0.5 mg % level, in bile, it has not been possible to demonstrate the presence of mesobilirubin in fistula, t-tube, or gall bladder bile from non-icteric humans. However, it was identified in the urine of a patient with severe hepatic failure and no gall bladder, thus indicating that a gall bladder is not essential to its production. In this situation, however, its occurrence is believed due to intestinal formation and enterohepatic circulation. Thus it is possible that mesobilirubin derived in this manner might on occasion be re-excreted in the bile, but none was observed in the present study.

Summary and conclusions. 1. Paper and column chromatographic methods of separating bilirubin and mesobilirubin are described. 2. Use of these methods permits detection of mesobilirubin at the 0.5 mg % level in the presence of much larger amounts of bilirubin. 3. The spectroscopic distinction of biliverdin and mesobiliverdin (glaucobilin), and of their respective zinc complexes, is discussed. 4. Glaucobilin is readily formed from d- or i-urobilin, in addition to mesobilirubin; glaucobilin formation on $FeCl_3$ oxidation is thus not specific for mesobilirubin as suggested by others. 5. Mesobilirubin has not been detected in human bile but was definitely identified in a urine sample containing larger amounts of bilirubin. Its occurrence in the urine is believed to relate to an enterohepatic circulation.

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Depression of Plasma Albumin by Steroid Therapy.* (28162)

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Albumin has been reported to bind estrogens avidly(1). This protein is responsible for 90 or more per cent of the orientation in human plasma of estradiol and estrone sulfate (2). It has been shown that Premarin (mixed, conjugated equine estrogens) increases survival of men with myocardial infarction(3). From these observations one might infer that a deficiency of certain estrogenic substances or of albumin exists in these patients. Since plasma albumin concentration is significantly depressed in the aging, arteriosclerotic subject(4), it was of interest to attempt to learn if the metabolism of the albumin molecule is altered by an increased plasma level of certain steroids.

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Methods and materials. Human albumin standard. A preparation of human albumin was donated by Cutter Laboratories, Berkeley, Calif. The preparation was globulin-free as determined by paper electrophoresis.

Albumin assay–electrophoretic. Separation of plasma albumin was made by the Durrum paper electrophoresis cell. The albumin band

TABLE I. Effect of Enovid on Plasma Albumin Concentration.

Exp No.	Normal Subject	Age (yr)	Albumin (g/%)	Enovid Subject	Age (yr)	Albumin (g/%)
1	MP	23	3.65	PH	20	2.72
2	RK	26	4.30	JD	20	3.30
3	ES	20	4.15	MJ	29	2.84
4	RR	22	4.44	KG	25	3.73
5	KL	21	3.93	BA	24	2.70
6	JG	23	4.15	JH	23	3.75
7	BP	22	4.50	BS	25	3.77
8	CO	24	4.60	RE	26	3.90
Avg		22.6	4.22		24.0	3.34

Per cent change of Enovid group with respect to controls = 20.9, $P < 0.002$. In each experiment a normal was run simultaneously with the experimental subject, assuring identical assay conditions. Each value is the result of duplicate or triplicate analyses. Maximum mean deviation in analyses on a given sample was less than 2%.

was subsequently stained, eluted, and assayed spectrophotometrically. The method is detailed in the Beckman Technical Bulletin No. TB6050A, Procedure B.

Plasma samples. Normal plasma samples were obtained from 9 young women 20 to 26 years of age. Nine other female subjects, 20 to 30 years of age, received a minimum of 5 mg of norethynodrel† and 0.075 mg† of ethynylestradiol-3-methyl ether daily for at least 7 days prior to donation of the plasma sample. Most of the subjects had been on the cyclical birth control therapy for more than 5 months preceding the analysis. Plasma was drawn after an overnight fast of 8-12 hours. Anticoagulant consisted of 1 ml potassium oxalate (0.1M) per 9 ml blood. Duplicate or triplicate assays on a normal and experimental plasma sample were conducted simultaneously under identical conditions immediately following withdrawal of the sample.

Results. Daily ingestion by normal female subjects of 5 mg norethynodrel and 0.075 mg ethynylestradiol-3-methyl ether caused a drop in plasma albumin from a normal level of 4.22 to 3.34 g %, Table I. This represents a 20.9% ($p<0.002$) decrease. The hematocrit for the normal group averaged 46.7% compared to 42.4% in the steroid section. This dilution would reduce the albumin deficiency to 11.7% assuming, in the absence of information on total body blood volume, that one may correct for the 9.2% dilution.

Discussion. It is noteworthy that the plasma albumin deficiency, if corrected for hemodilution, is in the same order of magnitude as the albumin defect of the aging subject who has recovered from a myocardial infarct and who is not on anticoagulant therapy (4). This observation is of particular interest in view of the report(5) that this steroid therapy also produces changes in a number of blood coagulation factors, changes which mimic the direction and magnitude of change noted in aging, arteriosclerotic subjects. The possibility is raised by these data that certain forms of steroid metabolism may be responsible for the clotting defects observed in thrombotic disorders.

It was recently reported(3) that Premarin (mixed equine estrogens) significantly improved the survival of men with myocardial infarction who were under 55 years of age. Two other steroid preparations were without effect. In a group of older men, over 55 years of age at admission to the study, the advantage in survival rate was depressed or nonexistent. The role of albumin in steroid transport(1,2) and metabolism and its deficiency in the aging, arteriosclerotic subject(4) suggest the possibility that combined Premarin-albumin therapy in the older patients may be more beneficial than Premarin alone.

Summary. The effect of steroid therapy on human plasma albumin has been studied. Daily oral ingestion of 5 mg of norethynodrel and 0.75 mg of ethynylestradiol-3-methyl ether depressed albumin levels by 20.9% ($p<0.002$). A possible relationship of plasma albumin to the effectiveness of estrogenic therapy in older subjects recovering from a myocardial infarction is noted.

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Rejection of the Homotransplanted Dog Liver in the Absence of Hepatic Insufficiency.* (28163)

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Transplantation of a donor liver has repeatedly been attempted(1) particularly in dogs, such as abdominal implantation with one 6-day survivor of 40 animals(2), orthotopic transplantation with one 2-week survivor, and several 1-week survivors in 35 attempts(3) or with preliminary porta-caval shunting and cool saline perfusion(4) with one 3-week survivor and several 6-day survivors. These attempts to study the process of rejection were complicated by hepatic insufficiency and jaundice resulting from the destruction of the donor liver. Therefore, a method has been developed to transplant a whole liver into the pelvis of a recipient animal with its own liver undisturbed *in situ*. This report presents preliminary observations dealing with: a) surgical procedure; b) function of the homotransplanted liver during rejection as indicated by flow and constitution of its bile; c) microscopic changes in the rejected liver; and d) functional and structural response of the host liver as a reflection of the effect of the graft on the host.

Materials and methods. Using donor dogs about 10 kg in weight under intermittent positive pressure endotracheal Nembutal anesthesia, an aortic pedicle is prepared extending from above the celiac axis to the iliac bifurcation. A corresponding vena caval pedicle is constructed extending from the liver to the iliac veins. The portal vein is skeletonized to the superior mesenteric vein, dividing the coronary, splenic and pancreatic branches. In about 20 kg recipient dogs, a second team exposes the infrarenal vena cava, the entire left external iliac artery and the entire left common iliac vein. The hepatic artery connections of the donor dog are then traced to the celiac axis, ligating and excluding superior mesenteric anastomoses as well as left gastric and splenic arteries. The common duct is cannulated with a polyethylene tube and the gall-bladder resected. Hypothermic perfusion of the isolated arterial circuit of the donor liver is commenced with proximal and distal cross clamping, utilizing an external iliac artery as inflow tract. This perfusion with 500 ml of an aqueous solution containing 15% dextran, 5% dextrose and 1,000,000 units penicillin cools the donor liver and avoids intravascular coagulation.

The incision in the donor dog is then extended into the chest with division of the inferior vena cava at the atrium. The liver is separated from its diaphragmatic attachments and the superior mesenteric artery is divided at the aorta. The isolated sterile liver is then transferred to the recipient (Fig. 1). An end-to-side venous anastomosis between the suprahepatic donor vena cava and the infrarenal recipient cava is followed by an end-to-end anastomosis between donor aortic segment and recipient external iliac artery. An iliac-portal venous anastomosis is then performed and the donor liver is stabilized by envelopment beneath the Fallopian tube mesentery. This permits immediate liver perfu-

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