the hemolytic streptococcus can be cultivated from the throats of a majority of individuals with acute nephritis. It has been possible to demonstrate normal carriers for the hemolytic streptococcus. Furthermore, antibody active against the soluble streptolysin (hemotoxin) of *S. hemolyticus* was increased in cases of acute glomerulonephritis. There also was the same degree of skin reactivity to the nucleoprotein of the hemolytic streptococcus in the South as in New York. These bacteriological and immunological findings therefore substantiate the clinical data already reported and give still further evidence that acute glomerulonephritis in the South as in the North is usually the result of a hemolytic streptococcus infection.

No opportunity was available to evaluate the relative frequency of the hemolytic streptococcus in the throats of the population at large in New Orleans as compared with New York nor to determine its seasonal incidence. Information on both these subjects would be interesting and possibly helpful in solving the basic problem which still remains, namely the reason for the "normal" incidence of one type of hemolytic streptococcus disease (acute glomerulonephritis) in the presence of a decreased incidence of other types of hemolytic streptococcus disease (scarlet fever, rheumatic fever) in the South. All strains isolated in New Orleans were brought back to New York and are being compared culturally and immunologically with a series of hemolytic streptococci isolated from the throats of individuals in New York City.

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Vitamin C and Diphtheria Toxin.

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In previous work,[1] a relation between Vitamin C and diphtheria toxin was found. Freshly prepared highly labile toxin was used.

In the present work these findings were confirmed and extended using a standardized, stable toxin. In relation to this toxin, we have examined 2 different actions, (a) the effect on guinea pigs of preliminary feeding of excess Vitamin C (cevitamic acid) and of

[1] Harde, E., *C. R. de l'Acad. des Sc.*, 1934, **199**, 618; Harde, E., and Phillipe, M., *C. R. de l'Acad. des Sc.*, 1934, **199**, 738.

treatment with the vitamin,* and (b) the action *in vitro* of cevitamic acid on the toxin, on antitoxin and on mixtures of toxin and antitoxin.

A. Effect on guinea pigs of preliminary feeding of excess vitamin C (cevitamic acid) and of treatment with the vitamin. Four guinea pigs were fed excess spinach or cabbage in addition to the stock diet of hay, oats and carrots for 10 days. They were then injected with 1 M.L.D. of toxin and kept on the same diet. No increased resistance was shown as compared with controls fed the stock diet. In a second experiment, 5 guinea pigs were given 10 mg. of pure cevitamic acid in ½ to 1 cc. salt solution by mouth daily for 3 days. Three of these animals were also injected daily, intramuscularly or subcutaneously,† with 10 mg. of the cevitamic acid in a similar solution. These animals were then injected with 1 M.L.D. of standardized toxin, and the vitamin C treatment continued daily. In comparison with the control animals injected with 1 M.L.D. at the same time, no increased resistance was shown, all the animals dying in 4 to 5 days.

These results differed from those obtained in previous work using a freshly prepared toxin, in which 3 out of 6 animals survived, and 5 controls died. A freshly prepared toxin is very unstable, losing rapidly a certain part of its toxicity, and it may also be more sensitive to vitamin C.

Animals injected with toxin may be extremely sensitive to the shock of handling. Occasionally during the feeding one of these animals died suddenly. There is always the possibility of some of the liquid going into the bronchi. Autopsies have shown this in a number of cases. We have also noticed at autopsy that subcutaneous or intramuscular injections of vitamin C were not always completely absorbed.

In another series of experiments, therefore, the animals were handled with great care so that there was very little shock, only the vitamin C solution being fed and injections avoided. A salt solution of 10-20 mg. of cevitamic acid was given to each of 4 guinea pigs; after the first or second day, each was injected with 1 M.L.D. of standardized toxin, and the feeding continued daily. The controls died on the 4th day. All the treated animals lost weight and showed indura-

* We thank the Hoffman LaRoche Company for the Redoxon-Cevitamic Acid they kindly supplied us.

† These solutions were always rendered neutral to litmus using a sodium hydroxide solution and sterilizing by bringing to a boil. To some animals carotene was also given by mouth without any effect being noted.

tion, one died on the 5th day and 1 on the 6th day. To the 2 others, vitamin C feeding was continued until the 8th day. These animals survived 20 and 22 days, dying of pneumonia. A second series gave similar results. One animal died on the 3rd day with lung lesions, one on the 9th day, and the remaining 2 survived. The 4 controls died in from 3 to 5 days.

B. Action *in vitro* of cevitamic acid on toxin, on diphtheria antitoxin and on mixtures of toxin and antitoxins. To 1 or 2 M.L.D.'s of toxin in 1 cc. of salt solution was added 1 cc. of a neutral solution of cevitamic acid, 10 mg. per M.L.D. This was slowly mixed, care being taken not to bubble air through it. Four guinea pigs injected with these solutions showed no loss of weight and no local symptoms, while the controls died in 4½ days. This experiment was repeated with similar results. With 5 M.L.D.'s using 10 mg. of the vitamin per M.L.D., 2 guinea pigs survived and 2 died ten days after injection. With a toxin solution containing 10 M.L.D.'s and treated with the same amount of vitamin C and injected, 2 animals survived, while 2 animals injected with 10 M.L.D.'s to which 60 mg. of vitamin C had been added died after 4 days, and one guinea pig receiving 20 M.L.D.'s and 100 mg. of vitamin C died in 48 hours.

We are now testing whether prolonged contact or increased amounts of cevitamic acid are necessary for action on the toxin.

In the earlier experiments a partial immunity was found in animals that had survived these injections when tested with 1½ M.L.D.'s. In this work the surviving animals were injected with 2 M.L.D.'s 5 to 6 weeks after the first injection. They all died in 2 to 4 days, suggesting that there had been little if any immunity produced by the first injection.

If vitamin C is to be used clinically as an adjunct to antitoxin in the treatment of diphtheria, it is necessary to test its action on the antitoxin.

To a standard antitoxin was added 10 mg. per unit of cevitamic acid, dissolved in a neutral salt solution. This was thoroughly mixed but not aerated and allowed to remain for 24 hours at 4°C. protected from the light. Two guinea pigs were injected with this mixture to which was added ½ L+ of toxin, 2 with one L+, and 2 with 1½ L+. For controls a freshly prepared standard unit of antitoxin was used with similar amounts of toxin. The only difference in resistance between these groups was shown in the one L+ dose. The guinea pigs receiving the solution containing the vitamin C lived 2 days longer than the controls, which died between the 3rd

and 4th days. No deleterious action of Vitamin C on the antitoxin was shown. Experiments were then done to note if the vitamin had any effect on toxin antitoxin mixtures. One unit of antitoxin, one L+ of toxin, and 10 mg. of cevitamic acid in salt solution were made up to 4 cc. with a salt solution, carefully mixed without aeration and kept at room temperature protected from the light for one hour. Controls without the cevitamic acid were kept under the same conditions. Three guinea pigs were injected in each series. The controls died within 4 days while the animals injected with the cevitamic acid mixture showed no symptoms and survived.

A final experiment was made with unneutralized cevitamic acid. One hundred and fifty mg. of the vitamin was dissolved in one cc. of standardized toxin, 500 M.L.D.'s per cc. The pH was between 4 and 4.4. The mixture was kept for 18 hours at room temperature. The guinea pigs were injected with one, 1¼ and 2 M.L.D.'s of the treated mixture. They showed no effects of the injection while the controls died. Here the vitamin was neither heated nor neutralized and was only added in the proportion of one mg. to 3½ M.L.D.'s. Further experiments are necessary to determine what factors are responsible for this detoxification.

Summary. Vitamin C under certain conditions increased the resistance of guinea pigs to injection of 1 M.L.D. of a standardized diphtheria toxin. Injections of mixtures of toxin and vitamin C if the solutions had been in contact for 1 hour at room temperature before injection were less toxic. The guinea pigs that had survived these injections 5 to 7 weeks showed but slight if any immunity when tested by the injection of 2 M.L.D.'s of the toxin. Vitamin C caused no destruction of the antitoxic properties of antitoxin or of the slightly toxic toxin antitoxin mixture. Only clinical work can determine whether the increased resistance to diphtheria toxin shown by guinea pigs treated with vitamin C can be applied to human beings. While we were engaged in these experiments Dr. C. G. King told us that he and Dr. Bessey[2] were working in the same field and had also found a relationship between vitamin C and diphtheria toxin.

We wish to thank Mr. Andrew Mackey for his aid in carrying out the experiments.

[2] Bessey, O. A., and King, C. G., *J. Nutrition.* In press.