

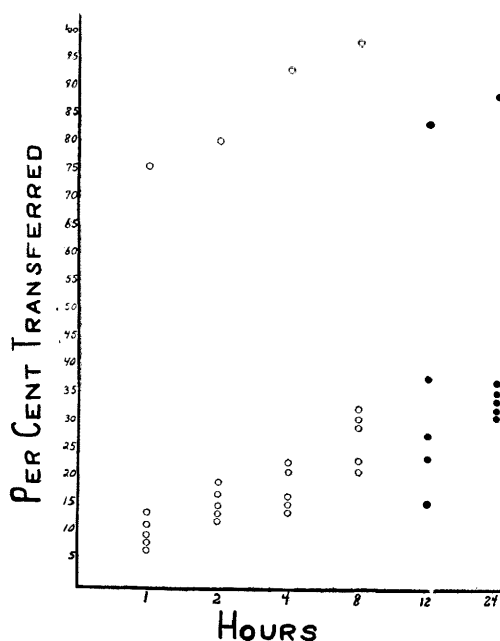


FIG. 1. % Cd^{115} transferred from plasma to human erythrocytes.

unknown. Without the application of more sophisticated technics, it is impossible to determine whether or not cadmium formed a stable complex with the constituents of the red cell surface or entered the erythrocyte proper. The former seems unlikely, particularly since similar studies with radioactive sodium chromate have indicated that most of the test isotope was bound to intracorporeal globin, and only insignificantly to hematin (11). Nevertheless, the numerous metabolic transfer systems of the erythrocyte require localization to the cell surface of enzymes which may bind cadmium to a variable degree. Many intracorporeal substances and

enzymes are capable of uniting with cadmium. Perhaps, as suggested for mercury, cadmium principally combines with thiols. Other reactions may involve amino, hydroxy, imidazole, or carboxyl groups.

Summary. The 24-hour in vitro transfer of radiocadmium, Cd^{115} , from plasma to human erythrocytes was investigated. Most of the isotope became erythrocyte-bound during the first 8 hours of incubation although subsequent kinetics were somewhat obscured by hemolysis. The magnitude of the shift approximated 30% of total cadmium, although the erythrocytes of one subject, who had recently hemorrhaged, exhibited a much greater affinity for the metal. These findings suggest the desirability of further studies to determine the value of cadmium as an indicator of erythrocyte survival.

1. Walsh, J. J., Burch, G. E., *J. Lab. Clin. Med.* 1959, v54, 59.
2. Burch, G. E., Walsh, J. J., *ibid.*
3. Simon, F. P., Potts, A. M., Gerard, R. W., *Arch. Biochem.*, 1947, v12, 283.
4. Gilman, A., Phillips, F. S., Allen, R. P., Koelle, E. S., *J. Exp. Pharm. Ther.*, 1946, v87, 85.
5. Kech, H., Smith, E., Shimp, N., Connor, J., *Cancer*, 1956, v9, 499.
6. Tietz, N. W., Hirsch, E. F., Neyman, B., *J.A.M.A.*, 1957, v165, 2187.
7. Margoshes, M., Vallee, B. L., *J. Am. Chem. Soc.*, 1957, v79, 4813.
8. Burch, G. E., Reaser, P., Ray, T., Threefoot, S., *J. Lab. and Clin. Med.*, 1950, v35, 626.
9. *Bull. of Scripps Metabolic Clinic*, San Diego, Calif., Aug. 1951, v2.
10. Granick, S., *Blood*, 1949, v4, 5, 404.
11. Nekeles, T. F., Weinstein, Erwin M., LeRoy, George, V., *J. Lab. and Clin. Med.*, 1953, v42, 358.

Received July 20, 1959. P.S.E.B.M., 1959, v102.

Comparison of Physiological Properties of Certain Phage-typable Coagulase-positive Staphylococci.* (25243)

WILLSON J. FAHLBERG AND JUDITH MARSTON

Dept. of Microbiology, Baylor University College of Medicine, Houston, Texas

Although the literature on physiological activities of Staphylococci is extensive, little

* Supported in part by U.S.P.H.S. Grant.

has been published concerning a comparison of these properties between different phage-typable groups. The purpose of our studies

TABLE I. Biological Activities of Certain Strains of *Staphylococcus pyogenes*.

	Phage type			
	(a) 44A 16 strains	(b) 44A/80/81 31 strains	(c) Other 9 strains	(d) Non-typable 34 strains
Free coagulase				
a. Tube	100	100	100	100
b. Glycine-tellurite	"	"	"	"
Bound coagulase	"	94.7	"	78.2
Pigment (orange)	"	89	"	96.8
Mannitol	"	100	"	100
Delta lysin	68	16	77.1	73
Alpha "	81	81.1	100	89
Beta "	6	3.2	11	28
Fibrinolysin	18.7	25	11.7	50
Phosphatase	100	100	100	100
Urease	18.7	.0	.0	23.5

was to determine whether there was any correlation, other than coagulase production, of biological activity and phage type.

Materials and methods. Ninety strains were collected of freshly isolated coagulase-positive staphylococci from human pyogenic infections. These organisms were phage typed, through the courtesy of Dr. J. V. Irons, director, Texas State Health Laboratory, Austin, according to the standard procedure. For our purpose the staphylococci were divided into 4 groups as follows: Those lysed by a) phage 44A, b) by both phages 44A and 80/81, c) by one or more phages other than 44A or 80/81, and d) those not lysed by any of the 25 standard phages. Ability of staphylococci to form "free" coagulase was measured by 2 procedures: The tellurite-glycine method of Zebowitz *et al.*(1) and standard test tube dilution procedure employing fresh rabbit plasma and supernatant of 24 hour culture of staphylococci grown in brain-heart infusion broth. Bound coagulase was measured by the slide method in accordance with Cadness-Graves *et al.*(2).

Alpha hemolysins as defined by McFarlan (3) were measured on 5% rabbit blood agar. Beta hemolysins were quantitated by determining their activity on sheep red blood cells, Smith and Price(4), and the culture filtrate heated to 55°C for 30 minutes thus destroying rabbit cell lysins and leaving about 50% of original beta lysin intact. Delta lysin activity was measured on human red blood cells after Marks and Vaughan(5).

Fibrinolysin activity was determined by procedure of Neter(6). The presence of the

enzyme urease was tested for using urea broth as outlined by Fusillo and Jaffurs(7). Phosphatase activity was measured by splitting of phenolphthalein from phenolphthalein diphosphoric acid in agar, see Hare and Thomas (8). Pigment production was assessed on Staph 110 agar (Difco) following 48 hours incubation at 30°C. Ability to utilize mannitol as source of carbohydrate was measured in mannitol broth (Difco) incubated 48 hours at 37°C.

Results. Table I shows the correlation of tube-plasma method of determining free coagulase and the tellurite-glycine agar was excellent. It further appears that it measures free coagulase and not bound. All free-coagulase-positive staphylococci produced phosphatase and utilized mannitol and the majority produced the characteristic golden pigment.

In accordance with observations of Elek and Levy(9) it appears that beta lysin production is not common to strains of staphylococci from human sources. Although 28% of non-typable group produced some sheep blood hemolysin, titers of activity rarely exceeded the 1:2 dilution. Close correlation of alpha lysin production with coagulase is a well-known fact. It, however, cannot be a sole criterion for pathogenicity as all our strains were isolated from a pathologic process and some failed to produce alpha lysin. We were unable to confirm the observation of Joiris(10) that human strains of staphylococci producing alpha lysin almost invariably produce delta lysin. Only 16% of Group b

(phage type 44A/80/81) produced both hemolysins.

It is of some interest that 50% of Group d (non-typable) strains exhibit fibrinolytic activity. This value is considerably higher than the 14% reported by Neter(6). While Fusillo and Jaffurs(7) defined a relationship between activity of coagulase and inactivity of urease among strains of staphylococci, our observations showed some urease activity in strains of Group a (44A) and Group d (non-typable).

Summary. Free coagulase can be measured adequately by either the tube-plasma method or the tellurite-glycine method. We were unable to establish any direct relationship between certain groups of phage-typable staphylococci and production of alpha, beta or delta hemolysins. Fibrinolytic activity was variable but 50% of Group d (non-typable) exhibited some activity.

1. Zebovitz, E., Evans, J. B., Niven, C. F., Jr., *J. Bact.*, 1955, v70, 686.
2. Cadness-Graves, B., Williams, R., Harper, G. J., Miles, A. A., *Lancet*. 1943, v1, 736.
3. McFarlan, A. M., *Brit. Med. J.*, 1938, v2, 939.
4. Smith, M. L., Price, S. A., *J. Path. & Bact.*, 1938, v47, 361.
5. Marks, J., Vaughan, A. C. T., *ibid.*, 1950, v62, 597.
6. Neter, E., *J. Bact.*, 1937, v34, 243.
7. Fusillo, M. H., Jaffurs, W. J., *ibid.*, 1955, v70, 481.
8. Hare, R., Thomas, C. G. A., *Brit. Med. J.* 1956, v2, 840.
9. Elek, S. D., Levy, E., *J. Path. and Bact.*, 1950, v62, 541.
10. Joiris, E., 1952, *Rev. belge. Path.*, v22, 185, as quoted by Elek, S. D. *Staphylococcus pyogenes* and its Relation to Disease, 1959 E. & S. Livingstone Ltd., Edinburgh and London.

Received July 22, 1959. P.S.E.B.M., 1959, v102.

Lowered Glucose Utilization, Phosphate Uptake and Reduced Glutathione of Erythrocytes Following Uretero-Caval Anastomosis.* (25244)

E. E. MUIRHEAD AND F. JONES (With assistance of B. Brooks)

Dept. of Pathology, University of Texas, Southwestern Medical School, Dallas

Following bilateral nephrectomy of the dog, glucose utilization, phosphate uptake and reduced glutathione content of erythrocytes are significantly lowered(1). Bilateral nephrectomy is also associated with a prominent shortening of the life span of erythrocytes(2). Anastomosis of one ureter into the inferior vena cava followed by contralateral nephrectomy is attended by the same degree of retention uremia as after bilateral nephrectomy, yet the life span of erythrocytes is significantly improved toward normal during uretero-caval anastomosis(3). These findings have suggested protection against *in vivo* hemolysis by intact renal tissue(3). With this background it became of interest to determine the state of glucose utilization, phosphate uptake and reduced glutathione content of erythrocytes in the uremia of the dog following uretero-caval anastomosis.

rocytes in the uremia of the dog following uretero-caval anastomosis.

Material and methods. Normal mongrel dogs were used. Under nembutal anesthesia one ureter was severed near the urinary bladder and connected to the inferior vena cava by means of a polyethylene tube. Through a purse string suture about a puncture of the vena cava the polyethylene tube was extended for 3 to 5 cm along the blood stream. Three to 4 days later the contralateral kidney was removed during ether anesthesia. Control blood samples were obtained prior to nephrectomy. The test samples were obtained 4 days following removal of the contralateral normal kidney. After the test sample was obtained the animal was sacrificed and the patency of the ureter and tube and state of the kidney were determined. In 5 of the 7 experiments the ureter and tube were open; there was no hydronephrosis and the kidney was of normal

*Supported by grant from Nat. Heart Inst., U.S.P.H.S.