34% of the glycerides are found in the α-lipoprotein fraction.

The values reported demonstrate definite species differences in the percentage distribution of lipids bound to α- and β-lipoproteins. The method employed, because of its simplicity and accuracy, enables further studies of the role of the lipoproteins in lipid metabolism in both human and animal studies. The probable significance of the blood lipids and, consequently, lipoproteins in atherogenesis make such studies of considerable importance.

Summary. Paper electrophoresis and ultracentrifugation have been employed to demonstrate the completeness of β-lipoprotein isolation from chick plasma by a polyanion precipitation technique employing Mepesulfate as the precipitating agent. Data are presented demonstrating that in the chick made hypercholesterolemic by cholesterol feeding, the increase in plasma cholesterol occurs almost entirely in the β-lipoprotein fraction. Lipid distribution in the α-lipoprotein fraction of plasma has been determined for chick, rabbit, guinea pig, rat, mouse and dog as well as for the human. The chick, rat, mouse and dog were found to have more than 50% of the total plasma cholesterol bound to the α-lipoproteins in contrast to man, the rabbit and guinea pig. Values are also presented for distribution of lipid phosphorus and glycerides.

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Unilateral Renal Ischemia and Erythropoietin.* (26992)

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The presence of a circulating erythropoietic stimulating factor (erythropoietin) in the plasma of anemic animals has been demonstrated by a large number of investigators (1). Hypoxia is considered to be the fundamental stimulus for erythropoietin production and therefore erythropoiesis(2). The concept has been developed by Jacobson and his associates that rate of erythropoiesis is determined by the relationship between oxygen tension in the blood and oxygen demand by the tissues (including the site of formation of erythropoietin)(3). The failure of nephrectomized rats, but not ureter-ligated rats, to respond to a single massive hemorrhage or injection of cobalt with an elevation of plasma erythropoietin has led this group to

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conclude that this factor or a precursor is manufactured in the kidneys(4). This finding has been supported by several investigators(5-6). However, other workers have disputed the above conclusion(7-9).

If one assumes the validity of this hypothesis that rate of erythropoiesis is controlled by amount of circulating erythropoietin, and that its production (or that of a precursor) by the kidney is controlled by the oxygen supply-demand relationships of this organ, any procedure that would cause the kidney alone to be hypoxic, and still provide a normal oxygen supply to the rest of the body, should increase the production of erythropoietin and evoke a polycytosis in the animal. Unilateral partial ligation of the right renal artery has been used in these experiments to produce a renal stagnant hypoxia.

Methods. Male Sherman rats, weighing 375-475 g were anesthetized with a light dose of nembutal (1.8 mg/100g) plus ether (1st exp.) or ether alone (2nd exp.). The right kidney was exposed, using a lumbar approach. The right renal artery was constricted by a sterling silver clip that was compressed by a modified surgical needle holder. Degree of closure of the needle holder was controlled by an adjustable setscrew. This procedure, devised by Schaffenburg(10), offered a means of regulating precisely the amount of restriction of the arterial flow and therefore the degree of ischemia that would be obtained. A closure of the needle holder of 0.017″ (0.45 mm) measured with a mechanic's feeler gauge was used for the first series and a closure of 0.011-0.013″ (0.29-0.34 mm) for the second series. In the latter group, the smaller animals received the slightly tighter clamping of the clips.

The rats were exsanguinated under ether anesthesia by cardiac puncture approximately 24 hours after the ligation of the renal arteries. The blood was collected in heparinized syringes and centrifuged for 15 minutes at 3000 rpm. The plasma was frozen and stored until assayed for erythropoietic activity in the starved rat, using the Fe^{59} red blood cell incorporation method recommended by Keighley, *et al.*(11). The plasma from each donor was injected on 2 consecutive days into a single recipient animal, in order to detect any possible variations in the erythropoietic response of each of the artery-ligated rats. Plasma from identical, but unoperated rats was similarly collected and assayed.

Food was withheld from 16-20 young Sherman rats (male in Exp. 1, female in Exp. 2) at zero hours. Either 2 or 2½ ml of the plasma to be assayed was injected I.P. into these animals at 24 and 48 hours. One microcurie of iron-59 (specific activity 8.9 or 4.1 mc/mg) as ferric chloride diluted in ½ ml of 1% sodium citrate was given I.P. at 72 hours. The rats were sacrificed with ether at 90 hours. About 2 ml of cardiac blood was withdrawn into heparinized syringes, and ½ ml of this blood was pipetted into tubes for counting in a well type γ-ray scintillation counter (Baird Atomic model 810) and scaler (Nuclear model 163). Duplicate microcapillary hematocrit values were also obtained.

Results. The renal artery constriction produced by application of the silver clips with the modified needle holder having a 0.45 mm clearance between the jaws caused a moderate restriction of renal blood flow, but the blood vessel ligation using the 0.29-0.34 mm needle holder closure evoked a severe restriction of circulation to the kidney. Grossly, the more loosely ligated kidneys appeared at the time of sacrifice to be slightly enlarged, perhaps edematous, and somewhat pale or ischemic. The kidneys having the more severe restriction of blood flow showed a variable degree of enlargement, were also edematous, usually had an ischemic cortex, and had, in most instances, a hemorrhagic medulla.

Plasma taken from these rats failed on 2 separate occasions to induce a significant increase in the 18 hour uptake of radioiron into the red blood cells of the recipients (Table I) when compared with similarly treated controls that received plasma from unoperated animals in a standard and sensitive bioassay for erythropoietin. The "artery-ligated plasma" also failed to produce a change in hematocrit, the microcapillary hematocrit values being essentially the same for both experimental and control groups.

Discussion. Both a moderate and a severe restriction of renal blood flow was im-

TABLE I. Erythropoietic activity of plasma from rats having a constricted renal artery. Plasma was assayed by measuring RBC radioiron uptake and hematocrit in the recipient animals.

		Rats having constricted renal arteries and their controls (Plasma donors)			90 hr starved rats (Plasma recipients)				
Exp. no.	No. of animals per group	Body wt (g) Before	Body wt (g) After	Degree of Renal A. constriction (mm)	Body wt (g) Before	Body wt (g) After	Plasma dosage (ml × 2 days)	RBC Fe^{59} uptake* (mean ± S.E.)	Hct (%) (mean ± S.E.)
1.	10	443.2	424.3	.45	191.4	141.6	2.5	3.68 ±.28	51.0 ±1.0
	10	428.5	—	None	200.6	151.6	2.5	3.13 ±.23	49.2 ± .7
2.	8	452.8	431.9	.29-.34	140.0	109.5	2.0	2.74 ±.71	48.2 ± .6
	8	431.8	—	None	137.6	107.2	2.0	2.58 ±.56	46.8 ± .9

* Uptake in 18 hours expressed as % of injected dose.

posed in these 2 experiments since, on the one hand, there was evidence indicating that renal tubular function may be essential for erythropoietin secretion by the kidney(12), while, on the other hand, it was considered necessary to constrict the renal artery tightly enough so that a stagnant hypoxia sufficiently intense for stimulation of erythropoiesis might result. The restriction of arterial flow imposed in the first experiment caused a noticeable degree of renal ischemia, and, in the majority of another group of rats, a permanent renal hypertension (unpublished data). While the hemorrhagic renal medullae, observed in the majority of the kidneys ligated in the second experiment, implied that arterial pressure had been reduced to such a level that renal shutdown had occurred, evidence of shutdown was not seen in 2 cases, and in no instance was renal blood flow interrupted. There was no indication of a correlation between erythropoietic activity of the unpooled plasma from the artery-ligated rats, and the observed condition of the tightly ligated kidneys. Since the unoperated, contralateral kidney should have been able to compensate for the loss of its partially ligated partner, renal function should not have been impaired to any significant extent, and it was not expected that these animals would become uremic.

Naets(5), basing his idea upon the "cell separation theory" of Pappenheimer and Kinter(13) believed that the renal tubular epithelium was acutely sensitive to blood oxygenation and might respond to a lack of oxygen by increased erythropoietin production. The failure of unilateral partial ligation of the renal artery to cause an increase in amount of circulating erythropoietin suggests that Naets' inference may not be correct and that the control over production of this factor may be exercised by some other organ. Seip (14) and Osnes(15) have suggested the hypothalamus as the site of this regulation, through either a nervous or neural humoral mechanism.

Recently, Kuratowska(16) has reported that the perfusion of rabbit kidneys with hypoxic blood induced the production of erythropoietin. A reticulocytosis and an increase in percentage of marrow erythroblasts was evoked in mice receiving boiled filtrates of the perfusate. Administration of filtrates of the fully oxygenated blood perfused through the rabbit kidneys caused only a slight reticulocytosis and no increase in marrow erythroblasts in the test animals. Nonperfused rabbit blood was also assayed. This work has been confirmed by Fisher and Birdwell(17), who perfused dog kidney-lung preparations with blood containing cobalt. The dialyzed plasma was assayed by measuring Fe^{59} RBC incorporation in starved rats. The possibility exists that the differences in response were due to the use of perfused kidneys by the former investigators and intact animals by ourselves.

The other possible explanations for this negative result are that partial ligation of an

artery may be an inadequate means of inducing renal hypoxia, or that insufficient quantities of erythropoietin are engendered by unilateral renal hypoxia over a 24 hour period. A moderately severe reduction in renal artery pressure may, by an interpretation of the "cell separation theory," cause an increase in the cell percentage of the normally cell-poor fraction of blood circulating through the peritubular epithelium, and the tissue would therefore receive an adequate supply of oxygen. A more severe and uncompensated restriction in arterial flow might result in failure of tubular function and inability to secret erythropoietin. However, there is evidence (unpublished data) that the juxaglomerular apparatus secreted renin in a group of rats having chronically and severely ligated kidneys. An extended rise in blood pressure was noted in these animals. Since ischemia was seen in even the series of moderately restricted kidneys, the tubular epithelium may very likely have been hypoxic.

Summary. Plasma taken from rats having moderately constricted or severely constricted right renal arteries failed on 2 occasions to induce a significant increase in the 18 hour RBC uptake of Fe^{59} when compared with similarly treated controls that received plasma from unoperated animals. Microcapillary hematocrit values were also the same for the experimental and control assay groups.

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Effect of Ribonucleic Acid Extracts upon Viability of Skin Homografts in the Rat.* † (26993)

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The phenomenon of "actively acquired tolerance" has been described as an induced state in which tissue homografts become immunologically acceptable to hosts which normally would react against them(1). In these now classic experiments, tolerance of skin grafts was produced by exposure of the hosts to foreign homologous tissue cells sufficiently early in foetal life. Further studies have demonstrated the induction of actively acquired tolerance of homologous tissues in newborn mice(2) and rats(3). An extensive series of investigations by the Minnesota group has provided ample evidence that in-

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† Abbreviations: EDTA, ethylenediaminetetraacetate; Tris, tris (hydroxymethyl) aminomethane; ATP, adenosine triphosphate.