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THE ORAL ADMINISTRATION OF ANTITOXINS FOR
PREVENTION OF DIPHTHERIA, TETANUS, AND
POSSIBLY SEPSIS, WITH SOME OBSERVATIONS ON
THE INFLUENCE OF CERTAIN DRUGS IN PREVENT-
ING DIGESTION AND PROMOTING ABSORPTION
FROM THE ALIMENTARY CANAL.*

CHARLES T. MCCLINTOCK AND WALTER E. KING.

(From the Department of Experimental Medicine, Parke, Davis & Co., Detroit, Mich.)

At the the 1902 meeting of the American Medical Association at Saratoga Springs, N. Y., one of us presented a paper entitled: "The Absorption of Albumins and Globulins."¹ This paper gave a summary of the results obtained up to that time in attempting to find the conditions under which sera were absorbed from the alimentary canal and what agents, if any, could be used to further this result. The conclusion reached at that time was stated thus: "These experiments so far appear to show either that antitoxin is not absorbed in the same proportion as the total albumins of serum, or is changed in being absorbed, the majority of the guinea-pigs dying when injected with several times the fatal dose of toxin, despite the large absorption of albumins."

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¹ *Jour. Am. Med. Assoc.*, 1903, 40, p. 635.

Since that report, the work has been continued, and we are now able to take series after series of guinea-pigs or rabbits, and by the oral administration of antitoxin save 100 per cent of the treated animals, when the antitoxin is administered before the toxin; while the untreated ones, receiving the same dose of poison, invariably die. There were many failures before reaching this stage of the work. In many cases in the earlier work, one or two pigs of a series of 10 would survive, the rest die; but gradually we appeared to learn the conditions necessary for success and something also as to the medicinal agents which would assist in promoting absorption of the antitoxic serum. The work is far from being completed. We find that there are a number of medicinal agents, on the one hand, tending to arrest digestion, on the other, helping to promote absorption, which may be used; but as to which of these and in what quantities it is best to use, we are as yet unable to say with certainty.

One may well ask: As we know that antitoxic serum may be injected hypodermically with good results why use an uncertain method?

It appears to the writers that there are two principal reasons for the attempt: (1) there is a certain amount of danger from any and all hypodermic medication. Death has been reported from the injection of one-eighth of a grain of morphine. A number of sudden deaths have followed the injection of antitoxic serum. We mean now deaths not due to contaminated or impure serum, but apparently due to an idiosyncrasy of the patient, the fatal results following in a few moments or hours after the injection.

Again, there are a number of very unpleasant skin and joint complications, urticaria, erythema, purpura, angioneurotic edema, that may follow hypodermic injection of even small quantities of serum. So far it appears that these phenomena are more prominent in prophylactic than in curative injections. In the 17 men and boys used in the following experiments, not one has shown any skin or joint disturbance.

(2) A large part of the cost of antitoxic serum is due to the exceptional care, the repeated careful tests that must be made in order to secure the fitness of the material for hypodermic administration. Roughly speaking, antitoxic sera, as they come from the animal,

do not cost more than one-half to one-third of what the product ready for hypodermic injection actually costs. Again, it is more than probable, although not yet demonstrated, that the serum to be given per mouth can be given in the dry form, and in such condition it will keep indefinitely. The high cost of liquid serum limits, to a certain extent, the prophylactic use of the serum, and if we should be fortunate enough to find serum of value in preventing suppurative diseases, tuberculosis, and other chronic diseases, where it would be advisable to keep the patient under treatment for some time, there would manifestly be great gain in being able to give this material per mouth rather than hypodermically.

The results thus far obtained do not warrant the use of antitoxin per mouth for curative purposes. The results are, and probably always will be, more uncertain, and, as reported by several observers, the effect is much slower than by the hypodermic method.

Practically, if a child has been in school and tomorrow one of the pupils in the room with the child develops diphtheria, it is not known how severe the exposure has been, and one hesitates as to the desirability of giving the hypodermic injection of serum as a prophylactic, but, could one give one or two doses of serum per mouth at a moderate cost, the matter would take on a different light. Or, here is a minor or dirt-infected wound, is it worth while to fear tetanus, and give a prophylactic dose of serum hypodermically? The practical answer to this, given by the profession, often is to take the chance. The serum is not given.

In the voluminous literature on serum therapy many references are made to the oral administration of antitoxins. A few cases are reported of a certain amount of immunity being produced when the antitoxin was given or taken per stomach. Here and there some clinical observer has reported good results following such administration, but practically the entire consensus of opinion is that the oral administration of sera is without value. It is generally believed that both toxin and antitoxin are not absorbed as such. Our experiments, however, lead us to the conclusion that both toxin and antitoxin are absorbed as such, unless they are destroyed by the secretions of the alimentary canal.

The specific objects in view in this investigation are:

1. To confirm work already done, showing that under certain conditions antitoxins can be absorbed.
2. To find under what conditions antitoxins would be absorbed in the greatest amount and with the greatest uniformity.
3. To ascertain whether the oral administration of diphtheria and tetanus antitoxins can be depended upon as safe and advantageous prophylactic measures.

TECHNIQUE

Guinea-pigs, rabbits, dogs, and men and boys have been used in the work thus far. In giving the antitoxin per mouth to guinea-pigs, several methods were tried. First, a small catheter was introduced into the stomach, and the serum was forced through this from a syringe or pipette. Later, the antitoxin was put into small capsules, and the pig was forced to swallow them. Finally it was found that the best way to administer the serum per stomach was to hold the mouth of the animal open with forceps, place the given amount of serum well back in the mouth very slowly, one drop at a time, and allow the pig to swallow it. By using care and patience and permitting the pig to close its mouth at intervals during the administration, in most cases not a drop of the serum was wasted.

Full-grown rabbits were treated by the above method very quickly and successfully. In case of small rabbits, the catheter sometimes had to be used.

The antitoxin was given to dogs by placing it in large gelatin capsules, and causing them to be swallowed by the dog.

ADMINISTRATION OF ANTIDIPHTHERIC SERUM, ALONE AND IN COMBINATION WITH VARIOUS DRUGS, TO GUINEA-PIGS PER STOMACH

A series of 121 pigs was used, divided into groups of six, with one control pig for each group. The following drugs, in small amounts, were added to the serum given to some of the groups: trikresol, opium, sodium bicarbonate, morphine sulphate, and chloroform. Practically all of these pigs were lost, the administration of a few hundred units of antitoxin alone and in various combinations with the above drugs not giving sufficient protection against the injection of two or three fatal doses of toxin. Finally, however, it was found that pigs treated with serum to which morphine, cocaine, and trikresol had been added could be saved from a few fatal doses of toxin although with no very great degree of uniformity. These pigs were injected with the toxin 12 to 24 hours after the administration of the serum. Fifteen pigs treated in this way with from 1,000 to 2,000 units of antitoxin withstood from 2 to 10 fatal doses of toxin.

The results of these tests which we naturally concluded were based upon the paralyzing action of the morphine and cocaine upon digestion led us to try the administration of antitoxin per intestine.

The technique of administration per intestine consisted in making an abdominal incision, securing a loop of small intestine, and injecting into it with a very small needle a given amount of serum. The intestinal loop was then replaced and the peritoneum and skin sewed up. Ether was used as the anesthetic.

The following series of pigs were treated with diphtheria antitoxin per intestine, and 15 hours after were injected subcutaneously with the toxin:

TABLE 1

Guinea-Pig No.	Units Anti-toxin	Fatal Doses Toxin	Result
1.....	600	5	Dead 6 days*
2.....	300	5	+
2.....	600	5	+
4.....	300	5	+
5.....	300	5	+
6.....	600	10	+
7.....	600	10	+
8.....	300	5	Dead 4 days*
9.....	300	10	+
10.....	300	15	+
11.....	300	20	+
12.....	300	25	Dead 3 days
13.....	300	35	+
14.....	300	40	+
15.....	300	50	Dead 5 days
16.....	300	60	" 5 "
17.....	300	70	" 5 "
18.....	300	25	" 3 "
19.....	300	35	" 3 "
20.....	300	40	+
21.....	300	50	+
22.....	300	60	+
23.....	300	70	Dead 3 days
24.....	300	25	+
25.....	300	35	+
26.....	300	40	+
27.....	300	50	Dead 4 days
28.....	300	60	" 3 "
29.....	300	70	" 4 "
30.....	300	80	" 3 "
31.....	300	25	" 4 "
32.....	300	35	+
33.....	300	40	Dead 3 days
34.....	300	50	" 4 "
35.....	300	60	" 3 "
36.....	600	40	" 2 "
37.....	900	50	+
38.....	1,500	70	+
39.....	2,100	100	Dead 2 days
40.....	600	20	+
41.....	900	25	+
42.....	1,500	35	Dead 4 days
43.....	1,800	40	+
44.....	2,100	45	+
45.....	2,100	45	Dead 6 days
46.....	2,400	50	+
47.....	300	15	+
48.....	300	20	+
49.....	200	25	+
50.....	300	25	+
51.....	300	30	Dead 3 days
52.....	300	35	" 3 "
53.....	300	40	+

With this series of 53 pigs 11 control pigs untreated and injected with 5 fatal doses of toxin, all died in from 2 to 4 days. Pigs 1, 8, and 16 died from peritonitis. It is possible that others died from shock.

Taking into consideration the delicacy of the operation and danger of death from shock and peritonitis, the results are fairly uniform, 30 pigs being saved out of the 53. It will be observed that many of these received enormous doses of toxin and comparatively small administrations of antitoxin.

Most of the pigs in the above series were given, per intestine, antitoxin to which was added small amounts of strychnin and potassium bicarbonate.

The result of the work in administering antitoxin per intestine demonstrates that absorption from the intestine and consequently protection from relatively enormous doses of toxin is possible. Our efforts were now directed toward administering the antitoxin orally and producing conditions such that the antitoxin would be unaffected by peptic digestion and pass into the intestine for the most part unchanged.

ORAL ADMINISTRATION OF ANTIDIPHThERIC AND ANTITETANIC SERA IN COMBINATION WITH VARIOUS DRUGS.

The specific purpose at this time in our work was to protect the antitoxin from peptic digestion, and cause it to pass into the intestine unchanged. A series of 104 pigs was used, divided into groups of 4 to 6, with a control pig in each group. Each pig received per stomach from 300 to 1,500 units of antidiphtheric serum in combination with various drugs. Fifteen hours after giving the serum each pig received five fatal doses of toxin subcutaneously. The following substances alone and in various combinations were added to the serum: cocaine, morphine, calomel, strychnin, opium, sodium bicarbonate, potassium bicarbonate, bismuth subnitrate, oil emulsion, and soap solution. Every one of these 104 pigs was lost.

Finally, after experimentation with several series of pigs, it was found that a combination of fluid opium and a saturated solution of salol in chloroform, added to the serum, and the mixture thoroughly shaken until an emulsion was produced, gave the best results. The

serum in all cases contained 0.04 c.c. of 1 per cent trikresol. The saturated solution of salol in chloroform and fluid opium were added in the proportion of one drop of the former to each c.c. and one drop of the latter to each 10 c.c. of antitoxin. The fluid opium concentrated contained 22 grains crystallized morphine in each fluid ounce, and was four times stronger than Tincture Opium U. S. P. Hereafter in this work all antitoxin given per mouth was prepared with the above ingredients.

Out of a series of 61 pigs treated by this method, 57 were saved, or 93.4 per cent. These pigs were kept without food for 24 to 48 hours before treatment, and 12 to 24 hours after administration of the serum were injected subcutaneously with the toxin; 15 control pigs, receiving the same amount of toxin as those treated, all died in less than five days.

The following table gives the result of this series with the amount of antitoxin and dosage of toxin used:

TABLE 2.

Group	No. Units Antitoxin	No. Fatal Doses Toxin	No. Pigs in Group	No. Control Pigs in Group	No. Treated Pigs in Group	No. Treated Pigs Lost	No. Treated Pigs Saved
44.....	600	3	4	1	3	0	3
45.....	1,200	3	6	1	5	0	5
48.....	1,200	3	6	1	5	1	4
49.....	1,000	3	12	2	10	0	10
52.....	1,500	3	10	2	8	0	8
54.....	1,500	3	10	2	8	2	6
55.....	1,500	2	6	2	4	0	4
56.....	1,500	2	8	2	6	0	6
57.....	1,500	2	12	2	10	1	9
Total...			74	15	59	4	55

Diphtheria antitoxin per stomach, rabbits.—Eighteen rabbits in groups of three were treated with antidiphtheric serum. With the exception of two groups which were injected with overwhelming doses of toxin in proportion to the high susceptibility and low resistance of rabbits to diphtheria, every animal was saved. In other words, out of 12 rabbits treated with 600 to 1,500 units of serum and afterward injected with one fatal dose of toxin for each 250 gr. weight, 100 per cent were saved. We feel safe in stating that, with proper care in treating and handling, rabbits can be saved from toxic doses of diphtheria by the oral administration of a reasonably small amount of antitoxin, without the loss of a single animal. All

control rabbits, injected with the same amounts of toxin as the treated ones, died in from two to four days. The results are given in the table below.

TABLE 3.

Rabbit No.	Weight Grams	Units Antitoxin	Fatal Doses Toxin	Result
1.....	1,855	Control	7.6	Dead 3 days
2.....	2,585	1,500	12.3	+
3.....	1,760	900	7.2	+
4.....	1,940	600	7.9	+
5.....	2,180	Control	10.7	Dead 2 days
6.....	2,585	600	12.3	" 3 "
7.....	2,115	450	10.4	+
8.....	1,790	300	9.1	Dead 3 days
9.....	1,500	Control	8.0	" 3 "
10.....	1,815	450	9.26	" 3 " (pregnant)
11.....	1,800	600	9.2	" 2 "
12.....	1,420	900	7.68	+
13.....	1,330	Control	6.32	Dead 3 days
14.....	1,015	900	5.06	+
15.....	1,375	900	6.5	+
16.....	2,060	900	9.24	+
17.....	580	Control	4.2	Dead 3 days
18.....	725	1,500	4.9	+
19.....	890	1,500	5.5	+
20.....	825	1,500	5.3	+
21.....	3,150	Control	9.0	Dead 2½ days
22.....	2,200	1,500	8.8	+
23.....	2,000	1,500	8.0	+
24.....	2,700	1,500	0.0	+

Diphtheria antitoxin per mouth, dogs.—Nine dogs, indicated in the following table, after treatment with the antitoxin, were injected with one fatal dose of toxin for each 500 to 750 gr. weight. Dogs 7 to 9 were injected with one fatal dose for each 500 gr. weight plus three fatal doses for each dog. All in this group died; however, two of them lived 7 and 8 days, while the control died in 4 days. Dog 5 was sick with distemper before treatment. Therefore, exclusive of the group of dogs which received the overwhelming doses of toxin, practically 100 per cent of the treated dogs lived.

TABLE 4.

Dog Number	Weight Grams	Units Antitoxin	Fatal Doses Toxin	Results
1.....	11,580	Control	15.4	Dead 7 days
2.....	7,370	4,500	9.8	+
3.....	7,870	4,837.5	10.5	+
4.....	5,600	Control	11.2	Dead 3 days
5.....	7,000	6,000	14.0	" 6 " (distemper)
6.....	7,020	Control	17.0	" 4 "
7.....	9,000	6,000	21.0	" 7 "
8.....	10,390	6,000	23.7	" 8 "
9.....	11,000	6,000	25.0	" 4 "
10.....	11,580	Control	15.4	" 7 "
11.....	14,000	9,000	17.0	+
12.....	9,700	9,000	13.0	+
13.....	11,400	9,000	15.3	+

Tetanus antitoxin per stomach, guinea-pigs.—In all, 97 pigs were used in testing the absorption of tetanus antitoxin. The table below gives those from the sixth group on. Many of those in the first groups were saved, but not until tests were made on several pigs was the correct dosage of antitoxin and toxin regulated, so that uniform results followed. The most of the pigs received administrations of serum on two or three successive days, in the meantime being kept without food. For instance, 10 units were given the first day, 10 units the second day, and the injection of the toxin was made on the second or third day.

TABLE 5.

Group	No. Units Antitoxin	No. Fatal Doses Toxin	No. Pigs in Group	No. Control Pigs in Group	No. Treated Pigs in Group	No. Treated Pigs Lost	No. Treated Pigs Saved
6.....	20	2	5	1	4	1	3
7.....	60	2	3	1	2	0	2
8.....	80	3	3	1	2	0	2
9.....	50	3	10	2	8	0	8
10.....	40	2	6	2	4	0	4
11.....	40	2	5	1	4	1	3
12.....	30	2	3	1	2	0	2
13.....	40	2	6	1	5	0	5
14.....	40	2	5	1	4	0	4
Total.....			46	11	35	2	33

Four of the above pigs, which are indicated as saved, showed symptoms of tetanus and recovered. All of the 11 control pigs died with tetanic spasms in less than eight days after injection. Ninety-four and two-sevenths per cent of the treated pigs were saved.

In Group 10, indicated above, four additional pigs were treated with 40 units of antitoxin exactly as the four pigs which, as shown in the table, were saved. The two fatal doses of toxin, however, instead of being injected after treatment, as in the case of the four pigs above, were injected just before administering the first 10 units of antitoxin with the following results:

G.-pig 7, symptoms 3 days, dead 5 days; G.-pig 8, slight symptoms 5 days, recovery; G.-pig 9, no symptoms; G.-pig 10, symptoms 3 days, dead 6 days.

This experiment strengthens the clinical observations that sufficient time must be allowed for absorption of antitoxin when given per mouth.

Gr.-pig	1, 30	fatal doses	diphtheria toxin	Sick 2 days, recovered
"	2, 30	"	"	"	Dead 2 days
"	3, 60	"	"	"	" 11 "
"	4, 60	"	"	"	+
"	5, 90	"	"	"	Dead 2 days
"	6, 90	"	"	"	Sick 2 days, dead 8 days
"	7, 15	"	tetanus	"	" 9 " recovered
"	8, 15	"	"	"	Dead 8 days
"	9, 30	"	"	"	+
"	10, 30	"	"	"	Dead 6 days
"	11, 60	"	"	"	" 3 "
"	12, 60	"	"	"	" 3 "

G.-pig	1, 30	fatal doses	diphtheria toxin	Dead 7 days
"	2, 60	"	"	"	" 3 "
"	3, 90	"	"	"	+
"	4, 15	"	tetanus	"	+
"	5, 30	"	"	"	Sick 3 days, recovered
"	6, 60	"	"	"	Dead 4 "

G.-pig	1,	$\frac{1}{2}$	c.c.	broth to which the drugs were added	+
"	2,	I	"	" " " "	+
"	3,	I	"	" " " "	+
"	4,	I		broth, drugs not added	+
"	5,	I		broth, " " " "	+
"	6,	I		distilled water to which the drugs were added	+
"	7,	I		" " " "	+

The following pigs received the toxins, to which the drugs were added, per mouth in three administrations, and before treatment were injected with relatively large amounts of the respective antitoxins:

[illegible]

G.-pig	1,	10	fatal	doses	tetanus	toxin	+
"	2,	50	"	"	"	"	+
"	3,	100	"	"	"	"	+
"	4,	200	"	"	"	"	+
"	5,	1	c.c.	1	per	cent	peptone	broth	+
"	6,	5	c.c.	1	"	"	+

Sick 3 days, dead 6 days

G.-pig	7,	200	fatal	doses	tetanus	toxin	with	drugs	.	.	Died	from	operation
"	8,	100	"	"	diphtheria	"	"	"	.	.	Dead	2	days
"	9,	100	"	"	"	"	"	"	normal	horse	serum	+	
"	10,	100	"	"	tetanus	"	"	"	"	"	"	+	
"	11,	200	"	"	"	"	"	"	"	"	"	+	
"	12,	1	c.c.	normal	horse	serum	+	
"	13,	2	c.c.	"	"	"	+	
"	14,	100	fatal	doses	tetanus	toxin	with	carbonated	water			+	
"	15,	100	"	"	"	"	"	"	"	"	"	+	

TETANUS ANTITOXIN PER STOMACH, RABBITS AND DOGS.

Experiments have been conducted upon a series of 20 rabbits with tetanus antitoxin, but up to the present neither positive nor negative results have been established. This is due to the failure thus far to find the correct amount of tetanus toxin to kill, without giving overwhelming doses. Symptoms have been produced in control rabbits, however, while treated rabbits of the same group have shown no symptoms.

Owing to the naturally high resistance of dogs to tetanus toxin, similar work with them has not been satisfactory. Several dogs have been experimented upon, none of which showed symptoms after the injection of enormous doses of toxin. It is interesting to note that one dog, weighing 15 kilos, has shown no symptoms after the injection of enough tetanus toxin to kill 1,270 guinea-pigs.

ABSORPTION OF DIPHTHERIA ANTITOXIN FROM ALIMENTARY CANAL OF DOGS.

Thus far the absorption of diphtheria antitoxin has been tested on four dogs. These dogs were starved for 24 hours previous to the operation, and the stomach emptied with apomorphine. The abdomen was opened, and the stomach tied off at the pylorus. Three sections of the intestine were used; one in the jejunum below the opening of the pancreatic duct; another at the end of the ileum; and the other in the rectum. The intestinal loops were 11 cm. long, their mesenteric attachments were unimpaired, rough handling was avoided, and, after the introduction of the serum, the organs were returned to the abdomen, the opening closed and the abdomen kept warm. After the serum had been in the organs for one hour, the amount remaining was determined by precipitating the remaining albumin, drying and weighing.

To the serum used in these experiments, trikresol, chloroform,

salol, or opium were added. The results are given in the appended table:

TABLE 6.

Antitoxin Mixture Diluted with Saline Solution	Stomach	Jejunum	Ileum	Rectum
Dog I. General Anesthesia—				
Percentage by volume of mixture absorbed..	Excess	14.3%	42 $\frac{4}{5}$ %	Excess
weight of albumins " " " " " "	"	0	12 $\frac{1}{2}$	"
Number of antitoxic units injected.....	3,955	396	396	396
" " " " absorbed.....	None	None	49	None
Dog II. Local Anesthesia—				
Percentage by volume of mixture absorbed..	Excess
weight of albumins " " " " " "	12 $\frac{1}{2}$ %
Number of antitoxic units injected.....	2,825
" " " " absorbed.....	57
Dog III. Local Anesthesia—				
Percentage by volume of mixture absorbed..	62 $\frac{1}{2}$ %	68 $\frac{1}{2}$ %
weight of albumins " " " " " "	25	12 $\frac{1}{2}$
Number of antitoxic units injected.....	452	452
" " " " absorbed.....	113	56
Antitoxin Mixture Not Diluted	Stomach	Jejunum	Ileum	Rectum
Dog IV. Local Anesthesia—				
Percentage by volume of mixture absorbed..	Excess	18%
weight of albumins " " " " " "	52.3%	28 $\frac{1}{2}$
Number of antitoxic units injected.....	2,768	2,768
" " " " absorbed.....	1,439	802

EXPERIMENTS WITH DIPHTHERIA AND TETANUS ANTITOXINS PER STOMACH, HOMO.

In conducting the following experiments the individual under treatment was bled from the median cephalic vein either before treatment with the antitoxin or a few weeks afterward. The serum was collected from this blood and, together with a given number of fatal doses of the toxin, was injected subcutaneously into guinea-pigs.

Each person swallowed a given amount of prepared antitoxin, in most cases on two or three successive days and the blood was again collected as before and tested as to its protective influence against the toxin in question, by injecting guinea-pigs with the blood serum and the toxin. A comparison of these results with those from injections of the human blood serum, before the individual took the antitoxin per mouth, indicated whether the diphtheria or tetanus antitoxin, which had been administered to the individual, had resisted digestion and had been absorbed in sufficient quantities to cause the protective antitoxin to be present in the blood of the individual.

The appended table illustrates the effect of the oral administration of the prepared serum *per os*.

TABLE 7.

DIPHTHERIA AND TETANUS ANTITOXINS, PER MOUTH, HOMO					GUINEA-PIG INJECTIONS BEFORE AND SEVERAL WEEKS AFTER ADMINISTRATION OF ANTITOXIN TO SHOW THAT THE HUMAN SERUM CONTAINED NO NATURAL PROTECTION. ALL PIGS KILLED BY TOXIN					GUINEA-PIG INJECTIONS A FEW HOURS AFTER ADMINISTRATION OF ANTITOXIN, SHOWING PROTECTION IN BLOOD OF INDIVIDUAL. ALL PIGS LIVED				
Case	Sex	Age	Kind of Antitoxin Administered	No. Units Antitoxin Administered	Before Treatment		After Treatment			No. cc. Serum	No. Fatal Doses Toxin	Time after Injec. Was Made	No. Units Antitoxin in i c.c. Blood of Individual Treated	No. Units Antitoxin in Total Quantity Blood
					No. cc. Serum	No. Fatal Doses Toxin	No. cc. Serum	No. Fatal Doses Toxin	No. Weeks after Taking Antitoxin					
1. W. E. K.	Male	29	Diph.	13,800	1 c.c.	5	2 c.c.	3	5 weeks	1 c.c.	5	72 hrs.	0.05	283.7
2. W. K.	"	15	"	13,800	1	5	2	3	5 "	1	5	72 "	0.028	128.3
3. L. T. C.	"	24	"	13,800	2	3	6 "	2	5	6 days	0.025	140.0
4. E. S.	"	7	"	6,000	2	5	6 "	2	10	82 hrs.	0.05	181.5
5. E. H.	"	16	"	6,000	2	7	7 "	2	10	5 days	0.05	180.0
6. E. H. H.	"	20	"	3,000	2	4	3 "	2	5	24 hrs.	0.015	84.0
7. R. S.	"	7	"	3,000	2	5	3 "	2	7	24 "	0.116	421.0
8. W. P.	"	7	"	6,000	2 c.c.	3	1	7	9 days	0.07	245.0
9. H. K.	"	19	"	4,500	2	5	1	5	72 hrs.	0.05	300.0
10. W. E. K.	"	29	Tet.	1,500	2	2	2	3	72 "	0.0000015	0.0084
11. W. P.	"	5	"	600	2	3	2	4	48 "	0.000002	0.008
12. W. K.	"	15	"	600	2	3	2	3	72 "	0.000003	0.0069
13. E. H.	"	16	"	1,500	1.5	3	1.5	3	9 days	0.0000045	0.01783
14. L. T. C.	"	24	"	900	2	2	2	2	60 hrs.	0.000001	0.005675

One additional case is still under observation. No experiment has been omitted, and thus far all the cases treated per mouth have given positive results. We believe the results to be trustworthy and accurate, because check pigs, in making injections of human serum and toxins, have been used in all cases.

Reference to the table on preceding page will show that in some cases the human serum apparently possessed some protection before taking the antitoxin or several weeks after taking it. Thus, in Case 9, 2 c.c. of the blood serum, before giving the antitoxin, protected against the diphtheria toxin up to five fatal doses. Five fatal doses killed. In Case 5, 2 c.c. of the serum protected against the toxin up to seven fatal doses. Seven fatal doses killed. This is in accordance with the observations of Wassermann,¹ who has found in normal human serum some protection against diphtheria toxin in a certain percentage of cases.

It will be observed, however, that, in those cases where some natural protection seemed to exist, as well as in those whose blood serum contained no protection against the toxin, a few hours or a few days after administering the antitoxin the blood serum exerted considerable more protective influence.

It is clearly possible that more antitoxin would have been absorbed from the alimentary tract in this series of experiments, had the serum been given on an empty stomach. Experiments on guinea-pigs, and rabbits certainly indicate that moderate starvation before administration of the antitoxin is of considerable aid in promoting absorption.

EXPERIMENTS TO DETERMINE AMOUNT OF DIPHTHERIA ANTITOXIN ABSORBED FROM SUBCUTANEOUS INJECTION.

Experiment 1. Dog.—Injected subcutaneously with 800 units of antidiphtheric serum. Forty-eight hours after, collected 40 c.c. blood from superficial epigastric artery. Weight of dog 12,620 gr. One-twelfth body weight = 1,050 c.c. blood in body of dog.

Supposing that all of the injected antitoxin appeared in the blood stream, there should have been 800 units antitoxin in 1,050 c.c. of blood, or 0.761 units in 1 c.c. One c.c. serum ought to have protected against 76.1 fatal doses of diphtheria toxin.

¹ *Zeitschr. für Hyg.*, 1895, 19, p. 408.

Guinea-pig injections:

G.-pig	1, control	5 fatal doses toxin, dead 3 days
"	2, 1 c.c. dog serum	48 hrs.	75	" " " " 2 "
"	3, 1	"	"	48	"	60	" " " " 2 "
"	4, 1	"	"	48	"	40	" " " " 4 "
"	5, 1	"	"	48	"	30	" " " " 4 "
"	6, 1	"	"	48	"	15	" " " " + "

By the above injections it was shown that less than 0.3 units of antitoxin were present in 1 c.c. of the blood of the dog, or 315 units in total quantity of the blood.

Experiment 2. Rabbit.—Injected with 400 units diphtheria antitoxin. After 48 hours, 30 c.c. blood collected from carotid artery.

By the same method of computation and testing, it was found that 1 c.c. of this rabbit blood should have protected against 432 fatal doses, while it actually protected against less than 100 fatal doses.

Experiment 3. Guinea-pig.—Amount antitoxin injected—40 units. Bled after 24 hours. One-tenth c.c. of blood should have protected against 20.9 fatal doses. One-tenth c.c. of blood did protect against less than 10 fatal doses.

Experiment 4. Homo F.—Male, age 7 years. Weight 27,272 grams. Injected in buttock with 1,040 units antitoxin. Twenty c.c. blood collected after 24 hours. One c.c. of blood should have protected against 46.1 fatal doses. One c.c. of blood did protect against less than 20 fatal doses.

Theoretically the blood should have contained 0.461 units of antitoxin per cubic centimeter. Tests showed that it contained less than 0.2 units antitoxin per cubic centimeter. After two weeks this blood still contained 0.18 units antitoxin per cubic centimeter.

Experiment 5. Homo B.—Male age 7 years, weight 24,500 grams. Injected in buttock with 1,040 units antitoxin. Twenty c.c. blood collected after two weeks and one day. One c.c. of the blood should have protected against 43.3 fatal doses. One c.c. of blood did protect against less than 12 fatal doses.

Less than 0.12 units of antitoxin was present in the blood, while theoretically 0.433 units should have been present.

Experiment 6. Homo H.—Male, age 7½ years, weight 27,000 grams. Injected in buttock with 480 units antitoxin. Twenty c.c. of blood collected after 24 hours. One c.c. of blood should have protected against 21.3 fatal doses. One c.c. of blood did protect against less than 12 fatal doses.

Less than 0.12 units of antitoxin was present in the blood, while theoretically 0.213 units should have been present. After two weeks and one day 1 c.c. of this blood was found to still protect against 10 fatal doses toxin.

Experiment 7. Homo E.—Male, age 7 years, weight 23,181 grams. Injected in buttock with 320 units antitoxin. Twenty c.c. blood collected after 24 hours. One c.c. of blood should have protected against 16.5 fatal doses toxin. One c.c. of blood did protect against less than 15 fatal doses.

Less than 0.15 units per c.c. were absorbed, while 0.165 should have been absorbed.

Experiment 8. Homo B.—Male, age 7 years, weight 23,591 grams. Injected in buttock with 1,040 units antitoxin. Twenty c.c. blood collected after 24 hours. One c.c. of blood should have contained 0.529 units antitoxin. One c.c. of blood did contain less than 0.38 units antitoxin.

This series of experiments is sufficient to show that, even with the subcutaneous injection of diphtheria antitoxin, only a portion of it appears in the blood.

We were not able to make a direct comparison of the results obtained by the two methods of administration of the antitoxin to homo. It will be noticed in the table illustrating the effect of oral administration, under column headed "Guinea-pig injections a few hours after administration, etc.," that the pigs withstood from 5 to 10 fatal doses of toxin, in case of diphtheria, when injected together with 2 c.c. of the human serum. However, in all but three of the cases treated with diphtheria antitoxin, the maximum amount of toxin that could be injected was not determined, owing to the difficulty of obtaining sufficient blood. Therefore, the maximum amount of antitoxin present in the blood of those cases treated with the antitoxin per mouth was not determined in all cases. Moreover, from these experiments it was clearly shown that a large amount of antitoxin given per mouth was not absorbed in proportion to the amount given, a reasonable amount of serum producing just as good protection.

None of those cases, included in our experiments in which the antitoxin was injected subcutaneously, received more than 1,040 units of serum, while some of the cases which were given the antitoxin per mouth received several thousand units. Experiment seemed to show conclusively that 3,000 units afforded as much protection as 6,000 or 13,000 units.

Taking these facts into consideration, and assuming that the oral administration be used only as a prophylactic, we believe the results obtained from administration per mouth compare very favorably with those obtained from subcutaneous injection. In the case of children, we find that the antitoxin is absorbed uniformly and in sufficient amount to give protection, when administered per mouth.

SENSITIZING ACTION OF HORSE SERUM UPON GUINEA-PIGS.

The recent publication by Rosenau and Anderson¹ of the Public Health and Marine Hospital Service, dealing with a newly identified poisonous action of blood serum, suggested to us the following experiment on guinea-pigs. Concerning the sensitization of guinea-pigs by

¹ No. 29. Pub. H. & Mar. Hosp. Service, *Hygienic Lab. Bull.* 1906.

administration of horse serum per stomach, the authors concluded that "guinea-pigs may be sensitized to the toxic action of horse serum by feeding them with horse serum or horse meat." Nevertheless their work shows that guinea-pigs do not appear to be nearly so susceptible to the toxic action of horse serum when given per stomach as when injected subcutaneously or intraperitoneally. Out of a series of 38 pigs, which were given per mouth relatively enormous quantities of horse serum by Rosenau and Anderson, but two died.

The following series of pigs illustrates the comparative sensitizing action of reasonable amounts of horse serum, when injected and when given per stomach to guinea-pigs.

G.-pig 1, control

" 2,	$\frac{1}{2}$ c.c.	antidiph. serum, injected	subcutaneously
" 3,	$\frac{1}{2}$ c.c.	"	"
" 4,	$\frac{1}{2}$ c.c.	"	"
" 5,	$\frac{1}{2}$ c.c.	"	"
" 6,	$\frac{1}{2}$ c.c.	"	"
" 7,	$\frac{1}{2}$ c.c.	"	"
" 8,	$\frac{1}{2}$ c.c.	"	"
" 9,	1 c.c.	"	"
" 10,	1 c.c.	"	"

Thirteen days afterward these pigs were treated as follows:

G.-pig 1, 6 c.c.	antidiph. serum, injected into peritoneum	No symptoms
G.-pig 2, 6	antidiph. serum, injected into peritoneum	Symptoms 5 min., recovery
G.-pig 3, 6	antidiph. serum, injected into peritoneum	" 3 " "
G.-pig 4, 6	antidiph. serum, injected subcutaneously	" 40 " "
G.-pig 5, 6	antidiph. serum, injected subcut.	" 25 " "
" 6, 6	" " " intraper.	" 3 min., dead 40 min.
" 7, 6	" " " subcut.	" 6 min., recovery
" 8, 6	" " " " "	" 15 " "
" 9, 6	" " " " "	No symptoms
" 10, 6	" " " intraper.	Symptoms 1 " "

Another series of 10 pigs was treated per stomach with the same amounts of serum and at the same times as the series above. None of these showed any symptoms from the second administration of serum.

A series of experiments was undertaken to determine the effects of artificial peptic digestion on diphtheria antitoxin. The results agreed entirely with those heretofore reported, namely, the antitoxin is destroyed by peptic digestion.

SUMMARY AND CONCLUSIONS.

1. Thus far our experiments to prevent or cure sepsis by the oral administration of sera, do not allow us to draw any conclusion as to their value. The experiments are being continued, and will be reported on later.

2. So far we have been able to show that the antitoxins for diphtheria and tetanus may be given per mouth and absorbed in sufficient quantity to show markedly antitoxic properties in the blood of the treated animal. This result has been obtained from the use of certain drugs with the serum: on the one hand, drugs that inhibit digestion; on the other, those that promote absorption. It remains to be shown what are the best drugs for this purpose and in what quantities. To this end our work is being continued as rapidly as possible.

3. The results of the experiments, showing that the toxins of diphtheria and tetanus are absorbed from the alimentary canal, provided digestion is inhibited, we believe to be noteworthy. It suggests a method for slow, gradual immunization of animals or men that is at the same time much easier and safer than hypodermic administration of toxin. It also suggests an explanation of many cases of toxemia, due to the absorption of putrefactive products where digestion is deranged.

4. When antidiphtheric serum alone is given to guinea-pigs per stomach, absorption of the antitoxin does not take place with any degree of uniformity.

5. Antidiphtheric serum alone given to guinea-pigs per intestine is absorbed in relatively large quantities. Small amounts of strychnin and potassium bicarbonate, added to the serum given per intestine, aids absorption to some extent.

6. Diphtheria antitoxin to which trikresol, salol, chloroform, and opium are added, when given to guinea-pigs, rabbits, and dogs per stomach, is absorbed in considerable amount and with reasonable uniformity.

7. Tetanus antitoxin, similarly prepared, administered to guinea-pigs per mouth, is equally as well absorbed.

8. Several hours must be allowed for absorption of antitoxin given per mouth.

9. Moderate starvation before and during administration of antitoxin per mouth enhances absorption.

10. Large amounts of tetanus and diphtheria toxins, administered alone per mouth, are rarely absorbed.

11. Preliminary work seems to show that tetanus and diphtheria toxins, which are prepared with chloroform, salol, trikresol, and opium, given per mouth, are absorbed with some degree of uniformity.

12. Diphtheria antitoxin placed in the stomach and different portions of the intestine of dogs, for a period of one hour, is absorbed in considerable quantities.

13. Diphtheria and tetanus antitoxins, to which trikresol, salol, chloroform, and opium have been added, given to human beings per mouth, are absorbed in sufficient quantities to show protective qualities in the blood serum of the individual.

14. When diphtheria antitoxin is injected subcutaneously into man, dogs, rabbits, and guinea-pigs, only a part of it appears in the blood.

15. The sensitizing action of horse serum, given to guinea-pigs per mouth is not nearly so great as when injected subcutaneously or into the peritoneum.

In conclusion we wish to express our sincere thanks to Dr. W. M. Donald for aid in obtaining clinical material in carrying out the experiments upon human subjects.