Course of Diabetes and Development of Cataracts After Injecting Dehydroascorbic Acid and Related Substances

JOHN W. PATTERSON

From the Department of Anatomy, School of Medicine
Western Reserve University, Cleveland, Ohio

Dehydroascorbic (1, 2), dehydroisoascorbic (2), and dehydroglucoscorbic (3) acids will produce a hyperglycemia in rats that persists for weeks. This has been called diabetes. Since dehydroascorbic acid occurs normally in man (4), it is of interest to consider the course of this disease and to further establish the diagnosis of diabetes.

Cataracts develop as a complication of human diabetes (5) and appear to be more common in those individuals who are poorly regulated. Cataracts occur in rats with experimental diabetes following pancreatectomy (6), or alloxan injection (7, 8). Those animals with the most severe diabetes develop cataracts the earliest (8). Therefore, an attempt is made to correlate hyperglycemia with the time at which cataracts occur following the injection of dehydroascorbic acid and related substances.

EXPERIMENTAL

Hyperglycemia was produced in male Sprague-Dawley rats (100-200 gm.) by the intravenous injection of dehydroascorbic, dehydroisoascorbic or dehydroglucoscorbic acid (1-3). (It has been found that these substances can be kept for a period of weeks, with little deterioration, if they are placed in rubber-stoppered test tubes and frozen immediately after preparation.) The animals were maintained on an unlimited diet of water and Friskies dog chow.

Blood sugars were determined once each week by a micromethod (9) and plotted on a graph. Small fluctuations were de-emphasized by averaging the blood sugars at 4-week intervals. The graphs of 6 rats that had received dehydroascorbic acid, 7 rats that had received dehydroisoascorbic acid, and 4 rats that had received dehydroglucoscorbic acid all showed a similar trend. The blood sugar level rose abruptly and gave a high average for the first 4 weeks which was then maintained for the period under study. This fact was particularly striking when data obtained on a total of 17 rats were incorporated in a single graph (fig. 1). Each point on this curve represented the average of 68 blood sugar values. Over a period of 7 months the blood sugar levels fell between 360 and 400 mg/100 cc. of blood. All of the rats included in the graph had a persistent hyperglycemia greater than 200 mg/100 cc.

After the animals had maintained a hyperglycemia for 7 to 11 months they
were placed in metabolism cages for a period of 5 days. During this period, the intake of food and water, and the output of urine and glucose (10) were determined daily. The results were averaged for a 24-hour period and incorporated in a graph (fig. 2). The glucoascorbic acid rat with the highest blood sugar died the third day so that in this case the average was for 2 days only. The terminal condition of the animal is reflected in the low food and water intake and low urine output. For better correlation the average of five consecutive weekly blood sugars obtained immediately prior to the studies in metabolic cages, and the weights of the animals at the time of these studies were also included in the graph. Essentially the same results were obtained regardless of the substance injected. In all cases the disease was characterized by hyperglycemia, glycosuria, polyuria, polydipsia, polyphagia and failure to gain weight. The severity of these signs correlated with the severity of the hyperglycemia.

The eyes of the rats were examined at weekly intervals and the time at which the lens became opaque was noted. Although the data presented in the first 2 figures represented the results on rats that had survived a minimum of 7 months, the data on cataracts also included results from animals followed for shorter periods. Some of the rats received sulffhydryl compounds 10 minutes after each injection of dehydroascorbic acid. These animals were controls in a sulffhydryl protection experiment (11) and the sulffhydryl was not expected to have any influence on the development of cataracts. This appeared to be true. The average blood sugar for the first 16 weeks of diabetes, or for as many weeks as the animal survived, was studied in relation to the time of cataract formation (fig. 3).

In those rats with high blood sugars, mature cataracts developed in 8 to 10 weeks and both eyes were involved at approximately the same time. In those rats with lower blood sugars cataracts developed more slowly and were sometimes unilateral or only developed in the second eye after several weeks had elapsed. Two rats with blood sugars of 295 and 263 mg/100 cc. have not developed cataracts in 24 and 28 weeks, respectively.

**DISCUSSION**

The hyperglycemia that develops, following the injection of dehydroascorbic, dehydroisoascorbic or dehydroglucoascorbic acid persists for many months and does not appear to change with time. Extrapolation of the hyperglycemia–time curve
(fig. 1) indicates that the hyperglycemia will persist indefinitely and is therefore permanent. The damage produced by these substances on the $\beta$ cells of the pancreatic islets of Langerhans (11) must be irreversible. Furthermore, the continued steady hyperglycemia is a strong indication that there are no new insulin producing cells being formed. In this respect the rat differs from the guinea pig which is capable of producing new $\beta$ cells (12). Rats should, therefore, serve as an excellent test animal in any attempt to isolate from the guinea pig the factor responsible for the formation of new insulin producing cells.

The permanent hyperglycemia resulting from the injection of dehydroascorbic, dehydroisoascorbic or dehydroglucoascorbic acid is accompanied by all the classical criteria for the diagnosis of diabetes mellitus. Inasmuch as these criteria are the natural outcome of persistent hyperglycemia, it is not surprising to note glycosuria, polyuria, polydipsia, polyphagia, and the failure to gain weight. Nor is it surprising that these factors correlate roughly with the severity of the hyperglycemia. With these findings the diagnosis of diabetes mellitus is definite.

Eight weeks or more after the production of diabetes by the injection of dehydroascorbic, dehydroisoascorbic or dehydroglucoascorbic acid, cataracts are noted in the rats. There is an inverse relationship between the time required for the occurrence of cataract and the extent of the hyperglycemia. The relationship is not expressed by a straight line equation but is approached by an equation for a hyperbola. This is consistent with the facts that a certain minimum time is required for the maturation of a cataract regardless of the height of the blood sugar and that a certain minimum blood sugar is required for the formation of cataract.

The general equation for a hyperbola is $(x - a) (y - b) = c$. It is possible to calculate the values of $a$, $b$, and $c$ by the method of least squares, which minimizes the sums of the squares of the deviation of the quantity $(x - a) (y - b) - c$ for each point. The resultant hyperbola for the data in figure 3 is described by the equation $(x - 5.7) (y - 225) = 1276$, where $x$ and $y$ represent the time in weeks that cataracts were first observed and the blood sugar in mg/100 cc, respectively. This equation indicates that cataracts should not appear before the week ending on day 40 and should not occur with blood sugars less than 225 mg/100 cc of blood. When these minimal values are subtracted from the blood sugar and time values, then the time required for cataract formation is inversely proportional to the blood sugar.
The minimal blood sugar for cataract formation is approximately 225 mg/100 cc. This is of some interest inasmuch as it is above the renal threshold and indicates at least partial saturation of the renal tubular reabsorption mechanism for glucose. It is possible that the saturation of a tubular reabsorption mechanism by glucose prevents the reabsorption of one or more essential substances that require the same pathway. Thus a deficiency could develop which results directly or indirectly in the formation of cataracts. Deficiencies of riboflavin (13), tryptophan (14), phenylalanine (15), histidine (15), leucine (15), threonine (15), and valine (16), are known to cause cataracts. Riboflavin is not depleted abnormally rapidly in diabetic animals (17), but poorly controlled diabetic patients may excrete abnormally high amounts of amino acids in the urine (18). That deficiency can develop by this mechanism under other circumstances is indicated by the fact that estrogens administered in large amounts to dogs can produce a vitamin-C deficiency in spite of the fact that a dog synthesizes its own vitamin C (19). In this situation the estrogen saturates the tubular reabsorption pathway usually used by ascorbic acid, thus causing its excretion with a resultant deficiency.

The minimum time required for mature cataract formation is 5.7 weeks. Since the time indicated on the graph is that of the week when cataracts were observed, it is apparent that they developed during the previous week. Thus an average of 0.5 week must be subtracted from the calculated value and the limit becomes 5.2 weeks or 36 days. This is in agreement with the results in alloxan (8) and in pancreatic diabetes (6).

Corrected for minimal values the product of the blood sugar value and the time required for cataract formation is a constant. Since the required blood sugar value is above the renal threshold the glycosuria produced should be approximately proportional to the hyperglycemia. Thus a similar relationship should exist if glycosuria is considered in place of the hyperglycemia. Charalampous and Hegsted (8) express the relationship between glycosuria and time of cataract formation as a straight line. It is felt that their data are better expressed by a hyperbola. This is particularly evident if their data for animals on a chow diet, which showed insignificant change and which is similar to the diet used for our animals, are added to the data for the controls (fig. 4). The calculated hyperbola is \((x - 39) (y - 8) = 314\). The standard
deviations of the distances of the points from the curve and straight line are 7.81 and 9.58 respectively. This would indicate a better fit around the calculated hyperbola. The limiting value of 39 days obtained from this equation agrees with the value of 36 days obtained from our data. Compared with results obtained in this laboratory the glycosuria values of Charalampous and Hegsted appear to be excessively high. The agreement of the two sets of data with respect to the minimum time required for mature cataract formation indicates that a hyperbola is a good first approximation for expressing the relationship between hyperglycemia or glycosuria and the time at which cataracts occur. Hence, the effect of any therapy for the prevention of cataracts must be evaluated from the hyperbola that describes the results obtained under standardized control conditions.

**SUMMARY**

Dehydroascorbic, dehydroisocorbic, and dehydroglucoascorbic acids all produce hyperglycemia in rats that persists with little change over a period of 28 weeks. The animals also have polyuria, glycosuria, polyphagia and polydipsia. They fail to gain weight normally. This establishes a diagnosis of permanent diabetes.

Cataracts occur after a period of weeks. The relationship between hyperglycemia and the time required for the appearance of mature cataracts is described by the equation $(x - 5.7) (y - 225) = 1276$ where $x$ and $y$ represent the time in weeks and the blood sugar in mg/100 cc. of blood respectively.

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**REFERENCES**