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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1. Caspersson, T. O., *Cell Growth and Cell Function*, W. W. Norton & Co., 1950.

2. Vendrely, R., and Lehoult, Y., *Compt. rend. acad. sci.*, 1946, v222, 1357.

3. Price, W. H., *J. Gen. Physiol.*, 1949, v33, 17.

lag period behavior, it was necessary to have an inoculum in a resting or decay stage but which contained a minimum of nonviable bacteria, since in the lag phase samplings up to one hour contain the same per cent of nonviable bacteria as the inoculum. Similarly, the inoculum must be large enough to come within range of the chemical technics employed. It was discovered that an inoculum large enough to allow accurate duplicate determination within the first hour was too large to permit normal growth in the logarithmic phase. Inocula were prepared by incubating for 24 hours 1 ml of a previous culture in 100 ml of medium in 250-ml Erlenmeyer flasks at 37°C. For lag phase determinations, 50 ml was spun down in a refrigerated centrifuge and inoculated into 500 ml of medium. For logarithmic phase determinations, 25 ml was so treated. The growth curve was followed by means of turbidity measurement at a wave length of 600 mμ on a Coleman Junior Spectrophotometer. Samples of 100 to 200 ml were removed at selected times, spun down, washed with distilled water and made up to 20 ml. Aliquots were removed for total nitrogen determinations. The samples were then washed with cold 10% trichloracetic acid (which, by inactivating nuclease activity, preserves the nucleic acid molecule relatively intact) and with 95% alcohol. It was found desirable to modify the washing procedure by resuspending the centrifuged bacteria in 1 or 2 ml water before adding the trichloracetic acid or alcohol to prevent clumping. The bacteria were stirred for 10 minutes with each washing. The samples were then hydrolyzed with two 5-ml portions of 5% trichloracetic acid in a water bath at 90°C. The hydrolysates were combined and centrifuged a second time to remove particulate matter and made up to 18 ml with 5% trichloracetic acid. Aliquots were now taken for determination of PNA, DNA, nucleic acid P, and nucleic acid N.

PNA was measured by the orcinol method (4), photometric measurement being made at a wave length of 670 mμ. The procedure was tested by measuring known mixtures of PNA and DNA standards, a correction factor(5) being calculated on the basis of an orcinol-DNA curve. DNA was measured by the diphenylamine method(6), the photometric readings being made at 600 mμ. Commercially prepared PNA and DNA of Nutritional Biochemical Laboratories were used as standards in these two reactions. The preparations were about 90% nucleic acid as calculated from phosphorus determinations. The impurity was determined chromatographically to be largely or entirely adenine, which does not interfere with either the orcinol or the diphenylamine tests. No free phosphate was found. Phosphorus was determined according to the method of Fiske and Subbarow(7). Total nitrogen and nucleic acid nitrogen were measured as ammonia by Nesslerization. The reagent used in Kjeldahl digestion was 1 ml of selenium oxide and 1 ml of a saturated solution of cupric sulfate made up to 100 ml with concentrated sulfuric acid. The photometric measurements were carried out at a wave length of 520 mμ.

Results. The significant data obtained are shown in Table I. The nucleic acid values (in Columns III and IV) have been related to total nitrogen of whole bacteria washed with distilled water. The ratio of nucleic acid N to nucleic acid P (Column V) tends to be somewhat higher than the theoretical 1.7. Total nucleic acid content of the inocula are determined in two ways: by calculation from the nucleic acid P (Column VI); and by addition of the PNA and DNA content as determined colorimetrically (Column VII). Price(3) reports agreement between nucleic acid colorimetry and phosphorus determinations within ± 15%. Ten out of the thirteen sets of determinations agree within this range.

Discussion. Our values agree with those of Price(3) on *Staphylococcus muscae* in having equal amounts of the 2 nucleic acids in a

4. Mejbaum, W., *Z. physiol. Chem.*, 1939, v258, 117.

5. Schneider, W. C., *J. Biol. Chem.*, 1945, v161, 293.

6. Dische, Z., *Mikrochemie*, 1930, v8, 4.

7. Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, v66, 375.

TABLE I. Pentosenucleic Acid and Desoxypentosenucleic Acid Values for *Staphylococcus aureus* of the "L" Strain Over the Early Stages of the Growth Cycle.

Sampling time (min.)	Incr. of optical density at time of sampling	DNA/ total N	PNA/ total N	Nucleic acid N / Nucleic acid P ratio	Total nucleic acid (mg) From P values	Total nucleic acid (mg) PNA + DNA
0	—	.64	.60	1.68	.22	.15
0	—	.59	.49	1.61	.28	.15
0	—	.59	.72	2.50	.16	.14
37	.016	.51	1.02	2.05	.21	.17
40	.020	.62	1.47	1.83	.18	.20
55	.024	.69	1.04	2.45	.14	.14
55	.039	.67	.95	1.80	.21	.21
70	.024	.86	1.44	1.61	.19	.20
90	.056	.76	1.54	1.95	.22	.25
93	.036	.81	1.54	2.10	.19	.20
125	.089	.65	1.41	1.81	.22	.21
185	.089	.80	1.34	2.00	.20	.21
185	.091	.67	1.15	2.00	.22	.23

resting culture. It will be noted, however, that as growth proceeds, both the DNA and PNA concentrations increase, the latter more sharply, to a peak at 1-1½ hours of growth, after which declines take place. The values of Vendrely and Lehoult(2), related to mg of total nitrogen, are 0.625 mg PNA and 0.2 mg DNA. Their very low DNA value suggests that one extraction fails to remove all the relatively insoluble DNA.

Summary. The concentrations of pentosenucleic acid and desoxypentosenucleic acid per total nitrogen content at significant stages in the growth cycle of *Staphylococcus aureus* were determined. The concentrations were equal in resting or decay cultures, rose to a peak at 1-1½ hours of growth and thereafter declined. At peak concentrations, the PNA content was about twice that of DNA.

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Effect of Plaster Cast on Tourniquet Shock.* (18339)

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Experimental tourniquet shock in rats is probably initiated by local fluid loss(1). The following study was made to determine whether shock could be delayed or prevented by applying a cast to reduce the edema. Previous reports indicate that local pressure is beneficial for both rats and dogs in tourniquet(2-4) and traumatic shock(5).

Method. This has been described previously (1). The technic results in severe shock which causes death within a few to several hours. Adult white male rats weighing about 200 to 250 g were used. Circulation in the left hind extremity was occluded for 5 hours by tightly wound rubber bands. Food and

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1. Koletsky, S., and Gustafson, G. E., *J. Clin. Invest.*, 1946, v25, 744.

2. Root, G. T., and Mann, F. C., *Surg.*, 1942, v12, 861.

3. Swingle, W. W., Remington, J. W., Kleinberg, W., Drill, V. A., and Eversole, W. J., *Am. J. Physiol.*, 1942, v138, 156.

4. Mylon, E., Winternitz, M. C., and deSüto-Nagy, G. J., *Am. J. Physiol.*, 1943, v139, 313.

5. Duncan, G. W., and Blalock, A., *Arch. Surg.*, 1942, v45, 183.