic acids were determined in the feces by using the spectrophotometric method of Kier,¹² along with the modifications suggested by Mosbach et al.¹³ and Haust and Beveridge.¹⁴

Results and Discussion

EXPERIMENT 1

The results are presented in table 1.

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TABLE 2

Effect of Dibenzylamine on the Elevation of Serum Cholesterol in Monkeys Fed a High-Cholesterol, High-Fat Diet

Group	Monkey no.	Serum cholesterol, mg./100 ml.						
		Initial level	Increase from the initial level					
			15 days	30 days	60 days	75 days	90 days	105 days
1 Control	1	49	173	250	401	457	457	..*
	2	93	219	314	417	535	639	562
	3	175	386	391	481	561	499	522
	Mean	106	259	318	433	518	532	542
2 Dibenzylamine-supplemented	1	93	136	207	317	366	375	..*
	2	103	132	214	481	636	629	496
	3	63	87	237	398	485	581	521
	Mean	86	118	219	399	496	528	509

*These two animals were killed at the end of 90 days on the experimental diet.

The mean serum cholesterol values in the three groups, before initiation of the high-fat diet, were 149.0 ± 9.13 ,* 137.1 ± 6.42 , and 143.6 ± 7.87 mg./100 ml. By the end of five weeks on the high-fat diet, the serum cholesterol levels had increased in all the animals and averaged 179.4 ± 8.74 , 166.7 ± 10.70 , and 176.6 ± 7.68 mg./100 ml., respectively, in the three groups. During the experimental drug-administration period, the control group of monkeys (group 1) showed a mean elevation of 0.86 mg./100 ml. On the other hand, group 2, receiving the Dibenzylamine, showed a mean depression in serum cholesterol of the order of 13.7 mg./100 ml. The administration of the phenoxyethyl analogue of Dibenzylamine, G-D 131, to the animals of group 3 also brought about a reduction in serum cholesterol of the order of 21 mg./100 ml. Analysis of variance of the changes in serum cholesterol during the drug administration period showed that the differences due to the administration of the drugs were statistically significant at the 1 per cent level ($F = 9.74$, Degrees of Freedom 17 and 2) and the differences due to the mode of feeding of the diet were found to be not significant ($F = 3.76$ for Degrees of Freedom 17 and 1). Hence, combining the dietary groups irrespective of whether the diet was fed as a single dose or divided doses, it was found by *t*-test that, while the

control animals did not show significant changes during the experimental period, those receiving Dibenzylamine and G-D 131 as supplements showed a significant ($P < 0.02$ and $P < 0.001$, respectively) depression in serum cholesterol. The values of the Dibenzylamine- and G-D 131-fed groups were not significantly different. Since the increase in the serum cholesterol brought about by a high-fat diet was significantly lowered, even with the administration of the analogue of Dibenzylamine (G-D 131, which does not possess the adrenergic blocking property), it would appear that the hypocholesteremic action of Dibenzylamine is independent of its adrenergic blocking effect. It may be pointed out that the addition of these drugs did not have any influence on the body weight of these animals over the experimental period.

EXPERIMENT 2

The results of this study are given in table 2. The mean serum cholesterol levels of the two groups over the different durations of feeding have been plotted in figure 1. It can be seen from table 2 and figure 1 that the hypocholesteremic effect of Dibenzylamine could be seen even when the diet contained cholesterol. The increase in serum cholesterol was retarded by the presence of Dibenzylamine in the diet of monkeys on a high-cholesterol, high-fat diet, during the first 60 days, but with the continuation of the experiment for

*Standard error.

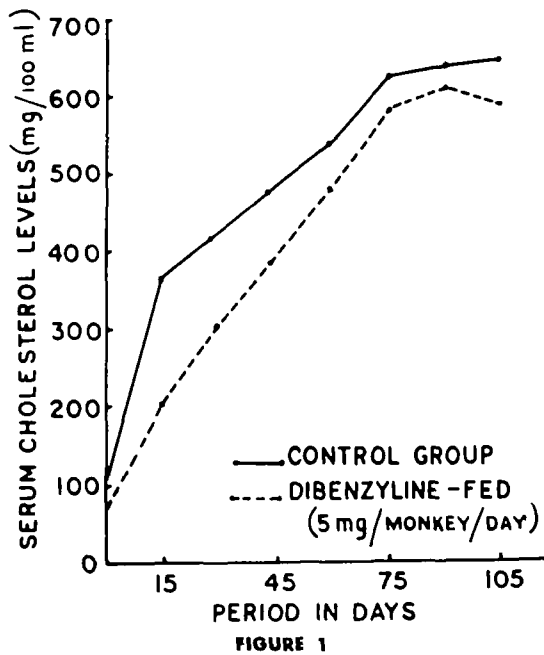


FIGURE 1
Effect of Dibenzyline on the increase of serum cholesterol levels in monkeys on a high-cholesterol, high-fat diet.

longer periods, the differences in serum cholesterol between the control and supplemented groups tended to narrow.

None of the monkeys killed at the end of the experimental period showed any histological evidence of atheroma in the aorta, even though the maximum serum cholesterol levels reached in the different animals ranged from 468 mg. to 739 mg./100 ml. It should be pointed out that the hypercholesteremia induced in these animals is of a high degree, considering the level of dietary cholesterol (0.5 per cent) fed. Cox et al.¹⁵ have reported development of atheromas in monkeys whose mean cholesterol levels were maintained between 256 and 393 mg. per cent for six months or more. In the present experiment, where, between 30 days and 105 days of the experimental period, the monkeys had a mean cholesterol level well exceeding 500 mg. per cent, the duration of this hypercholesteremic state (viz. 2½ months) was probably not enough for atherogenesis.

EXPERIMENT 3

The results of this investigation are indi-

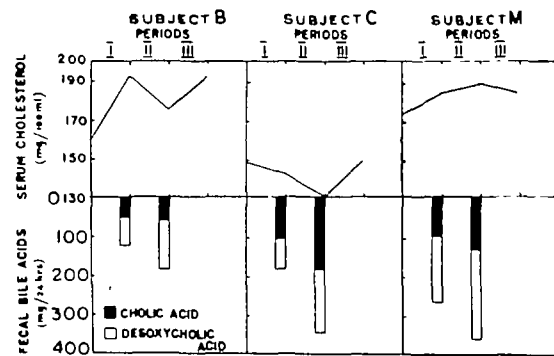


FIGURE 2

Effect of Dibenzyline, 10 mg./day, on serum cholesterol and fecal excretion of bile acids in normal human subjects on a high-butterfat diet. The duration of each period was 11 days. The serum cholesterol values plotted are the mean of two determinations done 8 and 11 days after each dietary period. Period I is the initial butterfat period; period II, the Dibenzyline-supplemented period; period III, the placebo-supplemented period. Deoxycholic acid values include chenodeoxycholic acid also, representing the dihydroxycholic acids.

cated in figure 2. In this investigation, in which human volunteers were used, two of the subjects, B and M, responded to the high-butterfat intake with an increase in serum cholesterol (from 160 to 193 mg. per cent in B and from 173 to 184 mg. per cent in M), which was reduced following Dibenzyline supplementation in one (to 175 mg. per cent in B), but not in the other (189 mg. per cent in M). Following the withdrawal of the drug, the serum cholesterol in subject B, which had shown a fall, returned to the pre-Dibenzyline level (192 mg. per cent). The third subject (C) showed no increase in serum cholesterol with the experimental high-fat diet as compared with the value on his home diet, but showed a lowering (from 142 to 130 mg. per cent) when Dibenzyline was given in addition, and a prompt return to a higher value (149 mg. per cent) following withdrawal of the drug.

All three subjects showed an increase (M, 36 per cent; B, 48 per cent; and C, 87 per cent) in the fecal excretion of total bile acids, cholic and dihydroxycholic (deoxycholic plus chenodeoxycholic) acids during the Dibenzyline-

supplemented period, at the end of which two of them showed a reduction in serum cholesterol levels. Subjects B and C, who responded to the administration of the drug by way of definite decreases in their serum cholesterol levels, showed greater increase in the excretion of dihydroxycholic acids than in that of cholic acid (68 per cent and 21 per cent, respectively, in B, and 99 per cent and 78 per cent in C). Subject M, on the other hand, showed equal increases (35 to 37 per cent) in the excretion of both cholic acid and dihydroxycholic acids. The fact that subject M showed an increase in fecal bile acids during the Dibenzylamine period and yet did not show a decrease in serum cholesterol may suggest that, but for the Dibenzylamine administration, his serum cholesterol would have continued to rise to a significantly higher level on the high-butterfat diet. The excretion of the Liebermann-Burchard chromogens was not affected to a significant extent (no change in B and 11 per cent decrease in M and C) due to the administration of Dibenzylamine. These observations suggest that the hypocholesteremic action of Dibenzylamine may be mediated, at least partly, through an increase in the synthesis of bile acids from cholesterol and their consequent enhanced fecal elimination.

As stated before, these investigations were originally planned to discover the possible role of emotional stress and the consequent increased circulating epinephrine in the causation of cholesterolemia. Since the adrenergic blocking agent used here, Dibenzylamine, had hypocholesteremic action unconnected with its action of adrenergic blockade, the elucidation of the aspect of emotional stress and cholesterol levels was not possible in this study. The study has, however, demonstrated the hypocholesteremic effect of Dibenzylamine and its analogue.

The relative merit of Dibenzylamine as compared with the other well-known hypocholesteremic agents may be investigated in patients with hypercholesterolemia. It was noted that as low as 10 mg. of the drug given for a

short period of 11 days was quite effective in reducing the moderate hypercholesterolemia induced by a high-fat intake in our subjects. Even on this low dosage level of Dibenzylamine, evidence of adrenergic blockade was present—the feeling of nasal congestion and a moderate sense of fatigue being complained of by all three subjects. There was, in addition, a slight fall in the blood pressure.

Summary

The effect of an orally effective adrenergic blocking agent, Dibenzylamine, on serum cholesterol levels was studied in human subjects and in monkeys on high-fat diets. In addition, the effect of the phenoxyethyl analogue of Dibenzylamine, G-D 131, was also investigated in monkeys.

The studies showed that the increase in serum cholesterol level brought about by a high-fat diet in monkeys could be considerably reduced by supplementation with Dibenzylamine. This hypocholesteremic action was also observed with the analogue of Dibenzylamine, G-D 131, which does not possess the adrenergic blocking property. It appears, therefore, that the hypocholesteremic action of Dibenzylamine is independent of its adrenergic blocking activity. When a high-fat diet which also contained a high amount of cholesterol was used, Dibenzylamine retarded the increase in serum cholesterol of monkeys for a considerable length of time.

Administration of Dibenzylamine, 10 mg. daily for 11 days, brought about a fall in serum cholesterol in two of the three human subjects and arrested the further increase in serum cholesterol in the third subject on a high-butterfat diet. All the subjects showed increased fecal elimination of cholic and dihydroxycholic acids during the Dibenzylamine-supplemented period, suggesting that the hypocholesteremic effect of the drug is at least partly mediated through increased elimination of cholesterol as bile acids.

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Book Review

The Human Pulmonary Circulation: Its Form and Function in Health and Disease, Peter Harris and Donald Heath. Baltimore, Williams & Wilkins Co., 1962, 354 pages. \$13.00.

This is the fourth monograph on the pulmonary circulation which has appeared in recent years. The outstanding feature of this one is the excellent correlation between the pathology and clinical physiology of the human pulmonary circulation. The first third of the book consists of a systematic discussion of clinical measurements of pressure, flow, blood volume, resistance, and impedance in

the pulmonary circulation. The rest of the monograph is devoted to various diseases in which the pulmonary vessels are abnormal in structure and function.

The authors should be congratulated for possessing the ability to sort out the settled facts from controversies. The latter were minimized by devoting the monograph to studies of the human lung and omitting the studies on other species. Some readers will undoubtedly wonder if there is any important functional difference between the pulmonary circulation of man and animals. They will have to secure an unbiased answer elsewhere.

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