Point of Irreversibility of Galactose Cataracts in the Rat.* (29832)

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It has recently been demonstrated in diabetic and galactose-fed rats that the appearance of lens cataract, in the form of a white opacity visible to the naked eye, is associated with a disruption of the fiber membranes(1). On the basis of studies made in the early cataractogenic period various theories have been suggested as to the etiology of cataract formation(2,3,4). The validity of these theories depends on the success with which they can be correlated with the final event. Therefore, it seemed of importance to determine the time at which the cataractogenic process became irreversible on the assumption that this point would be useful in identifying the major etiological pathway. This paper deals with the point of irreversibility of galactose cataracts.

Methods. Male Sprague-Dawley rats, 27 days of age, were placed on a diet consisting of 65% ground chow and 35% galactose. With unrestricted feeding the median time for cataract formation on this diet was 18 days. The time of cataract appearance was determined by making daily observations with the naked eye and noting the day on which the lens became opaque. Dulcitol and ATP were determined by previously reported methods(1).

Results. Cataracts frequently appear in one eye before the other(5,6). The appearance of unilateral cataract establishes that the animal has been exposed to an adequate cataractogenic diet. Placing the animal on a normal diet at this time permits one to observe the clear eye to determine whether it is possible to reverse the cataractogenic process. This was done with 130 rats that had been used for dietary studies(2). The time required for development of a second cataract on a cumulative percentage basis is shown in Fig. 1. By the 7th day 5% and by the 14th day 70% of the animals had bilateral cataracts.

This observation was confirmed by placing 40 rats that had been on a galactose diet for 14 days on a normal diet and awaiting the appearance of cataracts. The cumulative per cent of possible cataracts after 0, 1, 2, 3, 4, 5, 6 and 7 days was 5, 12, 30, 38, 53, 75, 80 and 85%. The mean time was 4 days or a total of 18 days after initiation of the galactose diet and equal to the mean time for cataract appearance if the rats are maintained continuously on a galactose diet.

To determine the point of irreversibility animals were taken off galactose on different days, placed on a normal diet and observed for cataract development 21 days after the start of the galactose diet. Irreversibility is first manifest on about the 10th day and is relatively complete by the 14th day (Table I).

The levels of lens dulcitol and hydration, as determined by wet weight, are elevated during galactose feeding and the level of ATP is decreased. With the replacement of the galactose diet with a normal diet these parameters return to normal (Tables II and III).

Discussion. The cataractogenic process involves a series of steps. An early step that is definitely linked with galactose cataracts in humans and experimental animal is galactosemia(2). A number of changes occur in the lens which are definitely the result of galactosemia. The levels of selected enzymes (7,8,9), ATP(10), glutathione(11), and proteins are decreased(4). The levels of dulcitol

TABLE I. Percent Cataracts at 21 Days After Variable Days on Galactose.

Days on galactose	No. of lenses	% cataracts
8	12	0
10	24	17
12	24	38
14	40	85

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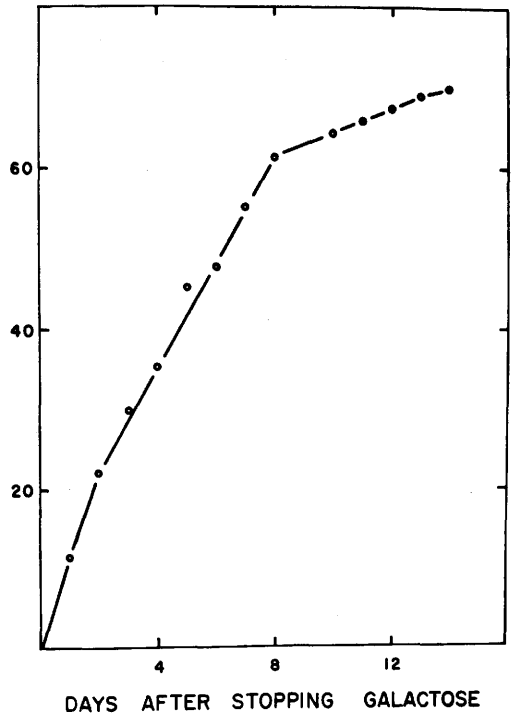

FIG. 1. Time required for development of a second cataract following appearance of unilateral galactose cataract and restoration of the animals to a normal diet, on the basis of the cumulative percent with 130 rats.

(12), and lens water are increased(3). The link between these changes and the appearance of cataracts is a matter of hypothesis.

The data reported here demonstrate that the cataractogenic process becomes irreversible several days before appearance of a white cataract, and provides a basis for separating precataractous lenticular changes into 2 groups on the basis of trends during the irreversible period. After the point of irreversibility, and following the institution of a normal diet, abnormal changes will either revert towards normal levels or continue on their abnormal course. If the former, the changes may be regarded as early steps which bring about an irreversible process or as irrelevant parallel phenomena which are unrelated to the cataractogenic process. If the latter, the changes may be regarded as late steps and their significance evaluated in relationship to the final step of fiber membrane disruption.

On the basis of this analysis, the lowering of lens ATP, the increase in dulcitol, and the lens swelling must be considered as early, or perhaps irrelevant, steps. In each of these instances the values return toward normal when the animals are placed on a normal diet during the irreversible period. Cataracts occur when the ATP level is relatively normal, the dulcitol level is low and the lens is not swollen.

Thus, it becomes clear, for this and other reasons(13), that the swelling and bursting of lens fibers following the accumulation of dulcitol, which has been implicated in the development of early vacuoles(3), is not directly related to the sudden final breakdown of the fiber membranes. Although the appearance of vacuoles and the appearance of a white opaque lens have both been called cataracts and used as investigative "end-points" they seem to be separate processes without a cause and effect relationship. This, however, does not mean that an increased dulcitol level and/or lens swelling may not produce a white opaque lens through the mediation of additional steps.

The basis for the irreversibility of the cataractogenic process is not established. Since the disruption of the fiber membrane is associated with the appearance of cataracts, since structural components and transport substances in the membrane are related to proteins, since the net increase in lens protein

TABLE II. Changes in Lens Weight and ATP After Stopping Galactose Feeding on the 10th Day.

Days after galactose	Wt (mg)	ATP (mg/100 g)
0	30.6 (10)*	63 ± 20† (5)
1	26.3 (7)	88 ± 21 (3)
2	26.4 (9)	121 ± 20 (4)
3	25.4 (7)	123 ± 10 (3)
4	23.1 (7)	110 ± 5 (3)

* No. of lenses shown in parentheses.
† ± Standard deviation.

TABLE III. Decrease in Dulcitol Level After Stopping Galactose Feeding on 14th Day.

Days after galactose	No. of lenses	Dulcitol (mg/100 g)
0	4	1160 ± 140*
1–2	5	690 ± 160
3–4	3	420 ± 100
5–6	3	150 ± 90
7–	4	180 ± 40

* ± Standard deviation.

stops on or about the 8th day(13) or approximately the point of irreversibility, and since interference with protein synthesis is a late step in theories of cataractogenesis, it is tempting to speculate that the point of irreversibility is coincident with the time when the restoration of protein synthesis is no longer possible.

Summary. Galactose-fed rats with unilateral cataracts develop cataracts in the second eye even though they are placed on a normal diet. Rats fed a 35% galactose diet for 14 days, followed by a normal diet, develop cataracts in a median time of 18 days just as if they were continuously on a galactose diet. The cataractogenic process becomes irreversible after 10-14 days of galactose. Restoration of a normal diet during the irreversible period results in a return of lens hydration, dulcitol and ATP to normal levels. It is suggested that changes such as these may be placed in the early or late period of cataractogenesis depending on whether they tend to return to normal or to continue their abnormal trend. Thus, the vacuoles seen after 2-4 days of galactose which are reported to be due to fiber swelling and bursting develop parallel with but are not the cause of the later white opaque cataracts.

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Studies on Inhibition of Brain 5-Hydroxytryptophan Decarboxylase by Phenylalanine Metabolites.* (29833)

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The conversion of 5-hydroxytryptophan (5HTP) to 5-hydroxytryptamine (5HT) is catalyzed by the enzyme 5-hydroxytryptophan decarboxylase (5HTPD)(1) and requires pyridoxal phosphate as a cofactor(2, 3). Davison and Sandler(4) have shown by *in vitro* studies that phenylpyruvic acid, phenyllactic acid, and phenylacetic acid inhibit 5HTPD. Huang and Hsia(5) have reported that this inhibition is competitive and substrate-dependent at pH 8 in rat kidneys.

The present communication describes experiments carried out to determine the inhibition of brain 5HTPD by phenylalanine derivatives.

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Materials and methods. For these studies, weanling guinea pigs were killed by decapitation, and a 20% homogenate of the brain was prepared. Brain 5HTPD was determined by the fluorometric method of McCaman and Robins(6). The final incubation mixture contained: 2-amino-2-methyl-1,3-propanediol buffer, 0.15 M, pH 8.05; bovine plasma albumin 0.05%; pyridoxal phosphate, 0.3 mM; isonicotinic acid-2-isopropylhydrazide (Hoffmann La Roche), 0.1 mM; DL-5-HTP (Sigma), 0.06-1.25 mM; and 0.25 ml homogenate in a total volume of 1.0 ml. Studies were performed in triplicate with 5 separate substrate concentrations. For the inhibition studies, 1.8×10^{-2}-5.4×10^{-2} M of phe-