patients so that minor differences in caloric balance will be recognized. Cohen *et al*(10) reported that a patient with carbohydrate induced lipemia sustained a reduction in plasma triglycerides on a starch diet. However, review of these data shows that the patient lost approximately 2 kg during each dietary period in which starch was given, with no weight loss in 2 similar periods of unsaturated fat and sucrose diets. Although this weight change appears to be minor, results in the present study indicate that it is of sufficient magnitude to be an important factor in the observed decrease in triglyceride level.

*Summary.* The comparative effects of dietary starch and dextrose on plasma triglyceride levels were studied on a metabolic ward in 2 lipemic patients. Plasma triglycerides were elevated when these subjects ingested a virtually fat free diet. Substitution of starch for the high dextrose formula produced no further change in plasma triglycerides. Caloric restriction in one patient resulted in a marked decrease in triglyceride even though he continued to eat an 85% carbohydrate, 15% protein formula. Both patients complained of a feeling of fullness and had trouble finishing an isocaloric high starch diet. These observations show that isocaloric starch diets did not decrease plasma triglycerides but that caloric restriction while a high carbohydrate diet is fed, will result in lower plasma triglyceride levels. They suggest that, unless careful supervision of dietary intake is maintained, the effects of carbohydrate substitutions in diet may be related to changes in caloric balance rather than to the type of carbohydrate ingested.

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## Correlation of Arachidonic Acid of Sterol Esters with Restrictive and Susceptibility to Naturally-Occurring Atherosclerosis in Pigeons.* (31612)

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The correlation of the arachidonic acid content of serum sterol esters to atherosclerosis has been reported(1,2). Swell and his co-workers(2) have hypothesized, from a study of several species of animals, that the level of arachidonic acid in the serum cholesterol esters may be related to their susceptibility to atherosclerosis. In the present work, the possibility of such a relationship has been studied in 2 breeds of the same animal species (pigeons) which exhibit a marked difference

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in their susceptibility to naturally occurring aortic atherosclerosis(3).

*Materials and methods.* Two hundred and forty pigeons, 60 male and 60 female Show Racer (SR) and an equal number of male and female White Carneau pigeons (WC) were used. The former breed is resistant to naturally occurring aortic atherosclerosis while the latter is susceptible. They were fed Purina‡ pigeon pellets *ad libitum*, until they were sacrificed at 8 months of age. Serum samples from each 10 pigeons of the same sex and breed were collected and pooled. Liver samples were pooled in like manner. The thoracic aorta from the aortic arch to a point 2 mm distal to the coeliac bifurcation were obtained. Each aorta was cut into a proximal and a distal portion at a point one centimeter proximal to the bifurcation. This was done for several reasons. First, atherosclerotic lesions are present predominantly in the distal segment of the aorta; this segment is also much more active in incorporation of acetate-1-$C^{14}$ into the lipids than the proximal portion (4). Second, the fatty acid composition of the sterol esters of aortic plaques and those of non-diseased aortas differ markedly(5). Third, although no grossly visible aortic lesions were found in these pigeons, microscopic changes and fatty streaks have been observed in pigeons of comparable age in previous studies(6). Due to the small amount of lipids present, proximal aortic segments from each 30 pigeons of the same sex and breed had to be pooled. The distal segments also were pooled.

Lipids were extracted from all tissues(5) and the sterol ester fraction was isolated by preparative thin layer chromatography using Silica gel HR§ and a solvent system composed of hexane‖:diethyl ether:glacial acetic acid, 73:25:2 (v/v/v)(7). All solvents except ethanol and diethyl ether were re-distilled. The component fatty acid methyl esters of the sterol esters were prepared(8) and analyzed in duplicate in a dual-column gas-liquid chromatograph. The 6-foot ⅛-inch stainless steel columns were packed with 15% (w/w) diethylene glycol succinate polyester on acid-washed Chromosorb W.¶ The column temperature was programmed from 155°C to 210°C at a rate of 2°/min using helium, at a flow rate of 40 ml/min, as the carrier gas. Fatty acids were quantified using the disc integrator of the recorder. Comparisons of the fatty acid values were made between males and females within breed, and between birds of the same sex of the two breeds. Student's t-test(9) was used to evaluate the significance of differences. In the tables, brackets connect the values which differ significantly from each other, and the probability values are shown in the footnote of each table.

TABLE I. Major Fatty Acid Composition of Sterol Esters of Serum and Liver.

| | Percent of total fatty acids (No. of carbons : double bonds) | | | | | |
|---|---|---|---|---|---|---|
| | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 20:4 |
| Serum | | | | | | |
| *SR-♂ | 8.8 | 2.5 | 3.2 | 27.0 | 54.5 | 3.1 |
| SR-♀ | †8.8 | 2.5 | 2.8 | 25.7 | 56.8 | 3.4 |
| WC-♂ | 9.0 | 3.0 | 2.9 | 26.9 | 55.8 | 2.4 |
| WC-♀ | 10.1 | 2.6 | 3.1 | 27.6 | 53.6 | 3.0 |
| Liver | | | | | | |
| SR-♂ | 10.8 | 2.9 | 7.6 | 54.7 | 20.2 | 3.8 |
| SR-♀ | 9.0 | 2.2 | 6.5 | 52.2 | 21.7 | 5.0 |
| WC-♂ | 16.6 | 3.0 | 9.0 | 49.9 | 17.5 | 2.8 |
| WC-♀ | 15.1 | 2.9 | 9.2 | 51.6 | 17.3 | 2.9 |

* SR, Show Racer; WC, White Carneau.
† $P < .001$ —— $P < .005$ --- $P < .010$ —○— $P < .025$ —×—

*Results and discussion.* The fatty acid compositions of sterol esters of serum and liver are shown in Table I. The serum sterol esters of SR males have significantly higher ($P < 0.025$) percentage of arachidonic acid than WC males. Although no significant difference is observed in this fatty acid between SR and WC females, the value tends to be higher in the former. The values for palmitic acid in both SR females and WC males are significantly lower ($P < 0.025$ and 0.010 respectively) than those in WC females.

More and greater significant differences in

‡ Ralston-Purina Co., St. Louis, Mo.

§ Brinkman Instruments, Inc., Westbury, N. Y.

‖ Skellysolve B from Skelly Oil Co., Kansas City, Mo.

¶ Applied Science Laboratories, Inc., State College, Pa.

the fatty acid values of sterol esters between breeds are found in the liver (Table I) than in serum. The liver sterol esters of SR females have a significantly higher ($P<0.01$) content of arachidonic acid than the WC females. Although the values of this acid in the males of the two breeds do not show a significant difference, the value for SR males tends to be higher than that for WC females. Both the male and female SR have significantly lower ($P<0.001$ and 0.005 respectively) palmitic acid than their counterparts in WC. In addition, the liver sterol esters of the SR females contain significantly lower ($P<0.005$) proportion of stearic acid than those of the WC females.

The arachidonic acid contents of both serum and liver sterol esters of SR tend to be higher than those of WC even though no consistent significant difference is observed between both sexes of the two breeds. The present finding suggests that the susceptibility to atherosclerosis may be inversely related to the level of arachidonic acid in not only serum sterol esters, as hypothesized by Swell and his associates(2), but also in liver sterol esters. It may be interesting to note that the arachidonic acid contents of both serum and liver sterol esters in decreasing order are SR females, SR males, WC females, and WC males. However, the significance of this observation, if any, is not known.

The aortas exhibited no grossly visible lesions at necrospy, as would be expected of pigeons of this age (8 months)(10). The fatty acid values for the proximal and distal portions of the aortas are presented in Table II. There is only one significant difference ($P<0.05$) which could readily be due to the operation of chance alone. The most abundant fatty acid in both the proximal and distal portions of the aorta is linoleic with one exception. In the WC females oleic acid predominates. At any rate this finding does not seem to be related to aortic atherosclerosis since previous study(11) has shown that male and female pigeons do not differ in their susceptibility to atherosclerosis.

Schrade and his co-workers(12) compared the fatty acid composition of serum cholesterol esters in human beings with clinically

TABLE II. Major Fatty Acid Composition of Sterol Esters of Aorta.

| | Percent of total fatty acids (No. of carbons : double bonds) | | | | | |
|---|---|---|---|---|---|---|
| | 16:0 | 16:1 | 18:0 | 18:1 | 18:2 | 20:4 |
| Proximal aorta | | | | | | |
| *SR-♂ | 20.8 | 5.4 | 7.8 | 25.2 | 32.1 | .5 |
| SR-♀ | 21.5 | 4.9 | 9.1 | 25.6 | 33.9 | 1.2 |
| WC-♂ | 20.4 | 7.8 | 8.4 | 25.3 | 28.6 | 1.4 |
| WC-♀ | 15.2 | 4.5 | 5.5 | 40.6 | 28.4 | 2.0 |
| Distal aorta | | | | | | |
| SR-♂ | 18.3 | 2.8 | 8.5 | 24.1 | 36.7 | 3.0 |
| SR-♀ | 17.6 | 2.9 | 8.6 | 27.9 | 34.6 | 3.1 |
| WC-♂ † | 20.0 | 4.3 | 9.0 | 25.0 | 35.8 | 2.4 |
| WC-♀ † | 16.2 | 2.8 | 7.8 | 36.2 | 31.6 | 3.3 |

* SR, Show Racer; WC, White Carneau.
† $P<.05$ —

manifest atherosclerosis and healthy subjects of the same age group. They found that the linoleic and arachidonic acid proportions were lower, and palmitic and palmitoleic acid proportions higher in the cholesterol esters of the serum of patients with atherosclerosis. The differences for linoleic and palmitic acids were statistically significant ($P<0.05$). While direct comparisons of the fatty acid composition of tissues from different animal species may not be valid, it can be noted that the differences found between the fatty acid values of the liver sterol esters of the atherosclerosis-susceptible WC pigeons and those of the atherosclerosis-resistant SR pigeons are in the same direction as the differences reported between the patients with atherosclerosis and healthy subjects. In the liver cholesterol esters of WC pigeons there are lower proportions of linoleic and arachidonic acids and higher proportions of palmitic, palmitoleic, and stearic acids.

*Summary.* The levels of arachidonic acid in cholesterol esters of both serum and liver from atherosclerosis-susceptible White Carneau pigeons are significantly lower than those of the atherosclerosis-resistant Show Racer pigeons. This finding, together with that of others, suggests that the susceptibility to atherosclerosis may be inversely related to the level of arachidonic acid of sterol esters of serum and liver. In addition, it was found

that the differences in the proportions of other fatty acids of cholesterol esters of liver between the two breeds of pigeons correspond with changes found in serum cholesterol esters in patients with atherosclerosis and healthy humans.

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## Immunoglobulin Responses to Experimental Human Infection with Adenovirus Types 26 and 27. (31613)

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Adenovirus types 26 and 27 have not been isolated in association with naturally occurring illness. A previous study has shown that experimental administration of these serotypes to human subjects caused an acute conjunctivitis and a prolonged, asymptomatic enteric infection(1). Each agent evoked the appearance of both homotypic and heterotypic antibody responses, with demonstrable resistance to illness after rechallenge with the same virus and after cross-challenge with the other adenoviral type. Moreover, the degree of restistance could be correlated with the level of circulating antibody.

A recent study of volunteers experimentally infected with adenovirus types 1 or 4 or inoculated with soluble antigens prepared from these serotypes has shown the appearance, during the first 3 weeks after inoculation, of antibodies in the 3 major immunoglobulin classes, IgM, IgA and IgG(2). IgM antibody activity was short-lived, while IgA and IgG antibodies were more durable. Production of heterotypic IgG antibody was also demonstrated.

The present investigation has been carried out in order to study the immunoglobulin response to experimental human infection with adenovirus types 26 and 27, which are not known to be associated with naturally-occurring illness. The immunoglobulin nature of the heterotypic antibody response was also studied, as were the immunoglobulin classes of the antibodies formed after rechallenge with homotypic virus and cross-challenge with heterotypic virus.

An unexpected finding was the fact that 19 S IgM hemagglutination-inhibition and neutralization antibodies could not be detected after fractionation of serum by either sucrose density gradient ultracentrifugation or sephadex G-200 gel filtration.

*Materials and methods. Clinical procedures and inocula.* Volunteers were adult male inmates of Federal correctional institutions selected and evaluated according to procedures previously described(1). For the present