**ANAEROBIC GLYCOLYSIS IN RATS AFFECTED WITH
RETINITIS PIGMENTOSA
ITS SIGNIFICANCE IN RELATION TO VARIOUS FORMS OF PRIMARY
PIGMENTARY DEGENERATION OF THE RETINA***

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RETINITIS pigmentosa in man is an hereditary disease of unknown aetiology. It is characterized by a chronic and progressive course. Although not clinically manifest at birth, it reveals itself in childhood by the presence of night-blindness, and in adolescence by the onset of retinal changes. It frequently results in blindness in middle or advanced life. Pathologically, it is essentially a degeneration of the retinal neuro-epithelium, particularly of the rod cells, and its established features are an attenuation of the retinal blood vessels, optic atrophy, and, almost invariably, widespread pigmentary changes in the retina. Secondary cataract develops as a later complication.

The occurrence of a pigmentary degeneration of the retina in the rat was reported by Bourne, Campbell, and Tansley (1938). These workers showed that the retinal lesion was inherited as a Mendelian recessive condition, with no evidence of sex-linkage. It commenced at an early age as a primary degeneration of the neuro-epithelium, particularly affecting the rod cells, and secondary changes affecting other retinal layers. In the case of pigmented retinae, the migration of pigment granules through the retina was observed. The close similarity between the clinical picture shown by the rats carrying this hereditary retinal lesion, and the human form of retinitis pigmentosa was pointed out, and led these authors to describe the lesion as "retinitis pigmentosa" in rats.

The close similarity in morphology between the condition in man and the retinal lesion in the rat suggested that an investigation into the retinal metabolism in these animals might help to elucidate the aetiology of the disease, which might have some bearing on the disease in man.

Anaerobic glycolysis is so outstanding a feature of retinal metabolism that it was selected for the primary study in this investigation. Warburg, Posener, and Negelein (1924), working on the normal rat, found retinal anaerobic glycolysis to be extremely high; higher in fact than that found for any other mammalian tissue or even in the majority of carcinogenic cells. It is surprising that there is no record in the literature of any attempt to correlate this very high rate of metabolic activity with retinal function.

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Experimental Methods

The rats used in these experiments were bred from descendants of the original colony described by Bourne and others (1938) and maintained in the Research Department of the Birmingham and Midland Eye Hospital.

The colony was kept under normal conditions and fed a complete proprietary diet (British Flour Millers, No. 41), with daily supplements of milk and boiled cow's liver.

Since the condition is of the Mendelian recessive type, it was necessary to establish the pure line. A known affected animal was crossed with a normal animal to yield the F_1 generation. These were back-crossed with the affected parents to give the F_2 generation, 50 per cent. of which would normally be affected animals, and the rest carriers of the lesion. The affected animals were differentiated by the removal of one eye for sectioning, under anaesthesia. The affected rats, thus found by the presence of the degeneration in the retina, were then cross-bred. Inbreeding was continued for many generations and the strain bred true (F_3). To facilitate the dissection of the retinae for metabolic work, only black and white rats were used in this work. Animals of the F_1 generation were used as control animals.

For metabolic work, the rats were killed by breaking the neck. Death was instantaneous, and the eyes were immediately removed and placed in complete buffer solution. The cornea was slit at the limbus with a fine von Graefe cataract knife, and the lens extruded by gentle pressure with fine iris forceps. The whole of the cornea was next cut off with pointed scissors, after which stage the retina was clearly visible as a purplish membrane. The tissue was carefully loosened from the choroid and detached from its attachment at the optic nerve.

Two retinae from one animal were used for the estimation of anaerobic glycolysis. Immediately after being dissected out they were placed in 3 ml. buffer in a Warburg cup fitted with one side-arm. The buffer was Krebs-Henseleit isotonic bicarbonate saline (Krebs and Henseleit, 1932), containing 250 mg. per cent. glucose. The retinae in the flask were gassed for 10 minutes with a gas mixture of 95 per cent. nitrogen and 5 per cent. carbon dioxide.

Estimations of the rate of anaerobic glycolysis were made manometrically using the Warburg apparatus. The general technique adopted was that recommended by Umbreit, Burris, and Stauffer (1945).

It was found that the delicate structure of the retina made it impossible to obtain the dry weight of the individual pairs of retinae used in an experiment. From estimations made by recovering the tissue after one hour's shaking in the Warburg apparatus, it was found that losses of up to 30 per cent. in the dry weight were occurring. To show that there was no difference between the tissue weights of retinae from affected animals and those from normal controls, the retinal dry weights were measured in a separate experiment. The retinae were dissected out as described above, washed in isotonic saline, and placed in small glass cups, of average weight 120 mg. The retinae were dried for 24 hours at 110°C. After cooling in a desiccator, the cups were weighed on a Stanton semi-micro balance. The cups were freed from dried tissue with hot chromic acid solution, washed in distilled water, dried in a desiccator, and re-weighed. In a few experiments, the manometric estimation of lactic acid produced by the retinae was checked by a chemical estimation. The method used was the modification recommended by Umbreit and others (1945), from the method of Barker and Summerson (1941).

Procedure

(i) *Anaerobic Glycolysis*.—Black and white rats between the ages of 12 and 37 days were used for this study. Anaerobic glycolysis of the two retinae from one animal was measured in 84 normal and 112 affected rats. The results are given in Table I, expressed in $\mu\text{l. CO}_2$ produced in one hour by two retinae from one animal under the standard conditions described. The daily mean anaerobic glycolysis of all results is plotted against age in days in Fig. 1 (opposite).

TABLE I
DAILY MEAN ANAEROBIC GLYCOLYSIS IN NORMAL AND AFFECTED
RATS SHOWN AS MICROLITRES CO_2 PRODUCED IN ONE HOUR BY
TWO RETINAE FROM ONE ANIMAL (See also Fig. 1)

Age (days)	Control Rats (F_1)		Affected Rats (F_3)	
	Mean ($\mu\text{l. CO}_2$)	No. of Tests	Mean ($\mu\text{l. CO}_2$)	No. of Tests
12	141	4	126	8
13	153	8	133	8
14	146	12	139	18
15	169	7	146	8
16	184	7	162	7
17	186	8	172	9
18	185	4	180	5
19	198	5	183	2
20	206	1	179	6
21	204	2	173	4
22	216	2	179	3
23	203	3	176	3
24	229	3	184	2
25	216	1	169	3
26	227	1	178	2
27	217	4	182	3
28	210	1	168	3
29	214	2	156	2
30	204	4	168	6
31	—	—	159	4
32	222	2	154	3
33	—	—	142	1
34	—	—	128	2
35	—	2	—	—
37	228	1	—	—

In order to establish the real significance of the results for anaerobic glycolysis, the values shown in Table I were subjected to a statistical analysis (Table IA).

TABLE IA
STATISTICAL ANALYSIS OF RESULTS FOR ANAEROBIC GLYCOLYSIS
FROM THE AFFECTED AND CONTROL RATS

Age (days)	Actual Difference between Means of Two Groups	S.E.M.	<i>P</i>
12—15	15.43	4.6	<0.001
16—21	16.398	4.07	<0.001
22—30	41.074	4.0 approx.	—

FIG. 1.—Daily mean anaerobic glycolysis in pairs of retinæ, by age.

Metabolic results are normally presented in the “Q” notation. This system is a method of expressing the results of metabolic experiments, and in the case of anaerobic estimations is the amount of carbon dioxide, measured in $\mu\text{l.}$, produced by one mg. tissue (dry weight) in one hour under standard conditions. The carbon dioxide arises by the interaction of the lactic acid produced by the tissue under conditions of anaerobiosis with the bicarbonate buffer solution. Gas output is indicated by a plus sign preceding the quotient. In the discussion, the metabolic results are compared with those obtained from the retina of the rabbit by other workers after the systemic administration of retinal poisons. In order to facilitate this comparison, the present results are expressed in the conventional notation in Table II, by the adoption of the glycolytic values from Table I and the retinal dry weights from Table III (overleaf).

TABLE II
ANAEROBIC GLYCOLYSIS RESULTS EXPRESSED IN “Q” NOTATION

Age (days)	$Q_{\text{CO}_2}^{\text{N}_2}$	
	Control Rats (F ₁)	Affected Rats (F ₃)
12—15	+46	+44
16—21	+59	+53
22—25	+63	+52
26—29	+59	+53
32	—	+47

(ii) *Retinal Dry Weight.*—The separate estimations of retinal dry weight were made, as described, on rats at 14, 16, 21, and 25 days of age to cover the significant stages of the metabolic investigation. A few other estimations were made for 29 and 32 days (Table III, overleaf).

TABLE III

RETINAL DRY WEIGHTS

Each measurement (mg.) in normal and affected rats represents one pair of dried retinae taken from one animal (See also Fig. 2).

Age (days)	Retinal Dry Weight (mg.)			
	Control Rats (F ₁)		Affected Rats (F ₃)	
	Individuals	Mean	Individuals	Mean
14	3.05; 3.39; 3.27; 3.21; 3.41	3.27	3.33; 3.41; 3.19; 2.89; 2.92; 3.07; 2.97	3.11
16	3.28; 2.96; 3.19; 3.01; 3.09; 3.33; 3.05; 3.21; 3.50; 3.03	3.17	3.27; 3.21; 3.53; 3.15; 3.20; 3.18; 3.17; 3.22; 3.15	3.23
21	3.37; 3.53; 3.38; 3.12; 3.31; 3.43; 3.12; 3.46; 3.48; 3.59; 3.42	3.36	3.48; 3.66; 3.57; 3.58; 3.01; 3.55; 3.27; 3.51; 3.18	3.42
25	3.54; 3.53; 3.03; 3.36; 3.34; 3.59; 3.49; 3.42; 3.44; 3.36; 3.34; 3.64	3.42	3.62; 3.37; 3.19; 3.24; 3.49; 3.57; 3.40; 3.48; 3.53; 3.29	3.42
29	3.71; 3.76; 3.69; 3.63; 3.58	3.67	3.52; 3.30; 3.18; 3.26; 2.98	3.25
32			2.78; 3.11; 3.34; 3.02; 3.88; 3.85	3.33

In Fig. 2 the daily mean retinal dry weight is plotted against age for the period 14 to 32 days of age.

FIG. 2.—Mean retinal dry weight, by age.

The results for retinal dry weight were subjected to statistical analysis in order to check that the results obtained showed a truly significant difference between the two groups (Table IV).

TABLE IV
STATISTICAL ANALYSIS OF RETINAL DRY WEIGHTS

	DF	SS	MS
Between days F ₁	3	4,065	1355
Between days F ₃	3	5,643	1881
Between F ₁ and F ₃	1	12	12
Within (Residual)	65	17,620	271
Total	72	27,340	380

Within Days Variance		Means (mg.)	
F ₁ only	244	F ₁	= 331.8
F ₃ only	301	F ₃	= 331.0
		Both	= 331.4

(iii) *Lactic Acid Production*.—A series of experiments was performed to check that the retinal tissue produced only lactic acid from the glucose in the buffer. Table V gives the comparison of the amount of lactic acid found by this means with that calculated from the manometric technique.

TABLE V
COMPARISON OF LACTIC ACID, MEASURED MANOMETRICALLY,
WITH CHEMICAL ESTIMATION

Age (days)	Lactic Acid Equivalent (μ l. CO ₂)			
	F ₁		F ₃	
	Measured Manometrically	Measured Chemically	Measured Manometrically	Measured Chemically
12	148 145 —	160 152 —	— — 146	— — 169
13	—	—	138	145

Results

(1) *Anaerobic Glycolysis*.—The measurements of anaerobic glycolysis extending over the age range 12 to 37 days are shown in Table I. This age range was chosen to cover the period before the onset of the degeneration as seen histologically, up to the time when the dystrophy was fairly well advanced. Before 15 days, no histological sign of the dystrophy can be seen in the affected rats. The first abnormality seen in the affected animals is a pyknotic nucleus in the outer nuclear layer at about 15 days. By about 23 days of

age the whole visual cell layer is considerably disrupted. Some of the tissue is removed, probably by phagocytosis, until eventually the visual cell layer has lost its cellular structure entirely. At about 30 days of age thinning of the cells in the outer nuclear layer has begun. During the ensuing months the whole retina is considerably altered by progressive degeneration of its various layers until in the later stages it is almost unrecognizable.

(a) *From 12 to 15 Days of Age.*—In rats of 12 to 15 days of age it was found necessary to perform a large number of experiments, since there was considerable overlapping in the results. Fig. 1 shows that there is a difference between the two groups although this is smaller than in older animals. Since the analysis of the retinal dry weights over this age period showed no difference between normal and affected animals, it is clear that the metabolic difference must be significant. It is most interesting to find that, even at this early age when no sign of the degeneration can be seen under the microscope, the anaerobic metabolism is lower than normal in affected animals.

(b) *From 16 to 25 Days of Age.*—There was a marked difference between the anaerobic glycolysis of affected animals and that of normal controls in this age group. In fact, there was virtually no overlap between them. At 16 days the histological signs of the degeneration are so slight that no loss of tissue had occurred. The only abnormality was seen in the distal segments of the rod cells, which appeared to break down into an amorphous mass. At this age very few of the rod cell nuclei were pyknotic, although over the next few days the number of necrosed cells increased. The rate of metabolism did not alter appreciably as the retina became progressively disorganized. In affected animals the anaerobic glycolysis is about 18 per cent. lower than normal, but in both groups there was a slight increase in the rate of metabolic activity which, in the main, followed the growth curve. The retinal dry weights for both groups showed a similar small increase during this period, showing that some active growth was still occurring.

(c) *Over 30 Days of Age.*—Fig. 1 shows that a further drop in the results for anaerobic glycolysis occurred in rats over 30 days old. This fall is to approximately 36 per cent. lower than normal, or about twice the difference between the two groups at the earlier age. A parallel histological investigation was not carried out in this group but, from the description already supplied by the original authors, it would seem that this second fall in metabolism coincides with the complete disintegration of the visual cell layer.

(2) **Retinal Dry Weight.**—The results for anaerobic glycolysis show a clear difference between normal and affected retinae, but it was also necessary to show that this difference was not due solely to a simple difference in tissue weight. The results recorded in Table II and the statistical analysis given in Table III confirm that no significant difference is present in the weight of the retinae from either group of rats up to 29 days of age. A slight increase

due to the normal growth processes does take place in both groups and this is paralleled in the metabolic results. Both groups follow about the same upward curve.

Discussion

(1) Anaerobic Glycolysis.—In considering the results obtained from measurements of anaerobic glycolysis of the retinae of affected rats, it must be remembered that the retina consists of a number of distinct layers which are nourished from the blood stream in two different ways. The visual cell layer is supplied by the dense choroidal capillary network, via the pigmentary epithelial layer, whilst the inner layers receive their nutrition from the retinal capillary system. It is not unlikely that the metabolism of some of the retinal layers could be impaired whilst the remainder of the tissue still functioned normally.

This investigation has shown that the anaerobic glycolysis is about 18 per cent. lower in affected rat retinae than in normal retinae, at a time when the histological signs of the degeneration are not visible. It is possible that this fall in anaerobic glycolysis originates mainly in the outer visual cell layer, and that if it could be measured for this layer alone the result would be much lower still.

It is an interesting fact that *aerobic* glycolysis in the retinae from rats bred from the colony described in this paper has also been found to be appreciably lower in affected animals, at a time when the signs of the dystrophy are not visible histologically (Lucas and Newhouse, 1956).

The theory that the production of an enzyme is under the control of a single gene has been supported by much recent work (Beadle, 1945; Mitchell, 1950). It may be that, in the retinal lesion in the rat, the fall in anaerobic glycolysis is due to the fact that one of the enzymes concerned is absent or abnormal. If such a mechanism could be shown to be responsible for the metabolic abnormality, it would enable the lesion to be classified as an inborn error of metabolism (Garrod, 1923).

(2) Comparison of the Hereditary Lesion with Chemically-Induced Retinal Lesions.—The hereditary retinal lesion described above is closely simulated by others produced in the laboratory by treatment of animals with certain chemical agents. A similar chemically-induced lesion in man has also been reported. Comparisons with these lesions offer a fresh approach to the general problems of the causative factors involved, although it must be remembered that there are differences between the chemically-induced lesions and those which occur naturally.

The earliest example of a chemically-induced retinal lesion was described by Riehm (1929), who examined patients treated with intravenous injections of an iodine-containing antiseptic solution, septo-iodine. This toxic substance produced a severe loss of vision, which showed ophthalmoscopically the typical signs of a pigmentary degeneration of the retina.

Riehm (1929) followed up this work with a study of the effects of injections of septo-iodine in rabbits. Histologically, he found that within 4 days of the injection the visual cell layer had perished, and that by 6 weeks the whole outer nuclear layer had disappeared. Similar results were obtained by Sorsby (1941) by treating rabbits with injections of sodium iodate, one of the principal constituents of septo-iodine.

More recently, Noell and Chinn (1949) found that the intravenous injection of sodium iodoacetate in rabbits abolished the visual response to illumination, as seen by the disappearance of the electroretinogram. Further work by Noell (1951, 1952) ascribed this to a direct effect by iodoacetate on the retina. At lower doses this abolition of the electroretinogram was reversible, but when the amount injected was increased the effect became permanent. Noell concluded that the "impairment of sensory function preceded the structural changes in the retina", and later demonstrated that, in rabbits in which the electroretinogram had been permanently abolished, the visual cell layer had completely disappeared with preservation of the inner layers. Animals which recovered from the iodoacetate treatment showed no morphological changes in the retina. The effect of iodoacetate appeared to be a direct attack on the visual cells, any modification in the pigmentary epithelium being secondary.

Noell (1951) held the view that iodoacetate interfered primarily with anaerobic glycolysis of the retina, which he deduced from experiments combining the effects of anoxia with injections of this chemical. This showed that the least resistant element of the electroretinogram to iodoacetate was that which was least affected by lack of oxygen.

Noell (1956) stated that he had later confirmed this hypothesis by manometric measurements of the retinal metabolism of rabbits treated with iodoacetate. He found a value for anaerobic glycolysis of $Q_{CO_2}^{N_2} = +46$ in a retina which had lost its visual cell layer. His value for the normal rabbit retina was +69, which is comparable with the value of +60 obtained in the present experiment on normal rat retina. Again, the figure obtained for affected rat retinae between the ages of 12 and 30 days was +51, and this fell to +47 in rats over 30 days of age, which is close to the value obtained by Noell.

It must be stressed that, whereas Noell's figures were obtained 4 weeks after the electroretinogram had been entirely abolished and the visual cell layer had disappeared, anaerobic glycolysis was measured in the present study, at a time when the lesion was actually developing in the growing retina, and was therefore closely related to vital processes. The metabolism was low several days before there were any histological signs of degeneration. The latter first appeared at 15 days of age, and by about 30 days of age the visual cell layer was almost entirely removed by phagocytosis. The second fall in anaerobic glycolysis occurred at this point when the degree of damage

in the rat tissue can be compared with the effect produced by iodoacetate poisoning, described by Noell, working on the rabbit. The close similarity between the anaerobic metabolism in the rat and the rabbit, at this stage, is most striking.

In considering the relationship between the genetically-induced lesions and those produced in the laboratory, it is of interest to note that Noell has found that the distribution of the attack by iodoacetate on the retina is identical to that found in sections taken from the eyes of human patients with retinitis pigmentosa. He concludes that iodoacetate destroys the same metabolic system in the retina as is deficient in the naturally-occurring retinal lesion. This point is further strengthened by the results here reported in the rat, particularly since this hereditary lesion bears such a close resemblance to the human disease (Bourne and others, 1938).

(3) The Theory of Abiotrophy as applied to Retinitis Pigmentosa.—Abiotrophy (Gowers, 1902) is a condition in which a tissue attains normal maturity and subsequently undergoes a progressive retrogression. The affected tissue is usually one which is highly specialized and instances of abiotrophy are found in certain familial conditions of the nervous system of which retinitis pigmentosa is an example (Collins, 1919).

Bourne and others (1938) classified the lesion in rats as belonging to this class. Other workers have found similar lesions in animals to fall into this category: mice (Tansley, 1951); red setter dogs (Parry, 1953).

The acceptance of the human and animal forms of retinitis pigmentosa as an abiotrophy has been challenged by recent results based on the evidence of electroretinography (Karpe, 1949; Parry, Tansley, and Thomson, 1951; Bounds, 1953) and of the histological changes which occur with the development of the lesion (Lucas, 1954; Lucas, Attfield, and Davey, 1955).

The results presented above show clearly that the metabolic state of the retina, as demonstrated by the changes in anaerobic glycolysis, is depressed before any histological changes are apparent. These results, therefore, do not support the theory that, in the rat at least, the lesion is an abiotrophy.

Summary

(1) The disease retinitis pigmentosa in man is described and compared with a similar retinal lesion found to occur spontaneously in the rat.

(2) The results are presented of an investigation into the anaerobic metabolism of the retina from affected animals of from 12 to 35 days of age.

(3) No difference was found between the dry weight of normal and affected retinae, at least up to 30 days of age, and the decline in anaerobic glycolysis in affected animals is not due merely to the loss of retinal tissue.

(4) Anaerobic glycolysis in affected rats is significantly lower than in control animals. The decreased rate of metabolic activity is present *before* there is any histological evidence of tissue abnormality.

(5) The results are discussed in relation to various naturally-occurring retinal lesions and to lesions induced by toxic chemical agents.

(6) The low rate of anaerobic glycolysis in the retina of this rat colony is very similar to that found in studies of rabbit retinæ treated with iodoacetate injections.

(7) The literature on the theory of abiotrophy as applied to hereditary retinal lesions is discussed, and the evidence of metabolic studies added to that of other recent work.

(8) The conclusion is drawn that the term abiotrophy, in this context, adds little to the understanding of the nature of the disease, and is not supported by recent findings.

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