than, the estimations of Flexner and his associates.

Summary and conclusions. 1. Regarding the pregnant organism as a multi-compartment system the exchange rate of the water in amniotic fluid with the water of the maternal and fetal systems, was determined. By the application of trans-abdominal catheters, placed into the amniotic sac of pregnant women at term, amniotic fluid could be withdrawn at desired intervals without disturbing the continuity of the system. The mechanism of tracer (deuterium) exchange was demonstrated to be that demanded by theory, and the application of well known theoretic equations to the data so obtained permitted the calculation of absolute exchange rates. 2. In 2 experiments where the isotopic tracer was first placed in one compartment (mother) and then in the other (amniotic fluid) the exchange rates in both directions were found to be about 600 cc per hour. The observations of Flexner and his associates on the exchange rate of the water of the amniotic fluid are thus confirmed in principle.

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Effects of Sex Hormones on Serum Lipoproteins in Rabbits.* (20218)

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Ultracentrifugal analyses of blood serum in human beings have indicated differences in the lipoprotein pattern between male and female: beta-lipoprotein concentrations are approximately 25% smaller in women than men (1) and, "The incidence of measurable concentrations of the Sf 10-20 class is significantly higher in males from 20 to 40 years of age than in females of the same age group"(2). In similar investigations, Barr, *et al.*(3) using alcohol fractionation technic, found that administration of estrogens to atherosclerotic men resulted in an increase in alpha and a decrease in beta-lipoproteins. However, Glass, *et al.*(4), did not observe any change in the serum lipids or low density lipoproteins (Sf 12-20) following treatment with estradiol of 55-65-year-old male and female patients.

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There are thus indications that androgens and estrogens may have opposite effects on lipoproteins and, therefore, be important in the regulation of lipoprotein concentration. Since androgens have not been studied, the present investigation was undertaken in rabbits subjected to alternate treatment with stilbestrol and testosterone.

Methods. Nine sexually immature rabbits (5 males and 4 females) of mixed strain with an average body weight of 1.35 Kg were kept without treatment during a preliminary period of 15 days, at the end of which they were castrated. During the following 5 months they were treated with stilbestrol or testosterone; each period of treatment was followed by a control period in order to permit a complete resorption of the drug. Diethylstilbestrol in oil solution was injected subcutaneously at the daily dose of 1 mg; testosterone in aqueous crystalline suspension was injected at the

TABLE I. Effects of Diethylstilbestrol and Testosterone on Serum Lipoproteins and Cholesterol.

Date	Treatment	$-S_{1.21}$ in mg/100 ml serum	>70	40–70	20–40 (35)	1–10 (7)	Serum cholesterol in mg/100 ml
5/9	—				50 (12–128)	69 (12–95)	41 (27–65)
5/22	Castration				78 (32–95)	96 (76–104)	66 (44–101)
6/24–7/7	Diethylstilbestrol, 1 mg/day				37 (21–80)	97 (57–142)	47 (36–64)
7/15–7/30	Testosterone, 20 mg/day		16 (0–66)	7 (0–21)	56 (16–62)	122 (104–130)	50 (39–64)
8/25	—				80 (17–170)	103 (71–127)	62 (38–71)
9/12	—				81 (49–141)	102 (47–164)	70 (48–114)
9/26–10/8	Testosterone, 20 mg/day		20 (0–59)	16 (0–35)	77 (30–138)	97 (54–115)	71 (54–91)

dose of 20 mg. The animals were fed on commercial rabbit pellets and had tap water to drink. As indicated in Table I, blood analyses were performed at intervals in the course of the experiment. Twenty ml of blood were drawn from the femoral artery and the ultracentrifugal serum lipoprotein pattern determined at a density of 1.21 as described by Green, Lewis and Page(5). On the same sample, total serum cholesterol was estimated by the method of Kendall *et al.*(6).

Results. The normal lipoprotein pattern of immature rabbits' sera showed 2 main peaks; a less dense component (beta-lipoprotein) with a negative sedimentation of -S 35 and a more dense component ($alpha_1$-lipoprotein) –S 7 (Table I). The concentration of the lipoproteins and cholesterol showed great individual variations, probably due to the mixed strain of the animals; –S 35 averaged 50 mg (range 12 to 128 mg per 100 ml); –S 7 averaged 69 mg (range 12 to 95 mg per 100 ml); cholesterol averaged 41 mg (range 27 to 65 mg per 100 ml).

Following castration, there was an increase in the concentration of –S 35 and –S 7 components as well as in cholesterol; this increase averaged respectively of 28, 27, and 25 mg per 100 ml. After treatment with diethylstilbestrol, the –S 35 lipoprotein and cholesterol concentrations decreased to values near the precastration levels, while the –S 7 remained unchanged. No abnormal components were found as a result of castration or of estrogen treatment.

Following testosterone injections, the lipoprotein pattern was markedly altered by the presence of –S (40-70) and –S >70 components, which are not usually present in the serum of normal or castrate rabbits. These changes are significant since they were present during the 2 periods of treatment and disappeared when testosterone treatment was discontinued.

This effect of testosterone on lipoproteins is further demonstrated in one rabbit, which is not included in the table because of abnormal components (–S > 70 and –S (40-70)) present during the control period: the respective concentrations of these 2 components were 36 and 12 mg per 100 ml. Following castration, these 2 abnormal components disappeared, but reappeared during testosterone treatment to reach the following values: –S > 70, 142 mg and –S (40-70) 33 mg per 100 ml, which are higher than the precastration levels. On substitution of testosterone by stilbestrol injections, these components again disappeared.

Discussion. Glass *et al.* observed no effects in human beings on "low density" lipoproteins as determined by ultracentrifugation at a density of 1.06 from physiologic or pharmacologic doses of estradiol(4). This low density fraction, which was present only in one of our rabbits at the beginning of the

treatment, disappeared under the influence of estrogen. In the other rabbits the only effect noted was a decrease in the concentration of the –S 35 fraction. The absence of a concomitant increase in alpha$_1$-lipoproteins in rabbits contrasts with the results of Barr *et al.* (3), obtained by alcohol fractionation and confirmed by ultracentrifugation at a density of 1.21(7), in patients receiving estrogens. Nava and Zilli(8) also observed an increased concentration of serum alpha-globulin following treatment of normal healthy men and women with estradiol or diethylstilbestrol. This variation in response to estrogens is associated with difference of species and difference in lipoprotein pattern of rabbit and human serum. The most significant finding in the present experiments is the increase in the low density lipoproteins (–S (40-70) and –S > 70) following treatment with testosterone; it explains the sex difference and may offer a partial explanation for the greater susceptibility of male than female to atherosclerosis.

Summary. The serum lipoprotein patterns of immature rabbits were determined by ultracentrifugation at a density of 1.21. Two main peaks were resolved: one at –S 35 (20-40) and another at –S 7 (1-10). After castration, these animals were treated with diethylstilbestrol and with testosterone. Only testosterone altered significantly and regularly the lipoprotein pattern by the appearance of two abnormal components; one, –S > 70 and the second, –S (40-70); both disappeared on cessation of treatment.

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Observations on Preservation of Human Spermatozoa at Low Temperatures. (20219)

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The survival of human spermatozoa, after freezing at low temperatures, is not a new observation. Mantegazza(1), as recorded by Davenport(2), was perhaps the first to report this phenomenon. Jahnel(3), Shettles(4), and Hoagland and Pincus(5) have since shown, by various technics, that human spermatozoa will survive temperatures of –79°C to –269.5°C. In an attempt to evaluate the merits of rapid freezing and to reconcile the varied results of these reports, Parkes(6) showed that survival is far better when 1.0 cc of semen is frozen in ampoules rather than tiny amounts in fine capillary tubes. He states it was fairly certain that, under optimum conditions, a large number of human spermatozoa could survive prolonged freezing, but expressed considerable doubt that the speed of freezing and thawing was the primary factor

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