ducklings. It was rarely evident beyond a 1:4 dilution of the plasma, and was fully removed by heating the plasma for one-half hour at 55-56°C followed by centrifugation to remove the precipitate which formed. In infected ducks 3 months of age or older these heat-labile agglutinins generally decreased as the infection progressed, except in individuals which exhibited a strong innate resistance to the parasites. The heat-labile agglutinins disappeared completely at the peak of a severe infection. Heat-stable agglutinins appeared in the plasma of most of the infected birds. These, unlike the heat-labile agglutinins, were not demonstrable in undiluted plasma but often were active in plasma diluted as far as 1:1000. In ducklings infected when they were one month old or younger, heat-stable agglutinins did not appear. As in severely infected older ducks, the heat-labile agglutinins vanished at the peak of the infection. If such ducklings were saved from death by treatment with quinine the heat-labile agglutinins reappeared to a varying degree. Relapses, like initial infections, were accompanied by a lack of heat-labile agglutinins.

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Experimental Studies on Pathogenesis of Hemoglobinuric Nephrosis. (18337)

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The exact mechanism of the production of the renal lesion following the transfusion of incompatible blood is as yet unclear. It has become apparent that no one factor is responsible, but probably some combination of hemoglobin excretion, the pH of the urine, shock (renal anoxia), and the incompatible reaction *per se* is involved. Many factors have been investigated in the production of hemoglobinuric nephrosis. Among these factors are the "toxicity" of hemoglobin(1-11), the chemical form of the excreted hemglobin (7,12-14), the pH of the urine(1,6,13,14), dehydration(8,9,11,14,15), renal anoxia(16, 17), hemorrhagic shock(2,5), subcortical renal vascular shunt(18), and the incompatible transfusion reaction *per se*(19-21). It has been suggested(22,23,25) that hemo-

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TABLE I. Summary of Experiments, Giving Amount of Blood Removed, Blood Pressure Level During Shock, and Amount of Hemoglobin Infused.

Hemolysed blood	Dog No.	Total amt blood removed (cc/kg)	Approx. avg blood pressure level during shock (mm Hg systolic)	Amt hemoglobin infused (g/kg)	Time elapsed from hemoglobin infusion to full recovery (hr)
Without red blood cell stroma	1	44	60	0.9	20
	5	40	50	1.0	(died 40 min.)
	4	48	53	0.5	20
	6	44	55	0.6	20
	2	49	45	0.6	12
	1	40	48	0.6	8
With red blood cell stroma	7	50	48	1.0	(died 9 hr)
	8	40	53	0.9	12
	4	43	50	0.5	18
	8	40	50	0.7	(died 4 hr)
	12	34	54	0.6	10
	9	47	50	0.7	10
	14	46	54	0.7	(died 17 hr)
Avg		43.4	52	0.7	

globinuric nephrosis may be due to a combination of shock and hemoglobinuria. Two other factors of importance are dehydration and an acid urine, and in hemorrhagic shock both are present(5). Hence it seemed logical to use a procedure that combines all these known factors (shock, hemoglobinuria, acid urine and dehydration). This experimental procedure, moreover, reproduces in most part the sequence of events as it occurs in many human cases of hemoglobinuric nephrosis. Therefore, it was decided to produce hemorrhagic shock of sufficient duration and depth to produce renal ischemia, to follow this by an infusion of hemoglobin solution, and then to bring the animals out of shock by transfusion with their own blood.

Method. Two groups of dogs were used, one receiving dog blood, the red blood cells of which were lysed and the red blood cell stroma removed, and the other receiving hemolysed dog blood containing the red blood cell stroma. Three dogs were used twice. Dog No. 1 was used 2 times in the group receiving hemolysed blood with stroma, dog No. 8 2 times in the group receiving hemolysed blood without stroma, and dog No. 4 once in each group. At least 5 weeks elapsed before the procedure was repeated on any dog. An average of 0.7 g/k of hemoglobin (range of 0.5-1.0) was given; this approximates the amount liberated in one incompatible transfusion in the human. (About 1.0 g/k in a 70 kg man.) Hemoglobin solutions were obtained by rapid freezing of citrated dog blood at −20° F. and thawing just before use. Female mongrels were anesthetized with pentobarbital sodium, and put in hemorrhagic shock. At the end of 40 minutes(24) the prepared hemoglobin solution was infused; this was followed by transfusion of their previously removed citrated blood except for an amount equal to the volume of hemoglobin solution infused (Table I). Blood pressures were recorded throughout most of the transfusion period. Urinalyses and non-protein-nitrogen determinations were performed pre- and post-operatively.

Results. Thirteen dogs were used, 6 in the group receiving hemolysed blood without red cell stroma, and 7 in the group receiving hemolysed blood with stroma. One of the former group and 3 of the latter failed to survive. Of those animals that survived, the results in each group were similar. The in-

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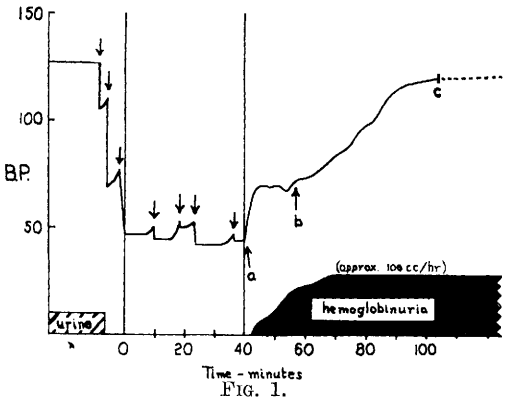

FIG. 1.

This diagram represents a typical recording of blood pressure (systolic, mm Hg) and urine flow during the procedure described in the text. Downward arrows indicate bleeding, the first such arrow representing the largest blood loss. The hemorrhagic shock period extends from 0-40 min. 'a'—start of hemoglobin solution infusion; 'b'—conclusion of hemoglobin infusion and start of citrated blood; 'c'—kymographic tracing discontinued.

fusion of the hemolysed blood was followed by an immediate rise in blood pressure to an average of 80 mm Hg systolic where it remained for about 10 minutes. A second slower rise occurred after a small amount of normal blood was infused, following which the blood pressure returned to normal in 15-20 minutes (Fig. 1). Return to full reactivity occurred within a period of 3-5 hours, and all animals were completely well clinically within 20 hours.

Flow of urine ceased at the onset of the shock period, and resumed in the post-shock period as soon as the blood pressure rose to 75-80 mm Hg systolic. At this time the urine was dark red-brown and remained so for 4-6 hours. For 5-6 hours after the infusion of the hemoglobin solution there was a marked diuresis, yielding as much as 100 cc/hr. All pre- and post-operative urines were acid in reaction. The latter were usually dilute (1.004-1.010) but occasionally animals excreted urines of specific gravity of 1.028-1.034. The exact amount of hemoglobin excreted was not determined. All post-operative urines contained 4+ protein; none contained glucose. Histologic examination of the sediment revealed granular, brown, amorphous material, and rare granular casts. The urine was completely normal within 24 hours. The blood non-protein-nitrogen ranged from 31-48 mg/100 cc pre-operatively, and from 38-48 mg/100 cc post-operatively; in no instance was there any significant elevation within one week after the procedure.

All the animals that died did so within 17 hours of the procedure. The death in the group receiving hemolysed blood without red blood cell stroma was probably due to anesthetic complications. Three dogs died in the group receiving hemolysed blood and red blood cell stroma. It is probable that these animals died in so-called 'irreversible' shock according to Wiggers' criteria(27). Autopsies on these 4 dogs revealed marked congestion of all the viscera and vascular engorgement. Histologic examination of the tissues was unremarkable except for congestion. The kidneys contained numerous hemoglobin casts in the tubules without evidence of tubular degeneration.

Three of the surviving dogs were sacrificed 2, 4, and 5 weeks respectively after the procedure and none revealed any gross or histologic evidence of any renal lesion.

Discussion. Thirteen dogs received hemolysed blood with or without the red blood cell stroma following a period of hemorrhagic shock. The length and depth of hemorrhagic shock employed was probably sufficient to produce almost complete cessation of renal blood flow and resultant renal stagnant anoxia (17). The fact that 4 dogs died of 'irreversible' shock is additional evidence that the hemorrhagic shock was of maximal length and depth compatible with survival. Despite this, none of the 9 surviving dogs developed any evidence of hemoglobinuric nephrosis, and the 4 dogs that died revealed no morphologic evidence of a renal lesion. The reasons for the failure to produce hemoglobinuric nephrosis could be inherent in the technic. Larger doses of hemoglobin, severe dehydration, and marked acidosis could be added alone or in combination to the described method. It is our belief, however, that those who have

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produced renal lesions comparable to hemoglobinuric nephrosis by using these extreme measures have gone beyond the rational pathogenetic sequence of events which usually occurs in the production of hemoglobinuric nephrosis in humans.

An important factor that is present in the production of this lesion in humans but was not included in our experiments is the incompatible transfusion reaction. True transfusion reactions in dogs, however, have been produced(22). None of these animals developed any evidence of hemoglobinuric nephrosis. It is possible, then, that some factor in the incompatibility reaction, plus hemoglobinuria, in addition to prolonged renal anoxia and/or hemorrhagic shock may be responsible for the production of such a lesion. At present we are investigating this line of reasoning.

Summary and conclusions. Prolonged hemorrhagic shock was produced in 13 dogs. This was followed by infusions of hemolysed blood with or without red blood cell stroma. The animals were then brought out of shock with transfusions of their own blood. Four dogs died within 17 hours of anesthetic complications or 'irreversible' shock. The 9 surviving dogs revealed no evidence of hemoglobinuric nephrosis, and were completely well within 20 hours. In this study the combination of prolonged hemorrhagic shock and hemoglobinuria failed to produce hemoglobinuric nephrosis.

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Nucleoprotein Content During the Staphylococcal Growth Cycle.* (18338)

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The key role played by nucleic acids in the processes of normal growth and neoplasia has been well demonstrated in recent years by the work of Caspersson and his group(1). Bacterial cultures offer excellent systems in which to trace the changes in nucleic acid concentrations with growth and it is the object of the present study to determine the concentrations of desoxypentosenucleic acid (DNA) and pentosenucleic acid (PNA) at significant stages in the growth cycle of *Staphylococcus aureus*. Previous investigations of the nucleic acid contents of staphylococcus have been sparse and somewhat contradictory. Thus, Vendrely and Lehoult(2) determined the nucleic acid contents of *Staphylococcus aureus* at a single growth stage (*i.e.* 20 hour cultures on agar) and found the PNA content to be 3 times as high as the DNA content. Price(3) in an analysis of a 22 hour slant of *Staphylococcus muscae* reported equal amounts of PNA and DNA, although in earlier phases of the growth cycle the PNA concentration was 2-2½ times that of DNA.

Methods. Determinations were carried out on *Staphylococcus aureus* of the "L" strain from the Department of Microbiology of Boston University School of Medicine. All cultures were grown in a medium prepared by adding water to 15 g of Bacto-Casamino Acid and 5 g of Bacto-Yeast Extract to a total volume of 1 liter. The medium was adjusted to pH 8 and autoclaved at 10 lb pressure for 20 minutes. In order to have reproducible

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