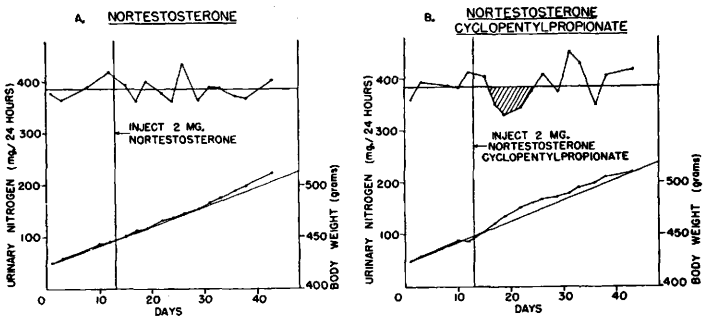

FIG. 4. Urinary total nitrogen and body weight changes of rats given single injections of (A) nortestosterone or (B) NTCP. Shaded area indicates nitrogen retention.

thylandrostenediol or androstanolone when given at 1/8 the dose of the 2 latter compounds. 3. 19-nortestosterone cyclopentylpropionate in a single injection of 2 mg produced retention of N lasting several days; unesterified nortestosterone, similarly given, did not result in significant N retention.

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Effect of Aluminum Hydroxide Gels on Experimental Hypercholesterolemia and Atheromatosis in Chicks.* (21087)

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It has been reported that aluminum hydroxide gel reduced cholesterolemia, delayed the onset of atheromatosis, and produced muscular weakness in chicks fed cholesterol(1,2). These effects were attributed to possible adsorption phenomena by alumina gel in the small intestine. The significance of these observations made it of interest to further investigate them.

Method. The regimen for producing atheromatosis was that of Dauber and Katz(3). One hundred White Leghorn cockerels certified to be free of salmonella infection were

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TABLE I. Experimental Design and Weight Changes (9 Groups).

Treatment group	No. of chicks	Treatment	Wt (g) Initial	Wt (g) Final
A	12	CD*	434	1601
B	12	+ 5% CSO	454	1678
C	10	CSO + 0.5% Ch	405	1647
D	10	+ 3% AHG (Am)	478	1320
E	10	+ Ch + 3% AHG (Am)	438	1124
F	10	+ 3% AHG (B-Gel)	499	1621
G	10	+ Ch + 3% AHG (B-Gel)	463	1585
H	12	+ Ch + 0.2% boric acid	468	1643
J	10	+ 2 mg CA† IM/day	431	1483

* CD = Control diet; CSO = Cotton seed oil; Ch = Cholesterol; AHG = Aluminum hydroxide gel (Am = Amphojel®); CA = Cortisone acetate.
† Cortone® Merck.

divided into 9 groups according to the outline in Table I. The reasons for including some of the diets are obvious. Cortisone and boric acid were also included; the former because of the reported effects on blood cholesterol, and the latter because of its tendency to accumulate in fat depots(4) and its possible effects on fat metabolism(5,6). The control diet consisted of Purina chick starter mash to which was added 2.5% cod liver oil and 0.1% chick granite grit. The cholesterol was suspended in cottonseed oil† and then mixed into the control diet. B gel‡ and Amphojel®,‡ which is a mixture of B gel with another alumina gel, were incorporated into the basic diet and passed through a fine screen to remove lumps. The chicks were housed in wire cages and given food and water *ad libitum*. The daily food consumption and weekly weight changes were recorded. Total cholesterol levels were determined weekly on blood obtained from the alar vein by a modification of Bloor's method(7). To facilitate sampling each group was divided into 2 equal subgroups. The blood samples within each subgroup were pooled for cholesterol determinations. The values for the 2 subgroups were averaged. At the end of 15 weeks the chicks were decapitated and exsanguinated. Heart, lungs, liver, kidneys, spleen, pancreas, adrenals, thyroids, testes, and parts of the small intestine were removed. The organs were weighed, fixed in formalin, and prepared for histological examination. Relative organ weights were compared statistically by analysis of variance for unpaired data(8). The aorta was freed to the bifurcation and cut at the level of the last rib; the thoracic portion was severed at the heart and the abdominal portion at the level of the renal arteries. The sections were mounted on corks and the intima stained with oil Red O according to the method of Lilli(9). They were graded without prior knowledge of treatment. The gross grading was correlated with the microscopic grading. Frozen sections stained with oil Red O from representative levels were taken at random to prevent identification of labels and graded 1 to 4. Grades 1 and 2 represent small and moderate amounts respectively of fat-stained material without displacement of the endothelium; grade 3, plaques with irregular elevations of the endothelium; and grade 4, ulcerated plaques.

Results. Effect of treatment on hypercholesterolemia. All groups on the cholesterol diet developed hypercholesterolemia after 2 weeks of treatment. This persisted throughout the experiment except in the group receiving Amphojel® and cholesterol, (Table II) in which the levels returned to normal at the fourth week and remained in this range until the treatment was altered. The failure to maintain hypercholesterolemia was preceded by decreased food consumption which eventually resulted in inanition with the clinical signs of muscular weakness, diarrhea, and small combs. Similar symptoms were also seen in the group receiving only Amphojel®. In the ninth week the regimen for both groups was changed so that they received the basal

† Puritan Brand—manufactured by Procter and Gamble, Inc.

‡ Manufactured by Wyeth Laboratories, Inc.

TABLE II. Effect of Treatment on Total Blood Cholesterol Levels and Food Consumption (9 Groups).

	Week of experiment														
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
T.B.C.	67	68	67	69	64	60	53	61	82	82	75	59	81	64	77	77
F.C.	.5	.7	.9	.9	.9	.9	.9	.9	1.0	1.2	1.3	1.5	1.8	2.0	2.1	2.3
T.B.C.	69	68	76	58	60	61	48	60	92	88	75	63	80	73	87	73
F.C.	.5	.7	.9	.9	.9	.9	1.0	1.0	1.1	1.3	1.4	1.6	1.8	2.1	2.2	2.4
T.B.C.	69	77	131	111	98	126	95	119	173	172	138	105	114	110	120	96
F.C.	.6	.7	1.1	1.1	1.2	1.2	1.3	1.3	1.5	1.7	1.9	1.9	2.1	2.0	2.1	2.0
T.B.C.	86	54	70	56	54	49	39	46	55	88	89	96	85	74	88	98
F.C.	.7	.8	1.0	.9	.8	.7	.7	.5	.4	.8	.9	.9	1.2	1.3	1.4	1.4
T.B.C.	77	105	172	149	86	92	66	64	58	85	139	98	113	112	126	172
F.C.	.7	.8	1.0	1.0	.9	.8	.8	.5	.4	.5	.9	1.0	1.2	1.2	1.4	1.6
T.B.C.	85	58	70	53	59	52	42	47	67	92	114	83	84	70	98	98
F.C.	.7	.8	1.1	1.1	1.1	1.2	1.2	1.2	1.3	1.4	1.4	1.4	1.5	2.2	2.3	2.3
T.B.C.	74	66	214	119	129	161	135	112	194	201	189	125	116	117	138	126
F.C.	.5	.5	.9	.9	.8	1.0	.9	1.0	1.0	1.3	1.3	1.5	1.7	1.8	1.9	1.8
T.B.C.	80	134	196	151	153	160	124	141	194	201	189	125	116	117	138	126
F.C.	.5	.6	.9	.9	.9	.9	.9	.9	1.0	1.2	1.4	1.4	1.6	1.7	1.7	1.9
T.B.C.	69	93	137	124	114	117	92	118	199	172	124	104	121	119	138	136
F.C.	.6	.8	1.0	1.1	1.2	1.2	1.3	1.3	1.4	1.4	1.5	1.5	1.8	1.9	2.1	2.2

T.B.C. = Total blood cholesterol, mg %. F.C. = Food consumption, kg/bird/wk.

diet for 2 consecutive days per week, resulting in the disappearance of all symptoms of inanition and accompanied by persistent hypercholesterolemia in group E.

Changes in the cardiovascular system. With the exception of group H all cholesterol-fed chicks showed a significant decrease in relative heart weights (g/kg body weight). There was no significant difference in the heart weight of the boric acid-cholesterol and the control groups. The results of the microscopic grading of aortic lipid content are found in Table III. There was a high degree of correlation between the microscopic and gross grading. Lesions were seen in the control chicks; cholesterol feeding resulted in a marked increase in severity of lesions. Neither of the alumina gels, boric acid nor cortisone acetate lessen the atheromata induced by the cholesterol feeding.

Gross and histological changes exclusive of the cardiovascular system. A significant increase in weight and fat deposits were seen in the liver of all chicks receiving cholesterol. There was also some increased fat deposition in the spleen, kidneys, adrenals, and pancreas of the animals receiving cholesterol. Chicks receiving Amphojel® had retarded development of the testes which was associated with decreased comb weights. Sections of the combs from the boric acid-treated group stained by the Ritter-Oleson(10) method showed decreased mucopolysaccharides. Boric acid administration also resulted in the appearance of dark brown subcutaneous fat which appeared normal histologically.

Discussion. Our observations that alumina gel *per se* did not prevent hypercholesterolemia or atheromatosis in cholesterol-fed chicks do not agree with those of Rodbard, Bolene, Peck, and Katz(1), and Rodbard and Bolene-Williams(2). In chicks receiving only the cholesterol diet 50% of the lesions were grades 1 and 2; those receiving alumina gel and cholesterol 75% of the lesions were grade 3 and 4. Our data indicate that the lower cholesterol values in group E were the result of decreased food consumption rather than to an adsorption phenomenon in the small intestine. These findings agree with those of Horlick and Katz(11) who demonstrated that the

TABLE III. Degree of Atheromatosis in Aortas (%). 9 groups.

Thoracic					Abdominal				
0	+	++	+++	++++	0	+	++	+++	++++
55	46					64	27	9	
42	58				50	50			
50	38	13				13	38	25	25
	100					83	16		
		60	20	20				60	40
	44	56			67	33			
		17	42	42		17	25	50	8
	27	18	36	9		27	27	18	27
		44	33	22		22	22	44	11

blood level of cholesterol in chicks was proportional to the cholesterol intake.

The muscular weakness syndrome(2) is commonly seen in chicks suffering from malnutrition(12) and may be a manifestation of encephalomalacia produced by inadequate diets, particularly those with vitamin deficiency and 2% fish oil(15). Our data indicate that the normal cholesterol values and the symptoms of this syndrome seen in the Amphojel®-cholesterol treated chicks were due to decreased food consumption rather than to a systemic effect of aluminum; from the ninth week on when the food intake became adequate no symptoms were present although the aluminum ingestion was increased 2-fold. The groups receiving B gel did not reject the diet throughout the experiment and did not develop the paralysis throughout the prolonged feeding of large amounts of aluminum.

Our chicks did not develop hypercholesterolemia as response to stress (inanition) which differs from the response of rabbits and humans(7,13,14). The significance of the findings that boric acid prevented the decreased heart weight that results from the administration of cholesterol in chicks cannot be evaluated from the present work; neither can the apparent decrease of mucopolysaccharides in the comb nor the brown color of the subcutaneous fat. The latter may be a confirmation of earlier reports(5,6).

Summary. 1. As long as the diet was ingested the addition of 3% aluminum hydroxide gel did not prevent hypercholesterolemia or atheromatosis in cholesterol-fed chicks. Neither did such a diet result in inanition or paralysis. 2. Normal cholesterol values, inanition, and paralysis developed with rejection of the diet. The stress of inanition did not produce hypercholesterolemia in chicks. 3. Neither cortisone acetate nor boric acid influenced the development of arterial lesions, although the latter had some effect on lipid deposition.

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