PREVENTIVE EFFECT OF s-METHYL METHIONINE SULFONIUM IODIDE ON DIETARY HYPERCHOLESTEREMIA IN RABBITS

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By

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In a previous paper (1), the authors called the attention that a sulfonium derivative of methionine, *s*-methyl methionine sulfonium iodide (MMSI) given by intraperitoneal injection, was effective to prevent the increase of serum cholesterol levels of rats fed on cholesterol supplemented diet.

The present study is put to verify this preventive effect of MMSI on dietary hypercholesteremia, with chemical and histological methods, in rabbits which have been laden cholesterol *per os*.

Experimental

Animals. Male albino rabbits, weighing approximately 1.5 kg, were haused and fed singly in wooden boxes with bottoms of hurdles.

Diet. All of animals were mentained *ad libitum*, on a diet composed of *Okara* (Waste product of *Tôfu* or soy-curd), wheat bran and fresh clover grass, for 60 days. The constitution and composition of the ration are shown in Table 1.

Table 1. Constitution and Composition of the Diet.

Constituents of the ration* (g/kg diet)		Composition of the diet (%)	
*Okara***	400	Moisture	65.92
		Crude protein	3.37
Wheat bran	400	Crude fat	3.27
		Carbohydrate	21.53
Clover grass	200	Fiber	4.40
		Ash	1.49

* Calcium carbonate was supplied at an amount of 1g/week for each animal.
** Waste product of *Tôfu* or soy-curd.

Test groups. Twelve rabbits were allotted into three lots, *i.e.*, the control lot (I), the cholesterol lot (II), and the MMSI lot (III). Each of them consisted of four animals. The animals of lots II and III were administrated with a dose of 0.4 g cholesterol, suspended in a 1 : 1 mixture of lard and olive oil every day, using stomach tube.

The animals of the lot II received an intravenous injection of aquous solution of sodium iodide of 100 mg in a dose, which contained approximately equivalent iodide ion in a dose of MMSI injection to the lot III. The animals of the lot III were injected with a dose of 200 mg MMSI into the veins of their ears, every other day throughout the experimental period. The constitution of the test groups is enlisted in Table 2.

Table 2. Constitution of the Test Groups
—Male rabbits : Fed for 60 days—

Lot	Number of animals	Administration of CH *per os*	Injection
I Control	4	None	None
II CH	4	0.4g/day	NaI 100 mg/dose*
III MMSI	4	〃	MMSI 200 mg/dose*

CH=Cholesterol.
* Intravenous injection, every other day.

Substances tested. MMSI was synthesized from DL-methionine by a modified method of the authors (1), which based on the procedure by Atkinson and Poppelsdolf (3). Sodium iodide was prepared from the potasium salt by cation exchange, percolating over *Amberlite IRC-120* resin in sodium type and crystallized from the elute.

Analytical procedure. Serum cholesterol was determined by the method of Zak *et al.* (4), every 10 days. Cholesterol in liver and aorta were determined by the method of Zak *et al.*, and of Gungbaum *et al.* (5), respectively. Liver and brain were analyzed for moisture, total lipid, saponifiable fatty acids and total unsaponifiable lipid by usual methods (1). Levels of serum protein were determined by the protein refractmeter. Relative concentration of haemoglobin in blood was determined according to the technique of Popov and Sobchuk (6).

Histological procedure. Liver, kidney and aortae (*Aorta descendans thoracia* and *A. abdominalis*) of each animal were fixed in 10-times diluted formalin, embedded in paraffin, sectioned to 8 micron slices and stained with Van Gieson stain or haematoxylin eosin double stain.

Besides these stainings, aortae were sectioned in freezing condition and stained with Sudan III.

Results

Growth. All animals of the three lots had normally grown, without showing any injurious effects of cholesterol feeding and MMSI or sodium iodide injection. The growth curves of the animals are shown in Figure 1.

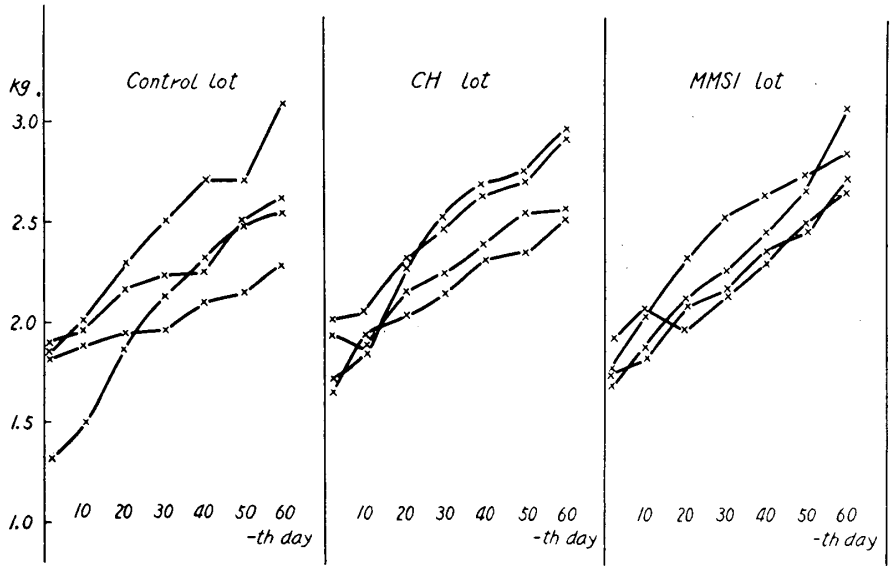

Fig. 1. Growth of the Rabbits
—Male rabbits: Fed for 60 days—

Weight of viscera and carcasses. All animals were sacrified by carotidal amputation without anaesthesia and dissected into individual organs. There were no remarkable deviations in the weights of viscera (Liver, kidney, spleen, heart, lung, brain and digestive tracts) in each lot and *inter se.*

Serum cholesterol. The cholesterol levels in the sera in total and free type were determined at 10 day's intervals. The data are presented in Table 3, and illustrated in Figures 2 and 3.

In these cases of two lots, the cholesterol lot (II) and the MMSI lot (III), it may be detectable that there is an apparent difference between the levels of them in both types of cholesterol. After 20 experimental days, this difference of cholesterol levels between these two lots are apparently found, namely, instead of rapid and high increase of serum cholesterol in the cholesterol lot, dull and soon ceiled development of the level is noticed in the MMSI lot. This phenomenon is more distinct in the case of the conjugated cholesterol than that of the free type. The level of the control lot had a little increase of both cholesterol throughout the feeding.

Lipids and moisture in liver. The contents of moisture, total lipid, saponifiable fatty acids, total unsaponifiable matter, and cholesterol of total and free types in liver, are shown in Table 4.

Tlabe 3. Cholesterol Levels in the Sera every 10 days (mg/dl)

Lot \ Days	0		10		20		30		40		50		58	
I Control	66		44		16		50		50		46		46	
Total	22	47±9*	36	37±2	50	40±9	25	38±7	62	40±10	50	45±2	66	55±7
	44		30		56		25		16		42		42	
	56		—		38		50		30		40		68	
Free	44		14		10		28		18		10		26	
	12	24±8	14	14±0	26	20±4	16	19±3	10	14±2	12	14±2	48	31±3
	12		14		14		16		16		18		22	
	26		—		28		16		12		16		28	
II CH	30		112		166		110		166		190		312	
Total	74	40±11	122	87±15	160	126±21	128	100±11	216	174±14	184	146±8	192	234±21
	30		52		78		78		152		94		230	
	26		62		98		88		162		116		202	
Free	30		38		60		40		106		130		242	
	34	24±5	48	36±5	30	38±10	98	53±15	210	104±38	130	96±20	134	146±34
	18		34		24		34		38		56		78	
	14		24		36		40		62		66		130	
III MMSI	—		72		104		64		78		100		62	
Total	54	34±10	78	68±5	84	99±6	94	83±7	88	88±4	146	115±13	146	105±18
	28		68		104		94		96		124		104	
	20		54		104		78		88		88		108	
Free	—		26		68		12		50		92		44	
	22	18±2	38	22±6	38	53±6	22	31±10	62	62±5	84	87±4	82	61±10
	18		12		52		58		62		86		72	
	14		12		52		30		72		84		46	

* Mean±Standard error of the mean

The moisture in livers of the control lot was higher than that of other lots, but the total lipid and the saponifiable fatty acids are lower than that of the cholesterol laden lots (II and III). The increase of total unsaponifiable matter

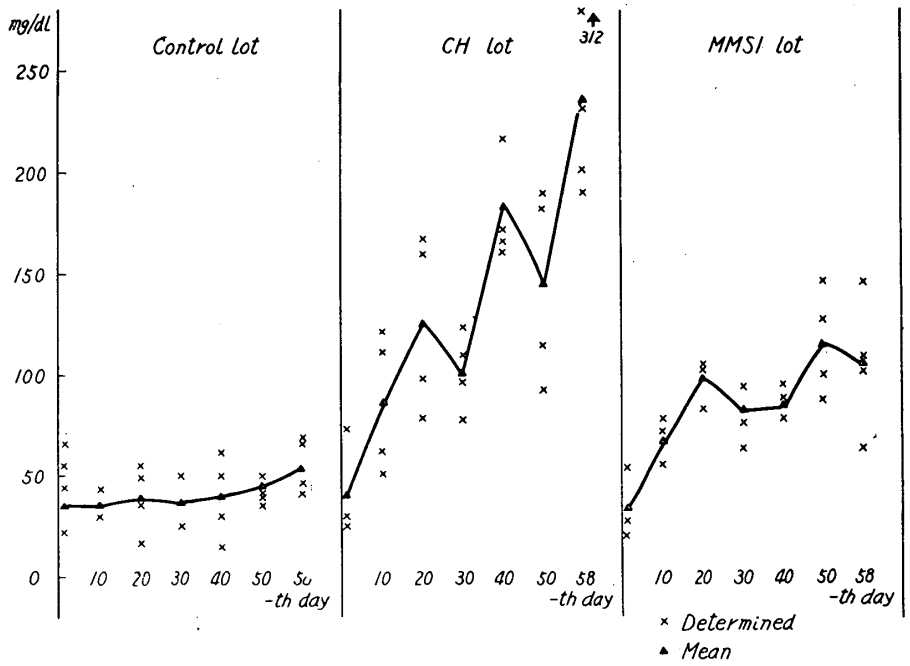

Fig. 2. Changes of Total Cholesterol Levels in the Sera.

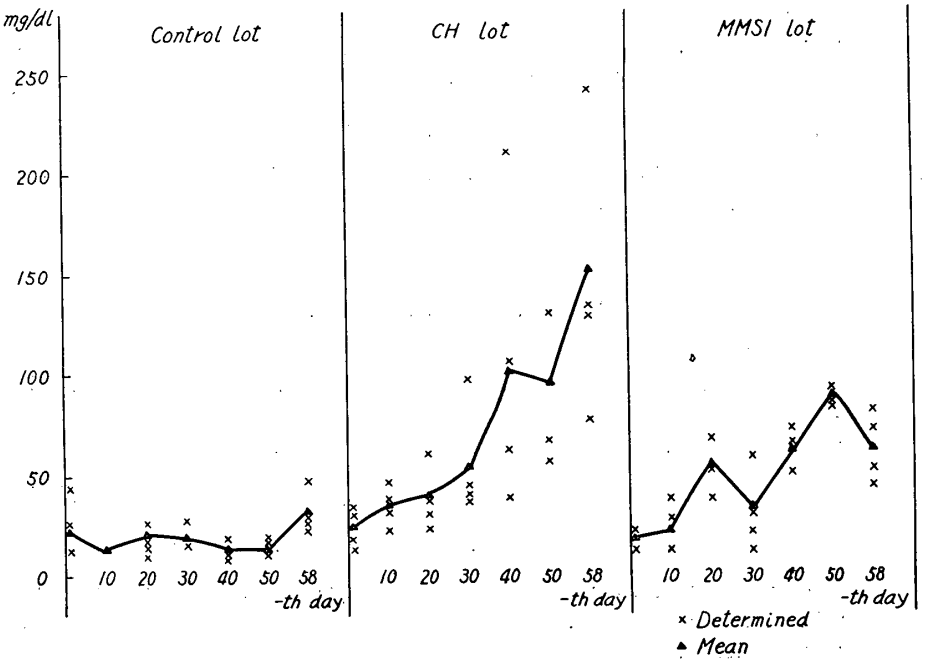

Fig. 3. Changes of Free Cholesterol Levels in the Sera.

in livers of the cholesterol lot and the MMSI lot is a matter of course, but the increase of saponifiable fatty acids may be a notable fact. There is no

significant deviation between the cholesterol lot and the MMSI lot in moisture, total lipid and saponifiable fatty acids. Cholesterol levels in livers are apparently different between the control and the other two lots. Morever, it may be an interesting fact that there is a significant difference between the cholesterol lot and the MMSI lot, especially in the free type.

Cholesterol in aorta. The cholesterol contents of aortae are presented in Table 5. The highest cholesterol content of them was found in the cholesterol lot, and the difference between the cholesterol lot and the MMSI lot was significant.

Table 5. Cholesterol Content in Aorta.

Lot	Cholesterol*			
	Total (mg/dkg)		Free (mg/dkg)	
I Control	105 118 77 93	98±5**	65 114 71 59	77±12
II CH	183 211 170 176	185±9	170 164 164 112	153±15
III MMSI	138 164 155 149	152±5	111 91 96 74	93±7

* Per fresh tissue.
** Mean±Standard error of the mean.

Lipids in brain. Total lipid, saponifiable fatty acids, and total unsaponifiable matter of the brains were determined, in combining the samples of every lot. There is no significant difference among them. These data are shown in Table 6.

Table 6. Lpids in Brain.

Lot	Fresh wt.* associated (g)	Total** lipid (%)	Saponifiable fatty acids** (%)	Unsaponifiable matter** (%)
I Control	39.5	13.16	4.98	5.51
II CH	43.0	12.75	5.64	4.65
III MMSI	40.5	14.08	4.05	4.32

* These brains from four animals in a lot were associated and treated for the analysis of lipids.
** Per fresh tissue.

Serum protein and haemoglobin in blood. The portein levels of the sera every 10 days and relative concentration of haemoglobin in the blood at the final day are shown in Table 7. There were found some deviations among

Table 4. Moisture and Lipids in the Liver.

Lot	Moisture (%)		Lipids*						Cholesterol* (mg%)			
			Total (%)		Saponifiable fatty acids (%)		Unsaponifiable matter (%)		Total		Free	
I Control	70.46	71.86±0.57	5.64	4.07±0.56	2.39	2.44±0.25	1.32	0.93±0.17	496	350±50	80	57±7
	71.52		3.88		3.15		0.46		353		50	
	72.29		3.13		2.22		0.94		239		45	
	73.16		3.16		2.01		0.98		312		52	
II CH	66.46	64.27±0.49	5.90	6.27±0.69	4.75	3.98±0.34	0.70	1.47±0.40	543	586±43	252	203±24
	64.44		7.74		4.23		2.63		615		221	
	62.84		4.49		3.15		1.22		494		203	
	63.35		6.96		3.75		1.33		639		134	
III MMSI	66.23	67.94±2.68	5.40	7.03±0.77	3.21	4.36±0.72	1.42	1.99±0.29	413	509±33	83	118±14
	75.70		6.30		3.40		2.83		478		106	
	66.58		9.03		6.64		1.90		614		145	
	63.25		7.32		4.20		1.84		530		136	

* Per fresh liver. ** Mean±Standard error of the mean.

Table 7. Serum Protein Levels and Relative Concentration of Haemoglobin in the Blood.

Lot \ Days	Serum protein levels (%)							Relative Hb. content*
	0	10	20	30	40	50	58	
I Control	5.7±0.5	5.3±0.6	7.0±0.9	6.5±1.1	5.8±0.2	5.2±0.1	6.2±0.2	0.95±0.16
II CH	6.1±0.2	7.1±1.6	6.8±0.2	7.5±0.2	7.0±0.4	6.8±0.4	7.2±0.3	0.81±0.18
III MMSI	7.3±0.6	7.4±0.8	5.6±0.5	7.5±0.5	6.7±0.1	6.8±0.2	6.9±0.4	1.11±0.11

* Data for the 60th day, determined following the method of Popov and Sobchuk (6).
** Mean±Standard error of the mean.

the searum protein levels, but the reason, why it had occurred, was obscure. The deviation of relative concentration of haemoglobin among these three lots is not significant.

Histological inspection. Liver, kidney and aorta of the animals were inspected histologically, under 100× fields. There is no detectable damege in any of these hepatic and renal tissues of all the animals.

All cases of the aortae of the lot II, which had been fed cholesterol and injected with sodium iodide, revealed severe pathological alternation of *Tunica intima, i.e.*, atheroma with thick sedimentation of sudanophilic substance in the fat stain, and, hypertrophic denaturation of the tissue in Van Gieson stain and haematoxylin eosin stain (*Vid.* the photographes of the sections).

Some cases of these aortae of the lot III, fed cholesterol and administrated MMSI, were also detectable of these pathological alternations, but the changes of these tissues were very slight ones. In Table 8, these histological inspections are summerized.

Table 8. Histological Examination of Liver, Kidney and Aorta.

Lot	Animal No.	Aorta			Liver		Kidney	
		Sudan III	H-E	V.G.	H-E	V.G.	H-E	V.G.
I Control	1	None	None	None	None	None	None	None
	2	〃	〃	〃	〃	〃	〃	〃
	3	〃	〃	〃	〃	〃	〃	〃
	4	〃	〃	〃	〃	〃	〃	〃
II CH	5	Fat+++	Atrm+++	Atrm+++	None	None	None	None
	6	+++	++	+++	〃	〃	〃	〃
	7	++	++	+	〃	〃	〃	〃
	8	+	+	+	〃	〃	〃	〃
III MMSI	9	None	None	None	None	None	None	None
	10	Fat+	Atrm+	Atrm+	〃	〃	〃	〃
	11	None	+	+	〃	〃	〃	〃
	12	〃	None	None	〃	〃	〃	〃

Sudan III : Sudan III stain. H-E : Haematoxylin eosin double stain.
V.G. : Van Gieson stain.
None : No histological alternation is detectable, under 100× field.
Fat : Sudanophilic deposition on *Tunica intima*.
Atrm : Atheromic hypertrophy of *Tunica intima*.
+ : Number of the cross represents the approximate degree of sudanophilic denaturation or hypertrophic change of the tissue (*Vid.* the photogrophes).

Conclusion and Discussion

These data of the determination of serum cholesterol levels and histological inspection of aortae lead us, with certainty, for supporting of the view that MMSI might act as a preventive agent against dietary hypercholesteremia.

The mechanism that MMSI is effective for depressing serum cholesterol

has not been proposed, but no considerable trial to dissolve it, has been introduced. Interpreting the fact, however, that MMSI is more effective for depressing the level of total cholesterol than for the free one, the authors provide for two processes where MMSI may be involved in cholesterol metabolism.

The first process to be supposed is that MMSI may be involved in the reaction to form the conjugated or esterified cholesterol from the free or alcohol one, and disturbs the esterification, depressing the levels of cholesterol in the serum as a whole.

Another assumption for the rôle of MMSI is that it may accelate the degradation of esterified cholesterol, and the level of the serum cholesterol, consequently, will be reduced.

The administration of MMSI at a level used in this experiment, did not cause unfavourable changes, such as interferance of the growth, fatty infiltration of the liver, pathlogical alternation of the hepatic and the renal tissues, this sulfonium derivative of methionine, therefore, may be no hazard or of very less toxic substance, in the present dosage, and these observations agreed with the detection of Bersin *et al.* (7), who had administrated some methyl methionine sulfonium salts to mice orally and intravenously, and then, concluded that these salts were very little toxic.

The deceased or slight deposition of sudanophilic substance on *Tunica intima* of aortae of the animals, received the injection of MMSI, may be a notable fact in this experiment.

Associating to the present study, it shall be tested, further, to evidence the effectiveness of MMSI for clearing of the scall, which has sedimented on the inner wall of aorta.

Since these animals in the lot II, furthermore, which received the injection of sodium iodide, had not reduced their serum cholesterol levels, and showed thick deposition of sudanophilic substance in the aortae, the potentiality of MMSI to prevent hypercholesteremia and atheromic denaturation of the aorta may be associated with methyl methionine sulfonium cation, but not with the anion of iodine.

Summary

1. Male albino rabbits were fed for 60 days, and laden 0.4 g cholesterol every day *per os.* A lot of the animals was administrated with intravenous injections of *s*-methyl methionine sulfonium iodide (MMSI) at a dose of 200 mg every other day, throughout the experimental period.

2. There was apparent less serum cholesterol of the MMSI lot than that of the lot administrated with equivalent sodium iodide.

The increase of serum cholesterol in the MMSI lot was dull and soon

ceiled, but rapid and steady increase was observed in the latter lot. Content of cholesterol in the aortae was also paralled to that of serum cholesterol. In liver, there was a significant decrease of cholesterol level of the MMSI lot.

3. In the aortae of the MMSI lot, no or very slight atheromic change was detected, but a severe one in the sodium iodide lot.

4. These results offered some support of the veiw that MMSI may act as a preventive effect against hypercholesteremia induced by cholesterol feeding, and, this potentialty may be associated with methyl methionine sulfonium cation.

Acknowledgement

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Plate 1.

Explanation of Figures

Histological sections of the aortae (*Aorta descendans thoracica* and *A. abdominalis*) after 60 days of the experiments: Stained with Sudan III. 100×.

1-4. Control lot; No sudanophilic sedimentation on *Tunica intima* is detectable.
5-8. CH lot; Atheromic denaturation of the aortae, *i.e.*, thick deposition of sudanophilic substance is observed. (Fed cholesterol 0.4 g/day, and injected NaI 200 mg every other day.)
9-12. MMSI lot; None or less depositions of sudanophilic substance are detectable. (Fed cholesterol 0.4 g/day, and injected MMSI 100 mg every other day.)

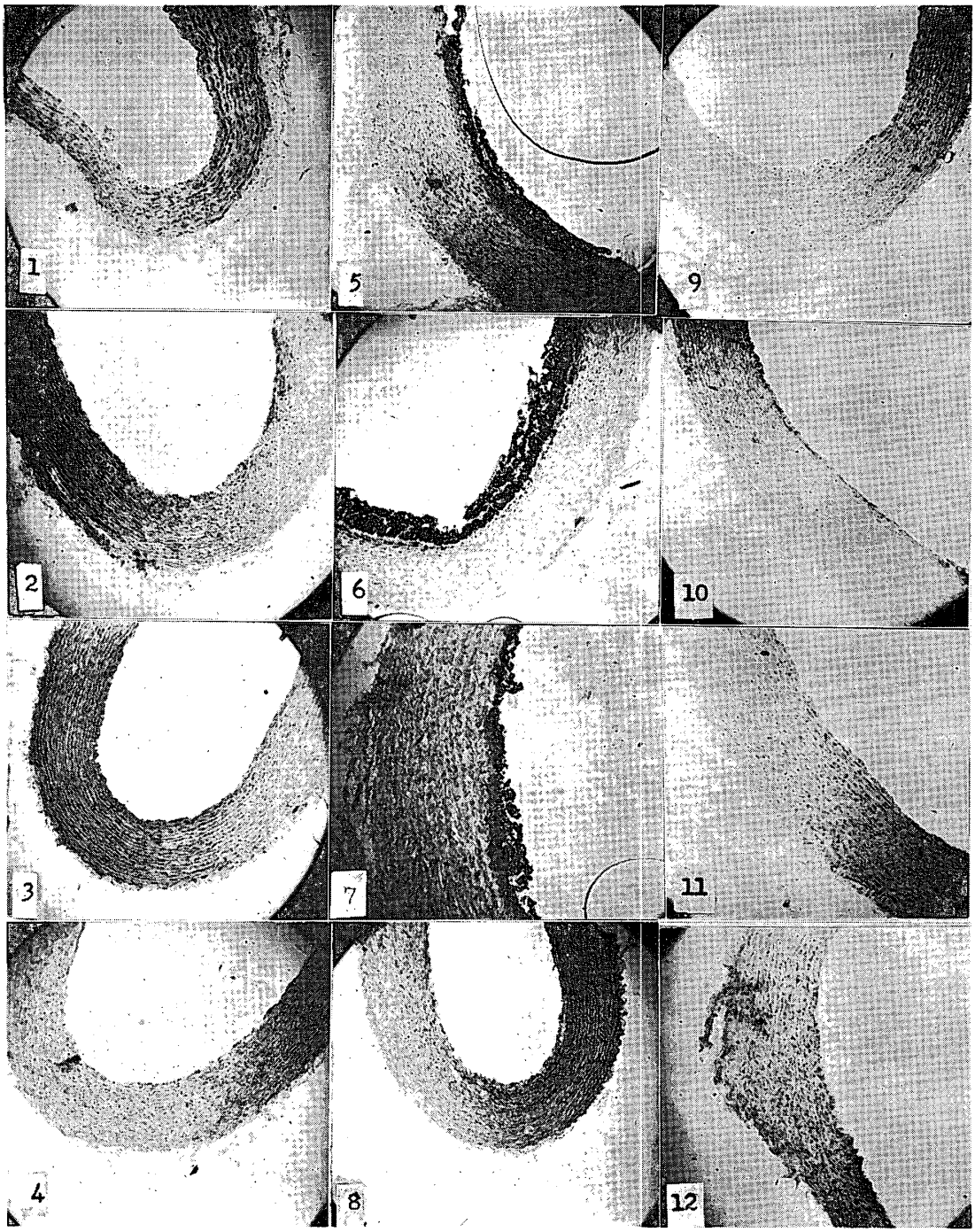
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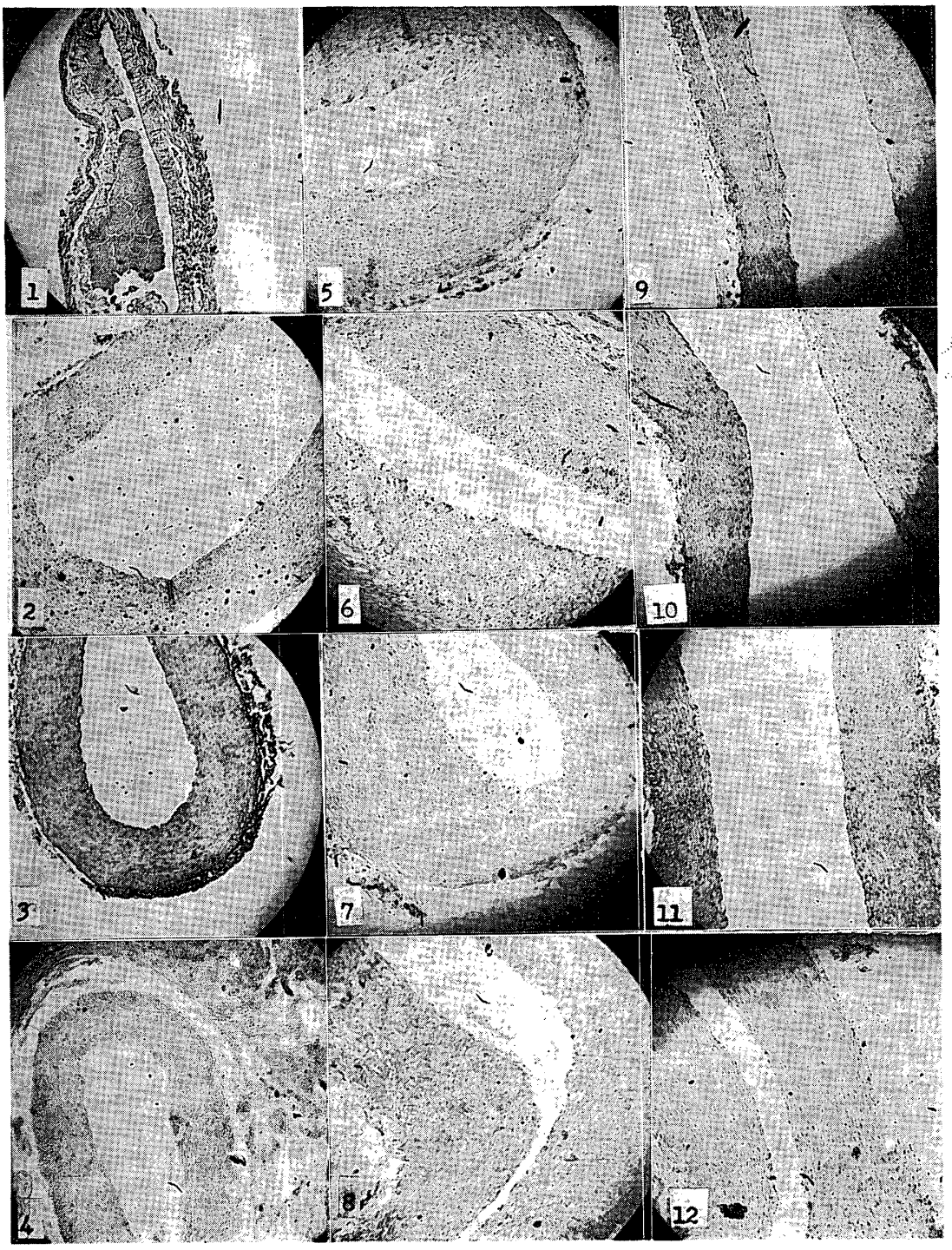
Plate 2.

Explanation of Figures

Histological sections of the aortae (*Aorta descendans thoracica* and *A. abdominalis*), after 60 days of the experiments: Stained with haematoxylin and eosin. 100×.

1-4. Control lot; No changes are detectable in *Tunica intima*.
5-8. CH lot; Atheromic denaturation of *Tunica intima* with highly hypertrophic alternation of the tissue is remarkable. (Fed cholesterol 0.4g/day, and injected NaI 200 mg every other day.)
9-12. MMSI lot; None or less hypertrophic alternations are detectable. (Fed cholesterol 0.4 g/day, and injected MMSI 100 mg every other day.)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