Effect of Indoor Housing Conditions on Serum Cholesterol Levels of White Carneau Pigeons. (28719)

CHARLES H. EADES, JR.,* VICTORIA B. SOLBERG, IRENE C. HSU AND GEORGE E. PHILLIPS (Introduced by W. M. Govier)
Department of Biochemistry, Warner Lambert Research Institute, Morris Plains, N. J.

Hoffman(1) has reported that male and female pigeons have seasonal cyclic variations in serum cholesterol, with a maximum in April and a minimum in August. The pigeons used in his studies were kept in a large outdoor enclosure with access to an indoor loft. For his data, he sacrificed several pigeons monthly for a year. In an effort to determine whether or not pigeons will show these effects when closely confined, we have maintained a control group of White Carneau pigeons under indoor laboratory conditions for 18 months. Our pigeons were not kept in fly-pens, nor were they exposed to any outdoor living conditions once they were brought into the experiment. Periodic serum samples obtained without sacrifice of the birds during this study revealed a pattern of serum cholesterol values definitely different from that observed by Hoffman(1). The results are reported here.

Procedure. Old (5-7 years) female White Carneau pigeons were obtained from the Palmetto Pigeon Plant, Sumter, S. C., in May, 1961. They were housed in a 4-deck "finishing battery"† having 2 compartments per deck. Each compartment measured 29½" by 24½" by 14" high and had a ¾" wire mesh floor with dropping pans below. Feeder and water troughs were external. Six pigeons were housed in each compartment. The sharp tips of the beaks were removed to prevent injury to the birds while they were establishing a "pecking order" among the groups. One of the 2 groups of 6 pigeons that comprise this study was in a top compartment and the other was in the third down on the opposite side of the battery.‡ During our study the fluorescent lights were turned on at 8:00 A.M. and off at 5:00 P.M. daily. The temperature was maintained at 76 ± 2°C and the humidity about 40%. There were no windows in the room. The pigeons received water and Purina Pigeon Chow Checkers *ad libitum* but a record of the food consumption was made. Since 12 pigeons were serving as control for several other experimental groups receiving drugs orally in pill form, they received a placebo§ pill daily by mouth. For serum, blood was drawn bi-weekly from alternate wings for about 7 months and then every 4 weeks for the remaining 11 months of the study. Weight records were also kept. Serum cholesterol was determined by the method of Abell *et al*(2).

Results. The pigeons weighed 426-523 g at the start and 484-664 g at the end with a mean food intake of 30.6 g per day. The weight gain was probably due to lack of exercise. The individual serum cholesterol values for several pigeons are plotted in Fig. 1 with the means of all 12 birds summarized in Table I. These data illustrate the wide variation of serum cholesterol values obtainable from individual pigeons from time to time and also the great difference observed among pigeons. Since we received the pigeons in May from the Palmetto Pigeon Plant where they were in outdoor fly-pens, the serum cholesterol values indicated that they were still on the elevated portion of the seasonal cycle but apparently on the descending slope when viewed in relation to the data presented by Hoffman(1). Of the 12 pigeons, only No. 1190, No. 1335 and No. 8066 had peak serum cholesterol elevations which persisted for several months. However, these

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† Oakes Manufacturing Co., Inc., No. 324.

‡ This was done to randomize the positions of these 2 groups with respect to others for which they served as controls.

§ The placebo pill contained 3.9 mg methylcellulose, 7.8 mg starch, 64.3 mg lactose, and 1.6 mg magnesium stearate. We assume that this placebo does not exert any effect on serum cholesterol.

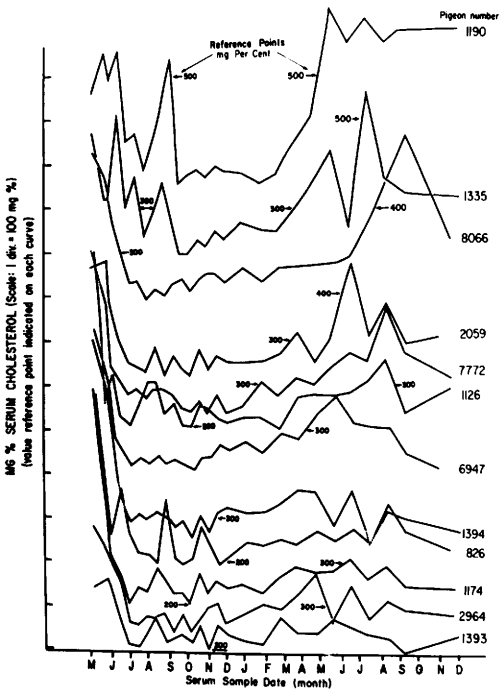

FIG. 1. Individual serum cholesterol values of old female White Carneau pigeons determined during 18 months of housing indoors under laboratory conditions. Each curve has mg% reference points indicated to show the serum cholesterol level.

peaks did not occur in April, as might be expected, but were delayed until June, July and even September. The other pigeons had somewhat erratic serum values also. Pigeon No. 2059 had a "spike" value in June that is difficult to interpret as a peak because it is a single high value not surrounded by rising and falling values over the several adjoining months. A general slowly rising slope with a small "hump" might be the interpretation for No. 1126, No. 2059, No. 6947, and No. 7772. On the other hand, these "hump" values do not approach the original high values found at the start of the study. In the other pigeons, No. 826, No. 1174, No. 1394, No. 1393 and No. 2964, the serum values dropped from the original high values and stayed low with only the choppy effect produced by slight variations from month to month. When the values from all 12 pigeons are averaged for each sampling period, as shown in Table I along with the standard error of the mean, we do not observe the seasonal cycle, but a fall from the original serum values and after 8 to 9 months the beginning of a very slow rise with a hump in August, 6 months later, this hump being due to values primarily from 3 pigeons only. Since pigeons that are allowed free access to flying areas and are exposed to seasonal changes in amount and intensity of daylight, and to changes in temperature and humidity, possess the seasonal cycle of serum cholesterol, and we do not observe this cyclic phenomenon in our pigeons, it must be concluded that putting pigeons into confining pens and regulating the environment to relatively constant conditions of light, darkness, temperature and humidity disrupts the "natural" sequence of metabolic events that leads to seasonal variations in serum cholesterol. It appears that in some pigeons the cycle is altered and delayed, whereas in others — probably most of the pigeons — the cycle is completely abolished, and serum cholesterol values vary in response to a variety of other uncontrolled variables. Therefore, if pigeons are to be used in testing drugs for their effect on serum cholesterol levels, more information on the factors that control the natural variation of serum cholesterol must be obtained. It is obvious from these results that individual pigeons of the same breed respond differently to the change from fly-pen to indoor housing conditions and that neither relatively constant serum cholesterol levels nor consistent cyclic variations can be depended upon. The

TABLE I. Mean Serum Cholesterol Values for 12 White Carneau Pigeons for 18 Months.

Sample date	mg %	Sample date	mg %
5/10/61	486 ± 31	11/ 7/61	249 ± 12
5/18	528 ± 41	11/21	267 ± 10
5/25	431 ± 24	12/ 5	252 ± 10
Break in rapid fall of values in most pigeons		1/ 2/62	265 ± 9
		1/30	260 ± 11
6/ 1	364 ± 21	2/27	274 ± 7
6/19	337 ± 27	3/28	301 ± 13
7/ 5	261 ± 13	4/24	315 ± 17
7/18	266 ± 15	5/22	342 ± 35
8/ 1	254 ± 17	6/19	346 ± 27
8/15	283 ± 16	7/17	344 ± 36
8/29	286 ± 20	8/14	369 ± 28
9/12	276 ± 27	9/11	332 ± 36
9/26	237 ± 9	10/17 to 11/28	300 ± 31
10/10	229 ± 10		
10/24	271 ± 10		

results lead one to speculate that individual pigeons vary more than is desired in this area of metabolism and thus are somewhat limited as a test system for drugs or conditions that modify serum cholesterol levels.

Summary. Old White Carneau pigeons maintained under regulated light (9 hr fluorescent light, 15 hr darkness), temperature (76 ± 2°C), and humidity (about 40%) conditions and in groups of 6 in cages that do not permit flying for a period of over 18 months do not have the seasonal cyclic variations in serum cholesterol possessed by pigeons that have free access to flying areas and are exposed to yearly weather conditions.

1. Hoffman, R. A., *Endocrinology,* 1960, v67, 311.
2. Abell, L. L., Levy, B. B., Brody, B. B., Kendall, F. E., *J. Biol. Chem.,* 1952, v195, 357.

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Reduced Antibody Forming Capacity During the Incubation Period of Passage A Leukemia in C_3H Mice.* (28720)

RAYMOND D. A. PETERSON, RONEE HENDRICKSON AND ROBERT A. GOOD
Pediatric Research Laboratories, Variety Club Heart Hospital, University of Minnesota, Minneapolis

The present investigation was designed to study the immunologic capacity of mice during the incubation period of Gross's passage A lymphatic leukemia. Mice infected with the virus during the newborn period remain healthy until approximately 3 months of age when an acute lymphatic leukemia develops in almost all of the animals(1). Until that time, even careful histologic examination will not disclose leukemia.

The motivation for this experiment was development of an experimental model which might clarify the clinically observed association between malignancy of the lymphocytic system and immunologic capacity. At least 4 children with the congenital sex-linked type of agammaglobulinemia have developed lymphocytic malignancies(2,3,4). In addition, several cases of acquired agammaglobulinemia have been followed, often years later, by a lymphoma(5) or reticulum cell sarcoma(6). Adults with chronic lymphatic leukemia frequently have a decreased capacity to develop circulating antibody(7,8,9). Patients with Hodgkin's disease often have impaired ability to express cellular allergy as manifested by delayed hypersensitivity(10,11,12). The proposed experimental model held promise of clarifying whether lymphocytic malignancy results in immunologic deficiency because malignant cells crowd out antibody producing cells, or whether it might do so by a more basic effect on the immunologic mechanism.

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Materials and methods. Animals: C_3H_f/Bi strain mice, obtained from the original colony of Dr. J. J. Bittner, were used. They were weaned at 3 weeks of age and separately caged. Experimental and control groups were identically managed except for administration of the virus.

Passage A virus: Leukemic C_3H mice, representing the 32nd passage of Gross's passage A leukemia originating in AKR mice, were obtained from Dr. Ludwik Gross in June, 1962. The virus was passaged in C_3H/Bi mice as reported by Gross(13) until used in the 35th passage for the present experiment. The experimental mice were given an intraperitoneal injection of 0.3 ml of a cell free filtrate of the virus on the 3rd or 4th day of life.

Antigen and assay method: The antigenic stimulus was T_2 bacteriophage (*E. coli* host). The phage was grown and partially purified by standard methods(14). Each mouse received an intraperitoneal injection of 10^{10} phage particles at either 3 or 9 weeks of age, and was bled *via* the orbital sinus 14 days later. Antibody content of each serum was