As a control for the method, radioactive phosphorus in the form of sodium phosphate was injected intravenously into three pouched dogs. The blood was observed to be radioactive for over 45 minutes but the gastric pouch juices secreted during this period exhibited no radioactivity, according to the criteria adopted in these experiments.

Summary. The foregoing observations show that "tagged" chloride ions when injected into the general circulation are identified in the acid gastric juice of the stimulated stomach in dog and in man as quickly as 60 to 120 seconds after injection. Presumably at least some of these ions in the juice were derived from the HCl present. Tagged chloride ions continued to circulate in the blood for more than one hour after intravenous injection and were transported into the gastric juice continuously during this period. Such ions did appear in the urine but were detected at irregular intervals and in relatively low concentration.

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Availability of Staphylococcal Antitoxin After Intramuscular Injection into Normal Monkeys and Men.*

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The increasing use of staphylococcal antitoxin[1, 2] makes it advisable to study its rate of absorption and concentration in the blood after injection of therapeutic doses. In accordance with previous work on diphtheric antitoxin[3] and antipneumococcal serums,[4] daily estimations were made of the titers of 6 "normal" human subjects, who were not suffering from staphylococcal infections and of 9 normal monkeys *(M. mulatta)* after intramuscular injections of Squibb's concentrated antitoxin. Some of each group were injected into the gluteal muscle with 1000 units per kg, while others received 2 such doses, 24 hours apart (Table I). *Alpha* antistaphylolytic serum

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1 Baker, L. D., and Shands, A. R., *J. A. M. A.*, 1939, **113**, 2119.

2 Stookey, P. F., and Scarpellino, L. A., *So. Med. J.*, 1939, **32**, 173.

3 Glenny, A. T., and Hopkins, B. E., *J. Hygiene*, 1924, **22**, 12-36; 208-222.

4 Finland, M., and Brown, J. W., *J. Immunol.*, 1938, **35**, 245.

TABLE I.

Summary of Data on Normal Monkeys and Persons Injected Intramuscularly with Varying Doses of Concentrated Staphylococcal Antitoxin (Squibb) and Rabbits* Injected Intravenously with Diphtheric Antitoxin.

Animal or patient No.	Wt, kg	Plasma volume, cc	Units of antitoxin per kg	Max. increments of serum titers (Units *alpha* antistaphylolysin per cc)	Sums of conc. of antibody 9 days (Areas)	Avg of areas	Antibody available in total plasma vol.	Avg
				Normal monkeys (*M. mulatta*).				
229	3.0	162.0	1000	25	110	113.5	17,800	19,160
253	3.4	183.6	1000	26	131		24,050	
243	2.5	135.0	1000	14	86		11,600	
244	2.75	148.5	1000	29	137		20,340	
252	4.0	216.0	1000	19	103		22,000	
254	2.7	145.8	2000	35	170	233.8	24,800	32,620
230	2.6	140.0	2000	45	278		39,030	
255	2.0	108.0	2000	38	270		29,160	
251	3.2	172.8	2000	38	217		37,500	
				"Normal" human subjects.				
1. M.R. (g)	57	3,078	1000	8.5	48	43	147,700	141,550
2. R.G. (a)	66	3,564	900	6	38		135,400	
3. N.C. (c)	56	3,024	2100	15	101	93	305,420	288,115
4. J.F. (m)	59	3,186	2000	25	85		270,810	
				Normal rabbits* (injected intravenously with 750 units diphtheric antitoxin.				
83 f	3.24	129.6	231.0	5.25	12.03		1,558	
109 f	2.84	113.6	264.0	7	15.90		1,806	
110 f	2.81	112.4	267.0	8	15.07		1,694	
94 y	0.91	36.4	824.0	29	34.17		1,244	
84 y	0.40	16.0	187.0	16	79.24		1,268	

(g) gastric ulcer; male, age 59.
(a) arthritis; male, age 31.
(c) non-specific conjunctivitis; male, age 25.
(m) moron; male, age 17.
*Illustrative data selected from tables of Glenny and Hopkins.[8]
y very young animals.
f full grown; almost mature rabbits.

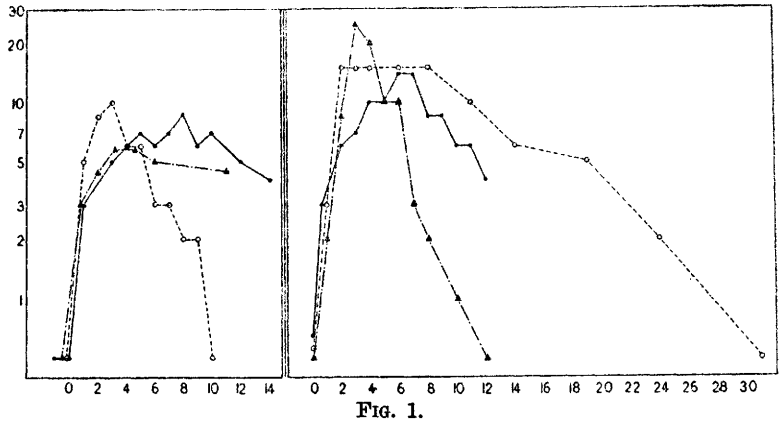

FIG. 1.

Rate of absorption of antistaphylococcal antitoxin after intramuscular injection into "normal" human subjects. Left graphs, 3 persons injected with 1000 units per kg; right graphs, 3 injected with 2000 units per kg. Ordinates: logarithms of units of *alpha*-antistaphylolysin per cc of serum; abscissæ, days after injection.

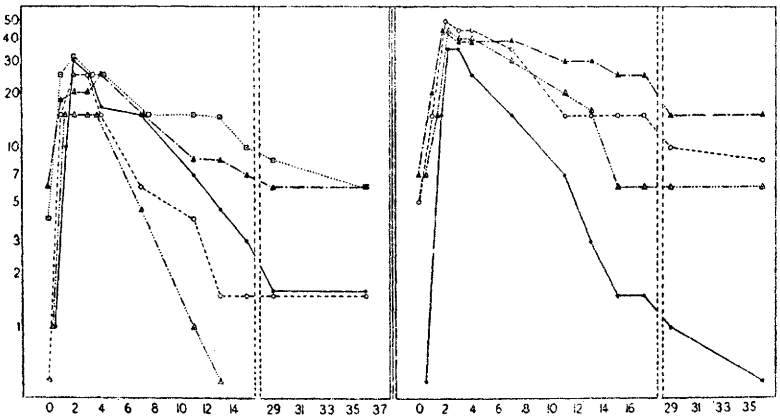

FIG. 2.

Rate of absorption of antistaphylococcal antitoxin after intramuscular injection of normal monkeys (*M. mulatta*). Left graphs, 5 monkeys injected with 1000 units per kg. Right graphs, 4 monkeys injected with 2000 units per kg. Coördinates as in Fig. 1.

titers were determined by the method of the National Institute of Health.[5]

Figs. 1 and 2 show striking uniformity in the rates of absorption of *alpha*-antistaphylolysin. Semi-logarithmic curves present 3 phases. In humans, phase A, the stage of absorption, lasted 3 to 8 days. Phase B, the period of maintenance of maximal serum titers, 1 to 6 days, the average being 3 days. Phase C, the stage of elimination of antibody,

[5] National Inst. Health, Washington, D.C., B-1199, Jan., 1936, U. S. Standard Staphylococcus Antitoxin.

extended from 2 to 4 weeks. The maximal increments in titer after 1000 units per kg of antitoxin, varied from 6 to 10 units. After 2000 units, maximal titers varied from 15 to 25 units. In monkeys injected with similar doses, phase A lasted 1 to 4 days; phase B, 3 to 6 days; phase C, from 4 to 5 weeks. The maximal increments were much higher than in humans—the titers ranging from 15 to 30 units after 1000 units of antitoxin and 35 to 50 after 2000 units per kg. It must be emphasized that the natural titers of monkeys are higher than those of humans. In the latter they were less than 1 unit whereas in monkeys they varied from less than 1 to 7 in this series, but may reach as high as 20 or more units per cc.[6]

In most instances, the serum titers reverted to their natural levels after elimination of the passively transferred antibody.

Because of individual variations in maximal serum titers and in the time of elimination of the antitoxin, it became necessary to develop a method of indicating the total available circulating units of antitoxin during a given period of time as the result of the injection of a given dose. By plotting on standard graph paper, concentrations of *alpha* antistaphylolysin per cc of serum as ordinates and time, in days after injection, as abscissæ, and integrating these curves, the areas underneath represent the summation of concentrations of antibody that have left their storehouse in the reticulo-endothelial system[7] and appeared in the blood stream during the period of observation. The areas may be conveniently determined by weighing the paper under the curve, counting the units squares or by use of a planimeter.

When 5 monkeys were each injected with 1000 units per kg, the average concentration of available antibody during 9 days was 113.5 units per cc (Table I). In 4 other monkeys similarly injected with twice the dose per kg, the average was double or 233.8. In human subjects the values for similar dosage and time-intervals were approximately 43 and 93 respectively, the ratio being again approximately 1 :2. Multiplying these values by the weights and the factor 54, one obtains the number of units of antitoxin appearing in the total plasma-volume.[8] Thus, in persons receiving 1000 units per kg, the total available circulating units in the course of 9 days was 141,550, whereas in 2 others receiving approximately twice this dose per kg, the total was about twice as large, or 288,115. The corresponding average values for monkeys are 19,160 and 32,620. In all instances,

[6] Weiss, C., *J. Immunol.*, 1939, **37**, 185.

[7] Sabin, F. R., *J. Exp. Med.*, 1939, **70**, 67.

[8] Brown, G. E., and Keith, H. Z., quoted in Peters, J. P., and van Slyke, D. D., *Quantitative Clinical Chemistry*, Williams and Wilkins, Baltimore, 1935, **1**, 725.

therefore, irrespective of the maximal titers or the duration of elimination, the sum of the concentrations of antibody during a given period of time (area under the curve) or the antibody content of the total plasma volume was directly proportional to the dose injected per kg weight.

This new procedure was also applied to data selected from Glenny and Hopkins.[3] They injected a constant dose of diphtheric antitoxin (750 units) into each of 5 rabbits weighing from 0.400 to 3.240 kg. Although the maximal titers and duration of elimination varied, as reflected in the sums of the concentrations of antibody during 9 days (12 to 79), nevertheless the antibody available in the total plasma was practically identical in all animals. In other words, with a given total dose injected, the turnover of antibody in the plasma is the same.

Preliminary observations on *beta* antistaphylolysin[9] revealed a uniform rate of absorption but the serum titers reached were very small, never exceeding 1 to 6 units, in normal persons and monkeys respectively. This may be explained by the low concentrations in commercial antitoxin which did not exceed 15 and 80 units per cc in Lederle's and Squibb's antitoxin respectively.

Summary and Conclusions. After intramuscular injection of staphylococcal antitoxin the rate of absorption is quite uniform in normal monkeys and humans. The antibody available in the total plasma-volume during a given period of days after injection of a specified dose (per kg weight) may be determined from the area under the curve, formed with time intervals as abscissæ and daily concentrations of antibody per cc as ordinates, multiplied by the plasma volume. This new method of calculating available circulating antibody has also been applied successfully to the data of Glenny and Hopkins[3] on diphtheric antitoxin and hence may be of general immunological interest.

[9] Smith, M. L., and Price, S. A., *J. Path. and Bact.*, 1938, **47**, 361.