mitral valve can also be obtained by hypothermic asystole at about 10°C at a very reduced systemic and coronary rate of blood flow(13) and because of the continuous oxygenation of the heart and all other organs left cardiotomy is not limited in time. For open surgery on the aortic valves the coronary flow has to be discontinued and asystole during hypothermia of about 20°C by cooling of oxygenated blood appears to be a desirable condition.

Summary. Hypothermia was produced in dogs by circulating, oxygenating and refrigerating venous blood in a small, plastic pump-oxygenator. The by-passed, contracting, hypothermic heart could be safely deprived of coronary flow for 15 minutes at 28°C and for at least 33 minutes at 20°C. During cardiac arrest induced by intracoronary injection of potassium citrate the safe period of myocardial ischemia could be prolonged to at least one hour at 20°C.

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Age Related Changes in Plasma Cholesterol of the Chicken.* (23261)

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The incidence of spontaneous atherosclerosis in the chicken is reported to increase with age(1). Since atherogenesis can be stimulated in the chick by hypercholesterolemia(2), the role of plasma cholesterol in spontaneous atherosclerosis, particularly in relation to changes of plasma cholesterol with age, becomes a matter for speculation. Data from a number of sources can be organized to suggest that plasma cholesterol increases rapidly with age in the hen. Thus 58 three-year-old hens(3) yield average value of 257 mg %, and combined data on 55 two-year-old hens (4,5) give 197 mg %. For 41 pullets between 6-9 months, grouped results(6,7,8) average 121 mg %. On the other hand, Kaishio(9) found no change in plasma cholesterol of hens between 9 and 81 months of age, but his average value of 80 mg % is considerably lower than most other published data. In males, there are no changes in plasma cholesterol up to 15 months of age(10), apparently the oldest birds studied.

Considering that plasma cholesterol is highly variable, particularly in the female, and may be affected by breed, diet, season, and reproductive state, as well as by analytical technic, it seemed desirable to restudy the problem under more uniform conditions.

Procedure. Male and female White Leg-

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horn chickens were housed on the floor and fed a standard all-mash ration. Four generations from a closed breeding flock were used, making it possible to reduce environmental effects by almost simultaneous measurements on birds varying from 1 to 5 years, as well as to make repeat measurements on some groups over years. Total plasma cholesterol was determined by the method of Zlatkis, *et al.*(11) on non-fasted animals.

Results. The results of 240 determinations on hens from 8 to 57 months old are arranged in 4 age groups in Table I. Analysis of variance indicates no significant differences among these groups, and there are no obvious changes related to age. The average plasma cholesterol for the entire series was 197 mg %.

The 149 determinations made on males from 8 to 45 months old are grouped in 5 categories in Table II. Statistical analysis indicates significant differences: in particular the 35-38 and 45 month groups differ from each other and from the 3 younger groups, but the latter are not different from one another. Thus in the male, plasma cholesterol apparently increases rapidly after the second year of life. The 209 mg % value for 45-month-old males may be questioned, since it is based on only 4 birds. This same group, however, had plasma cholesterol levels of 133 mg % at 17 months, and 158 mg % at 38 months, which are in complete agreement with average values of larger numbers at these ages.

Plasma cholesterol did not appear to be influenced to any appreciable extent by season in either sex or by reproductive state in the females.

Discussion. Our results on the hen agree with Kaishio's findings(9) to the extent that plasma cholesterol does not increase with age, although our average value of 197 mg % is more than twice his. The apparent increase in plasma cholesterol of the hen with age, as derived from pooled data in the literature, may be due to differences in method, breed, diets and modes of management.

TABLE I. Relationship of Total Plasma Cholesterol to Age in the Adult White Leghorn Female Chicken.

Age (mo)	8–14	26–29	50	57
No. birds	96	103	27	14
Avg plasma chol. (mg %)	189	209	182	198
Pooled S. E. of mean*		8.9, P >0.05		

* From analysis of variance.

TABLE II. Relationship of Total Plasma Cholesterol to Age in the Adult White Leghorn Male Chicken.

Age (mo)	8-12	14-16	23-25	35-38	45
No. birds	32	40	50	23	4
Avg plas. chol. (mg %)	142	143	134	155*	209*
Pooled S. E. of mean†		3.5, P <0.01			

* Significantly different from all other groups. First 3 groups not significantly different from one another.

† From analysis of variance.

We have no explanation for the rapid increase in plasma cholesterol of the male after the second year of life. Comparison with the results of aging studies in other species is difficult. In man, the change of plasma cholesterol with age is subject to conflicting interpretations(12). It may be worthy of note, however, that in the aging rat, changes in fat metabolism occur particularly in the male (13).

These data indicate that the increase in incidence of atherosclerosis with age in the female, or in the male up to 2 years of age, is not necessarily associated with a concomitant increase in total plasma cholesterol.

Summary. The average total plasma cholesterol level of hens from 8 to 57 months old was 197 mg % and did not vary with age. The cholesterol level of males remained relatively constant at 139 mg % between 8 and 25 months, but by 35-38 months it had risen significantly to 155 mg %. At 45 months of age, a small group of males had a level of 209 mg %.

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Extractable Heparin Level in Rat Tissues Under Normal and Experimental Conditions.* (23262)

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Much of the work on the study of mast cells and their relation to the histamine and heparin content of tissues has been carried out on rats(1,2). There is, however, little available information on normal heparin levels in different rat tisues, or on properties of rat heparin. Obviously there is a need for the establishment of normal levels of extractable heparin in different organs of the rat and a comparison of the product of extraction with an accepted standard. Levels for extractable heparin have been determined for 6 different organs of the rat, and the product has been compared with beef heparin in regard to antithrombic, metachromatic and anticoagulant potencies.

Materials and methods. Rats. Female rats (Wistar strain) of approximately 180 g were fed a standard laboratory diet of Purina Chow. Animals were killed by decapitation, the skin immediately removed and stored at –20°C until extracted. *Cortisone.* A saline suspension of cortone acetate (cortisone acetate Merck), 25 mg/ml was given subcutaneously in daily doses of 10 mg/animal. *Carbutamide* (BZ-55), Eli Lilly and Co., Indianapolis, Ind. A solution was prepared by dissolving 4 g of the material in 100 ml of alkaline solution and adjusting pH to 7.5. Daily subcutaneous injections of 50 mg/animal were given.

Heparin extraction. The method used was that described earlier(3) with the following additional step found desirable, when dealing with rat skin. After first alcohol precipitation the dried product was suspended in 1 ml of buffered saline/g of original tissue. The pH was adjusted to 8.0 and approximately 20 mg of trypsin/g of original tissue were added. This mixture was incubated one hour at 38°C and then treated with 80% phenol. The heparin was precipitated from the aqueous layer with 95% ethanol. For further purification the precipitate was dissolved in saline and the solution treated with benzidine as previously described(3). *Heparin assay.* (a) *Antithrombin Assay.* This was carried out according to the method of Jagues and Charles (4). (b) *Metachromatic Assay.* This was carried out as described by Jagues, Monkhouse and Stewart(5). (c) *Anticoagulant Assay.* Blood was withdrawn from a normal healthy dog by a siliconed syringe and needle and immediately placed in test tubes containing varying amounts of standard and unknown heparin solutions. The clotting times were noted and the potency of the unknown then expressed in terms of the standard heparin. *Heparin standard.* All values are expressed in terms of beef heparin kindly supplied by the Connaught Medical Research Laboratories. Unless otherwise stated the values given in Tables refer to those obtained by antithrombin assay.

Results. Normal extractable heparin levels in rat tissue. In Table I are shown the aver-

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