TABLE I. Mean Brain Weight, Protein, and 5-HT of Normal Male Rats Exposed for 4 Hours to Air Alone and to 6 p.p.m. Ozone.

Group	g brain/100 g body wt	g protein/100 g brain	μg 5-HT/g brain
Control	.72 ± .06	14.8 ± 1.4	.63 ± .14
Ozone	.78 ± .07*	13.7 ± 1.3	.39 ± .10†

* $P < .01$. † $P < .001$.

possibility was considered that hypercapnia resulting from pulmonary edema might cause brain edema and that this might produce an apparent decrease in brain 5-HT concentration by dilution. That some brain edema may occur is indicated by a slight increase in mean brain weight per 100 g body weight of the ozone-exposed group; however, no significant change is noted in brain protein content. The increase in brain weight (8%) is not sufficient to account for the marked decrease (38%) in brain 5-HT. These results are summarized in Table I.

Discussion. Brain 5-HT of ozone-exposed rats is lost and not merely diluted by the development of brain edema. This finding presents a series of problems. First is that of the nature of the 5-HT release from the brain and whether it is similar to that induced by reserpine. Next is the question of the fate of the released 5-HT, *i.e.*, if it is destroyed immediately in the brain or if it may be transported to the lung thereby accounting for part of the increase in this organ. If the later, the transport mechanism should be studied. Finally, the depression observed in ozone-exposed rats may be related, in part at least, to the loss of brain 5-HT; this should be clarified. These problems are now under investigation, and it is hoped that answers will soon be available.

Summary. A significant decrease in brain 5-HT of rats exposed to 6 p.p.m. ozone for 4 hours has been demonstrated. This was an actual loss of 5-HT from the brain, not merely an apparent decrease resulting from dilution by brain edema although some edema occurred.

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1. Skillen, R. G., Thienes, C. H., Cangelosi, J., Strain, L., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 178.

2. Bogdanski, D. F., Pletscher, A., Brodie, B. B., Udenfriend, S., *J. Pharmacol. Exp. Therap.*, 1956, v117, 82.

3. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., *J. Biol. Chem.*, 1951, v193, 265.

4. Bourdillon, R. B., Fischer-Williams, M., Smith, H. V., Taylor, K. B., *J. Neurol. Neurosurg. & Psychiat.*, 1957, v20, 79.

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Effect of Homologous Blood Components and Heterologous Lysed Red Blood Cells on Dog Renal Dynamics.* (26867)

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Peripheral intravenous infusion of homologous lysed red blood cells produced renal vasoconstriction in dogs(1). These changes in renal dynamics varied with rate of increase in plasma hemoglobin concentration rather than level acheived. On the contrary, no significant renal response occurred when the injection was made into the portal vein(2). Since rates of hemoglobin infusions and plasma hemoglobin concentrations were comparable in the peripheral infusion and portal infusion studies, it was thought possible that passage through the liver altered the effects of these solutions on glomerular filtration rate and effective renal plasma flow. In these experiments, lysed washed dog red blood cells were used, containing red blood cell stroma and other materials of the red blood cells in

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addition to hemoglobin. Although the vasoconstriction might be caused by hemoglobin alone, the possible effects of other red cell components need investigation and are the subject of the present report. In addition, since these components vary from species to species, it was thought advisable to study the effects of heterologous lysed red blood cell infusions when given by peripheral, as well as, portal infusion.

Method. Female mongrel dogs were prepared and measurements of GFR (glomerular filtration rate) and ERPF (effective renal plasma flow) with creatinine and para-aminohippurate, respectively, were done as previously described(1). Femoral artery blood pressures were recorded directly utilizing a Statham gauge and a Sanborn Twin Viso recorder.

A protein free solution was prepared aseptically from dog's red blood cells. The whole blood was spun and washed 3 times with saline in a refrigerated centrifuge. The protein was then precipitated from the red blood cells with alcohol, using 4 cc 95% ethyl alcohol to 5 cc of red blood cells. The precipitate was separated from the solution either by centrifugation or with a Buchner funnel. The solution was Seitz filtered and frozen until used.

For preparation of red blood cell stroma, the washed red cells were lysed in cold distilled water and the pH adjusted to 5.8 with CO_2. The stroma was separated from the solution using a Sharple's centrifuge, washed with 250 cc distilled water, resuspended in a minimal amount of distilled water and frozen.

To prepare the partially purified hemoglobin solution, the washed cells were brought to a pH of 5.5 (0.1 N HCl) with constant agitation. The precipitate was separated by centrifugation and the supernate slowly adjusted to a pH of 7.4 with 1 N NAOH. The solution was Seitz filtered and frozen.

Whole blood was collected with aseptic technics from the sheep, horse, and cow and 50,000 units of aqueous penicillin and 50 mg streptomycin were added to each pint of blood. Each bottle was cultured and discarded if contaminated. The human blood was collected in the hospital blood bank.

TABLE I. Effect of Intravenous Infusion of Blood Components on GFR and ERPF in Dogs.*

No. of dogs		I.V. infusion I	I.V. infusion II	Post-infusion I	Post-infusion II
		Protein-free filtrate			
3	GFR	−5	−7	−2	−1
	ERPF	−7	−7	+2	+3
		Red blood cell stroma			
4	GFR	−1	−7	−15	−2
	ERPF	+1	−12	−18	+1
		Partially purified hemoglobin			
4	GFR	−20	−36	+1	−4
	ERPF	−21	−44	+25	+46

* Figures reported as % of normal control.

Preparation of the lysed red blood cells, determination of plasma and urine hemoglobin, and peripheral vein and portal vein infusions were performed as previously reported(1,2).

Results. Protein free filtrate. The effect of intravenous infusion of a protein free filtrate of red blood cells is recorded in Table I. The slight decrease in GFR and ERPF during actual infusion is insignificant. No significant change occurred in urine volume or blood pressure.

Red blood cell stroma. The effects of the hemoglobin free red blood cell stroma are recorded in Table I. No effect was seen in the first infusion period and an insignificant decrease in GFR and ERPF in the second infusion period. A slightly greater decrease in both GFR and ERPF occurred in the first post-infusion period. No significant changes in urine volume or blood pressure were observed. The stroma solution contained Na 105 mEq. per liter, Cl 104 mEq. per liter, and K 0.3 mEq. per liter. Total protein measured 0.012 g %.

Purified hemoglobin. Partially purified hemoglobin solution (Table I) caused a significant decrease in GFR, ERPF and urine flow in both infusion periods. Rate of hemoglobin infusion was 153 mg/kg/min in the first period and 145 mg/kg/min in the second period. Plasma hemoglobin levels during the first and second infusion periods were 2.0 and 3.4 g % respectively and during the first and second post-infusion periods were 3.4 and 3.2 g % respectively. Blood pressure remained stable during the experiments. The hemoglobin solution contained Na 98 mEq.

TABLE II. Effect on Dog Renal Dynamics of Heterologous Lysed Red Blood Cells during Brachial Vein Infusion in 10 Dogs.*

	Brachial infusion		Post-infusion	
	Hb I	Hb II	I	II
GFR	−64	−57	−26	− 6
ERPF	−60	−54	+1	+28
Plasma Hb, g %	1.9	3.3	3.1	2.9
mg Hb/kg/min.	132	126	0	0

* GFR and ERPF reported as % of normal control.

per liter, Cl 75 mEq. per liter, and K 3 m Eq. per liter. The methemoglobin component averaged 18% of the total with a range from 5% to 25%.

Brachial vein infusion of heterologous lysed red blood cells. Table II is a summary of the effects of the brachial infusion group which consisted of 4 dogs in the human and 2 dogs each in the cow, horse and sheep lysed cell infusion experiments. All experiments are averaged as a group since the effects on renal dynamics were the same with all sources of heterologous blood.

Significant decreases in GFR and ERPF occurred during the infusion (Hb I and Hb II) of the heterologous lysed red blood cells in all experiments. Urine flows were uniformly depressed during infusion periods followed by post infusion diuresis as noted with homologous lysed red cell infusion.

Arterial blood pressure remained relatively stable throughout the experiments except in 2 instances. In one experiment using cow red cell lysate the pressure dropped from a control level of 102/65 to 77/45 over a 15-minute period and returned to slightly above normal upon completion of the infusion. In one experiment using horse red cell lysate the pressure dropped from 120/80 to 45/25 within 3 minutes and returned to 120/85 within 7 minutes after starting the infusion. An insignificant rise above control levels frequently appeared during post-infusion periods.

In all experiments with either homologous or heterologous red blood cell lysates the GFR and ERPF returned to normal levels in the first post-infusion period with one exception. In the first post-infusion period (Post Hb I) of the cow red cell lysate experiments, the GFR and ERPF were greatly decreased and in the second post-infusion period (Post Hb II) the GFR was still significantly decreased. As noted above in one of these dogs blood pressure demonstrated a moderate fall, and both manifested a marked increase in pulse, respiration, and developed purpuric lesions over the entire body. Oozing of blood occurred at the cut-down site where the arterial needle was inserted. Body temperature, recorded by a rectal thermometer, remained normal.

Portal vein infusion of heterologous lysed red blood cells. Table III is a summary of the effects of portal vein infusion. There were 3 dogs in the human and 2 dogs each in the cow, horse, and sheep lysed red blood cell experiments. A significant decrease in GFR and ERPF occurred in all experiments during portal infusion periods (Hb I and Hb II). A moderate decrease in urine flow followed by a post-infusion diuresis was noted as in previous experiments.

During the first infusion period of human lysed red cells blood pressure fell to shock level (46 mm Hg mean pressure) for 4 minutes in one experiment and to 58 mm Hg mean pressure for 2 minutes in another experiment. A similar drop was noted in one instance using cow red cell lysate in which the pressure fell to 70/37 within 3 minutes and returned to normal within 11 minutes. In one experiment using horse red cell lysate the pressure dropped from 133/92 to 70/56 in 4 minutes and gradually returned to 136/100 in 15 minutes after start of infusion. One dog's blood pressure, while receiving sheep red cell lysate, fell from the control level of 140/92 to 70/62 within 6 minutes and back to control level within 12 minutes of start of infusion. The rapid pulse and respir-

TABLE III. Effect on Renal Dynamics of Heterologous Lysed Red Blood Cells during Portal Vein Infusion in 9 Dogs.*

	Portal infusion		Post-infusion	
	Hb I	Hb II	I	II
GFR	−57	−53	− 3	+ 1
ERPF	−64	−49	+33	+35
Plasma Hb, g %	1.9	2.8	2.7	2.6
mg Hb/kg/min.	118	107	0	0

* GFR and ERPF reported as % of normal control.

ation, bleeding tendency, and decreased GFR and ERPF in the post-infusion period noted with brachial infusion of cow's lysed red blood cells did not occur in the portal infusion experiments. The blood used in both groups of experiments came from the same donor cow.

Since the human red blood cell has a high potassium content, potassium levels in the lysed cell solution were determined and found to be 21.5 mEq. per liter. Average potassium of the dogs' control serum was 3 m Eq. per liter and average levels for the periods Hb I, Hb II, Post Hb I and Post Hb II were 4.5, 5.5, 5.3, and 4.9 mEq. per liter respectively. As a result of this rise in serum potassium, it was felt necessary to test normal dogs' GFR and ERPF response to increasing levels of serum potassium. Control GFR and ERPF were determined in 3 normal mongrel dogs followed immediately by slow intravenous infusion of a potassium chloride solution containing 184 mEq. per liter. No decreases in GFR and ERPF were noted until serum potassium rose above 7.5 mEq. per liter. Thus, the increase in serum potassium noted, when human lysed cells were infused, was not the cause of the decreased GFR and ERPF.

Discussion. A previous report demonstrated that peripheral vein infusion of homologous lysed red blood cells in dogs caused an acute renal vasoconstriction which could be reversed or prevented with a vasodilator drug, magnesium sulfate(1). It was felt that the hemoglobin was the direct or indirect cause of the renal vasoconstriction. This was indicated by the demonstration that intravenous infusion of purified commercial human hemoglobin and autogenous hemoglobin in humans caused a decreased GFR and ERPF(3). Also, examination of renal biopsy or autopsy specimens revealed the same histologic renal damage in dogs following intravenous infusion of homologous hemoglobin solutions made from either hemolyzed red blood cells or crystalline hemoglobin(4). However, these reports did not deal with the acute renal effects of homologous hemoglobin in dogs nor the effects of red blood cell stroma without hemoglobin or a protein free filtrate made from homologous red blood cells.

From these present experiments it is apparent that a protein free filtrate of the dogs' red blood cells does not alter GFR or ERPF. The hemoglobin free preparation of red blood cell stroma was also without effect during the active infusion periods. However, it is interesting to note the decrease in both GFR and ERPF in the first post-infusion period. A significant decrease in both GFR and ERPF was demonstrated during infusion of the partially purified hemoglobin but the decrease was not as dramatic as with the lysed red blood cell solutions, even though the quantity of hemoglobin administered was quite high in both infusion periods (153 and 145 mg/kg/min) and plasma hemoglobin levels were as high as noted previously(1,2). These findings indicate that hemoglobin accounts for most of the renal vasoconstriction during infusion of homologous lysed red blood cell solutions but also suggest that the red blood cell stroma probably has an additive effect. This correlates well with the observation in rabbits that sudden death resulted during or immediately after injection of homologous hemolysed blood whereas if stroma was removed by centrifugation much larger amounts of the solution were tolerated (5).

It was noted that initial passage of the lysed homologous blood cells through the liver by direct portal infusion was accompanied by an insignificant effect on GFR and ERPF(2). This was interpreted as due to either a delay of the solution in reaching the general circulation or modification of the vasoconstrictor substance. Basis for believing that the liver specifically altered the homologous hemoglobin might be enhanced if differences in renal response could be demonstrated by infusions of heterologous lysed red blood cells through both peripheral and portal veins.

These experiments demonstrate that peripheral infusion of heterologous lysed red blood cells obtained from the human, horse, cow and sheep caused a significant decrease in GFR and ERPF similar to the effects of homologous lysed red cells. Peripheral infu-

sion of cow red cell lysate caused a decreased GFR and ERPF beyond the period of active infusion and was accompanied by tachycardia, tachypnea and a bleeding tendency. These signs and symptoms are quite similar to those seen in sensitized transfusion reactions in dogs or the reaction of dogs to massive transfusion of heterologous (human) whole blood(6).

All dogs which received the heterologous red cell lysates intraportally showed a significant fall in GFR and ERPF. In almost one half of these experiments a moderate decrease of peripheral arterial blood pressure was noted. The uniform fall in GFR and ERPF in these experiments is probably not due to the moderate vasodepressor effect since mean blood pressure remained above the critical level of 50-60 mm Hg below which the GFR and ERPF will start to fall(7). The interesting observations noted with the peripheral infusion of cows' lysed red cells did not occur during the portal infusion of this solution.

It is apparent that the 'protection' afforded the kidneys by passage of homologous lysed red cells through the liver is not present when the heterologous lysed cells are similarly infused. It would appear, therefore, that the 'protection' is not simply a mechanical slowing of the infusion's appearance in the general circulation. More likely a specific modification of the vasoconstrictor substance occurs in the liver when homologous hemoglobin is used. This mechanism is incapable of efficient modification of heterologous hemoglobin.

Summary. The renal vasoconstriction associated with peripheral intravenous infusion of homologous lysed red blood cells in dogs is primarily caused by hemoglobin although the red blood cell stroma appears to have an additive effect. Peripheral intravenous infusion of heterologous lysed red blood cells of human, cow, horse, and sheep origin cause a significant decrease in GFR and ERPF in dogs. Portal vein infusion of heterologous lysed red blood cells through the liver causes a significant decrease in GFR and ERPF in dogs and is frequently associated with moderate decrease in arterial blood pressure. These experiments suggest that the protection afforded the kidney by initial passage of homologous red cell lysates through the liver is due to specific modification of the hemoglobin, a process the liver is unable to perform with heterologous red cell lysates.

1. DeMaria, W. J. A., Harris, J. S., *Am. J. Physiol.*, 1955, v182, 251.
2. DeMaria, W. J. A., *ibid.*, 1960, v198, 1041.
3. Miller, J. A., McDonald, R. K., *J. Clin. Invest.*, 1951, v30, 1033.
4. Flink, E. B., *J. Lab. Clin. Med.*, 1947, v32, 223.
5. Barratt, J. O. W., Yorke, W., *Ann. Trop. Med.*, 1909, v3, 1.
6. Mueller, C. B., Mason, A. D., *Am. J. Clin. Path.*, 1956, v26, 705.
7. Mendelsohn, M. L., Szuter, C., *Am. J. Physiol.*, 1953, v173, 355.

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Monoenoic Fatty Acids in Dogfish Livers: Isomers of the C_{16}, C_{18}, C_{20}, and C_{22} Series. (26868)

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Recently, interest has been expressed in the structures of unique monoenoic fatty acids from vegetable(1) and marine-animal oils(2) and from human fecal lipids(3). Dogfish (*Squalus acanthias*) liver oil is a rich source of many monoenoic fatty acids(4) whose positional isomers have not been reported. We have isolated most of these fatty acids from the liver oil and have determined their structures by oxidative degradation of the double bonds followed by analysis of the resulting fragments as methyl esters by gas-liquid chromatography (GLC). This paper reports the principal structures of the C_{16},