Atheromatous Changes in Cholesterol-Fed Rabbits Treated with Eggplant Extract


Cholesterol-induced changes in rabbits were studied with and without administration of an extract of eggplant. An anemia, accompanied by atrophic gastritis, developed in the cholesterol-fed animals. Their atheromatous plaques, and elevated serum cholesterol, lipoprotein, a-lipoprotein, and neutral fat were not affected by the eggplant extract.

It was reported by Roffo that feeding dried eggplant (Solanum melongena L.), or injecting an alcoholic extract of the dried vegetable subcutaneously, caused a fall in the blood cholesterol levels of rabbits. The variation in the analytic values reported rendered the interpretation somewhat questionable. Later Herrmann found that eggplant, although less effective than choline, methionine, or inositol, lowered the blood cholesterol levels when administered to hens or to hypercholesteremic patients. Roffo attributed this action of eggplant to its cholagogue action. Later both Noble and Trelles Dueto confirmed the claim that eggplant is a cholagogue.

On the other hand, Wilkinson, Jackson, and Vogel in a test on 6 healthy male subjects, were unable to demonstrate any effect on serum cholesterol levels from feeding the dried equivalent of 180 to 360 gms. of fresh eggplant daily. In view of these conflicting findings and of the present interest in hypercholesteremia and its control, it was decided to reinvestigate this decholesterolizing action of eggplant.

METHODS

Forty rabbits (38 males and 2 females), varying in weight from 1065 to 3710 gms., and of mixed breeding, were listed in order of increasing weight, and subdivided into successive lots of five. With the aid of sets of random numbers, one rabbit was assigned from each of these lots to each of five treatment groups. In this manner the initial mean weights of the treatment groups were made approximately equal. The rabbits were housed in individual cages in an air-conditioned room. Wood shavings were used on the floors of the cages.

During the one week pre-experimental period, water and Purina rabbit feed in pellet form were allowed ad libitum. For the last three days the pelleted feed was removed in the evening. In the morning, the rabbits were given approximately 25 gms. of ground carrots which they had to consume before the pelleted feed was returned.

At the end of the pre-experimental period, blood samples of about 3 ml. were taken from the ear vein of each rabbit after 16 hours' fasting. The rabbits were allowed feed until evening and again starved overnight. On the following morning they were given approximately 25 gms. of ground carrots containing either (a) no addition (diet I), (b) 1 gms. of cholesterol U.S.P. (diet II), (c) approximately 6 gms. of eggplant extract (diet III), (d) 1 gms. of cholesterol plus 6 gms. of eggplant extract (diet IV), or (e) as (b) for the first 8 weeks and as (d) for the last 4 weeks. None of the rabbits was fed regular pelleted diet until all had eaten the supplement, in which it was occasionally necessary to mix a few ground pellets to improve palatability. The supplement usually was consumed in 2 to 3 hours, after which all animals received pelleted feed until it was again removed in the evening. This regimen was followed 7 days a week except on every second Monday, when blood samples were taken and body weights recorded.

Blood samples were held at room temperature for one hour, refrigerated for one hour, and centrifuged. A portion of the serum was stored in the frozen state in capped tubes for subsequent cholesterol determinations, and the remainder was stored at 4 C. for use in electrophoretic studies within the week.

Preparation of Eggplant Extract. Fresh eggplants were trimmed, chopped into conveniently sized pieces, and in 2 Kg. lots, blended with 1 L. of ethanol in a large Waring Blender until thoroughly macerated. The slurry was strained...
through several layers of cheesecloth and the residue was squeezed as dry as possible. The residues from two such extractions were combined and re-extracted in the blender with a second liter of alcohol. Again the residue was removed with the aid of cheesecloth. The combined filtrates were allowed to stand overnight and then filtered through two layers of Whatman No. 1 filter paper. Some 500 L. of extract were obtained from 245 Kg. of fresh, trimmed eggplant. This extract was concentrated in vacuo in a Flash Evaporator with continuous addition of extract and a bath temperature of 55 to 60 C. The final, thick, brown syrup occupied 9,700 nil. and had a specific gravity of 1.36. The finished concentrate was thoroughly mixed and stored in the refrigerator until required.

The material was conveniently dispensed with the aid of a large syringe, and 4.5 ml., corresponding to approximately 6 Gin., were mixed with the supplemental feeding of ground carrot per rabbit as required. This amount of extract was approximately equivalent to 110 Gm. of fresh eggplant.

Electrophoretic Separation of Serum Proteins and Lipoproteins. Serum samples were subjected to electrophoresis on agar with a slight modification of the apparatus and procedure by Giri. A diagram of the apparatus is shown in figure 1. The agar was prepared as a 1 per cent solution in pH 8.6 veronal acetate buffer of ionic strength 0.05. The sample of serum, 107, or 20A, was pipetted onto a strip of 3 MM Whatman paper 20 mm. long and 1 or 2 mm. wide and placed on the agar film. A potential of 150 volts, equivalent to approximately 4 volts/cm., was applied to the films, giving a current flow of approximately 3.5 ma. per film at room temperature. After three hours the current flow was cut off, the cover plates were removed, and the films were dried in a current of air. The filter paper connector strips were removed, and the cellophane bearing the dried agar film was stripped from the frame, suitably identified, and stored in a dry place until stained.

Staining. (a) Protein: Anido black was purified according to the method of Kawerau. The dye solution was prepared by dissolving 5 Gin. of dye in a mixture of 600 ml. of distilled water, 400 ml. of methanol, and 100 ml. of glacial acetic acid. The strips were immersed in the dye solution for 30 minutes and were then washed in three to four changes of 10 per cent acetic acid in methanol until the background was clear. The strips were pressed between dry filter papers. (b) Lipoprotein: The films were immersed overnight in a saturated solution of Oil Red O in 60 per cent ethanol. The stained strips were washed in three or four changes of 50 per cent ethanol and pressed between dry filter papers. According to Uriel and Grabar and the present findings, unlike the re-
sults of paper electrophoresis, no lipoprotein with a mobility similar to that of the β-globulins is found. Rather, most of the mobile lipid is associated with the albumin and α-protein fractions.

Interpretation. The 10X sample patterns, stained for protein, were scanned in the densitometer with light of 615 μm wavelength. The optical densities were plotted against distance in mm. on graph paper. The areas enclosed by the curves were cut out with scissors, subdivided as indicated into one or more fractions, and weighed for a roughly quantitative relative measure.

Cholesterol. Serum cholesterol was determined by the Henly modification of the method of Zlatkis, Zak, and Boyle. Cholesterol determinations were made on the 20X sample patterns, stained for lipid, were scanned with light of 505 μm wavelength. The optical densities were plotted against distance in mm. on graph paper. The areas enclosed by the curves were cut out with scissors, subdivided as indicated into one or more fractions, and weighed for a roughly quantitative relative measure.

Liver Lipid. The procedure used for the determination of liver lipid was that of Folch et al. Because of the alternatives permitted, the specific method is presented in detail. A 2.5 Gm. sample of liver was homogenized in CHCl₃:MeOH 2:1, transferred to a 50 ml volumetric flask, and the flask was filled to the mark with solvent. After mixing, the flask contents were filtered through No. 42 Whatman paper and 20 ml aliquots were transferred to glass-stoppered centrifuge tubes. After the addition of 4 ml of 0.04 per cent aqueous CaCl₂ solution and thorough mixing, the tubes were centrifuged. The upper layer was removed as completely as possible with the aid of suction, and the remaining lower phase was washed three times with a mixture of CHCl₃:MeOH·H₂O in the proportions 3:48:47. The lower phase was then treated with a little methanol to make a single phase, and poured into tared flasks. The washings, made with CHCl₃:MeOH·H₂O in the proportions 96:14:1, were added, and the whole was evaporated to dryness in the wate bath in vacuo at 60 C. The dried flasks were further dried in desiccators in vacuo and weighed.

Histologic Technics. Tissues taken for examination were fixed in 10 per cent formalin in normal saline. Sections were cut at 4μ and were stained with hematoxylin and eosin. Sections of aorta were also stained with Verhoeff's elastica stain. Lillie's modification of the ferrocyanide reaction of M. Perls was used on kidney and spleen sections.

Hematologic Technics. Red blood cell counts were made on a Spencer Bright-line hemacytometer. The Van Allen hematocrit method was used to determine the packed cell volume. Hemoglobin estimations were made with a slight modification of the pyridine-hemochromogen method of Rimington. Blood and bone marrow smears were stained with Giemsa.

After the animals had been 12 weeks on experiment, the final blood samples were taken, and the red cell count, the hematocrit and hemoglobin values were determined, and a blood smear was made. The rabbits were then decapitated and examined for gross pathology. A bone marrow smear was prepared, the liver and left adrenal were weighed, and portions of the liver and the adrenal were placed in sealed vials and frozen. Samples of liver, spleen, stomach, kidney, prostate, testes, pancreas, right adrenal, and heart with attached major vessels, were preserved for examination.

Results

Growth and Mortality. In table 1 are recorded the mean body weights of the five groups of rabbits throughout the test. The high standard deviations reflect the range of

<table>
<thead>
<tr>
<th>Weeks on diet</th>
<th>Diet</th>
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<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
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<td>2666±486 (8)</td>
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<td>2786±385 (7)</td>
<td>2800±509 (7)</td>
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*Survivors.
TABLE 2.—Oil Red O Stainable Material in Serum Electropherograms; Arbitrary Units ± Standard Deviation

<table>
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<tr>
<th>Weeks on diet</th>
<th>Diet</th>
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<th>III</th>
<th>IV</th>
<th>V</th>
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<tr>
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<td>548±140</td>
<td>276±74</td>
<td>514±163</td>
<td>565±140</td>
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<td>289±82</td>
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<td>302±91</td>
<td>780±249</td>
<td>364±166</td>
<td>802±234</td>
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TABLE 3.—Serum Cholesterol Mg./100 Ml.

<table>
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<th>Weeks on diet</th>
<th>Diet</th>
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<th>III</th>
<th>IV</th>
<th>V</th>
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<td>76±39</td>
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<td>752±325</td>
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<td>96±99</td>
<td>967±520</td>
<td>1228±395</td>
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<td>32±15</td>
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<td>43±18</td>
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<td>1831±749</td>
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<tr>
<td>12</td>
<td>31±10</td>
<td>1696±548</td>
<td>38±17</td>
<td>1457±855</td>
<td>1764±784</td>
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</table>

body weights in the animals at the start of the experiment, an unavoidable circumstance because of the difficulty of procuring rabbits. It is easily seen, however, that the treatments had no effect on the body weight of the test animals. Survival figures also are noted in this table. One animal on each of diets II, III and IV died during the experiment, under circumstances that in no instance could be directly related to the diets. Two succumbed to acute Pasteurella Infection. The cause of death in the third animal was not determined.

Serum Protein and Lipoprotein. The electrophoretic pattern suggested that feeding cholesterol tended to increase the β-globulin and decrease the γ-globulin fractions of rabbit blood serum. The individual variations were such that these differences did not prove to be statistically significant. The feeding of eggplant extract did not appear to have any influence on the results. It might be mentioned here that the technic used (electrophoresis in agar) leads to a higher percentage of protein designated as albumin than is the case when paper is used as the supporting medium. In part, at least, this may be the result of considerable "trailing" of albumin on paper and the lack of this effect in agar.

In the case of lipids, feeding cholesterol caused a tremendous increase in material stainable by Oil Red O, an increase which became progressively greater as the experiment continued. The changes occurred in the lipoprotein, in the α-lipoprotein, and in the neutral fat. The data, for total stainable material, in arbitrary units, are presented in table 2. It is apparent that feeding of eggplant extract along with the cholesterol or during the last third of the experimental period had no significant influence on the lipid level attained.

Serum Cholesterol. Feeding cholesterol caused a prompt increase in serum cholesterol levels which tended to show a further gradual increase as feeding was prolonged. The pre-experimental serum cholesterol levels were quite variable, and this variability persisted when the animals were fed cholesterol. The administration of eggplant extract appeared to have no effect on serum cholesterol levels (table 3).
ATHEROMATOUS CHANGES AND EGGPLANT EXTRACT

TABLE 4.—Adrenal Cholesterol

<table>
<thead>
<tr>
<th>Diet</th>
<th>Adrenal weight (mg.)</th>
<th>Cholesterol (mg./adrenal)</th>
<th>% Adrenal</th>
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<tr>
<td>I</td>
<td>160±57</td>
<td>11.7±7.5</td>
<td>7.26±2.9</td>
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<tr>
<td>II</td>
<td>340±77</td>
<td>25.4±3.5</td>
<td>7.33±2.2</td>
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<tr>
<td>III</td>
<td>380±65</td>
<td>16.0±6.2</td>
<td>7.89±2.8</td>
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<td>IV</td>
<td>391±75</td>
<td>24.4±3.1</td>
<td>6.66±2.0</td>
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<tr>
<td>V</td>
<td>399±115</td>
<td>25.4±1.8</td>
<td>6.97±2.3</td>
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Adrenal Cholesterol. Adrenal total cholesterol was significantly greater in amount in rabbits fed cholesterol (table 4). Feeding of eggplant extract had no influence on the results whether alone or with cholesterol. When the adrenal cholesterol content was calculated on a percentage basis it was apparent that the concentration in the adrenals was not significantly different between diets. The apparent differences in total adrenal cholesterol was caused by the hypertrophy of the adrenals of cholesterol-fed rabbits.

Liver Lipid. The alterations in liver weights caused by feeding cholesterol are shown, together with the lipid content in table 5. The increases in liver weight on diets II, IV, and V were significant only at $p = 0.1$. At the same time, this enlargement, when considered in conjunction with the very significant elevation in liver lipid concentration, indicated that feeding cholesterol resulted in a very marked increase in total liver lipid. Feeding eggplant extract appeared to have no influence on liver weight or lipid concentration.

Blood Picture. The results of the various determinations are presented in table 6. It is apparent that feeding cholesterol to rabbits caused a significant decrease in the red cell count, in hemoglobin concentration, and in percentage of packed cells. The mean corpuscular volume was not significantly altered, although there was some suggestion that the value was elevated in the cholesterol-fed animals. The mean corpuscular hemoglobin concentration was not affected. The color index was normal. The blood picture in the cholesterol-fed rabbits showed anisocytosis, poikilocytosis and polychromatophilia, megaloblasts, and normoblasts. "Tailed" and "racquet" erythrocytes were also noted.

In no instance did there appear to have been any influence on the blood picture from feeding eggplant extract. These results led to the conclusion that feeding cholesterol caused a macrocytic anemia in rabbits.

Gross Pathology

Atheroma Index. The feeding of cholesterol to rabbits had a profound effect on the incidence and severity of plaque formation in the rabbit aorta. Eggplant extract had no effect. The atheroma indices were derived according to a modification of the procedure of Gore
and Tejada. The aorta was divided into two areas for evaluation. Area 1 was 3 cm. long and included the aortic arch and a small portion of descending aorta. Area 2 was the remainder of the aorta to the aortic bifurcation. The aorta was opened on the dorsal surface, pinned flat on a block of wood with the intimal surface up, and was examined by the naked eye and with a stereoscopic microscope. Three grades only were used as follows: grade I, lipid patches, grade II, lipid streaks, and grade III, fibrous and lipid plaques. (Grouping was made according to the method of Gore and Tejada.) The indices so derived are presented in table 7.

Coccidial abscesses occurred in the livers of 4 control and 6 test animals.

Parasitic cysts (*Cysticercus pisiformis*) were present in the abdominal cavity of 2 test animals.

**Histopathology.** Microscopic changes in the aortas of the animals receiving cholesterol and eggplant extract were similar.

Clusters of foam cells were noted in the kidney and spleens of all except the control and eggplant extract group.

Several animals on diets II, IV, and V exhibited an atrophic gastritis. Arteries in the mucosa and submucosa of these animals were partially occluded with foam cells. The atrophy was most obvious in the pyloric portion of the stomach. It is interesting to note that this pathology was most marked in the animals that were markedly anemic, and that cholesterol-fed rabbits that were not anemic had no observable gastric atrophy. As the anemia was of the macrocytic type, it may have been related to the atrophic gastritis, although mucosal cells of the fundus were not obviously abnormal. Studies are being conducted to assess the validity of this theory.

A squamous cell carcinoma was observed in the stomach of 1 animal on diet IV.

The liver and adrenal changes were similar to those described by other workers.

**Summary**

Feeding cholesterol to rabbits during a 12 week period with or without the addition of eggplant extract had no effect on body weight or mortality and little influence on the serum protein level or distribution. Rabbits fed cholesterol had an elevated serum cholesterol and increased levels of lipoalbumin, a-lipoprotein and neutral fat. They showed an adrenal hypertrophy but no change in the concentration of cholesterol in the adrenals. Liver lipid levels were considerably elevated but liver weights were only slightly increased. Eggplant extract alone or fed with the cholesterol did not alter the results. An anemia developed in rabbits fed cholesterol. The measurements made indicated that this was a macrocytic type of anemia. Gross and microscopic pathological investigations revealed that most of the rabbits fed cholesterol developed atheromatous plaques while incidence in the controls was zero. Eggplant extract appeared not to alter the incidence of plaques.

Cholesterol-fed rabbits that developed anemia were affected with an atrophic gastritis. There appeared to be a relationship between the anemia and gastritis; the more anemic animals exhibited a marked gastric atrophy.

**Acknowledgment**

The authors wish to thank Dr. W. P. McKinley for his encouragement and continued interest.

**Summario in Interlingua**

La administración dietari de cholesterol a conílios durante un periodo de 12 septimanas, con o sin le addition de extracto de melongena, habeva nulle effecto super le pesos corporee e super le mortalitale e super le niveillo o le distribution del proteinas seral. Conílios recipiente cholesterol dietari habeva elevate niveillos seral de cholesterol e augmentate concentrationes de lipo-albumina, de lipo-
PROTEINA ALPHA, E DE GRASSIA NEUTRE. ISTE ANIMALS MONSTRAVA HYPERTROFIA ADRENAL SED NULL ALTERATION DEL NIVEL DE CHOLESTEROL IN LE ADRENALES. LE NIVELES DE LIPIDO HEPATIC ESSEVA CONSIDERABILMENTE ELEVATE, SED LE PESOS DEL HEPATE ESSEVA AUGMENTATE SOLMENTE PER LEVE GRADOS. EXTRACTO DE MELONGENA PER SE O IN COMBINATION CON CHOLESTEROL NON ALTERAVA LE RESULTATOS. LE CONILIOS RECIPIENTE CHOLESTEROL DISVELLOPPAVA ANEMIA. LE MESURATIONS EFFECTUATE INDICAVA CHE IL SE TRATAVA DI UN TIPO MACROCYTIC DI ANEMIA. INVESTIGATIONS PATHOLOGIE MACRO- E MICROSCOPIC REVELAVA CHE LE MAJORITATE DEL CONILIOS RECIPIENTE CHOLESTEROL DISVELLOPPAVA PLOSES ATHEROSCLEROTIC DURANTE CHE LE INCIDENTIA DI INSTE PHENOMENO ESSEVA ZERO IN LE ANIMALS DI CONTROLO. EXTRACTO DI MELONGENA NON PAREVA INFLUENZAR LE INCIDENTIA DEL PLOSES.

Le conilios che disveloppava anemia post le administration de cholesterol essaiva affilet per un gastritis atrophic. Il pareva existere un relation inter le anemia e le gastritis. Le plus anemic animales exhibiva plus marcate grados de atrophia gastric.

REFERENCES

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