change in resistance is limited largely to the quaternary ammonium compounds or possibly to those disinfectants characterized by long hydrocarbon chains. Further studies along this line are in progress.

Conclusions. Data have been presented to show that the resistance of *Staph. aureus* to quaternary ammonium compounds is materially increased by growing the test cultures in Difco "disinfectant testing" broth as compared with standard F.D.A. broth.

[1] Ruehle, G. L. A., and Brewer, C. A., *U. S. Dept. Agri. Circ.* 198, 1931.

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Gastrointestinal Tract of Guinea Pig and Elimination of Ascorbic Acid Given Intraperitoneally.

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In previous experiments it was found that adult guinea pigs receiving their ascorbic acid intraperitoneally in the proportion of 5 mg per 100 g of body weight excrete about one-fourth of it in the urine. Although some of the vitamin may be used up metabolically in some way in the tissues, it seemed probable that considerable loss due to destruction was occurring elsewhere. The present tests were performed to determine where this destruction, if any, takes place. A preliminary report of the results was made previously.[1]

Method. The investigations were conducted by administering the ascorbic acid intraperitoneally and determining at successive intervals the rate of urinary excretion of the vitamin, the amount present in the blood, liver, kidneys, spleen, and adrenals, and amount present in the contents of 4 different parts of the gastrointestinal tract, then correlating the latter data with observations made in another study[2] on the rate of movement of the contents through the gastrointestinal tract. In these latter studies it was found that in the guinea pig there is a rapid movement of the contents through the stomach and small intestine. Within a period of 2 hours these organs are practically emptied.

[1] Reid, M. E., *Proc. Fed. Am. Soc. Exp. Biol.*, 1947, **6**, 418.

[2] Reid, M. E., and White, W. C., *Am. J. Physiol.*, in press.

The present observations were made on 41 adult guinea pigs of an inbred strain. They were fed a commercial pelleted diet devoid of ascorbic acid. To ensure a sufficient supply of the B vitamins, 1% of powdered yeast was added to the pellets. The 29 male animals used in the tests had an average weight of 921 g, the 12 females an average weight of 771 g. Each animal was injected intraperitoneally each day with ascorbic acid (made up in 0.5% $NaHCO_3$, 15 mg asc. ac./ml) in the amount of 5 mg per 100 g body weight. The animals were maintained on this regime for at least a month before being used in the tests. At periods varying between 1 to 24 hours after injection, blood samples were taken from the heart, following which the animals were killed and the organs quickly removed. The liver, kidneys, spleen, and adrenals were frozen rapidly in dry ice and kept in the frozen state until the extractions with 10% metaphosphoric acid could be made. The contents of the stomach, small intestines, cecum, and large intestine were washed out separately with 10% metaphosphoric acid and centrifuged. The centrifugate was washed with another volume of acid and centrifuged. The first and second extracts were combined and made up to appropriate volumes. In the later phases of the study the washing of the centrifugate was omitted, the contents being made up to appropriate volume

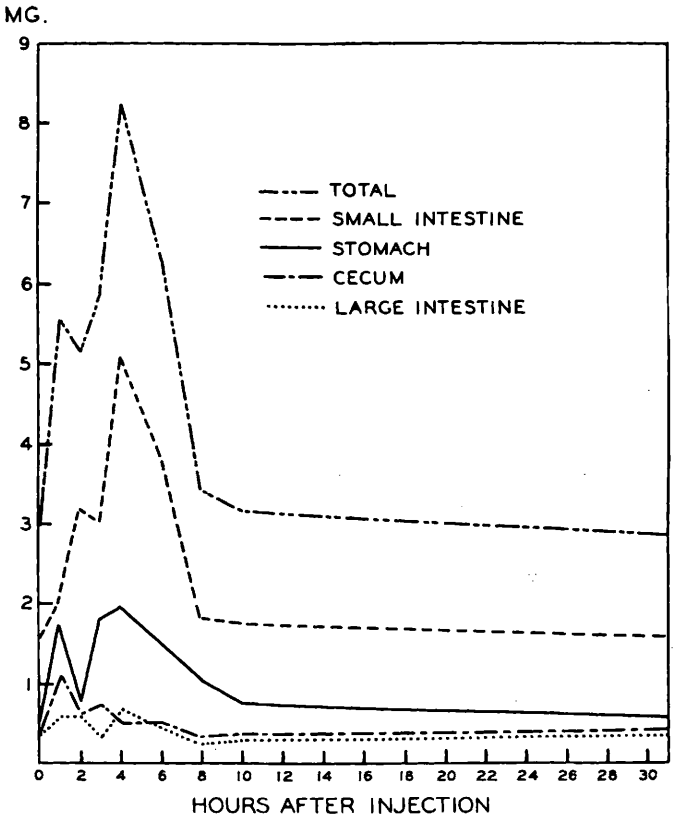

FIG. 1.

Total ascorbic acid (osazone method) found in contents of gastrointestinal tract at successive periods after intraperitoneal injection of the vitamin.

with the metaphosphoric acid before centrifuging with the results not significantly different from those obtained with the previous procedure. Assays were made by the indophenol method on these extracts and also on those of the organs. The extracts remaining after the indophenol tests, also the supernatant fluid from the whole blood samples prepared according to the Roe-Kuether technic[3] were frozen and kept in this state until the following day. Acetic acid was then added to make a final concentration of 10% acetic and 5% metaphosphoric acid and assays were made by the osazone method.

The number of animals studied in each group ranged from 3 to 8.

Results. Measurable osazone values were found in the contents of the gastrointestinal tract in all of the tests. Fig. 1 shows the average result (expressed as mg ascorbic acid/kg body weight) obtained with the osazone method. From a relatively small amount present immediately before injection, the ascorbic acid in the contents of the alimentary tract increased up to and through the fourth hour after injection and thereafter decreased slowly. After 10 hours, the amount had decreased to a level not much above the starting level. Most of the fluctuation with time is found in the contents of the stomach and small intestine. From 70 to 85% of the total amount of ascorbic acid in the contents of the digestive tract is found in the stomach and small intestine and more in the intestine than in the stomach. The indophenol values for the contents of the stomach and small intestine were usually lower than the osazone

[3] Roe, J. H., and Kuether, C. A., *J. Biol. Chem.*, 1943, **147**, 399.

TABLE I.

Ascorbic Acid in Blood and Organs (Osazone Method) at Successive Periods After Intraperitoneal Injection of the Vitamin.

No. of animals	Hr after last inj.	Blood		Liver		Kidneys		Spleen		Adrenals	
		Mg/100 ml	s.d.	Mg%	s.d.	Mg%	s.d.	Mg%	s.d.	Mg%	s.d.
4	0 (24)*	0.83	.10	30.7	5.4	11.8	2.1	59.9	1.2	145.4	9.0
4	1	4.29	.34	48.4	2.3	27.2	2.1	65.3	4.7	166.5	19.0
4	2	3.54	.18	58.5	4.6	20.6	1.0	60.5	3.4	144.8	8.5
4	3	2.29	.28	50.3	2.6	20.5	1.1	70.8	5.6	167.7	11.5
4	4	2.44	.29	51.7	3.4	18.0	2.2	68.4	4.3	146.6	14.7
4	6	2.11	.15	49.4	1.8	16.7	1.3	62.8	3.3	144.1	4.2
3	8	1.48	.16	51.4	4.0	15.3	1.4	66.6	5.9	141.0	4.3
3	10	1.31	.17	39.0	5.9	12.1	0.2	62.5	5.0	152.6	11.4
4	24	0.83	.10	30.7	5.4	11.8	2.1	59.9	1.2	145.4	9.0

* This group is identical to the 24-hour group reported below. It is included in this place for ease of comparison.

values, presumably because of the presence of some of the vitamin in the dehydro form. On the other hand, the indophenol values for the contents of the cecum and large intestine were always much higher than the osazone values, doubtless because of the presence of reducing substances other than ascorbic acid. The osazone method may give values somewhat higher than the true ascorbic acid because of the presence of small amounts of osazone-forming substances other than ascorbic acid such as uronic acids and diketogulonic acid. Since diketogulonic acid is known to be an oxidation product of ascorbic acid and since it forms an osazone, the osazone values obtained particularly in the contents of the cecum (where the digestion of cellulose with presumable liberation of uronic acids occurs) and large intestine may not be due entirely to ascorbic acid. The high values found in the contents of the small intestine and the low values in the much greater volume of contents of the cecum indicates that destruction of the vitamin must occur rather rapidly as the contents pass into the cecum.

Table I shows the ascorbic acid content of the blood and some of the organs at successive periods after injection of the vitamin as determined by the osazone method. The average value in whole blood 24 hours after injection is 0.83 mg %. Within 1 hour after injection it increases more than 5-fold then falls about 40% by the end of the third hour and remains at approximately this level through the sixth hour. By the tenth hour it drops to 1.31 mg % and thereafter decreases until it reaches the 0.83 starting level after 24 hours. The liver and kidneys both show marked increases in ascorbic acid content after injection. The kidneys gained 130% within an hour, lost some of it rather rapidly during the second hour and thereafter decreased slowly. The highest value for the liver was found at the end of the second hour. At the end of the 24-hour period the value was only half that found 1 hour after injection of the vitamin. The spleen showed only slight fluctuations with time. There were irregularities in the content of the adrenals but the variations do not appear to bear a close relation to the time

TABLE II.
Average Rate of Urinary Excretion of Ascorbic Acid.

Hr after inj.	% of total amt excreted
0- 1	4.4
1- 2	14.6
2- 3	27.2
3- 4	20.0
4- 6	28.9
6- 8	2.9
8-10	0.9
10-24	1.1
Total for 24 hr	100.0

of administration of the vitamin. The ascorbic acid values for all these organs are considerably higher than have been found by other investigators using the oral route of administration. The value for whole blood after 24 hours, however, is only slightly higher than that reported by Kuether, Telford, and Roe[4] for the blood of guinea pigs maintained on a diet of cabbage.

Table II summarizes the results of a study of the urinary excretion of ascorbic acid made daily for 6 successive days in each of 10 animals maintained under the experimental conditions previously described. Each of the values given in the table thus represents an average of 60 determinations using the indophenol method. In confirmation of previous results, approximately one-fourth of the total dose administered was found in the urine. The total excretion for the 24-hour period is expressed as 100%. The most active period of excretion extends from the second through the sixth hour. This is also the period of high blood level.

Discussion. When large doses of vitamin C are given intraperitoneally, a marked increase in the levels in the blood and certain tissues with considerable urinary excretion occurs. This contrasts to the situation when large doses are given orally in which case the blood and tissue levels remain much more nearly normal and there is no urinary excretion. It has been assumed by different investigators that the excess of orally administered vitamin C is not absorbed, but is destroyed in the digestive tract. Evidence to support this contention was obtained in the present studies in which the vitamin is given intraperitoneally. Considerable amounts appear in the contents of the stomach and small intestine, but very much less in the cecum and large intestine in spite of the fact that the rate of movement is such that the vitamin must be passed to these organs. A probable interpretation of these data appears to be that destruction does occur in the lower portion of the gastrointestinal tract as there is no definite evidence of reabsorption as judged by the blood studies.

Since as much as 15% of the injected dose may be found at one time (4 hours) in the stomach and small intestine and since the contents of these organs are being constantly and rapidly passed into the cecum and large intestine, it appears likely that the total amount destroyed by the gastrointestinal tract must be considerably more than 15%. This rate of destruction would be ample to account for the loss of vitamin C when given orally.

Summary. Ascorbic acid was found in the stomach and small intestine of adult guinea pigs maintained on a diet devoid of the vitamin. The amount present varied with the length of time after intraperitoneal injection of the substance, the maximum occurring at 4 hours. Small amounts only were found in the contents of the cecum and large intestine. The results suggest that the vitamin is excreted into the stomach and small intestine and is destroyed in the cecum.

The maximum content of ascorbic acid was found in the blood and kidneys 1 hour after injection, in the liver 2 hours after injection, and the maximum excretion in the urine occurred between the second and third hours after injection.

[4] Kuether, C. A., Telford, I. R., and Roe, J. H., *J. Nutrition*, 1944, **28**, 347.