# Adrenal Cholesterol Ester Fatty Acid Composition of Different Species.* (25990)

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It is well known that adrenal tissue contains a high concentration of cholesterol of which 80 to 90% is in the esterified form(1). Administration of ACTH to rats produces a marked drop in the cholesterol ester fraction of the adrenal(2). In view of these findings, it has been suggested(3) that adrenal cholesterol esters may be involved in steroid hormone biosynthesis, particularly during stress periods when the animal is called upon to produce increased amounts of steroid hormones. Rat adrenal cholesterol esters(4) have been shown to contain a high proportion of essential fatty acids and it has been suggested(5) that those acids play a role in formation of steroid hormones. In view of the scarcity of quantitative data on distribution of fatty acids in cholesterol ester fractions of the adrenal, it was of interest to examine the cholesterol ester fatty acid (CEFA) spectrum of different species utilizing the technic of gas-liquid chromatography.

*Methods and materials.* All animals used in this study were males with the one exception noted in Table I. The animals were as follows: rats (Carworth strain, 250-350 g), guinea pigs (Hartley strain, 300-350 g), chickens (White Leghorn, 1-2 years), geese (mixed breeds, 1-2 years), rabbits (albino, 6 months), dogs (mongrels, 1 year and older). All animals (5-6 animals per group) were maintained on the following diets: chickens and geese—cracked corn, dogs—Purina dog chow, rabbits and guinea pigs—Purina rabbit and guinea pig chow, rats—Purina rat chow. All the animals were allowed to become adjusted to the daily routine and were not subjected to stressful conditions before being sacrificed. The animals were sacrificed and adrenals removed as quickly as possible. Human adrenals were obtained at autopsy within 24 hours after death. Adrenals of each group of animals, except the dog and man, were pooled and lipid extracts prepared as described earlier(6). Cholesterol esters were separated from the other lipid components by chromatography on silicic acid(7). The isolated cholesterol esters were interesterified in HCl-methanol and methyl esters were sublimed according to the procedure of Stoffel *et al.*(8). Gas-liquid chromatography was carried out as previously described(9).

*Results.* The fatty acid composition of adrenal cholesterol esters is shown in Table I. There were marked differences in distribution of the fatty acids in the cholesterol ester fraction of the adrenals of different animals. Each species appeared to possess a specific characteristic adrenal CEFA pattern. The major fatty acid of the adrenal cholesterol ester fraction of the dog, man, goose, chicken and rabbit was oleic acid. In the case of the goose this acid accounted for 79.6% of total CEFA; the rat had the lowest amount of that acid (21.1%). Each of the species studied had appreciable amounts of palmitic acid (from 7.8 to 25%). There were also differences in proportion of the other saturated and monoenoic acids characteristic of the different animals. Adrenal CEFA composition of the rat is noteworthy since it has distinctively more polyunsaturated fatty acids than any of the other animals. A large proportion of CEFA of rat adrenal was arachidonic acid (23.1%). It was also observed that a fatty acid with a retention volume equivalent to a $C_{22}$ tetraenoic acid was present in substantial amounts (13.4%). This acid was also detected in the adrenal CEFA of man and dog. Acids with more than one double bond constituted up to 48.1% of total fatty acids in the rat. The dog was the only other animal that had appreciable amounts of polyunsaturated fatty acids (32.4%). That animal also

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TABLE I. Fatty Acid Composition of Adrenal Cholesterol Esters in Different Species.

| Fatty acid† | | Cholesterol ester | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Chain length carbons | No. double bonds | Rat (24)‡ | Dog (5) | Man (3)§ | Goose (6) | Chicken (6) | Rabbit (6) | Guinea pig (6) |
| | | | | % total fatty acids | | | | |
| 6 to 12 | | 1.6 ± .4* | 1.7 ± .8 | .9 | 1.0 | .8 | 1.7 | 5.4 |
| 14 | 0 | 2.4 ± .6 | 2.2 ± .9 | 3.9 | .3 | 1.2 | 2.9 | 6.2 |
| " | 1 | 1.2 ± .4 | .5 ± .5 | .6 | tr | .3 | 1.0 | 1.4 |
| 16 | 0 | 17.3 ±3.0 | 11.0 ±3.2 | 15.4 | 7.8 | 12.6 | 17.6 | 25.0 |
| " | 1 | 2.7 ±1.7 | 5.7 ±1.2 | 6.8 | 2.0 | 8.6 | 6.2 | 10.6 |
| " | 2 | .2 ± .0 | tr | .2 | tr | tr | tr | .9 |
| 18 | 0 | 4.3 ± .5 | 7.5 ±1.6 | 7.7 | 1.9 | 3.3 | 3.4 | 3.1 |
| " | 1 | 21.1 ±1.6 | 36.7 ±6.2 | 47.1 | 79.6 | 63.1 | 43.4 | 24.4 |
| " | 2 | 7.4 ± .9 | 17.0 ±3.6 | 2.9 | 4.8 | 5.2 | 11.4 | 9.4 |
| " | 3 | 2.5 ± .7 | .7 ± .4 | 1.0 | .2 | .8 | .9 | tr |
| " | 4 | tr | tr | 1.8 | tr | .8 | tr | " |
| 20 | 0 | 1.3 ± .5 | " | tr | " | .3 | 1.1 | 6.0 |
| " | 3 | 1.5 ± .6 | 5.5 ±1.7 | 2.7 | " | 1.2 | 2.1 | tr |
| " | 4 | 23.1 ±1.9 | 9.2 ±2.0 | 5.1 | 2.4 | 1.8 | 8.3 | 7.6 |
| 22‖ | 4 | 13.4 ±3.0 | 2.1 ± .9 | 3.9 | | | tr | |

* Stand. dev.

† Represents major acids found; small amounts of others were also detected.

‡ Represents No. of animals; values on rat represent avg of 4 pools of 6 rats, and on man and dog individual analysis. All animals were males except 3 geese.

§ Insufficient values for reliable stand. dev.

‖ Identification based on retention time.

had the highest concentration of $C_{20}$ trienoic acid.

*Discussion.* The present study shows that each species has a characteristic adrenal CEFA spectrum. The differences between the CEFA in the adrenal of the different species do not appear to be related to diet. Diets consumed by the animals were previously analyzed for fatty acid composition(10). All of the diets were low in fat (2 to 4%), with the exception of man, but dietary fat was high in linoleic acid (30 to 54%). All species had a smaller proportion of linoleic (4.8 to 17.0%) and some species (dog, man, goose, chicken and rabbit) had a higher proportion of oleic acid in their adrenal CEFA than was present in the diet. The fatty acid spectrum of the adrenal cholesterol esters does not bear a close similarity to that of the serum cholesterol esters in each species. In most of the species studied(10) the major fatty acid of the serum cholesterol ester fraction was linoleic acid and in the adrenal fraction oleic acid is the major acid. The only similarity which does exist relates to the fact that those species which have large amounts of polyunsaturated fatty acids (rat and dog) or oleic acid (chicken and goose) in adrenal CEFA also have large amounts of those acids in serum CEFA. Blood cholesterol esters of the rat, human and dog were checked for $C_{22}$ tetraenoic acid and only traces could be detected.†

The significance of the adrenal polyunsaturated fatty acid which may be a $C_{22}$ tetraenoic acid is not apparent. Further studies are underway to characterize that acid more completely. Whether specific cholesterol esters play an important role in biosynthesis of steroid hormones is not known. Preliminary experiments have indicated that there may be changes in proportion of arachidonic acid and the $C_{22}$ tetraenoic acid of the adrenal cholesterol esters following administration of ACTH to rats.†

*Summary.* The fatty acid composition of adrenal cholesterol ester fraction has been determined by gas-liquid chromatography in 7 species. Characteristic CEFA patterns for each type of animal were apparent. Rat and dog had the highest content of polyunsaturated fatty acids in the adrenal cholesterol ester fraction. The remaining species had considerably less of those acids and the major CEFA of dog, man, goose, chicken and rabbit

† Unpublished observations.

was oleic acid. In the dog, rat and human, an acid tentatively identified as a $C_{22}$ tetraenoic acid was found in the adrenal CEFA. The significance of these results in relation to hormone synthesis and polyunsaturated fatty acid metabolism is discussed.

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## Plasma Disappearance Rate and Tissue Distribution of Radioactive Cobalt Labelled Cyanocobalamin Injected into Various Animals.* (25991)

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In humans and laboratory animals, parenterally injected cyanocobalamin is rapidly cleared from the blood stream and approaches normal levels within 24-48 hours as determined by microbiological assay methods(1-3). With increased sensitivity attainable with cobalt-60 labelled cyanocobalamin, Miller *et al.* (4) demonstrated that an injected dose of the radioactive vitamin disappeared from the plasma more slowly in patients with chronic myelogenous leukemia than from the plasma of normal subjects. After 24 hours, sufficient radioactivity remained in the plasma to determine the slope of the exponential disappearance curve, which approximated a half life of 5 days. In female rabbits, Rosenthal (5) found that plasma disappearance curves beginning 48 hours after injection of cobalt-60 cyanocobalamin could be resolved into 2 components with apparent half lives of 4.1 days (Component A) and about 50 days (Component B) respectively. The similar plasma disappearance rate of cyanocobalamin in human subjects and rabbits prompted us to determine plasma disappearance rate of injected radio cyanocobalamin in a variety of animal species.

*Materials and methods.* White rabbits and white leghorn chickens weighing 2-3 kg were obtained from commercial sources. Mongrel dogs weighing 6 to 15 kg were obtained from the local pound. Healthy male human subjects were laboratory personnel weighing 64 to 86 kg. All laboratory animals were maintained on commercial animal feed; water was available *ad lib.* The human subjects ate their usual diets without restriction. The animals were injected with $Co^{60}$-cyanocobalamin intramuscularly into the left hind leg or intravenously *via* the ear vein in rabbits, wing vein in chickens and jugular vein in dogs. The human subjects were injected with $Co^{58}$-cyanocobalamin *via* the antecubital vein.

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