THE ACTION OF CHOLESTEROL ON UNSTRIATED MUSCLE AND BLOOD VESSELS AND ITS RELATION TO HYPERTENSION

By Sunita Inderjit Singh, M.D. and Inderjit Singh, F.A.Sc.

(From the Department of Physiology, Medical College, Agra)

Received October 8, 1955

Hypercholesterolæmia and atherosclerosis are rarely found during the initial stages of hypertension. Westphal (1925) found hypercholesterolæmia in 71 per cent. of his patients with hypertension. Wacher and Fahrig (1932) observed an increase in cholesterol of the blood serum in 75 per cent. of cases with hypertension. Though cholesterol is deposited in the intima of the arteries, the media may also contain it in excessive amounts, as there is no likelihood of any diffusion barrier between the intima and the other coats of the blood vessels. The question arises whether cholesterol acts as an inert substance, or whether it can cause tonic contraction of the arterioles. In the present research an attempt has been made to determine the action of cholesterol on unstriated muscle and blood vessels to elucidate its possible role in producing or modifying hypertension. Unstriated muscle is a sensitive tissue, and it is unlikely that it would not be affected by a cholesterol rich medium.

Experimental

The action of cholesterol was tested on (*a*) the excitatory mechanism; (*b*) contractile mechanism of the circular unstriated muscle of the stomach of dog and the frog, *Rana tigrina*. Cholesterol is insoluble in water, but forms an opalescent colloidal solution if the saline is saturated with chloroform. The action of cholesterol can thus be investigated, as chloroform enhances the irritability of certain unstriated muscle to chemical excitants which produce a tonic contraction (Singh, 1938 *b*). From this solution, cholesterol has a tendency to crystallise out.

The action on the contractile mechanism was tested by using dying and heat-killed muscles (Singh and Singh, 1949, 1950, 1954 *a, b, c*). They were immersed in various solutions for 24 hours.

The action on the blood vessels was tested by perfusing dog's hind legs as described previously (Singh and Singh, 1955).

RESULTS

Action on the excitatory mechanism.—Chloroform is a stimulant of unstriated muscle. It produces a tonic contraction and increases the excitability to other tonic stimulants such as potassium (Singh, 1938). It decreases the excitability to nervous stimulation and alternating current. Saline saturated with chloroform causes a tonic contraction of dog's stomach muscle (Fig. 1), of frog's stomach muscle (Fig. 2); it causes immediate slowing of the perfusion flow, so that the arteriolar muscle is also stimulated (Figs. 3, 4).

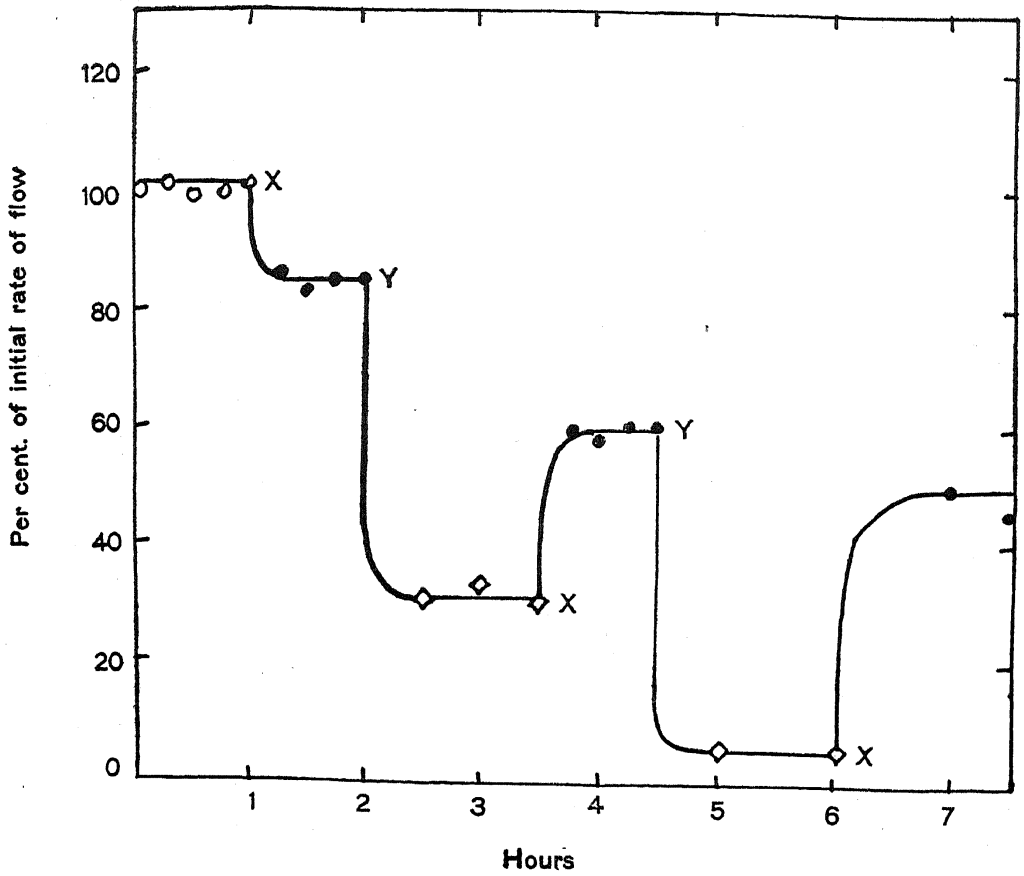

FIG. 3. Dog's hind limbs. At first the limbs were perfused with mammalian saline. Then with chloroform saturated saline at X. Perfusion with cholesterol saline at Y produced immediate retardation of flow.

Cholesterol is insoluble in water, but it forms an opalescent colloidal solution in the presence of chloroform; it tends to crystallise out form this solution. In these experiments a saturated solution of cholesterol was made in saline which had been previously saturated with chloroform. For perfusion experiments, the strength of cholesterol was 1 in 1,000, and for other

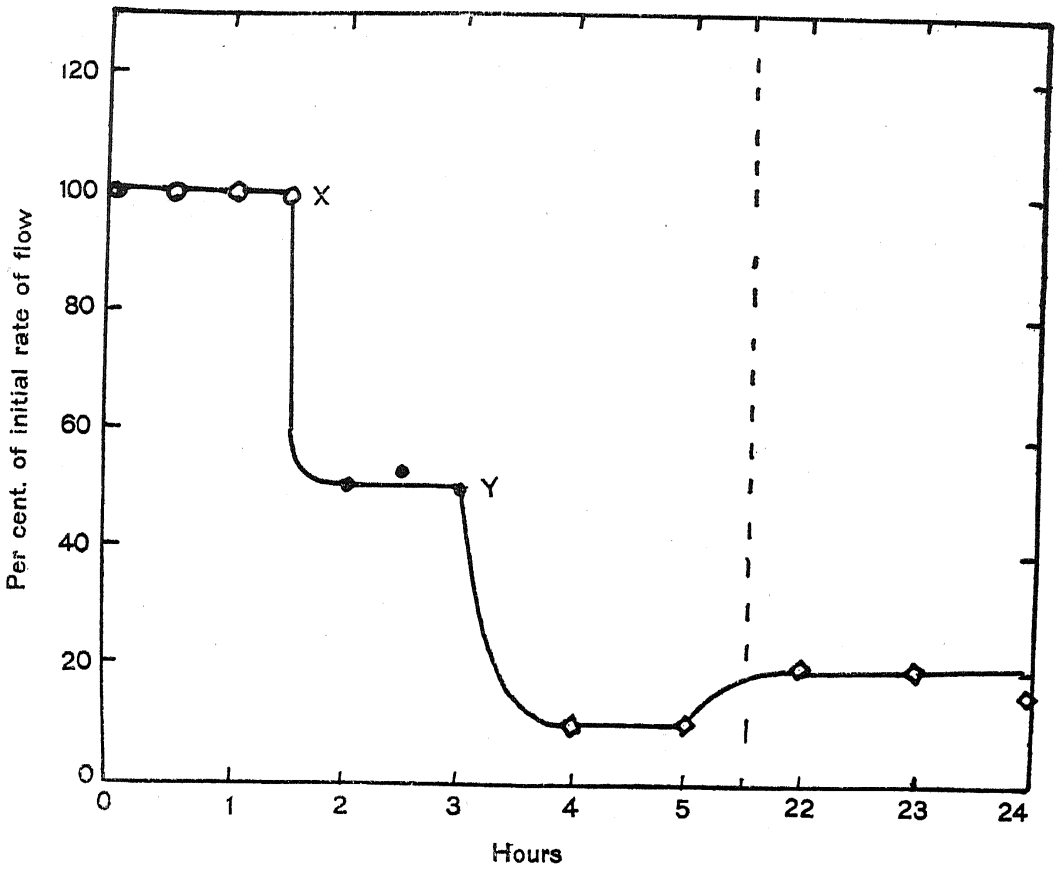

FIG. 4. Dog's hind legs. At first perfused with mammalian saline. Then saline saturated with chloroform was added at X. Next cholesterol was added at Y.

experiments 2 to 5 in 1,000. It is difficult to say how much of the cholesterol in solution is in an active form; probably a very minute concentration. Also it is difficult to say whether the colloidal form has any action as such, or whether it first passes into a non-colloidal solution, and then acts on the muscle. The strength of cholesterol in solution is therefore of no consequence in these experiments. From solutions chloroform tends to evaporate so that the cholesterol is precipitated; a little undissolved chloroform should therefore be kept in all solutions.

Cholesterol produces a tonic contraction of dog's and frog's stomach muscle, so that it appears to be a general stimulant of unstriated muscle (Figs. 1, 2). The contraction produced by cholesterol has very interesting properties. It is irreversible. The nature of tone changes, the contraction passing into alactic tone (Singh, 1949; Singh and Singh, 1948). Adrenaline, asphyxia or alternating current fails to produce any relaxation. In unloaded dog's stomach muscle, active relaxation after stimulation with potassium is abolished.

Action on the contractile mechanism.—This was tested on unloaded dying and heat-killed frog's stomach muscle, which were immersed in various solutions for 24 hours. In a first series of 6 experiments with dying muscles, using bicarbonate buffered saline at pH 8, cholesterol produced contraction or relative contraction in all muscles by 8 to 31 per cent. In 6 heated muscles, the contraction was from 8 to 13 per cent., so that the action was no doubt on the contractile mechanism (Table I). If the muscles were loaded, then no tension was produced, this probably being due to the fact that the action of cholesterol is very slow, so that any contraction is undone by the stretch, as it is a well known property of unstriated muscle, that if loaded, it slowly increases in length.

TABLE I

Frog's Stomach Muscle. Effect of Cholesterol on the Contractile Mechanism

Immersion for 24 hours

No. of experiments	Per cent. of initial length		Per cent. decrease of length in cholesterol
	In chloroform saline	In cholesterol	
	Dying Muscles		
1	111	90	21
2	110	90	20
3	100	92	8
4	100	88	12
5	122	91	31
6	120	100	20
	Heat-Killed Muscles		
1	113	100	13
2	112	104	8
3	113	100	13
4	126	108	18
5	110	100	10
6	133	120	13

In other series of 24 experiments with dying muscles, contraction occurred in 4 muscles, only by 6 to 10 per cent., relaxation in 7 muscles by 6 to 26 per cent., and the rest were unaffected. With 24 heated muscles, contraction occurred in 4 muscles by 7 to 11 per cent., relaxation in 3 muscles and the rest were unaffected.

The cause of these variable results was investigated. In 6 experiments with dying muscles, using isotonic potassium chloride, 0·154 *M* KCl as medium, cholesterol caused relaxation in all muscles by 7 to 27 per cent. (Table II). In similar experiments with 6 heat-killed muscles, relaxation

TABLE II

Frog's Stomach Muscle. Effect of Cholesterol on the Contractile Mechanism

Immersion for 24 hours. Saline used 0·154 *M* potassium chloride, which was saturated with chloroform

No. of experiments	Per cent. of initial length		Per cent. increase in length in cholesterol
	In chloroform potassium saline	In cholesterol potassium saline	
	Dying Muscles		
1	90	105	15
2	113	120	7
3	96	108	12
4	94	103	7
5	83	110	27
6	108	122	14
	Heat-Killed Muscles		
1	117	113	—4
2	100	120	20
3	108	114	6
4	112	123	11
5	124	112	—12
6	120	120	0

occurred in 3 muscles by 11 to 20 per cent., the rest being unaffected. It is clear therefore, that the effect of cholesterol depends upon the ionic medium.

In 6 experiments with dying muscles in isotonic sodium chloride medium, cholesterol produced no significant effect. Heated muscles also showed no change. In the presence of small quantities of calcium, usually contained in mammalian saline, out of 12 experiments, contraction occurred in 7 muscles by 10 to 21 per cent., relaxation in one muscle by 10 per cent. and the rest were unaffected. Heated muscle did not show any significant change. Thus the effect of cholesterol on the contractile mechanism of unstriated muscle is dependent upon the ionic balance of the muscle.

In acid media, phosphate buffered saline at pH 7 (6 experiments), pH 6 (6 experiments), pH 5·3 (6 experiments), cholesterol produced no significant effect. Magnesium, 0·001 *M*, did not have any significant effect on the action of cholesterol.

Action on blood vessels.—The action of cholesterol on the arterioles is identical with that on other unstriated muscle. In 6 perfusion experiments, cholesterol produced an immediate retardation of flow. The difficulty arises about the crystallisation of cholesterol from the solution. A few crystals might appear and retardation of flow might be due to blocking of the arterioles by such crystals. This, however, seems unlikely in these experiments, as shown by the following observations. The contraction is partially reversible, the spasm passing off on the removal of cholesterol (Fig. 3). The tendency to crystallisation is greater after standing for 24 hours, but the rate of flow increases, the initial spasm having subsided partially (Fig. 4). The contraction of arterioles produced by cholesterol is reversible, thus differing in this respect from its action on dog's stomach muscle; the contraction may, however, become more or less, irreversible. Direct microscopic observation of the guinea pig's arterioles in the mesentery show that cholesterol causes a permanent tonic contraction; chloroform may cause a temporary spasm.

DISCUSSION

Cholesterol leads to mechanical narrowing of the blood vessels by producing atheromatous patches. It is possible that it may be one of the agents in increasing vascular tone. As hypercholesterolæmia and atherosclerosis are rarely found during the initial stages of hypertension, cholesterol may complicate matters in the later stage of the disease. As cholesterol changes the nature of the tone and impairs active relaxation of smooth

muscle, it is quite possible that the irreversibility of hypertension in later stages might be due to the action of cholesterol.

The action of cholesterol on the contractile mechanism is enhanced by calcium. This may be of significance, as in arteriosclerosis, deposition of calcium occurs in the media. High concentrations of calcium have a direct effect on the contractile mechanism of unstriated muscle, causing it to contract. This may also be a contributary factor in causing an irreversible contraction of the arterioles.

The sequence of events in hypertension may be as follows. Increase in the sodium or decrease in the potassium content or some other factor leads to increase in the irritability of the nervous system. This leads to increase in tone of the vascular centres. The tone of the arterioles thus increases. Some vasopressor substance liberated in the blood stream may also produce similar action. Stimulation of the arteriolar muscle would increase their permeability, thus allowing sodium, calcium and cholesterol to enter. These act on the contractile mechanism to produce a tonic and later on an irreversible contraction of the arterioles. Cholesterol would enter the muscular coat from the side of the intima. Increase in the sodium content of the arterioles would also act on the excitatory system, thus rendering them more sensitive to chemical excitants in the blood.

What causes cholesterol to enter the coat of the blood vessels ? In text books of Pathology, this question is fully discussed. Mechanical factor no doubt plays an important part. In this connexion, it is interesting to note, that intermittent mechanical pressure increases the permeability of unstriated muscle to sodium. Thus blotting of the muscle intermittently, increases the gain of weight by the muscle in certain solutions, the gain of weight being due to the passage of ions and water into the muscle (Singh, 1938 *a*).

Summary

1. The action of cholesterol on the excitatory and the contractile mechanisms of unstriated muscle has been described.

2. Cholesterol causes contraction of dog's and frog's stomach muscle by action on the excitatory system. It changes the nature of tone, so that the muscle is unable to relax.

3. Cholesterol increases the tone of the blood vessels of dog's hind limbs.

4. Cholesterol causes contraction of the contractile mechanism of unstriated muscle; this contraction is dependent upon the ionic balance.

5. It is concluded that cholesterol may be active in later stages of hypertension, in increasing tonus of arterioles, and producing irreversibility.

REFERENCES

Singh, I. .. *J. Physiol.* 1938 *a*, **91**, 398.

———— .. *Ibid.*, 1938 *b*, **92**, 62.

———— .. *Proc. Ind. Acad. Sci.*, 1949, **21**, 190.

——— and Singh, S. I. .. *Ibid.*, 1949, **30**, 270.

———— .. *Ibid.*

———— .. *Ibid.*, 1950, **32**, 12.

———— .. *Ibid.*, 1954 *b*, **40**, 125.

———— .. *Ibid.*, 1954 *c*, **40**, 145.

———— .. *Curr. Sci.*, 1948, **17**, 321.

———— .. *Ibid.*, 1954 *a*, **23**, 126.

Wacher, L and Fahrig, C. .. *Klin. Wchnschr*, 1932, **11**, 762. Quoted from *J. Stambul*, "The Mechanisms of Disease," 1952, p. 262. Froben Press, Inc., New York.

Westphal, K. .. *Wchnchr. f. Klin. Med.*, 1925, **101**, 559. Quoted from *J. Stambul*, "The Mechanisms of Disease," 1952, p. 261. Froben Press, Inc., New York.

EXPLANATION OF PLATE FIGURES

FIG. 1. Dog's stomach muscle. Contraction due to chloroform and cholesterol. First stimulated with alternating current (12 volts/10 sec.) at A.C. Then saline saturated with chloroform was added. Next cholesterol dissolved in chloroform saline was added. It was then removed. Note the muscle does not relax.

FIG. 2. Frog's stomach muscle. Contraction due to chloroform and cholesterol. First chloroform saline was added; when the contraction began to decline, then cholesterol was added.

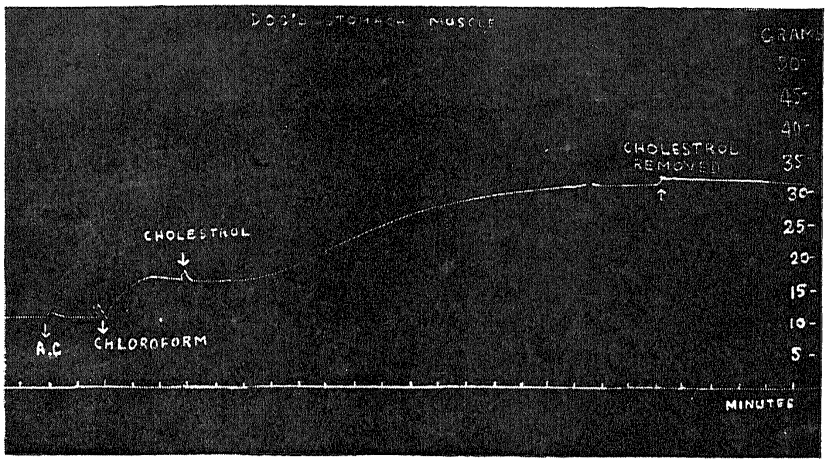
CHOLESTROL REMOVED
CHOLESTROL
A.C
CHLOROFORM
MINUTES

FIG. 1

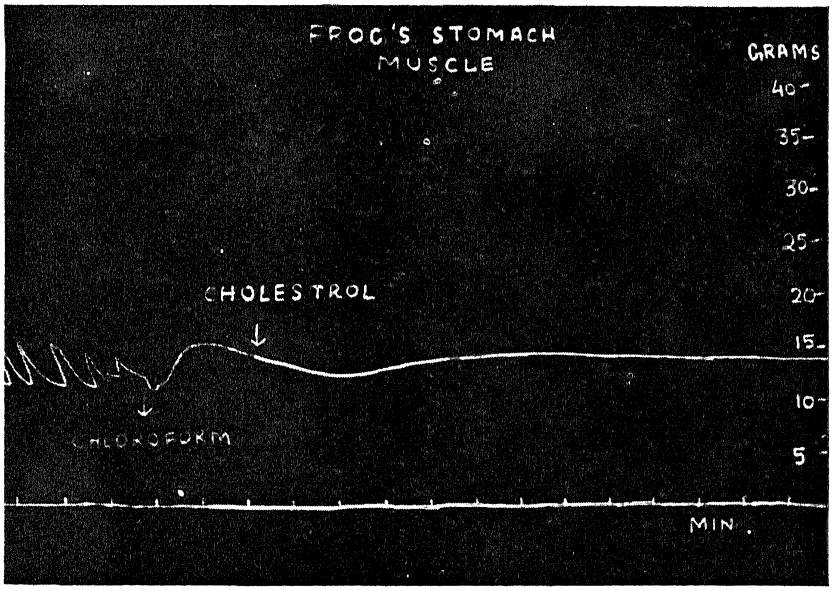
FROG'S STOMACH MUSCLE
GRAMS
CHOLESTROL
MIN.

FIG. 2