

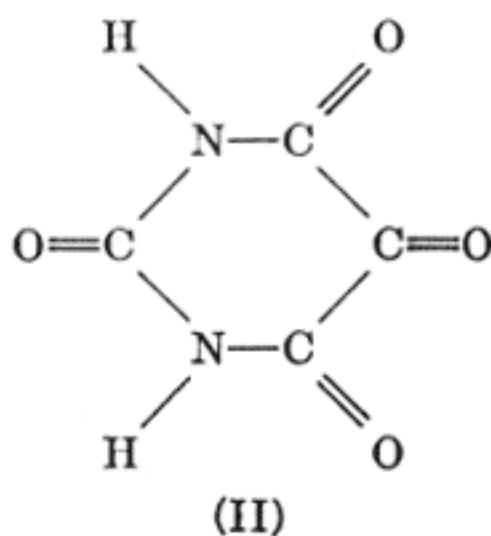
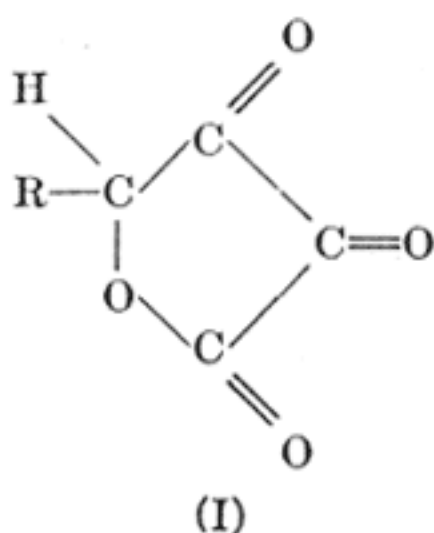
THE DIABETOGENIC EFFECT OF DEHYDROASCORBIC AND DEHYDROISOASCORBIC ACIDS*

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Ascorbic acid is known to exist in the animal body in a reversibly oxidized form as dehydroascorbic acid (I). Its chemical structure in the non-hydrated state is similar to that of alloxan (II).



The chemical properties of these two substances are strikingly similar. Both compounds are irreversibly decomposed under biological conditions. Alloxan has a half life of 1 minute (1) and dehydroascorbic acid has a half life of a few minutes (2). Alloxan is readily reduced to dialuric acid (3) with which it forms alloxantin (3). Dehydroascorbic acid is readily reduced to ascorbic acid (4) and also forms a similar intermediate compound (5). Alloxan (6) and dehydroascorbic acid (7) both form addition compounds with molecules containing the sulfhydryl group. Alloxan (8) and dehydroascorbic acid (9) both produce the Strecker reaction with amino acids and give the ordinary ketone reactions.

It would appear logical, therefore, to expect that there might be a relationship between alloxan and dehydroascorbic acid with regard to the production of diabetes. There is some evidence suggesting that dehydroascorbic acid may have diabetogenic properties. It is known that the dehydroascorbic acid level in the scorbutic animal is higher than normal, the dehydroascorbic acid-ascorbic acid ratio being as much as 20 times the normal value (10). Correlated with this is the fact that scorbutic guinea pigs have a decreased glucose tolerance (11) and show degeneration of the islets of Langerhans (12). Furthermore, ascorbic acid is known to act synergistically with alloxan (13, 14) and it seems probable that this effect

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is the result of the formation of dehydroascorbic acid. This is shown in the present work. It is also demonstrated that dehydroascorbic and dehydroisoascorbic acids produce diabetes in a manner similar to alloxan.

EXPERIMENTAL

Reagents—

Alloxan (Eastman).

Crystalline ascorbic Acid (Merck).

Dehydroascorbic acid was prepared immediately before each series of injections by dissolving ascorbic acid (1.08 gm.) in water (10 cc.) and shaking for 15 minutes with an equal volume of ether containing freshly sublimed quinone (0.66 gm.) (15). The ether layer was removed and the water solution was washed five times with an equal volume of ether. The excess ether was removed by suction and the solution used without further treatment. The yield is known to be excellent (16) and the solution was assumed to have about 100 mg. of dehydroascorbic acid per cc. The product was free of ascorbic acid by spectrophotometric examination and contained from 95 to 98 mg. of dehydroascorbic acid per cc. by polarimetric analysis. Slow decomposition produces a product with the opposite sign of rotation; thus these values are probably slightly low (17).

Dehydroisoascorbic acid was prepared from isoascorbic acid¹ by the procedure described above.

*Action of Dehydroascorbic Acid on Rats—*In male rats weighing 100 to 150 gm. the injection of 10 to 50 mg. of dehydroascorbic acid produces a characteristic reaction. Immediately after intravenous injection the rat becomes excited and runs aimlessly but rapidly around the cage for about 2 minutes. From time to time during this period, it may raise itself on its hind legs and vigorously rub its face with its fore legs. After this period of hyperactivity the rat collapses. It gradually starts to gasp for breath and slowly resumes normal respiration. There may be a slight serous discharge from the nose and mouth during the period of prostration. There is usually a brownish red discharge apparent around the eyes. With a dose of 20 mg. the animals appear normal about 10 minutes after the injection.

With larger doses the rats may not recover after the collapse. In a series of male Sprague-Dawley rats, 116 to 124 gm. in weight, that were injected intravenously with dehydroascorbic acid, there were no deaths in seven given 20 mg., one death in four given 30 mg., four deaths in eight given 40 mg., and four deaths in six given 50 mg. In the rats that died the heart continued to beat after respiration had ceased. At autopsy it was noted that

¹ Kindly donated by Dr. H. G. Luther of Chas. Pfizer and Company, Inc.

there was no frothing in the trachea, that a section of lung would float in water, and that the right ventricle of the heart was dilated with blood, whereas the left ventricle was small.

The characteristic hyperactive reaction could be produced with dehydroascorbic acid prepared by the oxidation of ascorbic acid with sodium hypochlorite, indicating that the effect was not due to an organic contaminant.

It is of great interest to note that the rats which had recovered from one injection of dehydroascorbic acid immediately tolerated a second dose several times the size of the initial dose, up to a maximum of about 1 gm. per kilo. This increased tolerance was noted as early as 10 minutes and as late as several hours after the administration of the initial dose. After a large second dose it was possible to give a similar large dose as much as a week later without killing the animal. The exact limits of this tolerance have not yet been determined. When the initial dose of dehydroascorbic acid was relatively large, there was little or no hyperactive response following the second dose. The rats became lethargic but occasionally they would bite an adjacent rat or object. With large doses respiration frequently became difficult. In those rats that died the autopsy findings were the same as those in rats that died following the initial dose. When the initial dose of dehydroascorbic acid was small, there was a second hyperactive response following the second dose. A small initial dose would not permit the administration of a large second dose.

Dehydroisoascorbic acid produced a similar hyperactive reaction in the rat, but a larger dose was required to produce the same effect and the lethal dose was much higher. As much as 160 mg. could be given as an initial dose with few deaths occurring. With this substance the animals did not tolerate a second dose that was greatly different from a large initial dose.

Synergistic Action of Dehydroascorbic and Ascorbic Acids with Alloxan—Male rats of Albino Farms stock were injected intravenously with alloxan without previous fasting and then, by the same needle, with ascorbic acid or dehydroascorbic acid. Diabetes was determined by the 48 hour blood sugar as in previous ascorbic acid studies (13, 14). Blood sugars over 150 mg. per cent were considered an indication of diabetes. When ascorbic acid was given with the alloxan, an alloxan dose of 20 mg. per kilo was necessary to produce diabetes in 44 per cent of the rats, whereas when dehydroascorbic acid was given with alloxan, an alloxan dose of 15 mg. per kilo was sufficient to produce diabetes in 58 per cent of the rats (Table I). Ascorbic acid with this smaller dose of alloxan had no effect on the level of blood sugar.

Diabetogenic Action of Dehydroascorbic and Dehydroisoascorbic Acids—

Blood sugars of male Sprague-Dawley rats were measured by a micro-method (18) before and from 2 to 14 days after intravenous injection of the test substances (Table II). The maximum dose of dehydroascorbic acid

TABLE I

Synergistic Action of Ascorbic Acid and Dehydroascorbic Acid with Alloxan after Intravenous Administration in Male Rats

| No. of rats | Weight | | Alloxan | Dehydro-ascorbic acid | Ascorbic acid | Diabetic | | Non-diabetic | |
|-------------|---------|---------|-------------|-----------------------|---------------|-------------|----------------------------|--------------|----------------------------|
| | Range | Average | | | | No. of rats | Average 48 hr. blood sugar | No. of rats | Average 48 hr. blood sugar |
| | gm. | gm. | mg. per kg. | mg. | mg. | | mg. per 100 cc. | | mg. per 100 cc. |
| 6 | 102-154 | 117 | 30 | 20 | | 6 | 392 | | |
| 4 | 120-158 | 130 | 30 | | 20 | 4 | 334 | | |
| 4 | 96-144 | 112 | 20 | 20 | | 4 | 375 | | |
| 9 | 100-134 | 121 | 20 | | 20 | 3 | 190 | 6 | 126 |
| 12 | 96-118 | 106 | 15 | 15 | | 7 | 242 | 5 | 113 |
| 12 | 88-116 | 104 | 15 | | 15 | | | 12 | 118 |
| 2 | 98-108 | 103 | 10 | 15 | | | | 2 | 108 |
| 2 | 102 | 102 | 10 | | 20 | | | 2 | 122 |
| 7 | 138-156 | 150 | 0 | 20 | | | | 7 | 126 |

TABLE II

Diabetogenic Action of Dehydroascorbic and Dehydroisoascorbic Acids after Intravenous Administration in Male Rats

| No. of rats | Weight | | Dehydro-ascorbic acid | Dehydro-isoascorbic acid | Average blood sugar before and after injection, mg. per 100 cc. | | | | | | | | |
|-------------|---------|---------|-----------------------|--------------------------|---|--------|--------|--------|--------|--------|--------|--------|---------|
| | Range | Average | | | 0 day | 2 days | 3 days | 4 days | 5 days | 6 days | 7 days | 8 days | 14 days |
| | gm. | gm. | gm. per kg. | gm. per kg. | | | | | | | | | |
| 4* | 116-120 | 118 | 1.1 | | 110 | 125 | 330 | 182 | 156 | 132 | 130 | 133 | |
| 4 | 130-138 | 133 | | 1.0 | 138 | 327 | 312 | 208 | 127 | 107 | 141 | 137 | |
| 2 | 130 | 130 | | 1.5 | 137 | 400 | 450 | 450 | 450 | 450 | 450 | 450 | 450 |
| 4 | 120-126 | 123 | | 1.4 | 116 | 246 | | 357 | | | 371 | | 327 |
| 2† | 150-163 | 156 | | 1.5 | 104 | 332 | | 400 | | | 425 | | 450 |

* 40 mg. were given each rat as a preliminary dose 10 to 30 minutes before the indicated dose.

† Fasted overnight.

that could be given after an initial small dose, administered 10 to 30 minutes earlier, was about 1.1 gm. per kilo. In the rats that survived the injections hyperglycemia developed on the 3rd day and persisted for 3 days. 1 gm. per kilo of dehydroisoascorbic acid produced essentially the

same temporary hyperglycemia as did dehydroascorbic acid. The injection of 1.4 gm. per kilo or more of dehydroisoascorbic acid produced a permanent diabetes. One rat, observed for 4 months, maintained a blood sugar of over 400 mg. per cent and four rats showed a similar high blood sugar level after 6 weeks. A number of rats with hyperglycemia died within 2 weeks; one died on the 17th day and two after 6 weeks. Terminally, the blood sugar values tended to drop.

The effect of repeated doses of dehydroascorbic acid is shown in Table III. On the 1st day an initial dose of 20 mg. was administered intravenously 10 to 30 minutes before the first of three or more daily intravenous injections of 80 mg. Mortality during this period of injection was about 25 per cent. Of fourteen rats surviving a series of three or four injections,

TABLE III

Diabetogenic Action of Repeated Intravenous Injections of Dehydroascorbic Acid in Male Rats

| Weight of rat | Dehydroascorbic acid* | No. of daily injections | Blood sugar before and after last injection, mg. per 100 cc. | | | | | |
|---------------|-----------------------|-------------------------|--|----------|----------|----------|---------|---------|
| | | | Initial | 2-3 days | 4-5 days | 6-7 days | 14 days | 21 days |
| <i>gm.</i> | <i>mg. per day</i> | | | | | | | |
| 119 | 60-80 | 10 | 148 | 450 | 400 | 410 | 310 | 375 |
| 112 | 80 | 3 | 129 | 450 | 450 | 450 | 450 | 450 |
| 149 | 80 | 3 | 127 | 435 | 380 | 315 | 400 | 176 |
| 113 | 80 | 3 | 87 | 450 | 140 | 185 | 113 | 100 |
| 127 | 80 | 3 | 123 | | | | 390 | 395 |
| 126 | 80 | 3 | 145 | | | | 280 | 270 |
| 128 | 80 | 4 | 141 | 450 | 450 | 450 | 450 | 450 |
| 154 | 80 | 4 | 114 | 430 | 330 | 355 | 257 | 108 |

* On the 1st day an initial dose of 20 mg. was injected intravenously 10 to 30 minutes before the indicated dose.

seven died with elevated blood sugars within 3 weeks, the highest mortality occurring about a week after the completion of the injections. The results on seven surviving rats along with one that received more injections are reported in Table III. At the end of 3 weeks there was a marked hyperglycemia in five of the eight rats. Smaller doses produced less striking results. However, one rat that received 60 mg. daily for 9 days showed an irregular hyperglycemia and a marked decrease in tolerance to glucose 40 days after the last injection. The intraperitoneal administration of 3.5 gm. of glucose per kilo produced an immediate rise in blood sugar which was maintained above 450 mg. per cent for 3 hours. The hyperglycemia resulting from daily injections of dehydroascorbic acid responded to the injection of small amounts of insulin.

DISCUSSION

A characteristic hyperactive reaction is produced in the rat by the intravenous injection of dehydroascorbic acid, and to a lesser degree by the injection of dehydroisoascorbic acid. Dehydroisoascorbic acid results from the oxidation of isoascorbic acid, which is a synthetic analogue of ascorbic acid and differs from it only in that it has the opposite optical configuration on carbon atom 5. Isoascorbic acid has only one-twentieth of the anti-scorbutic activity of ascorbic acid (19). Therefore, the fact that dehydroisoascorbic acid has less tendency to produce the hyperactive response of dehydroascorbic acid may indicate that the reaction occurring with dehydroascorbic acid is merely an exaggeration of a normal biochemical process.

There is an increased tolerance to a second dose of dehydroascorbic acid with a marked decrease in the characteristic hyperactive reaction that follows the initial dose. The increased tolerance is noted 10 minutes after the initial dose and is prolonged. The fact that a large second dose of dehydroascorbic acid produces less hyperactivity than a smaller initial dose seems to indicate that the hyperactive response is mediated by a substance liberated *in vivo* which is present in a limited quantity. Thus, if all of this substance is liberated at one time, death results, while liberation of most of it causes the characteristic reaction without death. The second dose then merely releases the small amount remaining without causing any effect. It is interesting that some of the effects produced by dehydroascorbic acid are similar to those produced by acetylcholine.

It seems likely that toxic manifestations previously reported for ascorbic acid (20) may have been due to the presence of dehydroascorbic acid.

Dehydroascorbic acid under the conditions used is more effective than ascorbic acid in acting synergistically with alloxan to produce diabetes. It is possible that the effect of ascorbic acid is the result of its conversion to dehydroascorbic acid by the oxyhemoglobin (21) of the blood which is released on hemolysis following the injection of alloxan (22). Dehydroascorbic acid possibly acts by lowering glutathione and thus having a sparing action on alloxan (23).

Permanent hyperglycemia is produced by the injection of 1.4 gm. per kilo of dehydroisoascorbic acid. In a dose of 1 gm. per kilo, a temporary diabetes is produced. In this same dosage dehydroascorbic acid produces essentially the same result as dehydroisoascorbic acid. The general toxicity of dehydroascorbic acid prevents the use of a higher dosage. However, repeated injections of dehydroascorbic acid seem to be capable of producing a permanent diabetes. It is believed that this is the first time that diabetes, seemingly permanent, has been produced by a known chemi-

cal substance that is not closely related to alloxan. The fact that dehydroascorbic acid occurs physiologically in man and is capable of producing permanent diabetes is also significant.

There does not appear to be a correlation between the hyperactive response following the intravenous injection of these compounds and their diabetogenic effect. On the basis of the temporary hyperglycemia produced with smaller doses, dehydroascorbic acid and dehydroisoascorbic acid seem to have about the same potency as far as the production of diabetes is concerned. However, dehydroascorbic acid is 4 or more times as effective as dehydroisoascorbic acid in producing a hyperactive response.

The mechanism of action of these substances is probably similar to that of alloxan (13, 24) which is thought to interfere with essential sulfhydryl enzymes of the β cells. The similarity in action to that of alloxan is indicated by the previously demonstrated (25) triphasic glucose response following the injection of dehydroascorbic acid. The action on the β cells with decreased insulin production is indicated by the ready response of the hyperglycemia to injected insulin.

It is too early to generalize about the essential chemical structure required for the production of diabetes, but the results with dehydroascorbic and dehydroisoascorbic acids lead to a greater variation in possible structure. In searching for a precipitating factor in human diabetes, it becomes necessary to broaden the search beyond the nitrogen-containing purines and pyrimidines to substances which may be derived from carbohydrates, and as a matter of fact to any substance that may cause an excessive oxidation of ascorbic acid to dehydroascorbic acid in the β cells of the islets of Langerhans.

SUMMARY

1. Dehydroascorbic acid produces a characteristic reaction in rats with an LD_{50} of about 320 mg. per kilo. Following a sublethal dose, rats will tolerate a second dose 3 to 4 times as large as the initial dose.

2. Dehydroascorbic acid is more effective than ascorbic acid in acting synergistically with alloxan to produce diabetes.

3. Dehydroascorbic acid is shown to be similar to alloxan in structure and chemical properties, and to produce a hyperglycemia of a few days duration following a dose of 1.1 gm. per kilo in rats. Three daily injections of 80 mg. are capable of producing what appears to be permanent diabetes in rats weighing about 120 gm.

4. Dehydroisoascorbic acid produces permanent diabetes in doses of 1.5 gm. per kilo.

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