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Serum Lipoproteins in Atherosclerosis-Susceptible and Resistant Pigeons.*† (25472)

HUGH B. LOFLAND AND THOMAS B. CLARKSON (Introduced by Camillo Artom)
Dept. of Biochemistry, and Vivarium, Bowman Gray School of Medicine, Wake Forest College, Winston-Salem, N. C.

White Carneau pigeons (both male and female) develop spontaneous atherosclerosis involving aorta and coronary arteries, while Show Racer pigeons appear resistant to atherosclerosis(1,2). Despite striking differences in incidence of the disease, the 2 breeds do not differ significantly in serum levels of total lipides, cholesterol (free or ester), or phospholipides(3). Studies on man(4,5,6) and experimental animals(7,8,9) indicated that atherosclerosis may be associated with changes in levels of serum lipoproteins, especially increases in beta-lipoproteins. Accordingly, we thought it of interest to compare atherosclerosis-susceptible and resistant pigeons for possible differences in levels of beta-lipoproteins, as determined by agar precipitation method of Boyle and Moore(10). It soon became apparent that enormous fluctuations occurred in the K-agar Δ‡ values obtained for female pigeons. These results, and those of a more detailed study of serum lipides in pigeons are reported here, together with observations on reliability of the K-agar precipitation technic.

Materials and methods. White Carneau and Show Racer pigeons ranging in age from 6 weeks to 7 years from Palmetto Pigeon Plant, Sumter, S.C., were maintained on commercial pigeon rations (mixtures of corn, peas, wheat, kaffir, and minerals). Blood samples were obtained from the alar vein. On one sample of serum (within one to 5 hours after drawing), the K-agar Δ values were determined(10). Another sample of whole blood was mixed with ACD§ solution and centrifuged, and the supernatant subjected to low-temperature alcohol fractionation, as described by Lever, *et al.*(11). This procedure yielded 2 fractions, one containing alpha-lipoproteins, and the other beta-lipoproteins. Lipides were extracted from each fraction with boiling alcohol and alcohol-ether, and purified with chloroform(12). "Total lipides" of each extract were determined by weight and an aliquot was used for determination of total cholesterol by the method of Zlatkis, *et al.* (13), as modified by Rosenthal, *et al.*(14). On another aliquot, lipide phosphorus was determined according to Stewart and Hendry (15).

Results. Values for K-agar Δ and total cholesterol in serum of sexually mature pigeons of the 2 breeds are shown in Table I. These birds were actively reproducing. No significant difference in levels of beta-lipoprotein exists among males of the 2 breeds. The extremely wide range of K-agar Δ values determined on female birds, however, suggested that these values were influenced by sequence of events in the ovulatory cycle, thus making difficult any comparison we could make between females of the 2 breeds. This suggestion is supported by results of K-agar Δ de-

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‡ K-agar Δ is defined as optical density of sample of serum mixed with buffer and a solution of K-agar, minus optical density of a blank consisting of serum plus buffer.

§ ACD Solution: Citric acid monohydrate 0.8%, sodium citrate ($2H_2O$) 2.2%, and dextrose 2.2%.

TABLE I. Serum Beta-Lipoproteins and Total Cholesterol in 2 Breeds of Pigeons.

Breed	No. of birds	Avg age	Sex	Beta-lipoproteins* Range	Beta-lipoproteins* Avg and S.E.	Total cholesterol, mg %†
White Carneau	18	2.8 yr	♂	20- 69	34.9 ± 2.8	407.2 ± 14.9
	11	1.4 "	♀	24-148	78.4 ± 16.4	468.5 ± 23.0
Show Racers	11	1.7 "	♂	13- 61	27.8 ± 4.1	390.3 ± 24.9
	18	3.3 "	♀	19-512	173.7 ± 44.8	434.7 ± 25.7
White Carneau	130	6 wk	Mixed	11- 90	40.4 ± 3.8	401.4 ± 15.9

* K-agar Δ value (see footnote 1). † Avg value and stand. error of mean.

terminations on 130 six-week-old White Carneau pigeons of both sexes. From these results, also included in Table I, it appears that sexually immature birds have serum lipoprotein values very similar to those of older males.

To gain more information on changes in serum lipides during the ovulatory cycle, these changes were followed systematically in 2 pairs of mated White Carneau pigeons. Beta-lipoprotein cholesterol (expressed as % total cholesterol) and K-agar Δ values for one pair of birds are shown in Fig. 1. In the male, only about 30% of total cholesterol is carried in the beta fraction, and low K-agar Δ values are found consistently. The female, on the other hand, shows a sharp rise in both beta-lipoprotein cholesterol and K-agar Δ values approximately 30 days following egg laying. It appears that there is a shift in transport of cholesterol from alpha to beta fraction at this time. Shortly after these changes occurred, a new egg was laid.

Further evidence for the striking difference between male and female in quantitative aspects of lipide transport was obtained by subjecting serum samples, taken 30 days after egg laying, to electrophoresis in Spinco Model H Electrophoresis apparatus (Fig. 2). Differences between male and female patterns are obvious, and are similar to those described for chickens by Moore(16).

In addition to cholesterol, serum total lipides and phospholipides also undergo a shift from alpha to beta lipoprotein (Fig. 3, in which the values (obtained from a different female pigeon) are expressed as per cent of lipide component present in the beta-lipoprotein). In the female, however, there is no positive correlation between K-agar Δ values and total cholesterol in serum. Whereas serum total lipides and phospholipides rise sharply at time of egg laying, there is no accompanying rise in serum total cholesterol (Fig. 4). Boyle and Moore state that in humans K-agar values are positively correlated with total serum cholesterol. It might be pointed out, of course, that there is no assurance that the substances precipitating with K-agar in human serum are qualitatively and quantitatively identical with those obtained by the same technic from pigeon serum.

Discussion. It has long been known that in serum of birds, lipides are raised considerably during egg-laying activity(17,18,19,20). Our data suggest that female pigeons, during their reproductive life, undergo regular periodic shifts in lipide transport from alpha- to beta-lipoprotein. These shifts are accompanied by increases in total phospholipides and by marked decreases in cholesterol/phospholipide ratios. It seems logical to assume that the changes observed are under the influence of changes in estrogen production. Somewhat similar changes in distribution of lipides between various lipoprotein fractions in serum of hens during the ovulatory cycle are also apparent from data of McIndoe(19). This author describes appearance of a new lipoprotein fraction just prior to egg-laying. This fraction may possibly be the precursor of a dense lipoprotein isolated from egg yolk by Schjeide and Urist(20).

Pick, *et al.*,(21) reported that cholesterol-fat fed chicks exhibited high cholesterol/phospholipide ratios, and extensive coronary atherosclerosis. When these chicks were treated with estrogen, they were free of coronary lesions and the cholesterol/phospholipide ratio was lowered, suggesting a protective action of estrogen against coronary atherosclerosis. It appears that this view cannot

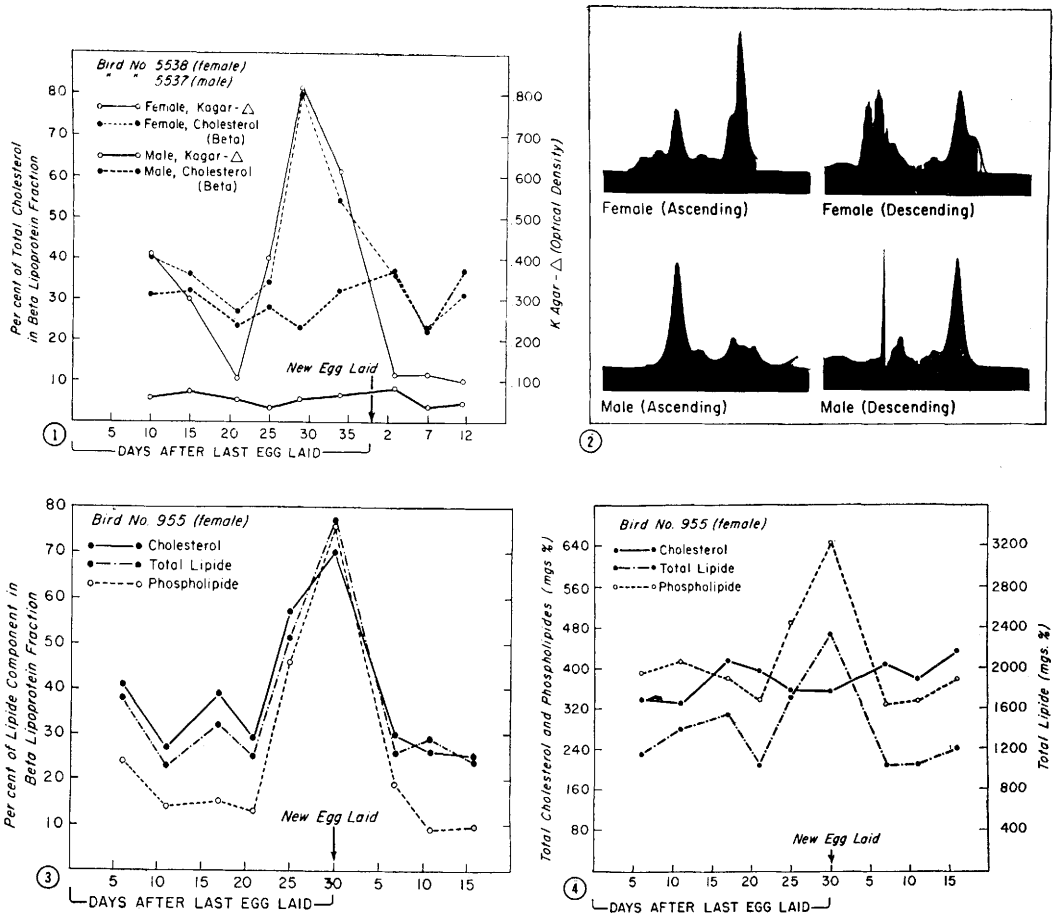

FIG. 1. Comparison of K-agar △ values, and beta-lipoprotein cholesterol in one pair of White Carneau pigeons during an ovulatory cycle.

FIG. 2. Electrophoresis of serum from male and female White Carneau pigeons. Blood samples drawn 30 days after last egg was laid.

FIG. 3. Changes in % of cholesterol, total phospholipides, and total lipides carried in beta-lipoproteins during ovulatory cycle of female White Carneau pigeon.

FIG. 4. Changes in total cholesterol, total phospholipides and total lipides in serum of a female White Carneau pigeon during an ovulatory cycle.

be easily extended to spontaneous atherosclerosis of pigeons. We previously reported lack of sex difference (hence estrogen protection) in incidence of aortic atherosclerosis in White Carneau and Autosexing King pigeons (3), and recently included coronary atherosclerosis in the same age group(2). Furthermore, from previous(3) and present data, it appears that although cholesterol/phospholipide ratio decreases periodically in female pigeons, these birds are not protected against atherosclerosis. Differences between results of Pick *et al.*,(21) and ours might be a reflection of the fundamental difference between the disease produced by cholesterol feeding and that occurring spontaneously in pigeons.

We compared these findings with the statement that in human beings, high levels of beta-lipoprotein cholesterol in serum may be associated with occurrence of myocardial infarction(5). In man, estrogen administration results in increased concentration of cholesterol in alpha-lipoproteins(22), while in the female pigeon the response to increased production of estrogen is in the opposite direction. Lack of a sex difference in incidence of atherosclerosis in pigeons makes doubtful any causal relationship between altered distribu-

tion of lipides in lipoproteins and atherogenesis in this species.

Summary. 1) Using the agar precipitation method and low temperature fractionation for determination of beta-lipoproteins, amounts of beta-lipoproteins, total lipides, phospholipides, and cholesterol in serum of atherosclerosis-susceptible and resistant breeds of pigeons were compared. The agar precipitation method seems a reliable method for estimating levels of beta-lipoprotein cholesterol in serum. 2) No significant differences were seen when male birds of the 2 breeds were compared. Lipoprotein levels of females varied within wide limits, probably due to phospholipemia associated with egg-laying activity. Such activity is accompanied by increased proportion of cholesterol in beta-lipoproteins. At time of egg-laying, total lipides and total phospholipides (but not total cholesterol) are increased in the serum. Thus, such cyclic changes appear to produce a more "favorable" (*i.e.*, lowered) cholesterol/phospholipide ratio. In contrast to cholesterol-fat fed chicks, female pigeons of susceptible breed are not protected against aortic or coronary atherosclerosis.

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Renal Tubular Excretion of Riboflavin in the Chicken.* (25473)

BARBARA R. RENNICK (Introduced by Jay Tepperman)
Dept. of Physiology, State University of N. Y., Syracuse, N. Y.

The renal tubule has been demonstrated to be capable of excreting a number of organic bases by an active transport system. Tetraethylammonium(1,2), n-methylnicotinamide (3), tolazoline (Priscoline)(4), choline and thiamine(5) represent a few of these compounds, most of which are quaternary ammonium compounds. Studies of competitive inhibition among this series indicate that the substances are transported by a common system. Excretion by the renal tubule of the chicken has been demonstrated for all of these substances. This study describes the renal tubular excretion of riboflavin (6,7-Dimethyl-9-1(d-l'-ribityl-) iso-alloxazine) in the

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