etheral sulphate and glucuronide conjugation. The butyryl side chain appears to survive intact, and no evidence of oxidation to more complex phenols was obtained.

3. Etheral sulphate conjugation at the 5-hydroxyl group leads to 5-butyryl-2:4-dihydroxyphenyl hydrogen sulphate, isolated as its potassium salt. This was converted into 5-hydroxy-2:4-dimethoxybutyrophenone, and the synthesis of this substance is described.

4. Glucuronic acid conjugation at the 4-hydroxyl group leads to 4-butyryl-2:5-dihydroxyphenyl glucosiduronic acid, which was not isolated but was converted into 4-hydroxy-2:5-dimethoxybutyrophenone. The isolation and synthesis of this substance is described.

5. Dogs readily tolerate a daily dose level of 0.5 g./kg. for 1 year, and rats a daily dose level of 1.5 g./kg. for 2 years, with negligible storage of 2:4:5-trihydroxybutyrophenone in fat, liver, brain and kidneys.

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Metabolism of the Developing Retina

2. THE EFFECT OF AN INHERITED RETINAL DEGENERATION ON THE DEVELOPMENT OF GLYCOLYSIS IN THE RAT RETINA*

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An inherited retinal degeneration which bears a striking resemblance to human retinitis pigmentosa has been described in rats (Bourne, Campbell & Tansley, 1938) and other animals (Tansley, 1933; Parry, 1953). The defect is inherited as a simple Mendelian recessive, and histologically the condition is characterized by the degeneration of the whole of the outer retina between the inner nuclear layer and the choroid.

Graymore (1959) observed a dramatic increase in glycolysis in the retina of the developing rat, towards the end of the second week of life, coinciding approximately with the time at which the

eyes opened. Since this surge in activity did not appear to be light-dependent (Graymore, 1958*a*), it was suggested that it might be a reflexion of the stage of histological development, possibly being a manifestation of the differentiation of the visual cells. This idea gains support from the observation that subsequent destruction of the visual cells by intravenous injection of iodoacetate leads to a much reduced rate of retinal glycolysis (Graymore & Tansley, 1959*a, b*), and it has been suggested that the visual cells may make a major contribution to the high rate of retinal glycolysis.

The development of glycolysis in rats exhibiting the inherited retinal degeneration has been studied to determine whether or not metabolic changes accompanied the cellular breakdown. Such studies

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might help to compare the iodoacetate-induced and inherited degenerations which Noell (1953) believes may be identical.

METHODS

Animals. The animals used were albino and black-and-white rats of either sex, aged between 5 days and 7 months, all of which were bred from the original stock used by Bourne *et al.* (1938), and maintained subsequently at University College London and the Institute of Ophthalmology. The animals were fed *ad lib.* on diet 41 of Bruce & Parkes (1946). Black-and-white rats of either sex and corresponding ages were used as controls.

Histology. The procedure was that described by Graymore & Tansley (1959a).

Measurement of anaerobic glycolysis. The animals were killed either by dislocation of the cervical vertebrae or by decapitation, according to size, and the retinae removed intact within 2 min. of the death of the animal as described by Graymore (1958b). One to two retinae were incubated for 1 hr. at 37° in Warburg flasks containing 4 ml. of Krebs-Ringer bicarbonate buffer (Krebs & Henseleit, 1932) gassed with $N_2 + CO_2$ (19:1, v/v) and fortified with 200 mg. of glucose/100 ml.; evolution of CO_2 was measured manometrically. Previous experiments in which both CO_2 evolution and lactic acid accumulation were measured in the same flask had shown that the former provided a satisfactory measure of glycolysis. After incubation, the protein content of the flasks was estimated by the micro-Kjeldahl method, and the glycolytic value was expressed as μ l. of CO_2 liberated/hr./mg. of protein ($q_{CO_2}^N$).

RESULTS

Fig. 1 shows the changes in the rate of glycolysis during the development of normal and degenerating retina. Up to the eighteenth day there is little difference between the experimental and the

Fig. 1. Change in the levels of retinal anaerobic glycolysis during the development of normal rats (broken line) and rats having the inherited retinal degeneration (continuous line). Vertical lines represent the s.e.m. Where no vertical line is given the s.e.m. is too small to show. Number of animals per group (left to right): controls, 3, 4, 2, 2, 1, 2, 6; experimental, 4, 2, 5, 6, 4, 3, 3, 3, 3, 4, 2, 4.

control groups, although the retinae from the affected stock do not show maximum activity until 18 days ($q_{CO_2}^N$, 53.5 ± 2.6), whereas in normal animals the maximum is attained by about 14 days ($q_{CO_2}^N$, 50.6 ± 6.5). After 18 days there is considerable divergence. The retinae from the control animals maintain their mature value, whereas the glycolytic activity in the experimental group falls abruptly, returning to a value similar to that in the immature retina by the end of the fourth week ($q_{CO_2}^N$, 28.5 ± 6.1). This value remains approximately constant for the following 10 weeks, but then there is a further decline in activity, the last value recorded being 13.0 ± 1.6 at 7 months.

Histology. Histological examination of retinae from the affected stock showed that the course of the degeneration was essentially the same as reported originally by Bourne *et al.* (1938). Development is quite normal during the first 2 weeks of life, and although scattered pycnotic rod nuclei can be seen by the end of the second week, they do not make any significant contribution until about 3 weeks of age. The general nervous differentiation is complete by 18 days, although the rod nuclei are not completely differentiated until 5–7 weeks. Until the end of the third week of life, therefore, the histological pattern of the developing retina is comparatively normal; the visual cells attain full maturity and then begin to degenerate. At 5 weeks the peripheral region of the retina is still comparatively normal, apart from an unusually high number of pycnotic nuclei, but the outer nuclear layer of the central retina is becoming thin and disorganized and many of the remaining cells are dead. By 14 weeks the whole of the outer retina, comprising the rods and their nuclei and the outer fibre layer, has disappeared, resulting in the close apposition of the inner nuclear layer and the choroid.

DISCUSSION

The times of onset of the biochemical and histological deviations are not sufficiently separated to allow of a clear-cut decision about which is the cause and which is the effect. The death of cells which in normal health exhibit a high rate of glycolysis would produce the observed effect, but the speculation that degeneration may result from failure of some particular glycolytic enzyme is also attractive in view of the genetical nature of the condition. Cell death might then be the result of inability to survive on a much reduced rate of glucose utilization. Some support for this latter concept is gained from a consideration of the differing rates at which the two phenomena proceed; the decline in glycolytic activity is very abrupt, whereas cell degeneration continues over a period of weeks. At 28 days, when glycolysis has

returned to the immature level, large areas of the retina are still unaffected and over the next few weeks, whilst the degeneration continues, the glycolytic level remains fairly constant.

The difference between the time courses of the two phenomena could be explained on the basis of a considerable delay between actual cell death and cellular disorganization and degeneration, but it is not easy to reconcile this view with evidence from the iodoacetate-poisoned retina. Treatment of healthy, mature rats with a carefully chosen dose of sodium iodoacetate results in a fall in the glycolytic activity of the whole retina and total destruction of the visual cells (Graymore & Tansley, 1959*a*, *b*). This ability of iodoacetate to destroy the visual cells has been observed previously in other animals (Schubert & Bornschein, 1951; Noell, 1952), and Noell (1953) stressed the histological similarity of the chemically induced and hereditary lesions in the monkey, cat and rabbit. From this standpoint it is of interest that the glycolytic levels of retinæ from rats having the hereditary degeneration lie between 28.5 ± 6.1 and 33.8 ± 4.1 from the fourth to the fourteenth weeks of life, and the glycolytic level of retinæ from normal mature animals 1 week after destruction of the visual cells with iodoacetate is 30.15 ± 0.83 . This indicates a further point of similarity between the two conditions.

In contrast with this, however, the visual-cell debris is removed completely within 7 days of treatment with iodoacetate. This suggests that in the present investigation cell death may be a gradual process, and that it may be preceded by the glycolytic inhibition. The greater rapidity of the iodoacetate-induced destruction could be accounted for in terms of the wider action of the inhibitor; iodoacetate is not very specific in its action although its ability to inhibit glycolysis is particularly marked.

It should be noted, on the other hand, that Sorsby & Nakajima (1958) were unable to produce degeneration in the retina of the rat or rabbit by intravenous injection of other established glycolytic poisons or respiratory inhibitors, although some degree of degeneration was achieved by using a combination of fluoride and cyanide. This appears to argue against the idea that a simple glycolytic lesion might produce retinal damage, but apart from the combined fluoride and cyanide experiments relatively small numbers of experiments were carried out and the difficulties of establishing correct dosage levels is a complicating factor. In this Laboratory, for example, the determination of the precise level of iodoacetate required to produce retinal damage necessitated the analysis of a very large number of animals (Graymore & Tansley,

1959*a*). The further fall in activity in retinæ from the affected rats after 14 weeks probably reflects gross changes in the remaining layers of the retina; histological examination of retinæ from animals at this more advanced stage showed evidence of adhesion, some distortion and vascular changes.

It is not possible at present to draw any firm conclusions about the precise relationship between glycolytic inhibition and visual-cell degeneration; more information is required about other aspects of retinal metabolism under these conditions.

SUMMARY

1. The changes in the rate of anaerobic glycolysis in retinæ removed from rats having an hereditary visual degeneration have been compared with those seen in the normal rat retina. The developmental patterns are very similar up to the third week of life, but thereafter the glycolytic activity of the degenerating retina falls rapidly.
2. Both the histological and the biochemical changes commence at approximately the same time, although the course of the biochemical change is much more rapid.
3. The significance of these findings is discussed.

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