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EXPERIMENTAL DEGENERATION OF THE RETINA*

III. INHIBITORS OF GLYCOLYSIS AND OF RESPIRATION AS INDUCING AGENTS

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ONE of the grounds on which disturbance of glycolysis has been assumed to be responsible for experimentally induced degeneration of the retina is the observation recorded by Noell (1951) that injection of sodium iodoacetate abolishes the electric response of the retina much more markedly in mammals than in other species. This finding is suggestive in view of the fact that the mammalian retina is much more dependent on glycolysis than the retina of the lower vertebrates. Furthermore, the high glycolytic activity of the retina raises the possibility that retinal degeneration might arise from interference with this activity; indeed, the action of sodium iodoacetate in inducing experimental degeneration has been ascribed to its possible effect on glycolysis. Since iodoacetate is an outstanding thiol reactor, other such reactors were studied and their effects have been recorded previously (Sorsby, Newhouse, and Lucas, 1957; Lucas, Newhouse, and Davey, 1957). With one exception, none of the thiol reactors used produced any experimental lesions in the rat or rabbit, the exception being bromoacetate, an analogue of iodoacetate, and this gave only a mild lesion in the rabbit and none at all in the rat. The present study was undertaken to test whether inhibitors of glycolysis and respiration—other than thiol reactors—produce retinal damage in the experimental animal *in vivo*. The literature contains several tentative negative results. Thus Karli (1954) failed to obtain degeneration of the retina in the rabbit by the use of sodium fluoride and of phloridzin, and Babel and Ziv (1956) also failed to obtain any results—ophthalmoscopic, electroretinographic, or histological—from the use of fluoride and glyceraldehyde. In the present investigation, a representative series of inhibitors of glycolysis and of respiration was tried in the rat and the rabbit.

Techniques and Agents Used

The procedures in injecting the agents, and the ophthalmoscopic and histological techniques employed in assessing results, were essentially the same as in the previous study. The agents and dosages employed are listed in Table I (opposite). The one innovation introduced was electroretinography carried out on the rabbits. The findings will be recorded separately by one of us (A.N.).

Results

Ophthalmoscopically and histologically none of the agents produced any changes in the retina in the rat or rabbit. As most of these agents are largely

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TABLE I

INHIBITORS OF CARBOHYDRATE METABOLISM ADMINISTERED INTRAVENOUSLY TO RATS AND RABBITS

	Substance	Rats		Rabbits		
		No. of Animals Treated (a single dose)	Dose* (mg./kg.)	No. of Animals Treated	Dose* (mg./kg.)	No. of Full Doses Given
Mainly or Exclusively Inhibitors of Respiration	Sodium cyanide ..	—	—	1	0·5	1
	Sodium oxalate ..	2	75	2	50	2†‡
	Sodium malonate	3 (*a*)	1,500	1	700	2†
	Sodium maleate ..	2	3,660*	1	250	1†
	Sodium fluoracetate	1	2·0	1	0·25	2†‡
	Phenosafranin ..	1	10	4	5·0–6·0	3; 3; 3; 2‡
	Congo red ..	2	200	1	250	1†
	Triiodobenzoic acid	2	75*	1	200*	1
	Malononitrile ..	—	—	2	10	1; 2†
	Sodium azide ..	2	20	1	12·5	2†‡
Mainly or Exclusively Inhibitors of Glycolysis	DL-Glyceraldyehde	1	117*	1	300	1†
	Methyl glyoxal ..	2	100	1	50	3
	Sodium fluoride ..	2	30	1	65	2‡
	Nicotine	2 (*b*)	0·5–1·0	1	3·0	3

* Maximum tolerated dose, except that for the three agents carrying a * sign; the dose was limited by solubility and the maximum tolerated dose could not be established.
† In addition to the maximum tolerated dose, one or more submaximal doses were administered, generally before the maximum dose.
‡ Found dead overnight after the last injection.
(*a*) One further animal received 2,500 mg./kg. and survived for 30 minutes; histologically negative.
(*b*) Two further animals received 2 mg., and two animals 4 mg. subcutaneously; histologically negative.

inhibitors of either glycolysis or of respiration, these negative results raised the possibility that retinal damage might be produced if inhibitors of both functions were administered simultaneously. For this purpose the combined use of two such inhibitors was tried as shown in Table II (overleaf).

It should be noted that some of these agents are known to have other effects too. The doses used were generally more than half the dose used for each agent separately and were mixed together just before injection. It will be seen that the combination of a respiratory inhibitor like *p*-chloromercuribenzoate with fluoride and mapharside—both known inhibitors of glycolysis—gave no retinal damage. Similarly, respiratory inhibitors like malononitrile and cyanide combined with inhibitors of glycolysis like fluoride, mapharside, and glyceraldehyde, though given to the limit of tolerance, gave no results, except that a marked retinal degeneration set in when the combination of cyanide and fluoride was used. At first with small doses of these agents (0·25 mg. and 35 mg./kg. respectively) there was no ophthalmoscopic lesion, but a localized reaction was observed when the same animal received a larger dose (0·60 mg. and 45 mg./kg.). A fairly similar dose administered as a single injection in a further rabbit gave the fundus appearances shown in Fig. 1 and the histological changes shown in Fig. 2 (overleaf).

Ophthalmoscopically, the course and appearance of this experimental degeneration were very similar to those produced by sodium iodate. The effect, however, was not always obtained. These substances—generally in a dose of 0·5 mg. cyanide and 50 mg. fluoride—given to 30 more rabbits produced an immediate mortality of eight and only three positive results amongst the 22 survivors. Fifteen of the nineteen unresponsive rabbits produced no further positive result from two or three repeated injections. In all, there were, therefore, five positive results in a series of 24 survivors.

TABLE II

SIMULTANEOUS INTRAVENOUS INJECTION INTO THE RABBIT OF INHIBITORS OF GLYCOLYSIS AND OF RESPIRATION

Agents Used	No. of Animals	Doses (mg./kg.)	No. of Days Observed	Remarks
Sodium fluoride and p-chloromercuribenzoate	1	45 and 15	4 (*a*)	No retinal damage.
Mapharside and p-chloromercuribenzoate	1	1·5 and 15	15	No retinal damage.
Sodium fluoride and Malononitrile	2	40 and 5	3 (*b*); 4	No retinal damage.
Mapharside and Sodium cyanide	1	1·5 and 0·65	7 (*b*)	No retinal damage.
DL-glyceraldehyde and Sodium cyanide	1	210 and 0·65	5	No retinal damage.
Sodium fluoride and Sodium cyanide	1	35 and 0·25	14	No retinal damage after the first dose. A second combined dose of 0·6 and 45 mg./kg. respectively gave a retinal reaction within 3 days; this was rather more localized than the reaction obtained with a single combined dose of 0·50 and 50 mg./kg. respectively.
Sodium fluoride and Sodium cyanide	1	50 and 0·50	18	
Sodium fluoride and Sodium cyanide	22	37–65 and 0·37–0·5	4–28	Retinal damage from one injection in three rabbits.
Sodium fluoride and Sodium azide	1	60 and 8	8	Dose repeated and animal observed for 4 days more; no retinal damage.
Sodium azide and Malononitrile	1	10 and 10	8	Dose repeated and animal observed for 4 days more; no retinal damage.

(*a*) Destroyed because of paralysis of hind-legs. (*b*) Found dead.

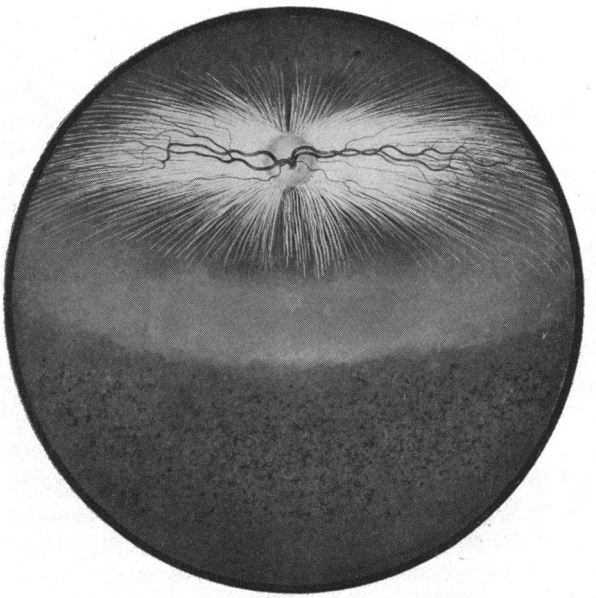

FIG. 1.—Fundus of rabbit 18 days after a single intravenous injection of 0·5 mg./kg. sodium cyanide simultaneously with 50 mg./kg. sodium fluoride. Below a zone of oedema or coagulation necrosis abutting on the disc, there is a wide zone of extensive pigmentary degeneration, which began to develop 3 days after the injection.

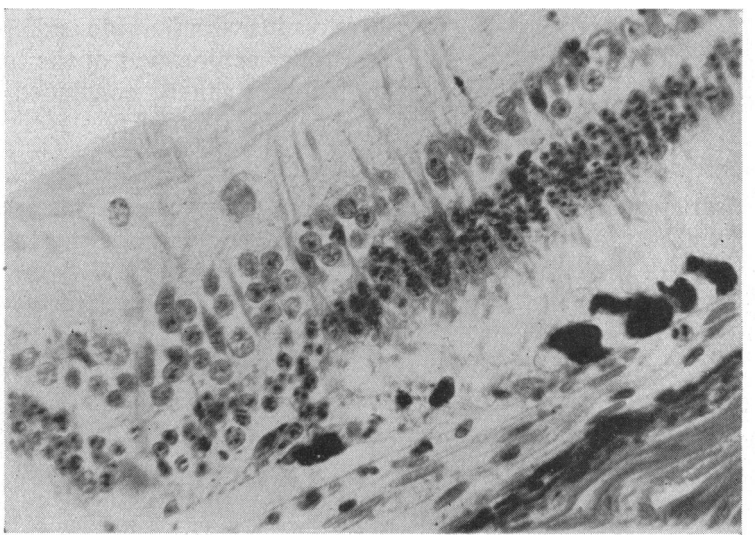

FIG. 2.—Section of the retina shown in Fig. 1. Note the extensive breaking up of the cells of the pigmented epithelium and of the visual cells, most marked to the left. A chorio-retinal adhesion with invasion of pigment is present at a break in the outer limiting membrane. The section is of retina near the disc.

Two single attempts to produce retinal degeneration by the use of azide together with fluoride, or with malononitrile were both negative.

Discussion

The present study confirms the scattered observations recorded in the literature that the administration of carbohydrate inhibitors does not produce retinal damage in the experimental animal. It carries matters further in bringing out that neither inhibitors of glycolysis or of respiration give any result. The Pasteur phenomenon suggested the possibility that the failure of these specific inhibitors might be due to the fact that the remaining carbohydrate system would act for both itself and the system excluded by the inhibitor. The results recorded with the combined use of cyanide and fluoride lend some support to this assumption, but the negative results with other similar combinations raise considerable difficulties. For the moment it is of some significance that experimental degeneration of the retina has been achieved by a procedure deduced from the established fact that the retina is highly dependent on glycolysis and respiration. The possibility arises that sodium iodoacetate induces degeneration of the retina by virtue of its being an inhibitor of both respiration and glycolysis—a reading not altogether supported by electroretinography which shows a different response as between iodoacetate and cyanide and fluoride combined. Moreover, this assumption would not explain why ethyl iodoacetate, which is almost as effective as sodium iodoacetate as an inhibitor of respiration and of glycolysis, fails to produce retinal degeneration; nor would it explain why sodium iodate which does not inhibit either function, readily produces a lesion. The low proportion of positive results with fluoride and cyanide in combination does in any case call for a fuller exploration of the individual inhibitors of glycolysis and respiration before it can be concluded that the effect recorded here is indeed due to the combination of two agents.

Summary

(1) A series of inhibitors of respiration and of glycolysis was used intravenously in rats and rabbits in sublethal doses without producing any degeneration of the retina.

(2) The combined use of sodium cyanide and sodium fluoride—typical inhibitors of respiration and of glycolysis respectively—produced a marked retinal degeneration ophthalmoscopically and histologically in five out of 24 animals treated.

(3) As yet other similar combinations have given no result; the theoretical implications are indicated.

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