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Atherosclerotic Response of "Aggressive" and "Passive" Groups of Chickens To a Cholesterol Enriched Diet.* (23443)

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The possible effect of various emotional factors upon development of hypercholesteremia and atherosclerosis in laboratory animals also given a cholesterol enriched diet rarely has been studied. It was thought advisable to attempt such a study in respect to one emotional factor, namely, degree of aggressiveness as observed in young male chickens. This species was considered for such a study because of the well known pecking order (which might be considered a form of aggression) often observed in a flock of chickens(1,2) and susceptibility of chickens to dietary induced atherosclerosis(3).

Methods. In order to study male chickens ostensibly exhibiting either "aggressiveness" or "passiveness", a total flock of approximately 2500 male chickens (Avg. age: 6 weeks) was observed carefully by one of us (C.A.) for a number of hours both before, during and after feeding periods. A preliminary selection of 100 most "aggressive" chickens and 100 most "passive" chickens then was made. The characteristics of aggression were considered to be (1) insistence and speed of obtaining proffered feed, (2) over-all general physical activity and (3) agitation and belligerent resistance when handled. Passive characteristics were considered to be the converse of the preceding traits.

Following this preliminary selection, the 2 groups were housed in respective coops and were observed daily for 2 weeks during which time, the 30 most "aggressive" and 30 most "passive" chickens were selected from each. After this selection, the chickens were tagged with leg bands. Each group then was housed in 2 coops (approximately 5 feet square). The "aggressive" chickens were removed from their coops every 2 weeks, intermingled and then replaced in the coops. This was done in order to prevent a few from gaining group ascendancy. The "passive" chickens in the other 2 coops were treated similarly.

The animals were fed twice daily with a standard poultry mash (Albers Pullet Grower) enriched with cottonseed oil (5%) and cholesterol (2%). Routinely, the "passive" chickens were fed first and time allowed for feeding was one hour. At the end of this

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TABLE I. Atherosclerotic Response of "Aggressive" and "Passive" Chickens to Cholesterol Enriched Diet.

	"Aggressive group"	"Passive group"
Final: Avg wt (oz)	84 ± .94† (80-91) ‡	77 ± 2.98† (59-84) ‡
Comb wt (g)	29.4 ± 1.26 (22.6-35.6)	21.6 ± 1.98 (10-26.9)
Testes wt (g)	17.8 ± 1.2 (12.1-24.8)	13.2 ± 1.7 (8-22)
Avg plasma cholesterol (mg/100 ml)	348 ± 40.1 (180-599)	525 ± 76.9 (166-905)
Avg aorta cholesterol (mg/100 g)	1264 ±226 (929-3709)	1957 ±294 (1035-3150)
Degree of coronary atherosclerosis (%)*	45 ± 8.5 (1-88)	47 ± 7 (14-80)

* This has been calculated as the ratio of No. of vessels exhibiting Sudanophilic staining to total No. of arteries and arterioles observed.

† ± stand. error of mean.

‡ Range.

period, the amount of food ingested was calculated, then this exact amount was proffered to the herd of "aggressive" chickens. The average amount of mash ingested was approximately 3.5 lbs per feeding. It was of interest that the "aggressive" fowls regularly consumed their portion of food approximately 15 minutes more rapidly than the "passive" animals. Despite this arbitrary equivalence of food intake, it was observed at the end of the seventh week of feeding that the "aggressive" group exhibited a greater gain in weight. Therefore, an additional pound of food per feeding was given to the "passive" group for the remaining 8 weeks.

Blood samples were drawn from the axillary vein and the serum was analyzed for cholesterol on the first, 8th and 15th week of the experiment, using the method of Zak *et al.*(4). The average plasma cholesterol of each group was computed by averaging the 3 cholesterol values thus obtained. Weights were recorded on the first, 4th, 8th and 15th weeks.

During the course of the experiment some of the "aggressive" chickens were observed to exhibit a more passive type of behavior and conversely, some of the "passive" group later exhibited aggressive characteristics. At the conclusion of the experiment then, 12 "aggressive" and 9 "passive" chickens were obtained that had exhibited a consistent behavior pattern from the beginning.

After the 15th week the chickens were killed by bleeding. The testes, upper comb, heart and a segment (4 cm) of the arch and thoracic segments of the aorta were removed from each chicken. The weight of the testes and combs were recorded. The aortic segments were analyzed for cholesterol by previously described methods(5). Three transverse sections were obtained of the entire left ventricle (base, middle and apex) and were stained with Sudan IV. The coronary vasculature (arteries and arterioles) in these sections then were examined microscopically for Sudanophilic staining. A ratio of the number of vessels so stained to the total number of vessels observed (approximately 80 in each heart) was obtained.

Results. As might be expected, average weights of the 12 "aggressive" chickens and of the 9 "passive" chickens were approximately the same (Table I). It was of interest that secondary sex characteristics of the aggressive group (as indicated by weights of comb and testes) appeared to be more pronounced.

Rather surprisingly, average plasma cholesterol of the "passive" group was approximately 34% higher than that of the aggressive group (Table I). Similarly cholesterol content of aortic segments of the "passive" group was also higher (35%) than that of the "aggressive" group. It is, however, somewhat questionable whether these differences are significant in view of the relatively small number of animals and the wide range of individual values of both plasma and aortic cholesterol contents.

Despite the above possible differences in development of secondary sex characteristics and cholesterol contents of plasma and aorta respectively, no difference was observed (Table I) in extent or degree of coronary atherosclerosis as indicated by the ratio of affected to total number of normal vessels seen.

Summary. A group of male chickens ex-

hibiting "aggressive" and another group exhibiting "passive" behavior were studied during and after a period of high cholesterol and fat feeding. Although the "aggressive" groups exhibited more pronounced evidences of androgen activity, their plasma and aortic cholesterol contents were perhaps less than those of the "passive" group. The extent and degree of coronary atherosclerosis was the same in the two groups.

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Altitude Stress: Its Effect on Tissue Citrate and Salmonellosis in Mice.* (23444)

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Mice exposed to simulated altitude of 20,000 ft for 3 weeks or for 6 weeks show a uniform percentage decrease in tissue citric acid content and in their susceptibility to salmonellosis(2). Attempts to verify these findings in guinea pigs native to the Andean altiplano compared to those native to the coastal plain of Peru were not convincing for tissue citrate because of the wide range of individual values found within groups(3). The present communication presents results of studies on mice subjected to simulated altitude for a period of 3 to 4 months along with additional measurements designed to elucidate possible causes for the observed changes in tissue citrate.

Methods. Mice were exposed to simulated altitude in decompression chambers according to the schedule and technic previously described(1) with one important modification. Rather than control pressure within the chamber by a relay, an adjustable needle valve was substituted. The pressure of a desired altitude could be maintained during continuous operation of the pump with a variation in 24 hrs of approximately 3 cm of mercury (± 1,000 ft). Daily, the chambers were brought to atmospheric pressure while the cages were cleaned and fresh food and water were added. After about 15 to 20 minutes the animals were again at simulated altitude. The method of Ettinger *et al.*(4) was used for citric acid assays on blood, liver, spleen, kidney, duodenum, diaphragm, heart, and lung. Each analysis was done on the pooled tissues of 3 mice. Animals were killed by decapitation. The blood for assay was collected in a paraffined watch glass and transferred by tuberculin syringe to tungstic acid, the protein precipitant. The tissues were dissected quickly from each animal in 75 to 150 mg amounts and placed directly on dry ice. They were weighed in the frozen state, returned to the dry ice until all tissues were collected, and then they were homogenized in cold tungstic acid using a teflon in glass homogenizer. CF 1 female mice were used in all experiments. They were fed Purina dog chow *ad libitum* and water was available at all times.

Results. Citric acid in tissues of control mice. The mean values of citric acid (μg/g wet wt) for each of the tissues are given in column 2, Table I. These agree closely with

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