EXPERIMENTAL DEGENERATION OF THE RETINA

IV. DIAMINODIPHENOXYALKANES AS INDUCING AGENTS*

BY

ARNOLD SORSBY† AND AKIRA NAKAJIMA‡

Royal College of Surgeons of England and Royal Eye Hospital

RAISON and Standen (1954, 1955) and Collins (1954) have independently drawn attention to the high schistosomicidal activity of the diaminodiphenoxyalkanes. Clinical trials with one of these agents, 1:7 di(*p*-dimethylaminophenoxy) heptane, has apparently been carried out and has given retinal complications (Grant, 1956, 1958). Experimental evidence that the allied 1:5 di(*p*-aminophenoxy) pentane produces retinal lesions has been given by Edge, Mason, Wien, and Ashton (1956), who recorded that the monkey, dog, and cat (when given 100–400 mg./kg. by mouth or by subcutaneous injection, or 25–50 mg./kg. intravenously) developed fundus changes simulating retinitis pigmentosa. They could not obtain any lesions in the mouse, rat, guinea-pig, rabbit, or ferret, treated with single oral doses up to 400 mg./kg. (about two-thirds the mean lethal dose in the mouse). An account of the histological changes induced by this agent in the cat retina has been given by Ashton (1957). In a study on the toxicity of the alkane derivatives, Goodwin, Richards, and Udall (1957) have stressed the retinotoxic effects of these agents when administered by mouth or by subcutaneous injection. Large and repeated doses given by mouth had no apparent effect on the eyes of mice, rats, or guinea-pigs, but gave ready results in the cat, especially with primary and secondary amines; the effects of tertiary amines seemed less constant. Apart from histological studies, they used the Zewi technique of assessing resynthesis of rhodopsin to determine retinal damage; here, too, they found the primary amines the most and the tertiary the least toxic.

The present study was undertaken to test a fairly wide range of diaminodiphenoxyalkanes, the rabbit being used as the test animal and the agents being injected intravenously.

Methods and Agents Used

Seven primary amines in the diphenoxyalkane series, two secondary methyl amines, seven tertiary methyl amines, and two tertiary ethyl amines were used. In all these compounds the amine was in the *para* position. Two further agents were also employed, one being a tertiary methyl amine in the *ortho* position and the other a tertiary hydroxyethyl amine in the *para* position. The structural relationship of these agents and the doses employed are shown in Table I (overleaf), which

* Received for publication September 16, 1957.

† Wernher Group for Research in Ophthalmological Genetics. (Medical Research Council).

‡ Department of Ophthalmology, Jutendo University, Tokyo. Working in London 1956–57 as a British Council Scholar.

also shows that most animals received a single injection of the agent, though some were given several injections, generally to step up the dose. In a few cases, injections were given in two or more divided doses because of the acute toxicity of the agent. The tolerated dose of the different agents varied rather widely, as did the physical properties. Thus one particular agent, 1:8 di(*p*-aminophenoxy) octane, was barely soluble even in its hydrochloride salt. As for the acute toxicity of the agents, the best tolerated were the primary amines, whilst most of the remaining

TABLE I
DOSAGE AND EFFECTS OF DIAMINODIPHENOXYALKANES

Where <i>n</i> =	No. of Animals	Dosage: Single Intravenous Injec- tions unless Other- wise Indicated (mg./kg.)	Acute Toxicity	Duration of Observation (days)	Retinal Damage	Lethal Doses (mg./kg.) where estab- lished
Primary amines: NH_2 $\text{O}-(\text{CH}_2)_n-\text{O}$ NH_2						
2	1	50	Slight	14	—	120
	1	100	Moderate	27	—	
3	1	50	Slight	13	++	
	1	5	„	16	?±	
5	3	40	Slight	21; 2; 5	++	̄ 100
	1	60	Slight	40	++	
	1	5	„	16	+	
6	1	40	Moderate	17	++	̄ 40
	1	25	Slight	3	++	
	1	5	Slight	10	++	
	1	0.3	„	32	?±	
7	1	100	Slight	7	++	
	1	60	Slight	21	++	
	1	40	Slight	3	++	
	1	10	Slight	10	++	
	1	5	Slight	10	++	
8	1	10	Slight	6	++	14
	1	3	Slight	17	++	
	1	1	„	5	—	
9	1	56	Severe	2	++	̄ 60
	1	36	Slight	17	++	
	1	8	Slight	7	++	
	1	4	„	4	++	

contin.

TABLE I—continued

Where <i>n</i> =	No. of Animals	Dosage: Single Intravenous Injec- tions unless Other- wise Indicated (mg./kg.)	Acute Toxicity	Duration of Observation (days)	Retinal Damage	Lethal Doses (mg./kg.) where esta- blished
Secondary methyl amines: $\text{CH}_3\text{HN} \langle \text{C}_6\text{H}_4 \rangle \text{O}-(\text{CH}_2)_n-\text{O} \langle \text{C}_6\text{H}_4 \rangle \text{NHCH}_3$						
5	1	50; 50; 63; 90	Marked ⁽¹⁾	29	—	110
	1	75	Marked	30	—	
7	1	30; 50; 60	Marked	10	—	
	1	75	Marked	25	+	

⁽¹⁾ Paralysis of hind legs with 90 mg./kg.

Tertiary methyl amines: $\text{N}(\text{CH}_3)_2 \langle \text{C}_6\text{H}_4 \rangle \text{O}-(\text{CH}_2)_n-\text{O} \langle \text{C}_6\text{H}_4 \rangle \text{N}(\text{CH}_3)_2$

4	1	20	Marked	10	—	40
5	1	100; 80; 100	Marked	58	—	160
6	1	16; 25	Slight	30	—	
7	1	160	Severe ⁽²⁾	2	—	? < 50
	1	28*; 36	Slight	38	+	
8	1	4.5; 13*	Slight	11	—	< 10
9	1	6.6*; 23*; 23*	Slight	52	?±	< 10
10	1	8; 15*	Slight	33	—	

⁽²⁾ This rabbit receiving 160 mg./kg. survived, but had to be destroyed 2 days later because of paralysis of hind legs.

* Divided over two or more doses, all injected within an hour.

Tertiary ethyl amines: $\text{N}(\text{C}_2\text{H}_5)_2 \langle \text{C}_6\text{H}_4 \rangle \text{O}(\text{CH}_2)_n-\text{O} \langle \text{C}_6\text{H}_4 \rangle \text{N}(\text{C}_2\text{H}_5)_2$

4	1	25; 43	Severe	4	—	
7	1	200	Slight	28	+	350
	1	250	Slight	5	—	
	1	300	Severe	10	—	

Other tertiary amines:

Methyl amine in *ortho* position: $\langle \text{C}_6\text{H}_4 \rangle \text{N}(\text{CH}_3)_2 - \text{O}-(\text{CH}_2)_n-\text{O} \langle \text{C}_6\text{H}_4 \rangle \text{N}(\text{CH}_3)_2$

5	1	33	Severe	31	—	40
---	---	----	--------	----	---	----

contin.

TABLE I—continued

Where <i>n</i> =	No. of Animals	Dosage: Single Intravenous Injec- tions unless Other- wise Indicated (mg./kg.)	Acute Toxicity	Duration of Observation (days)	Retinal Damage	Lethal Doses (mg./kg.) where estab- lished
Hydroxyethylamine: $(\text{HOCH}_2\text{CH}_2)_2\text{N} \langle \text{C}_6\text{H}_4 \rangle \text{O}(\text{CH}_2)_n\text{O} \langle \text{C}_6\text{H}_4 \rangle \text{N}(\text{CH}_2\text{CH}_2\text{OH})_2$						
7	1	200	Slight	28	—	
	1	150	Slight	27	—	

agents, especially the secondary methyl amines and the tertiary ethyl amines, were particularly difficult to use, as convulsions and paralysis of the hind legs were readily precipitated. The experimental and ophthalmoscopic techniques employed were essentially those recorded in the previous studies on inducing agents of experimental degeneration of the retina (Sorsby, Newhouse, and Lucas, 1957; Sorsby and Nakajima, 1958). The material was fixed in acid Zenker's solution and embedded in ester wax after dehydration in 2 ethoxy-ethanol (Chesterman and Leach, 1956). Electroretinographic records are reported separately by one of us (A.N.).

Results

The results obtained with these twenty agents show a fairly simple pattern. Of the seven primary amines, six gave a marked and rapidly developing retinal lesion when the agent was given in a single intravenous dose. The seventh primary amine used—the one with the lowest carbon chain—gave an indefinite result ophthalmoscopically and histologically. Of the remaining thirteen agents, all but three proved negative, and the three positive results were not readily obtained. As will be seen from Table I, the positive result obtained with the secondary methyl amine with seven carbon chains was reached with a sub-lethal dose, repeated smaller doses giving no effect. Likewise, the positive result obtained with one of the tertiary ethyl amines was balanced by negative results in two animals receiving higher doses but observed over a shorter period. The positive result obtained with the tertiary methyl amine was not marked. The striking findings in this series were therefore confined to the primary amines, the one with two carbon chains excluded.

The ophthalmoscopic picture was fairly constant with the six primary amines that gave a positive result, and was very similar to that observed with iodate and iodoacetate. Within 2–3 days there was loss of translucency of the retina, showing itself ophthalmoscopically by an appearance simulating oedema of the retina; some narrowing of the arteries was common and pigmentary proliferation began to set in within 5 days and tended to progress

for the next 2–3 weeks. The fully developed picture was generally present within 21 days. Histologically the visual cells and pigmented epithelium were most affected. Figs 1 and 2 are fairly typical of these appearances. In contrast, the positive results obtained with one of the secondary amines and two of the tertiary amines were ophthalmoscopically slow in onset, taking some 3 weeks to show anything definite, whilst histologically the lesion was mild.

FIG. 1.—Rabbit retina 21 days after injection of 60 mg./kg. 1:7 di(*p*-aminophenoxy) heptane. H and E $\times 400$.

Note total disappearance of visual cells and disruption of cells of pigment epithelium. There is also considerable invasion of pigment into the inner layers of the retina.

FIG. 2.—Rabbit retina 7 days after injection of 8 mg./kg. 1:9 di(*p*-aminophenoxy) nonane. (a) Retina away from periphery. The cells of the pigment epithelium are swollen and disrupted. There are only minor changes in the visual cells.

(b) Retina towards periphery. There is loss of the visual cell layer; the pigment epithelium is swollen and disorganized. H and E $\times 400$.

Discussion

The results recorded bring the new group of schistosomicidal drugs into line with the other agents that give retinal damage in the rabbit on a single intravenous injection: sodium iodate (Sorsby, 1941), sodium iodoacetate (Schubert and Bornschein, 1951; Noell, 1951), sodium bromoacetate (Lucas, Newhouse, and Davey, 1957), dithizone (Butturini, Grignolo, and Baroncelli, 1953), and cyanide and fluoride in combination (Sorsby and Nakajima, 1958). Edge and others (1956) failed to obtain a retinal lesion in the rabbit on oral administration of the primary amine they studied, and they considered that the rabbit, as well as several other animals, was not susceptible to this agent. As the present results show, the rabbit is readily susceptible when intravenous injections are used. In experimental degeneration of the retina induced by sodium iodate, sodium iodoacetate, sodium bromoacetate, dithizone, and cyanide and fluoride in combination, sub-lethal doses have to be employed. Table I suggests that the primary diaminodiphenoxyalkanes differ in this respect from these agents, for the doses producing retinal damage are well tolerated, and retinal damage appears to be produced by injections of quite small quantities.

The present study—like the parallel study by Goodwin, Richards, and Udall (1957)—is of interest in that it brings forward a substantial series of analogues producing retinal damage. Previous studies have not gone much beyond establishing sodium bromoacetate as the one analogue of sodium iodoacetate—and that with more limited efficacy.

The fact that the most definite results were obtained with the primary amines in this series suggested that the noxious agent might be the *para*-aminophenoxy grouping. On this assumption, *p*-aminophenol and its two immediate analogues, *p*-anisidine and *p*-phenetidine, as also 4:aminophenol butyl ether containing four carbon chains and another substance with 7 carbon chains were tried experimentally. Table II (opposite) shows that, though sub-lethal doses were employed, no retinal damage was obtained ophthalmoscopically or histologically with any of these five agents. These negative findings are parallel to an observation recorded by Goodwin, Richards, and Udall on the hair of mice. They had noted that one of the toxic effects of the alkane derivatives on mice was loss of hair. No such effect was obtained by them when they used *p*-aminophenol.

It is not clear whether there is any significance in the fact that amongst the thirteen secondary and tertiary amines only the three substances with seven carbon chains gave positive results.

The observations recorded in Table III (overleaf) have not helped to elucidate the nature of the action of the diaminodiphenoxyalkanes. It will be seen that a modification in the central carbon chain as represented by the first agent listed (47C53) does not interfere with the retinotoxic effect of the primary alkanes. In contrast, the intact carbon chain and a

TABLE II
OBSERVATIONS ON RETINO-TOXIC EFFECTS OF *p*-AMINOPHENOL AND FOUR DERIVATIVES

Agent	Structural Formula	Lethal Dose (mg./kg.)	No. of Animals Used	Dose (mg./kg.)	No. of Injections	No. of Days Observed	Retinal Damage
<i>p</i> -aminophenol ..	<chem>NC1=CC=C(O)C=C1</chem>	770	3	(1) 1,170 (2) 970 (3) 1,700	8 3 3	20 13 10	None None None
<i>p</i> -anisidine ..	<chem>NC1=CC=C(OC)C=C1</chem>	300	2	(1) 660 (2) 320	6 2	24 18	None None
<i>p</i> -phenetidine ...	<chem>NC1=CC=C(OCC)C=C1</chem>	100 (a) 150 (b) 300 (c) > 50 (d)	1 1 2 1	180 250 (1) 100 (2) 290 110	2 2 1 2 2	1 24 3 27 12	None None None None None
4:aminophenol-butyl ether	<chem>NC1=CC=C(OCCCC)C=C1</chem>	50	1	50	2	10	None
11C54(d) Burroughs Wellcome ..	<chem>NC1=CC=C(OCCCCCO)C=C1</chem>	75	1	50	1	11	None

(a) In saline suspension. (b) In alcohol suspension.
(c) Dissolved in dilute HCl. (d) Purified by dissolving in dilute HCl and adding charcoal, and then filtered.

modification in the structure of the amino grouping, as represented by pentamidine, gives no retinal damage. The central carbon chain is also intact in the succeeding five agents and again there is no retinal damage. The last two observations in this Table show that it proved impossible to prevent the retinotoxic effect of one of the primary alkanes by a possible blocking effect of *p*-aminophenol, and by a possible 'detoxicating' effect of sodium thiosulphate.

Summary

(1) Attention is drawn to the clinical and experimental findings by other observers on retinal damage produced by diaminodiphenoxyalkanes—agents that are being investigated for the treatment of schistosomiasis.

(2) Seven primary amines in the diphenoxyalkane series, two secondary methyl amines, seven tertiary methyl amines, and three tertiary ethyl amines, in all of which the amine was in the *para* position, as also one tertiary ortho-methyl amine, were tested in the rabbit by intravenous injection for their retinotoxic effect.

(3) Six of the seven primary amines gave marked degeneration of the visual cells and pigment epithelium after a single intravenous injection of quite small and well tolerated doses. Of the remaining agents, one secondary methyl amine, one tertiary methyl amine, and one tertiary ethyl amine—all with seven carbon chains—proved damaging to the retina, though the damage was relatively slight and obtained only with sublethal doses. The tertiary methyl amine that gave a retinal lesion—1:7 di(*p*-dimethylaminophenoxy) heptane—is the agent known to have produced damage in man.

TABLE III

FURTHER OBSERVATIONS BEARING ON THE MODE OF ACTION OF THE DIAMINODIPHENOXYALKANES

Agent	Structural Formula	Lethal Dose (mg./kg.)	No. of Animals Used	(mg./kg.) Dose	No. of Injections	No. of Days Observed	Retinal Damage
47C53 (Burroughs Wellcome) ..		?	1	22	1	18	++
Pentamidine* ..		c. 20	1	20	1	42	—
Dodecamethylene diamine ..	NH ₂ (CH ₂) ₁₂ NH ₂	c. 10	1	10	1	8	—
Dinitriles with central carbon chains	CN—(CH ₂) ₃ —CN CN—(CH ₂) ₄ —CN CN—(CH ₂) ₁₁ —CN	c. 150 ? 25 > 100	1 1 1	328 (a) 25 (b) 469 (c)	6 2 7	55 5 60	— — —
Synthalin ..	NH ₂ C:NH(CH ₂) ₁₂ NH . C:NH ₂	5	2	3½ and 4	1	26 and 8	—
Primary diphenoxy-alkane n = 8 with p-aminophenol ..		14 770	1	5 and 160	1	18	++
Primary diphenoxy-alkane n = 8 with Sodium thiosulphate	 Na ₂ S ₂ O ₃	14 ?	1	5 and 1,100	1	18	++

* Used as the isothionate salt.

(b) In two doses of 10 and 15 mg./kg.

(a) In six doses of 10, 15, 26, 37, 90, and 150 mg./kg.

(c) In seven doses of 10, 20, 36, 53, 90, 190, and 70 mg./kg.

(4) The possibility that the *p*-aminophenoxy grouping was responsible for the retinal damage was not borne out by trials with *para*-aminophenol and four of its derivatives with carbon chains.

(5) There is little to suggest that either a long carbon chain or the diphenoxy grouping is essential for a retinotoxic effect. In its context the amine grouping is probably important.

We wish to thank Dr. O. D. Standen and his colleagues, Dr. R. S. F. Hennessey, Dr. L. G. Goodwin, and Dr. V. Udall, of the Wellcome Laboratories of Tropical Medicine, for the considerable trouble they have taken in supplying us with some of the agents used in this investigation and for helpful discussions. We are likewise obliged to Mr. S. Ellingworth of Imperial Chemical Industries (Pharmaceuticals Division).

We are indebted to Messrs. May and Baker for a supply of 1:5 di(*p*-aminophenoxy) pentane. We are obliged to Dr. D. R. Lucas for the histological reports and to Mr. R. Harding for technical assistance.

REFERENCES

- ASHTON, N. (1957). *J. Path. Bact.*, **74**, 103.
 BUTTURINI, U., GRIGNOLO, A., and BARONCELLI, A. (1953). *G. clin. Med.*, **34**, 1253.
 CHESTERMAN, W., and LEACH, E. H. (1956). *Quart. J. micro. Sci.*, **97**, 593.

- COLLINS, F. D. (1954). *Biol. Rev.*, **29**, 453.
- EDGE, N. D., MASON, D. F. J., WIEN, R., and ASHTON, N. (1956). *Nature (Lond.)*, **178**, 806.
- GOODWIN, L. G., RICHARDS, W. H. G., and UDALL, V. (1957). *Brit. J. Pharmacol.*, **12**, 468.
- GRANT, W. M. (1956). Personal communication.
- (1958). "Drug Intoxications and Chemical Injuries", in "Systemic Ophthalmology", ed. A. Sorsby. 2nd ed., Butterworth, London.
- LUCAS, D. R., NEWHOUSE, J. P., and DAVEY, J. B. (1957). *Brit. J. Ophthalm.*, **41**, 313.
- NOELL, W. K. (1951). *J. cell. comp. Physiol.*, **37**, 283.
- RAISON, C. G., and STANDEN, O. D. (1954). *Trans. roy. Soc. trop. Med. Hyg.*, **48**, 446.
- (1955). *Brit. J. Pharmacol.*, **10**, 191.
- SCHUBERT, G., and BORNSCHEIN, J. (1951). *Experientia (Basel)*, **7**, 461.
- SORSBY, A. (1941). *Brit. J. Ophthalm.*, **25**, 58.
- and NAKAJIMA, A. (1958). *Ibid.*, **42**, 558.
- , NEWHOUSE, J. P., and LUCAS, D. R. (1957). *Ibid.*, **41**, 309.