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EXPERIMENTAL DEGENERATION OF THE RETINA

I. THIOL REACTORS AS INDUCING AGENTS*

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USING electroretinography in attempts to elucidate the relative significance of aerobic glycolysis and of respiration in the metabolism of the retina in the intact animal, Noell (1951, a, b) found that intravenous injections of sodium iodoacetate—an inhibitor of glycolysis—extinguished within a few minutes electrical response to illumination of the retina in the cat and rabbit. In confirming these findings, Schubert and Bornschein (1951) drew attention to the extensive destruction of the rod and cone and the outer nuclear layers of the retina induced by the iodoacetate injection—observations also recorded by Noell (1952, a, b). That the relationship between changes in the electrical response and histological damage is not direct is seen from the fact that only functional and no anatomical disturbances are observed after injections of azide (Noell, 1952, a), and of sodium fluorite (Babel and Ziv, 1956). The histological damage induced by iodoacetate has been repeatedly confirmed (Karli, 1952 and 1954; de Berardinis and Bonavolontà, 1952; de Berardinis, 1953; and Babel and Ziv, 1956), but the mode of action remains obscure. Remarking on the known ability of iodoacetate to react with thiol groups, Noell (1952, b) observed that many intracellular activities might be affected by this agent. The present investigation was undertaken to determine whether the injurious action upon the retina is shared by other compounds reactive for thiol groups when given intravenously to the rabbit and rat.

Methods and Agents Used

With sodium iodoacetate in a dose of 29 mg./kg. in the rabbit, ophthalmoscopic and histological lesions essentially similar to those reported by previous observers were obtained. Like Karli, we found it unnecessary to give more than one injection. In the rat, a dose of 42 mg./kg. had to be used, but the effect was less consistent, the lesions being either mild or entirely absent.

The various thiol reagents administered are listed in the Table (overleaf). Usually only one injection was given, but some animals received two or three similar injections, generally on consecutive days. The single doses given represented more than 80 per cent. of the minimum lethal dose in nearly every case.

In the rabbit, reagents were injected in the ear vein; in the rat, the injection

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TABLE
THIOL REAGENTS ADMINISTERED INTRAVENOUSLY TO RATS AND RABBITS

Substance	Rats		Rabbits	
	Number Treated	Dose (mg./kg.)	Number Treated	Dose (mg./kg.)
Sodium monobromoacetate	8 1	32 72(*a*)	6 4	32 45(*b*)
Sodium monochloroacetate	2	117(*a*)	3	82
Ethyl iodoacetate	3 6	10 13(*b*)	1	21(*a*)
Iodoacetamide	4	14	3 2	37 67(*b*)
Bromoacetyl bromide			1	81(*a*)
Phenacyl bromide...	3 2	20 24(*b*)	1	5
1-Fluoro 2:6-dinitrobenzene	3	19		
Sodium *p*-chlormercuribenzoate...	6 5	8 11(*b*)	1	19
Mercuric chloride...	2	5		
Sodium arsenite	1	3(*a*)	1	7
3-Amino 4-hydroxyphenyl arsenoxide hydrochloride	2	21(*a*)		
3-Amino 4-hydroxyphenyl dichloroarsine hydrochloride	5 2	20 20(*b*)	1	58(*a*)
Sodium o-iodobenzoate	3 3	43 86(*a*)	1	272
Iodine	2 4	6 18(*a*)	1 1	3 3(*a*)
Phenol indo 2:6 dichlorophenol...	3	200(*a*)	1	200(*a*)

Doses were the maximum tolerated as a single injection, except that:

(*a*)=two maximal doses and in a few instances a third maximal or fractional dose.
(*b*)=two or more sub-maximal doses.

was made into the femoral vein through a small incision made under ether anaesthesia. All reagents were made up in aqueous solution or suspension buffered to pH 7·4, concentrations being adjusted to give injection volumes of 2 to 5 ml. in rabbits, and 0·5 to 1·0 ml. in rats. Acids were neutralized with sodium hydroxide; iodine was administered dissolved in propylene glycol.

Ophthalmoscopic examinations were carried out by the direct method in the rabbit, and by the indirect method in the rat. Animals were kept under observation for some 7 to 10 days before being destroyed for histological study. The eyes were fixed in acid Zenker solution: rabbit eyes were usually incised at the

equator and cotton wool wick inserted; rat eyes were fixed intact. Embedding was carried out in paraffin wax, and sections were cut at 5μ.

Results

In the rat, in which sodium iodoacetate produces a mild and inconstant effect, none of the agents used produced any ophthalmoscopic or histological changes. In the rabbit, the results were also negative, except that bromoacetate caused both ophthalmoscopic and histological changes. These changes, obtained with a dose of 32 mg./kg., were similar to those obtained with iodoacetate in doses of 29 mg./kg., differing mainly in being rather milder and largely restricted to the central area. They are described in detail elsewhere in this issue (Lucas, Newhouse, and Davey, 1957).

Discussion

The thiol agents used—including, as they do, heavy metal derivatives, oxidizing agents, and alkylating agents—cover a representative range. That the results in the intact animal were negative except for bromoacetate suggests that the effect is characteristic of iodoacetate and its analogue rather than of thiol reagents as a group. Iodoacetate does not behave like other thiol reagents when used as an enzyme inhibitor (Smythe, 1936) or as a means of inducing developmental anomalies (Hicks, 1953). It can react *in vitro* with the NH_2-group of certain amino-acids (Michaelis and Schubert, 1934) and some of its inhibitory effects are not reversed by addition of thiol compounds (Barron and Singer, 1945). Some thiol-dependent enzyme reactions are inhibited as readily by iodoacetamide as by iodoacetate, yet the neutral amide does not damage the retina, a fact which again points to an unusual mode of action by the parent acid. That the latter is exceptionally effective against phosphoglyceraldehyde dehydrogenase (Cori, Slein, and Cori, 1948), an enzyme of particular importance to a tissue with the high glycolytic activity of retina, may be significant.

While the present work does not indicate that iodoacetate and bromoacetate damage the retina by virtue of being unselective thiol reagents, that possibility cannot be excluded. The influence of such factors as blood-retinal barriers, detoxication processes, and systemic toxicity may be important. As to systemic toxicity, it is of interest that most of the more powerful inhibitors are, in molar terms, too lethal to be administered in dosage comparable with that of sodium iodoacetate. Thus, in the rabbit, the maximal tolerated dosage (millimoles per kg. body weight) of sodium *p*-chloromercuribenzoate or sodium arsenite is 0·050, only a fraction of that of sodium iodoacetate (0·140) or bromoacetate (0·200). Body fluid concentrations of the heavy metal compounds and perhaps of other thiol reagents (*e.g.* phenacyl bromide) high enough to damage the retina may not therefore be feasible. In contrast, however, iodoacetamide, which is no more lethal than sodium iodoacetate, was without effect. These results

would suggest that sodium iodoacetate may owe its effects to a combination of low systemic toxicity and inhibitory properties of a restricted type.

Summary

Sub-lethal doses of a representative group of thiol reagents were injected intravenously into rabbits and rats to determine whether the injurious effect of iodoacetate upon the retina is shown by other inhibitors of sulphydryl enzyme systems. In the rabbit, sodium bromoacetate gave a lesion somewhat similar to that of iodoacetate; all other thiol reagents proved negative. In the rat, bromoacetate was ineffective, as were all other thiol reagents except iodoacetate, which itself produced only a mild and variable effect.

The failure of thiol reagents, other than sodium iodoacetate and bromoacetate, in producing retinal degeneration cannot be related to systemic toxicity alone, for on a molar basis some of the agents were more toxic and others less toxic than iodoacetate. The possibility that iodoacetate and bromoacetate act by virtue of properties other than thiol reactivity is discussed.

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