STUDIES ON EXPERIMENTAL HYPERCHOLESTEROLEMIA AND ATHEROSCLEROSIS

By

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Separate groups of rats and guineapigs were given cholesterol and vitamin C in addition to cholesterol for different periods. It was seen that in both the species of animals, vitamin C did not allow the increase in cholesterol level of blood, though there was no influence on the process of atherogenesis. Vitamin C also, to a certain extent, reduced the fatty metamorphasis in the liver. This finding suggests that Vitamin C can play a role in reducing hypercholesterolemia and thus may prevent the degenerative change in the blood vessels.

Atherosclerosis as described by Boyd (1962), Klotz (1943), Kimmelsteil (1943), Hirsch and Weinhouse (1943) and Vogel (1653) is a degenerative process of the intima of blood vessels. In the earlier stages of this process there is thickening and hyalinization of intima resulting in the loss of endothelial lining. In the later stages, there is a progressive increase of cholesterol, phosphatides and galactosides until atheroma is formed. Cholesterol is found in both forms, combined and free.

Gertler and Garn (1950) showed that the cause of atherosclerosis may be due to disturbances in cholsterol metabolism rather than increase in total plasma cholesterol concentration. Gofman (1950) and Sefik Kayaham (1960) showed higher SF 10-20 and increased b-lipoprotein in coronary disease.

Katz and Stamler (1953) and Gopalan and Ramnathan (1956) produced experimental atherosclerosis in dogs, chicks, geese, rats and monkeys by feeding them atherogenic diet. Chaikoff (1942) produced spontanous atherosclerosis by feeding them cholesterol. Functional changes in livers of guineapigs were shown by Chakravarti and Mukerji (1956) in addition to atherosclerotic changes. Booker *et al.* (1957), and Chakravarti and Mukerji (1957) noted that ascorbic acid and vitamin B_{12} prevented the rise of serum cholesterol in experimental hypercholesterolemia in rabbits. The present work was undertaken to show the effect of experimental hypercholesterolemia and vitamin C on the blood vessels and livers of rats and guineapigs.

METHODS

Adult albino rats and guineapigs formed the material for this work and each species was divided into three groups as follows :—

Rats :

Group I. Six female rats were kept on a stock diet (Control).

Group II. Six female rats were given the stock diet and also 1.5 per cent cholesterol mixed in wheat flour pellets.

Group III. Six female rats were given the stock diet, 1.5 per cent cholesterol and 30 mg vitamin C orally in water to each animal.

The average age of animals in each group was 5 to 7 months at the commencement of experiment.

Guineapigs :

Group I. Six adult male guineapigs were kept on the stock diet (Control).

Group II. Six adult male guineapigs were kept on the stock diet and also 1.5 per cent cholesterol orally.

Group III. Six male adult guineapigs were given the same diet as that of Group II, but in addition received 30 mg vitamin C in addition.

The average age of each group was 5 to 7 months.

The stock diet consisted of wheat flour 37.5 per cent Lucereine grass 42.0 per cent Bengal gram 17.0 per cent and Dalda 3.5 per cent; each rat and guineapig were getting 20 g to 30 g of stock diet daily respectively. Feeding was done in the morning and evening. Vitamin C was given to rats in water, care being taken that all the water was consumed by them. In guineapigs, it was administered by a pipette inserted into the mouth. The duration of vitamin C administration is given in Tables II, III, VI, and VII.

Estimation of Cholesterol.—Blood was taken from the heart by direct puncture through the chest and total cholesterol level in whole blood was estimated by *Bloor's method* i.e. the Leibermann Burchard reaction with acetic anhydride and sulphuric acid using Spectronic 20. Estimation of total cholesterol in whole blood was done at different periods in both the species of animals.

TABLE I

Showing cholesterol level and body weight of rats on different observations

Rat No	Age months	Body weight (G)	Initial cholesterol level (mg)	First observation			Second observation			Total days	Total Increase in cholesterol
				No. of days	Body weight (G)	Cholesterol (mg)	No. of days	Body weight (g)	Cholesterol (mg)		
1	6	195	100	53	200	105	32	200	100	85	0
2	7	220	110	53	220	98	32	214	100	85	−10
3	5	240	100	67	235	98	50	235	95	117	−5
4	6	205	90	83	203	100	40	200	120	123	30
5	6	232	98	62	230	95	61	235	95	123	3
6	6	200	120	82	205	124	40	210	125	122	5
Average	6	215.3	103	66.6	215.5	103.3	42.5	215.7	105.8	109.1	(+2.8)
Standard Error	0.26	7.45	4.28					6.51	5.39	7.70	

TABLE II

Showing cholesterol level and body weight of rats

Rat No	Age months	Body weight (G)	Initial cholesterol level (mg)	No. of days	Body weight (g)	Cholesterol (mg)	No. of days	Body weight (g)	Cholesterol (mg)	Total days	Total increase in cholesterol
7	5	200	135	50	230	177	28	235	202	78	67
8	6	190	140	52	255	208	57	250	174	109	34
9	5	215	105	49	250	150	70	234	200	119	95
10	5	275	100	49	305	183	59	310	190	108	90
11	5	127	100	39	180	105	57	180	180	96	80
12	6	230	105	96	215	170	22	270	170	118	65
13		250	100	30	266	110	88	265	180	118	82
Average	5.4	212.4	112.1	52.1	243.1	157.7	54.4	249.1	185.1	106.5	73.3
Standard error	0.2	17.99	6.62					15.12	4.72	5.67	7.75

TABLE III

Showing cholesterol level and body weight of rats on different observations

Rat No	Age months	Body weight	Initial cholestrol level (mg)	First observation			Second observation			Final observation			Total days	Total increase in Cholesterol
				No. of days	Body weight (g)	Cholesterol (mg)	No. of days	Body weight (g)	Cholestrol (mg)	No. of days	Body weight (g)	Cholesterol (mg)		
1	5	225	90	63	240	168	3	246	131	47	246	140	113	42
2	5	172	103	45	265	125	66	246	132	23	195	118	134	15
3	5	240	108	51	260	99	89	285	100	19	285	110	159	2
4	6.5	225	106	94	275	100	46	275	120	24	280	130	164	24
5	5	240	110	44	245	100	28	245	135	24	248	135	96	25
6	5	200	100	63	275	105	54	242	144	22	242	150	138	50
Avg.	5.3	217	102.8	60	260	116.1	47.5	256.5	127	26.5	249.3	130.5	134	26.3
S. E.	0.25	10.70	2.95								13.21	5.96	10.69	7.15

TABLE IV

Showing comparative increase in cholesterol and body weights of rats

Group	Rat No.	Initial cholesterol value (mg)	Total increase in cholesterol	% Increase in cholesterol	Initial body weight (g)	Total increase in body weight	% Increase in body weight
Group II stock diet and 1.5% cholesterol	7	135	67	49.6	200	35	17.5
	8	140	34	24.2	190	60	31.4
	9	105	95	90.4	215	19	8.8
	10	100	90	90.0	275	35	12.8
	11	100	80	80.0	127	53	41.7
	12	105	65	66.6	230	40	17.3
	13	100	82	82.0	250	15	6.0
Group III stock diet 1.5% cholesterol and 30 mg vit C	1	98	42	42.8	225	21	9.5
	2	103	15	14.5	172	23	13.3
	3	108	2	1.8	240	45	18.7
	4	106	14	14.8	225	50	22.2
	5	110	25	27.7	240	5	2.0
	6	100	50	50.0	200	42	21.0

TABLE V

Table showing cholesterol level and body weight of guineapigs on different observations

G. pig No	Age months	Initial body weight (g)	Initial cholesterol level (mg)	No. of days	Body weight (g)	First observation		Final observation		Total days	Total increase in cholesterol (mg)	Weight of lever
						Cholesterol (mg)	No. of days	Body weight (g)	Cholesterol (mg)			
1	7	600	95	62	598	100	33	605	100	95	0	23.5
2	7	630	120	63	632	115	40	632	118	105	10	24.5
3	6	590	100	80	600	112	41	592	98	121	— 5	25.8
4	6	610	98	80	615	95	29	612	100	109	—30	26.2
5	7	632	100	66	634	120	28	635	120	94	0	25.0
6	6	640	125	95	638	128	8	642	130	103	5	25.0
Average	6.5	617	106.3	74.3	619.4	111.6	29.8	619.7	111	104.1	6.6	25.0
S. E.	2.21	8.81	5.11					8.01	5.48	4.08	5.11	0.39

TABLE VI

Table showing cholesterol level and body weight of guineapigs on different observations

G. pig no	Age months	Initial body weight (g)	Initial cholesterol level (mg)	First observation			Second observation			Final observation			Total no. of days	Total increase in cholesterol (mg)	Weight of liver
				No. of days	Body weight (g)	Cholesterol (mg)	No. of days	Body weight (g)	Cholesterol (mg)	No. of days	Body weight (g)	Cholesterol (mg)			
1	7	613	65	59	655	106	28	755	200	55	755	280	142	215	—
2	7	760	100	59	810	180	28	752	215	55	825	240	142	140	37
3	6	641	105	59	690	200	28	700	255	79	600	500	165	395	35
4	5	591	130	58	640	162	28	685	182	92	540	266	178	136	38
5	7	600	100	16	610	200	11	615	320	81	630	320	168	220	32
6	6	435	100	15	435	380	—	—	—	—	—	—	15	280	—
Average	6.5	591.5	100	44.3	640	204.6	24.6	701.4	224.4	72.4	690.0	331.0	135.1	231.0	35.5
S.E.	0.33	33.48	8.47								42.81	46.5	27.12	39.57	1.32

[a] The animal died after first observation.

TABLE VII

Showing cholesterol level and body weight of guineapigs on different observations

G. Pig no	Age months	Body weight (g)	Initial cholesterol level	First observation			Second observation			Final observation			Total no. of days	Total increase in cholesterol	Weight of liver
				No. of days	Body weight (g)	Cholesterol (mg)	No. of days	Body weight (g)	Cholesterol (mg)	No. of days	Body weight (g)	Cholesterol (mg)			
7	6	650	94	57	670	112	54	800	130	30	850	175	141	81	—
8	6	640	80	53	685	112	9	670	132	75	700	160	137	80	—
9	7	450	96	53	560	100	35	720	150	63	720	164	151	68	30
10	7	475	96	44	600	100	35	790	160	53	792	157	132	79	40
11	7	700	85	29	738	110	59	787	162	28	800	170	115	85	43
12	6	660	90	29	670	200	59	715	220	28	730	220	115	130	350
Average	6.6	595.8	90.2	45.8	668.8	122.3	41.8	747	159.0	46.1	765.3	174.3	131.8	87.0	37.8
S.E.		0.21	43.09	2.66							23.56	9.52	5.90	8.88	2.6

TABLE VIII

Showing comparative increase in cholesterol, body weight and liver of guineapigs

Group	G. Pig no	Initial cholesterol value	Total increase in cholesterol	% Increase in cholesterol	Initial body weight (g)	Total increase body weight (g)	% In body weight	Average weight of liver (g)	Weight of liver on Postmortem	% Increase in weight liver
Group II	1	65	215	333	615	142	23.0	25	—	—
Stock diet	2	100	140	140	670	155	23.1	25	37	48
and	3	105	395	376	641	-41	6.3	25	35	42
1.5% Choles-	4	130	136	104	590	50	8.4	25	38	52
terol	5	100	220	220	600	15	2.5	25	32	28
	6	100	280	280	435	0	0.0	25	--	—
Group III	7	94	81	86	650	200	30.7	25	—	—
Stock diet	8	80	80	100	640	60	9.3	25	—	—
and	9	96	68	70	450	270	60.0	25	30	20
1.5% Choles-	10	96	79	82	475	317	84.5	25	40	60
terol	11	85	77	90	700	87	12.4	25	43	72
and	12	95	130	117	660	55	8.3	25	35	40
30 mg. vit. C										

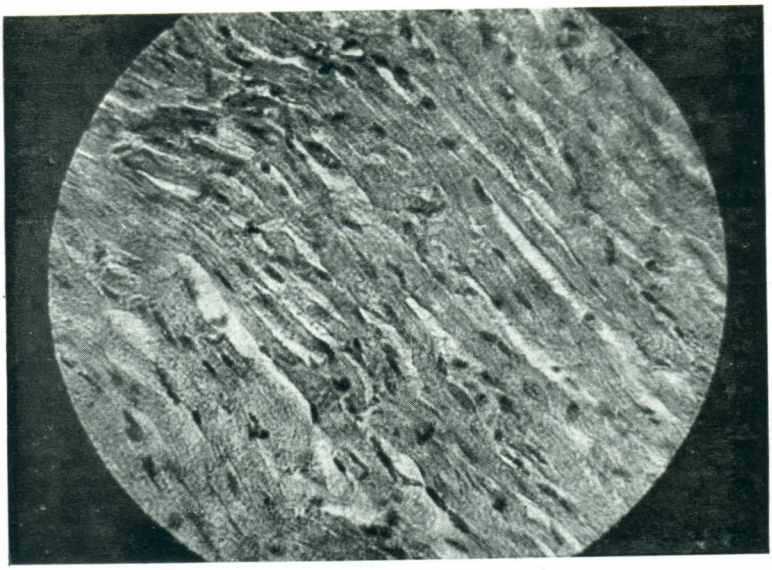

Fig. 1. Heart, rat. Control. H. E. Stain. Normal cardiac muscle fibres are seen. X600.

Fig. 2. Coronary artery, rat. Group II. H. E. Stain. Medial thickening of artery is seen. Heart muscle striations not well defined. X500.

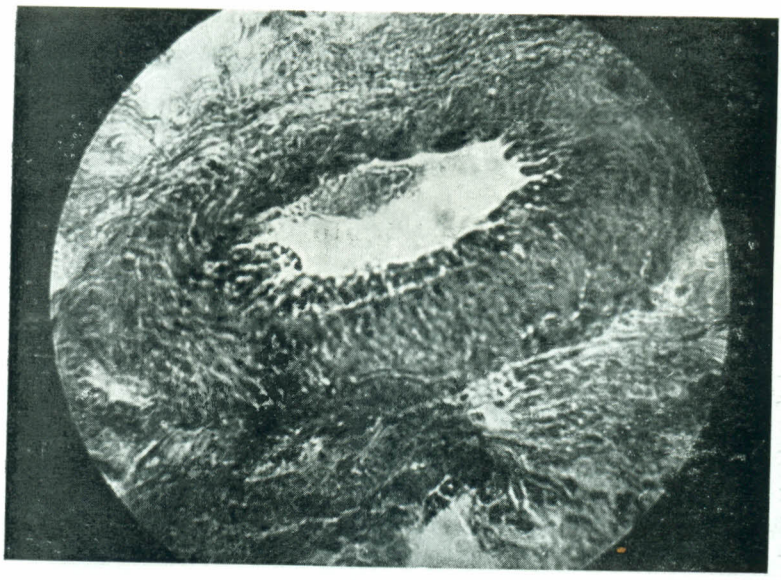

Fig. 3. Coronary artery, g. pig. Group III. H. E. Stain. Mainly medial thickening and slight intimal proliferation is seen. X500.

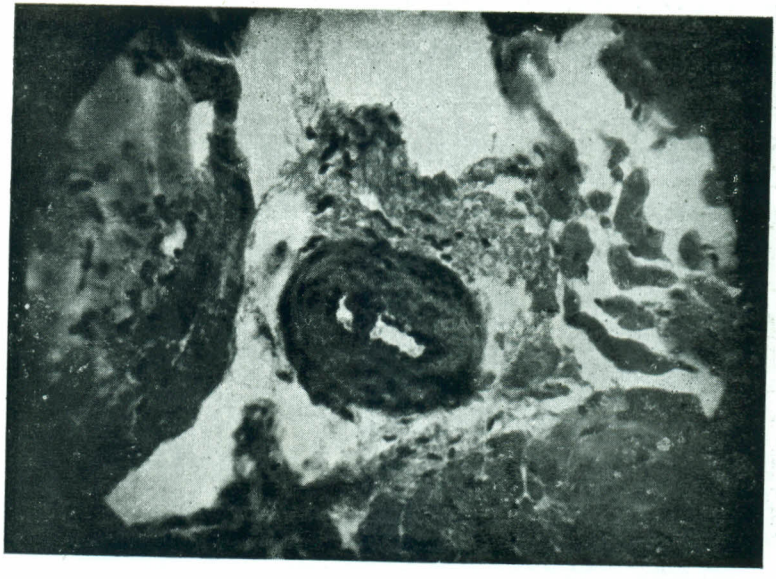

Fig. 4. Coronary artery, g. pig. Group III. H. E. Stain. Pronounced thickening of the wall of coronarary artery. X500.

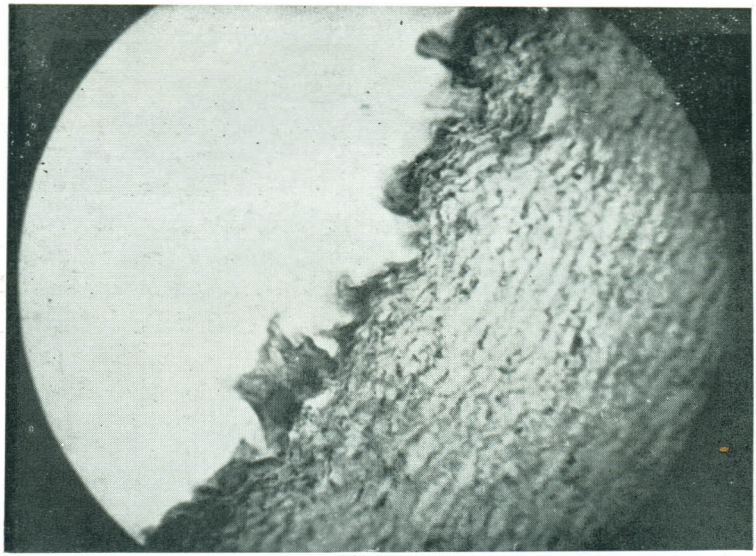

Fig. 5. Thoracic aorta, g. pig. Group II. Weigert's Stain. Intimal fibrous thickening is evident. X500.

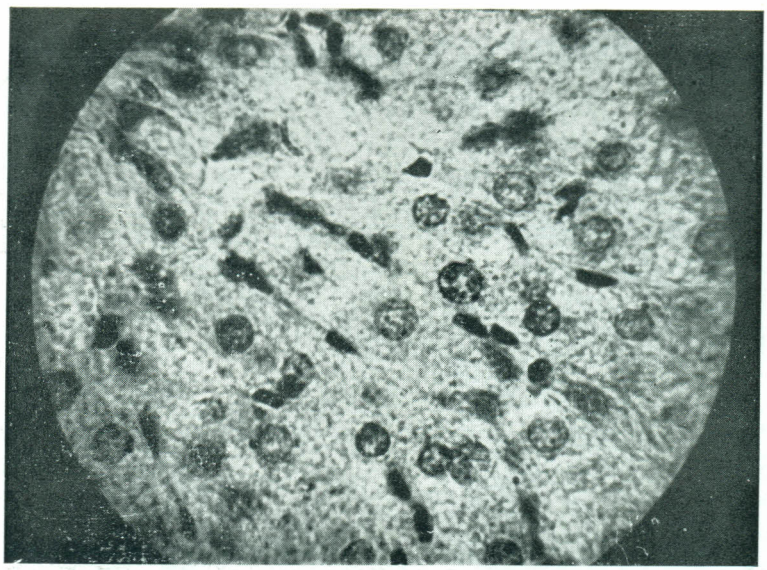

Fig. 6. Liver, g. pig Control H. E. Stain. Hepatic cells are seen in normal state. X1250.

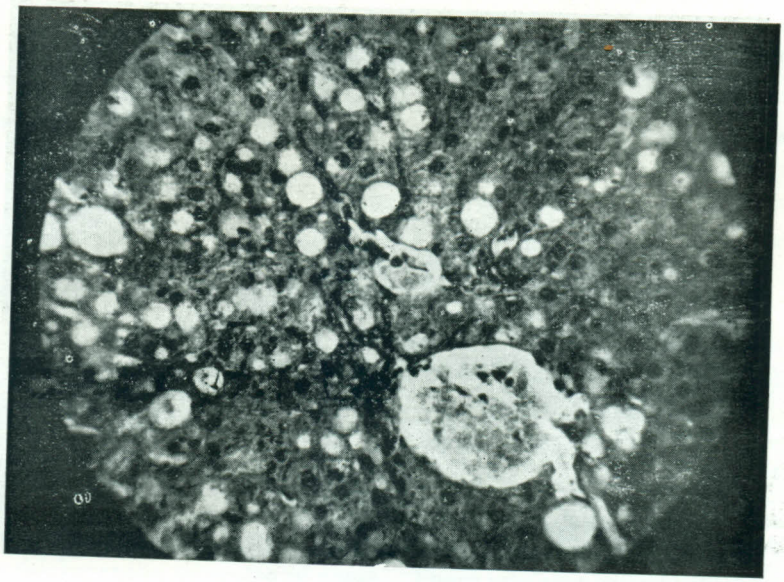

Fig. 7. Liver, g. pig. Group II. H. E. Stain. Many cells are fat loaded. Fatty metamorphosis is extending to center of lobule. X500.

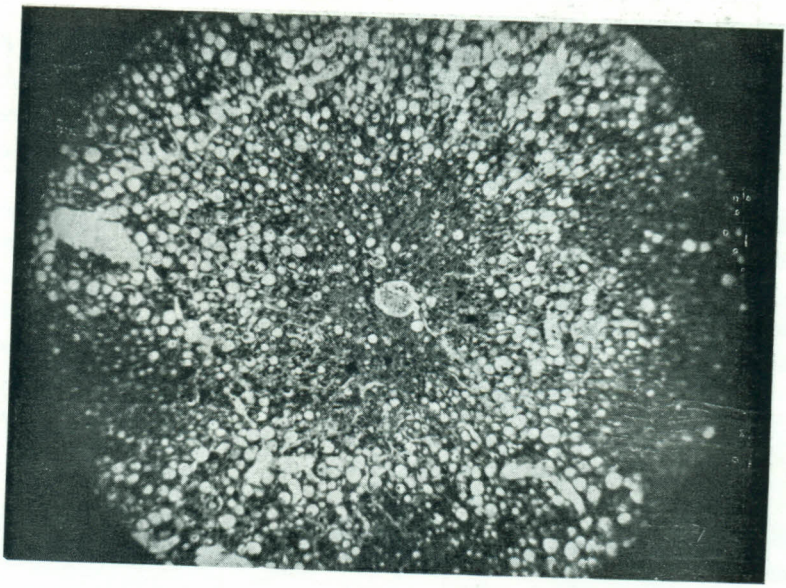

Fig. 8. Liver, g. pig. Group II. H. E. Stain. Liver lobule with fatty infiltration at the periphery and also extending center. X125.

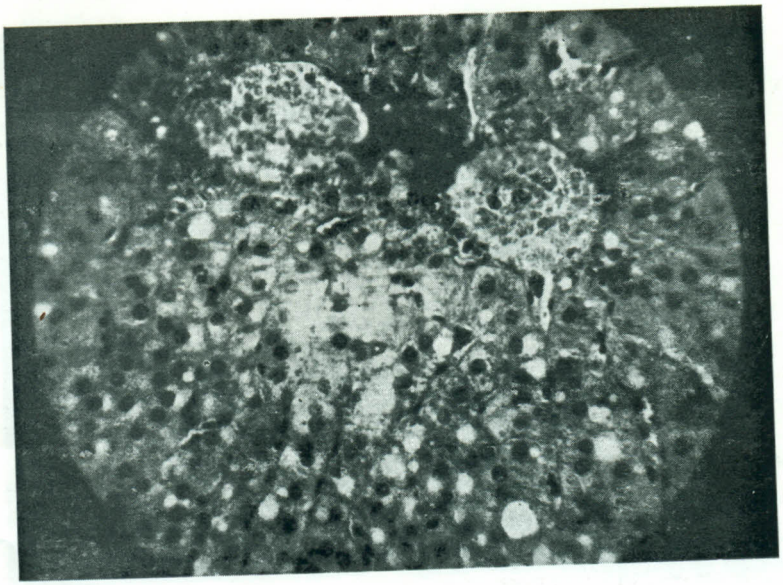

Fig. 9. Liver, g. pig. Group III. H. E. Stain. Fatty metamorphosis is confined to periphery of lobule, lever cells. Liver cells at the central part of lobule are normal. X500.

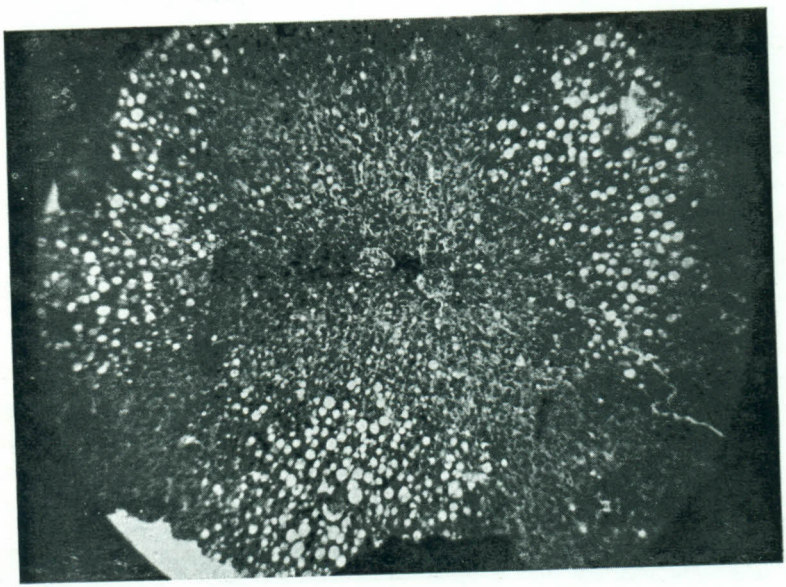

Fig. 10. Liver, g. pig. Group III. H. E. Stain. Central portion is having nomal cell but periphery is surrounded, by cells with fatty infiltration. X125.

Histological Findings.—Thoracic and abdominal aorta, heart and liver were fixed in formal-saline (5 per cent formaline) in 0.88 per cent saline with fragments of calcium carbonate. Paraffin blocks were prepared and were cut at 4 to 6 microns thick with a Spencer microtome.

Paraffin sections were stained with haematoxyline-eosin and von Gieson's stain.

RESULTS

Weights of rats of Group I and Group III (Table II and IV) increased, not due to increase in food intake but due to disturbed lipid metabolism, whereas the Group I (Table I) showed body weight of control animals. On postmortem, liver was found to be enlarged, though its naked eye appearance was normal. Heart was also enlarged with tortuous coronaries, aorta slightly thickened, though naked eye appearance of inner surface was normal.

Cholesterol level in Group II and III rats increased but increase in Group II was more than in Group III.

Striations in cardiac muscle fibres were found to be faint (Fig. 1 and 2). There was thickenening in media of aorta. There was also intimal thickening in some animals. Liver did not show any change.

Guineapigs also showed considerable increase in body weight in both the Groups (II and III) as compared to the body weight of Control Group I (Table V). On postmortem, liver was enormously enlarged and slightly yellowish. Heart seemed to be enlarged as compared to normal. Naked eye appearance of inner surface of aorta was normal. The cholesterol level in blood increased appreciably in Group II, but not so in the Group III (animals which were getting vitamin C in addition) Table V, VI, and VII.

Histological examination revealed intimal thickening and loss of endothelial lining in blood vessels (Fig. 3, 4 and 5). Liver showed gross lipid metamorphosis of second and third degree. In animals of Group II fatty metamorphosis was noted extending throughout the lobule (Fig. 7 and 8), whereas in animals of Group III (vitamin C fed) fatty metamorphosis was confined to the periphery of lobule (Fig. 9 and 10). These are histological appearances compared to normal pictures (Fig 6). Actual thickening of the wall or walls of blood vessels were not increased.

DISCUSSION

Administration of vitamin C reduced the rise of blood cholesterol level. This might be due to correction of fat metabolism or rapid disposal of cholesterol. There was fatty metamorphosis of second and third degree in the

liver of guineapigs, but none in the rats, and this was of reversible degree. Hepatic cellular degeneration was not seen. While fatty change was similar in both the Group II and III of guineapigs and appeared to progress from the periphery to central part of the lobule, this progressive change was less rapid in Group III guineapigs (vitamin C fed). This increase in body weight in both the species without any increase in food intake suggests disturbances in fat metabolism due to cholesterol feeding.

The changes noted in the blood vessels were mainly in thoracic aorta but very few in abdominal aorta There was medial fibrotic thickening of coronary vessels with narrowing of the lumen, both of which were favourable for occlusion or for prevention of compensatory dilatation in an emergency.

These findings show that there are no significant changes in the blood vessels in the rat, in spite of increase in blood cholesterol, supports the statement of certain workers that unlike guineapigs rats are resistant to atherosclerotic changes.

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