sumption. The difference in symptoms and lesions between turkeys and rats, however, indicates that there is some species difference not associated with age or feed consumption.

The turkey poult is highly susceptible to hock disorders as indicated by investigations of Scott(7), Hunt *et al.*(8) and Slinger *et al.* (9). While these researches showed that supplemental niacin, vit. E and phosphorus can prevent enlarged hock disorder under certain conditions it is apparent from the last two reports that the problem is a complex one. Hock disorder of uncertain etiology appears in many commercial flocks but usually in only a few birds.

While the turkey poult consuming low levels of BAPN develops abnormalities similar to those which appear under field conditions, it is impossible to say whether or not BAPN is a causative agent in field cases. If BAPN is involved, its source under practical conditions is yet to be located.

Summary. (1) Beta aminopropionitrile hydrochloride was toxic to turkey poults at levels as low as 0.01% of diet. (2) Paralysis, degeneration of anterior motor neurons and growth depression were the chief abnormalities observed at 0.25% and 0.125% while pericardial and pulmonary hemorrhage, ruptured aortas, leg and toe deformities, and growth depression were the primary symptoms at lower levels. (3) Preliminary attempts to alter severity of symptoms by reducing the methionine level or by adding vit. B_{12} or a high level of all B vitamins were not very successful, but further investigation of the B vitamins appears to be justified.

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Lipid Distribution and Metabolism in Two Areas of Aorta of Normal and Cholesterol-fed Rabbits. (22858)

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When cholesterol-fed rabbits are killed at successive periods gross, discrete atherosclerotic lesions appear first in the aortic arch and only much later is the rest of the aorta similarly affected. We have found a high incidence of atherosclerosis in the aortic arch only in rabbits on a high cholesterol diet for 60-70 days but rarely were lesions seen in aorta of rabbits only 40 days on the diet. Duff and McMillan(1) reviewed evidence indicating an importance of "local factors that operate in the vessel wall to determine the localization and to influence the subsequent development of individual atherosclerotic lesions." With due cognizance of the element of time as a factor in the development of atheromatosis, these observations raise the question of the nature of the factor(s) responsible for the localization and site of appearance of the earliest atherosclerotic lesions in the rabbits. The foregoing prompted us to study the distribution of lipids and phospholipid metabolism in 2 areas of the aorta, namely, the arch and the descending limb of the aorta of rabbits on the usual laboratory diet and rabbits kept for 40 and 70 days on a

TABLE I. Lipid Distribution and Specific Activities of Phospholipid in Aortic Arch and the Descending Aorta of Control and Cholesterol-Fed Rabbits for 2 Time-Periods.

Item	Aortic arch			Descending aorta			"P" value of diff. between aortic sections		
	Diet	Cholesterol-suppl. diet*		Diet	Cholesterol-suppl. diet*				
	a. Controls	b. 40 days	c. 70 days	a. Controls	b. 40 days	c. 70 days	a.	b.	c.
Part A	(15)	(5)	(6)	(15)	(5)	(6)			
Total lipid	10.16±.72	10.99 ±1.81	9.31 ±1.01	6.93±.76	5.96±.54	5.38±.91	‡	‡	‡
Phospholipid	.91±.05	.81 ± .09	.88 ± .13	.83±.04	.62±.07†	.74±.05	ns	ns	ns
Total chol.	.18±.02	.18 ± .03	.63 ± .22†(†)	.15±.01	.16±.01	.26±.04†	ns	ns	ns
Free "	.13±.01	.10 ± .04	.33 ± .25	.12±.01	.13±.01	.19±.01‡(‡)	ns	ns	ns
Ester "	.05±.01	.08 ± .01	.30 ± .14	.03±.006	.03±.01	.08±.03	ns	‡	ns
Neutral fat	9.03±.74	9.94 ±1.81	7.57 ±1.12	5.93±.75	5.14±.75	4.32±.83	‡	‡	‡
Part B	(12)	(4)	(4)	(12)	(4)	(4)			
S.A. $\times 10^3$	2.19±.18	2.77 ± .28	3.08 ± .34†	3.15±.31	3.62±.43	2.85±.67	†	ns	ns

* See text for description. Atheromata in aortic arch only of rabbits 70 days on high cholesterol diet; no atherosclerosis in group fed 40 days. All values are mean ± S.E. Figures in parentheses are No. of animals in each group. S.A. (specific activity) is % of inj. P^{32}/mg lipid phosphorus.

Significant differences between means of controls and each exp. group are indicated as follows: † "P" value = <.05 >.01. ‡ "P" values of 0.01 or less. Significant differences between means of two cholesterol-fed groups are indicated by "P" value in parenthesis.

high cholesterol diet. To allow an assessment of the relative status of lipid metabolism in the groups studied, lipid distribution and phospholipid metabolism of plasma and liver also were determined in these animals.

Materials and methods. New Zealand white female rabbits were used. At 10 a.m. and 4 p.m. each day of the experiment a rabbit pellet food (70 g), tap water and 10 g of shredded carrots were given to half the total number of rabbits under investigation while the remaining number of animals were given the same provisions except that the carrot portion was thoroughly mixed with 1 g of cholesterol (crystalline cholesterin, U.S.P., Merck*). This dietary regime was readily and entirely consumed by the rabbits each day for 40 and 70 days. Animals fed the normal (group *a*) and the high-cholesterol diet for the 2 periods (groups *b* and *c*) were killed on the same day; tissues for chemical and radioactivity analyses were obtained within a 10-day period from animals of all groups. Some rabbits of each group received by vein approximately 0.5 mc of radioactive phosphate (P^{32}) on the final day and killed 6 hours later. A large sample of heart blood was obtained and the animal then given an overdose of EVIPAL (n-methyl-cyclo-hexenyl-methyl-barbituric acid). The whole liver and aorta were removed; and the latter then cleaned of adherent tissue. The aorta then was divided into 2 sections: (a) the aortic arch—3.0 cm of vessel from the aortic ring; and (b) the remainder of the aorta. The 2 aortic sections were graded separately by visual judgment for severity of atherosclerosis from normal (zero) to most severe (4 plus). Chemical analyses of tissue lipid fractions and radioactivity (P^{32}) measurements of tissue phospholipid- and acid soluble-phosphorus were done by procedures previously reported(2) from this laboratory. Total lipid was calculated from weight of residue after evaporation of petroleum ether extract and drying to constant weight; lipid and acid soluble phosphorus by the methods of Youngsburg and Youngsburg(3) and Fiske and SubbaRow(4); cholesterol by the method of Schoenheimer and Sperry(5); and neutral fat was calculated by subtracting the sum of the values for phospholipid, free cholesterol and cholesterol oleate from the total lipid value. Measurements of radioactivity were done on duplicate samples of suitable aliquots for a minimum of 4000 counts employing a Tracerlab Autoscaler with

* We wish to thank Frederick K. Heath, for generous supply of cholesterol (Cholesterin, U.S.P., Merck).

TABLE II. Lipids Distribution in Plasma and Liver and Phospholipid Synthesis in Rabbits on a High Cholesterol Diet for 2 Time-Periods.

Item	Diet a. Controls	Cholesterol-suppl. diet* b. 40 days	Cholesterol-suppl. diet* c. 70 days	Diet a. Controls	Cholesterol-suppl. diet* b. 40 days	Cholesterol-suppl. diet* c. 70 days
Part A	Plasma lipids (mg/100 cc)			Liver lipids (g/100 g of tissue)		
	(15)	(5)	(6)	(15)	(5)	(6)
Total lipid	341.0±17.7	1656.0±385.3§	2448.0±553.2§	4.82±.11	5.52±.36	6.82±.49
Phospholipid	124.2± 8.9	319.6± 39.3§	453.2±103.2§	4.01±.14	3.90±.16	4.21±.14
Total chol.	82.3± 5.7	797.3±231.8§	1154.2±214.6§	.28±.007	.65±.13§	1.39±.28§(‡)
Free "	27.3± 1.6	206.9± 66.3‡	322.1± 55.6§	.24±.006	.33±.03§	.41±.06§
Ester "	55.0± 4.2	590.5±196.6‡	832.1±159.8§	.04±.006	.32±.11‡	.98±.23§(‡)
Neutral fat	94.7± 9.2	125.2± 60.7	232.9± 54.8‡	.51±.05	.78±.08‡	.57±.14
Part B	Summarized radioactivity data of plasma and liver phospholipid					
	Plasma phospholipid-phosphorus			Liver phospholipid-phosphorus		
	(12)	(4)	(4)	(12)	(4)	(4)
% I.D.†	10.89±1.01	26.43±1.85 §	32.10±3.15 §	10.11±1.58	12.09±1.83	20.36±3.97‡(‡)
S.A. × 10^3	2.42± .12	2.24± .28	2.22± .73	6.43± .36	8.00±1.11	12.23±2.83‡

* See text for description. Atherosclerosis found in aortic arch only in rabbits fed cholesterol diet 70 days; no atheromata in rabbits fed 40 days high cholesterol diet.

† % of inj. P^{32} incorporated in phospholipid, disintegrations/min.; for plasma expressed/cc × 10^5 and for liver expressed/g × 10^3.

Significant differences between means of control and exp. groups shown as follows:

‡ "P" value = <.05 >.01. § "P" value = <.01. Significant difference between two groups fed cholesterol diet indicated by "P" value shown in parenthesis.

S.A. (specific activity) is % of inj. P^{32}/mg lipid phosphorus. All values are mean ± S.E. Figures in parentheses indicate No. of animals in each group.

a Victoreen No. 1B85 Geiger tube.

Results. The lipid distribution and phospholipid specific activities of the arch and descending limb of aorta of normal and cholesterol-fed groups are presented in Table I. The mean weights of the two areas of aorta from the different groups of rabbits were similar. The aortic arch of normal-diet rabbits weighed .235 g (range .190-.310 g) and that of groups *b* and *c* weighed .214 g and .252 g, respectively. The descending limb of the aorta of normal-diet rabbits weighed .318 g (range .258-.420 g) and that of the 2 cholesterol-fed groups weighed .332 g and .376 g, respectively. In agreement with the signs of gross atherosclerosis detected in the 3 groups of animals, cholesterol concentrations were significantly elevated in the 2 aortic areas only in rabbits on the high-cholesterol diet for 70 days.

The results of special interest with regard to aortic lipid constitution may be seen from the comparisons of the concentrations of the different lipid fractions of the arch and the descending limb of the aorta in the same group of rabbits. The results show that total lipid and neutral fat concentrations in the descending limb of aorta were significantly less than that of the aortic arch in each of the groups. Further, the radioactivity data indicate that the rates of phospholipid synthesis in the 2 areas of aorta were significantly different in the normal-diet rabbits. However, this was not the case for the groups on the high-cholesterol diet. Specific activities of phospholipid of the aortic arch and the descending limb of aorta were similar for both cholesterol-fed groups. It may be seen that the latter findings were due to an increased rate of synthesis of phospholipid in the aortic arch, particularly in the rabbits of group *c*. This kind of change in aortic phospholipid metabolism was reported(6,7) to be associated with cholesterol deposition and the development of overt atherosclerosis. Thus, the distinction in rates of synthesis of phospholipid apparent in the aortic arch and descending limb of aorta of normal rabbits was eliminated with the development of gross atheromatous lesions in the longer-term cholesterol-fed rabbits.

The chemical and radioactivity data of

TABLE III. Plasma Lipids Relationships of Control and Cholesterol-Fed Rabbits for 2 Time-Periods.

Ratios	Diet a. Controls	Cholesterol-suppl. diet* b. 40 days	Cholesterol-suppl. diet* c. 70 days
	(15)	(5)	(6)
Free cholesterol/Total cholesterol	.34 ± .009	.26 ± .004§	.28 ± .008§(‡)
Total cholesterol/Phospholipid	.70 ± .05	2.34 ± .58 §	3.02 ± .59 §
Phospholipid/Neutral fat	1.49 ± .18	2.33 ± .79	2.63 ± .78
Total cholesterol/Neutral fat	1.00 ± .13	4.64 ±1.17 §	5.92 ±1.11 §

*‡§ See legend of Table II.

plasma and liver and plasma lipid relationships of the 3 groups of rabbits are presented in Tables II and III. Plasma cholesterol concentrations and phospholipid levels of groups *b* and *c* were not statistically different, but these lipids were 8 and 4 times greater, respectively, than the plasma levels of normal-diet rabbits. Further, the data presented in Table III show that plasma lipid relationships were not different in both cholesterol-fed groups with the exception of the ratio of free cholesterol/total cholesterol, while in all instances but one the ratios of plasma lipid fractions of both cholesterol-fed groups were markedly different from the plasma lipid relationships of the rabbits on the normal diet. The radioactivity data also show that plasma phospholipid synthesis was accelerated to a similar extent in rabbits 40 and 70 days on the cholesterol diet and in both groups the rate of formation of this lipid was considerably greater than that of the controls. On the other hand, the chemical and radioactivity analyses of liver lipid constitution show that total and ester cholesterol concentration and liver phospholipid synthesis were significantly greater in rabbits of group *c* than those on the cholesterol-supplemented diet for only 40 days.

Discussion. The 2 groups of rabbits on the cholesterol-supplemented diet were distinguished from each other with respect to the duration of the regime and also with regard to the presence of atherosclerosis. Only one rabbit of group *b* had grossly visible plaques in the aortic arch; this animal was discarded from the group for presentation of the data. On the other hand, every rabbit fed the high-cholesterol diet for 70 days presented gross lesions in the aortic arch only which were graded as 2-4 plus in severity. Aorta of rabbits on the regular laboratory food pellets alone were entirely free of gross signs of atherosclerosis.

Our findings show that the hypercholesterolemia, altered plasma lipid relationships and increased synthesis of plasma phospholipid in rabbits after 40 days on a high-cholesterol diet were not essentially different from that of rabbits on the same diet for 70 days and, in fact, were the same as the results obtained in rabbits on this dietary regime for 4 months (8). Wang *et al.*(9) found in cholesterol-fed rabbits a steady rise of blood cholesterol level until the 40th day of the diet and then a relatively maintained level of this lipid for the next 4 months. Since aortic atherosclerosis in cholesterol-fed rabbits is rarely evident before 60 days of this dietary regime, the evidence indicates that conditions favoring the development of atherosclerosis may exist for some time before the eventual evolution of gross lesions.

One implication of the time-lapse in the development of atherosclerosis is that factors (? metabolic) in the aortic wall are significantly related to the deposition of lipids in this tissue. It is known that in the cholesterol-fed rabbit discrete, local lesions develop first in the ascending limb of the aortic arch and these later enlarge, appear to coalesce, and progress down the aorta. In discussing these morphologic features, Duff and McMillan(1) suggest that local factors "operate in the vessel wall to determine the localization and to influence the subsequent development of individual atherosclerotic lesions." In this study evidence was presented that the neutral fat content of the aortic arch of normal rabbits was different from that of the remainder

of the aorta and the rate of phospholipid synthesis in the arch was significantly less than that of the descending limb of the aorta. It is of interest that Werthessen *et al.*(10) reported a concentration gradient of cholesterol over the length of the calf's aorta. Lower concentrations of cholesterol were found in the arch than at more distal sections of the aorta. Although these facts suggest some fundamental metabolic differences between certain areas of the aorta, their possible relationship to the pathogenesis of atherosclerosis must await further studies of arterial metabolism.

Summary. Lipid partition and phospholipid metabolism of the aortic arch and descending limb of aorta, plasma and liver were determined in groups of rabbits on a normal diet and 40 and 70 days on a cholesterol-supplemented diet. In all groups, the descending limb of aorta had significantly lower total lipid, primarily due to decreased neutral fat concentration, compared with the aortic arch. In normal rabbits the specific activity of phospholipid in the descending aorta was significantly greater than that of the aortic arch. However, this distinction in phospholipid synthesis between the two areas of aorta was not found in the groups of rabbits on the high-cholesterol diet. The radioactivity data indicated this was due to an increased rate of formation of phospholipid in the aortic arch of rabbits fed the cholesterol-supplemented diet, particularly those rabbits on the diet for 70 days, and probably was related to the development of atheromatous lesions in the arch only of these rabbits. The results were discussed in relation to the general problem of local factors in the arterial wall influencing the localization and subsequent development of atherosclerotic lesions.

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Induced Resistance in Mice to Intravenous Toxicity of Influenza Virus.* (22859)

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Protection of intact animals from rapidly appearing damage, usually referred to as toxicity, which follows inoculation with massive doses of influenza viruses has been reported (1-5), but attention has largely been directed to toxicity following intracerebral injection in mice, to lung consolidation caused by nasal installation of certain strains into mice, and to the pyrogenic effect of intravenous injection into rabbits. Beyond the initial description of intravenous toxicity of influenza virus in mice(6), little study has been applied to this phenomenon. When it was found, during studies of factors modifying host reactions to

* Opinions expressed in this article are those of the authors and do not necessarily reflect the views of the Navy Department.

† Some data in this report were included in thesis submitted to Graduate School of University of Wisconsin in partial fulfillment of requirements for degree of Doctor of Philosophy. Present address: Indiana University Medical Center, Indianapolis.