

Acta Ophthalmologica  
1948

Olof Røe (Oslo):

THE GANGLION CELLS OF THE RETINA IN CASES OF  
METHANOL POISONING IN HUMAN BEINGS AND  
EXPERIMENTAL ANIMALS

1. Introduction.

In a clinical study of methanol poisoning (1946) I have shown that severe acidosis is necessary for the development of amblyopia and amaurosis.

According to the literature of experimental methanol poisoning, severe acidosis has been demonstrated neither in herbivorous nor in carnivorous animals. Haskell and his collaborators (1921) experimented on dogs, in some of which they found a slight or moderate degree of acidosis, while in others there was no reduction of the alkali reserve. Severe acidosis was not found in a single case, nor was there any favourable effect from treatment with sodium bicarbonate. Loewy and Münzer (1923) found no appreciable reduction of the carbonic acid binding capacity in the blood of two rabbits and a dog given large doses of methanol.

leading omnivores

In human beings, Pick and Bielschowsky, MacDonald, Menne have found degeneration of the ganglion cells of the retina as the cause of amaurosis. Birch-Hirschfeld, Schwarzkopf, Alder and his collaborators have described changes in the ganglion cells in experimental animals, whereas others

Received Aug. 1st, 1947.

(Gersheimer and Verzár) have failed to find any pathological features in these cells.

When, on the one hand, severe acidosis is necessary for the development of amblyopia in human beings poisoned with methanol and when, on the other hand, severe acidosis has never been found in poisoned animals, one is tempted to conclude that the ganglion cells of the latter do not degenerate on poisoning with methanol.

The following account is given in order to elucidate this problem.

## 2. Material and Methods.

A histological examination was undertaken of the retina of 12 patients dying of methanol poisoning. Six of them are mentioned in my clinical study (ars. 67, 68, 71, 73, 76, 79), the other six died after I had completed my clinical examinations. In every case the diagnosis was confirmed by post-mortem examination and the chemical investigations.

The post-mortem examination was made from 12 to 24 hours after death, and in nine cases the eyes were not fixed till this moment. After they had been enucleated, a window was cut with a sharp knife in the equator region to facilitate diffusion of the fixation solution (4% formaldehyde) within the eye. The preparations were embedded in celloidin and stained with thionin.

In the remaining three cases the formaldehyde solution was injected into the vitreous body 45 minutes, 1½ hour and 2 hours respectively after death. Fully 1 ml. of the fixation solution was injected after the aqueous humour had been aspirated through a small opening in the cornea. At the post-mortem examination the eyes of these patients were treated in the same way as those of the other patients. Fibrillary staining was carried out only in the cases in which fixation was effected early, for in such an examination early fixation is particularly important (Turner). Some of the bullus and optic nerve was embedded in paraffine and stained with Bodian's modification of the Bielschowsky-Maresch silver impregnation method, van Gieson's connective tissue stain and Holzer's glial stain. Another part of the optic nerve was stained with sudan and with Spielmeier's medullary sheath stain (gelatine frozen section).

One eye of a patient who died of air embolism served as a control of the preparations fixed relatively late, the preparation being fixed about 24 hours after the patient's death. The preparations from the patients whose eyes were injected with formalin directly after death were compared with a preparation from a patient who had died of fracture of the skull and whose eye had been injected

with formalin one hour after death. Some of the bullus and optic nerve was embedded in paraffine and stained in the same way as the preparations which had been fixed early in the cases of methanol poisoning. Here, too, the optic nerve was stained with sudan and Spielmeier.

The experiments were conducted on 21 rats and five rabbits, 12 of the former and all the latter being subjected to a histological examination of Nissl-stained preparations of the ganglion cells of the retina. The animals were not killed till they had received so much methanol that they were moribund or deeply comatose. The methanol was administered through a gastric tube in a concentration of 40 vol. %.

Here, too, a 4% solution of formaldehyde was used for fixation, alcohol having been found in some preliminary tests to be most unsuitable, as it made the preparations shrink much with the result that the retina became detached and lacerated. Poljak also insists that alcohol is most unsuitable for fixation of the eye when, as usually happens, it is placed, either intact or opened, in the fixation solution.

Hitherto the alkali reserve of a few methanol-poisoned rabbits has been examined, but it does not seem to have been examined in rats. It seemed therefore advisable for me to examine this phenomenon in the rabbits and in some of the rats, blood from the former being obtained by puncture of the heart, in the latter by exsanguination. I experienced great difficulty in taking blood from the ears of the rabbits' ears as their cardiovascular circulation evidently failed on the administration of large doses of methanol, and their ears did not become hyperaemic on massage.

## 3. Histological Findings in the Ganglion Cells of the Human Retina.

### A. Fixation 12-24 Hours Post Mortem.

*Normal persons:* The nuclei of the ganglion cells are circular or oval, usually occupying a central or slightly eccentric position in the large cells, and touching on the outer limit of the cytoplasm in the small cells. The colour of the nuclei is light blue and fairly homogeneous. The nucleolus usually lies in a central or only slightly eccentric position, never in the periphery of a nucleus.

As might be expected with late fixation, the cytoplasm contains practically no tigroid substance. Birch-Hirschfeld (1900) found the first changes in the tigroid substance two hours

after death, and almost complete tigrolysis five to six hours after it.

*Methanol-poisoned persons:* The appearance of the ganglion cells may resemble the first stages of retrograde degeneration, particularly with regard to the large cells, many of which are swollen and almost completely circular, A wreath

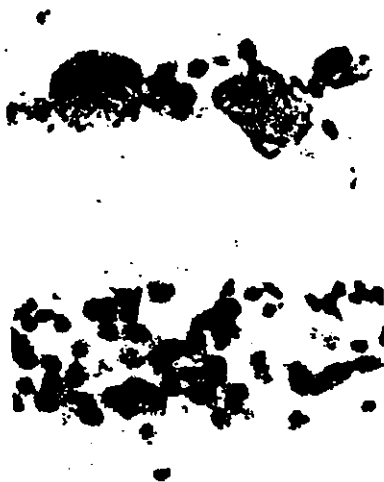


Fig. 1.

Preparation from a patient who died from methanol poisoning. Volatile reducing substance in blood 0.226 % calculated as methanol which was found in urine and contents of the stomach. Two ganglion cells with tigroid fragments in the periphery are seen, the nucleus of the one cell is not included in the section. That of the other cell is flattened and in the periphery. Position of the nucleolus peripheral. Thionin ( $\times 600$ ).

Nissol  
Bodies

of tigroid fragments is always to be seen in the periphery, whereas the central portion of the cytoplasm is as colourless as in a normal preparation. It would therefore seem that the post-mortem breaking down of the tigroid substance is less complete in methanol-poisoned persons than under normal conditions.

But what more than anything else is indicative of a serious

lesion of the ganglion cells is the appearance of their nuclei which, particularly in the large cells, are pressed flat and squeezed out into the extreme periphery of the cells (fig. 1).

In many cells it looks as if the nuclei are to some extent outside the bodies of the cells, being not infrequently irregular and notched in outline. Partly on account of shrinking of the



Fig. 2.

Preparation from a man, aged 63, died of fracture of the cranium. Fixation 60 minutes post mortem. A ganglion cell with circular nucleus and nucleolus in the centre. Thionin ( $\times 600$ ).

nuclei, and partly on account of swelling of the cytoplasm, there is often a great change in the relative size of the nucleus and the whole cell. The nucleolus is always in the periphery of the nucleus. While the colour of the nucleus in thionin-stained preparations from normal persons is, as already pointed out, light blue, it is bluish-grey in methanol-poisoned persons, and the colouring is not so homogeneous. The nucleus contains markedly blue granules some of which are quite large. This is particularly noticeable in the small ganglion cells.

B. Fixation 45 Minutes to Two Hours Post Mortem.

*Normal persons:* The large cells usually show profusion of tigroid substance which is, as a rule, evenly distributed throughout the cytoplasm. But not infrequently there is more tigroid substance in the neighbourhood of the nucleus than elsewhere. The nucleus is circular or oval, its outline is even, and its nucleolus is almost in its centre (fig. 2). The colour is a deeper blue than in the preparations fixed comparatively late.

In the small ganglion cells it is not rare for the nucleus to approach the outer limits of a cell, but it is never in the extreme periphery.

*Methanol-poisoned persons:* The central portion of the cytoplasm contains a dust-like tigroid substance and no well-defined granules. In the periphery there is always a wreath of large and small tigroid granules. The nucleus is angular or



Fig. 3.

Preparation from a man, aged 35, who died a few minutes after admission to hospital. Alkali reserve 9 vols.%. Methanol found in urine and contents of the stomach. A ganglion cell with an angular nucleus in the periphery. Nucleolus in the periphery. Fixation 70 minutes post mortem. Thionin (X 600).

flattened, being entirely in the periphery, and with a nucleolus always in its periphery (fig. 3). In other words, we find in the main the same changes just described in the preparations fixed comparatively late. The essential difference depends on the fact that the central portion of the cytoplasm is somewhat more deeply stained in the preparations fixed early than in those fixed later. Further, the granules staining blue in the nucleus come out more clearly in the preparations fixed early, and they are also most prominent in the small ganglion cells in these preparations.

The marked changes seen in the nuclei in all the preparations from patients dying of methanol poisoning indicate the existence of a serious lesion of the ganglion cells. As early as 1896, Nissl insisted that changes in the tigroid substance are a less serious manifestation.

In the subjects of methanol poisoning no fibrillary structure was visible in the ganglion cells, and in the cytoplasm a finely granulated mass could be seen taking its place. No pathological changes could be found in the connective tissue, glia or medullary sheaths.

Most of the nuclei of the cells in the inner nuclear layer showed on thionin staining markedly blue granules, such as are seen in the small ganglion cells.

4. The Ganglion Cells of the Retina of Rats.

The first experiments concerned 11 albino rats, none of which showed signs of acidosis, on poisoning with methanol. Large doses had a marked narcotic effect, and about 10 ml. per kg. body weight proved fatal.

The alkali reserve was measured in nine rats receiving 8 ml. methanol per kg. body weight, in five rats after 24 hours, and in four after two days. There was no reduction of importance of the alkali reserve (table 1).

The first 11 rats and rat nr. 21 underwent a histological examination, and rat nr. 12 served as a control.

Rats' ganglion cells are small and most of them are not very rich in tigroid substance, most of which is not infrequently situated in the periphery. There are usually some fine granules close to the nucleus which is usually in the periphery

600 ml / 4 L.  
Bios.  
Plasma  
or 1570  
MeOH  
in 60 Kg  
Humay

Table 1.  
Alkali Reserve in Rats after Poisoning with 8 ml. Methanol per kg. Body weight.

Rat's nr.	Weight in gram	Alkali Reserve vol. %	
		2. day	3. day
13	350	49	
14	450	55	
15	420	59	
16	410	60	
17	290	56	
18	390		47
19	480		55
20	370		49
21	270		53

of the cell. The shape of the nucleus is circular or oval (Fig. 4).

In none of the preparations from poisoned rats could I find nuclei whose appearance corresponded to my descriptions of findings in man.

##### 5. The Ganglion Cells of the Retina of Rabbits.

Like the rats, the rabbits showed no sign of acidosis. The alkali reserve of all five rabbits was measured, and was found to be normal or a trifle reduced, as indicated in table 2.

This table also shows that the reaction of the pupils to

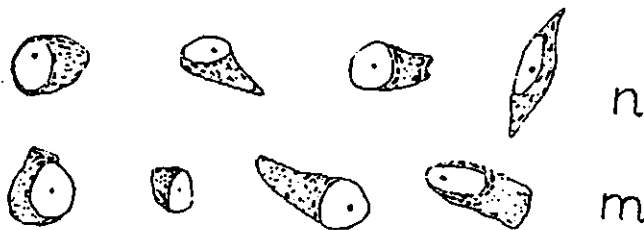


Fig. 4.

Diagrammatic sketch of the ganglion cells of the retina of a normal animal (n) and of a rat poisoned with 8 ml. methanol per kg. body weight (nr. 21).

Table 2.  
Methanol Poisoning in Rabbits.

Rabbit's nr.	Weight in kg	Day of test	Al methanol per kg body weight	Alkali Reserve vol. %	Reaction of pupils	Remarks
1	1	1	4	54		
		2	3	49	++	
		3	5	45	++	7. day, time 22:
		4	4	47	++	Rabbit moribund.
		5	5	53	++	Pupils not reacting to light.
2	1.1	7	7	67	++	Alk. res. 79 vol. %
		1	7	59		2. day's evening:
		2	7	46	++	Comatose. Pupils not reacting to light.
3	1.25	3	5	48	+	
		1	8	58		
4	1.6	2		38	+	
		3		67	++	
5	2.4	1	10			Coma, 2. day:
		2		54	+	Moribund. Killed.
		3				

++: Pupil reaction normal.

+: Pupil reaction weak.

light did not cease till the rabbits were moribund or deeply comatose (nrs. 1, 2, 4), and it recurred quickly on recovery from coma (nr. 2).

The content of tigroid substance in the ganglion cells of the retina of rabbits varies just as much as in man, and is, as a rule, greatest in the large cells in which it is usually distributed evenly throughout the cytoplasm. In the small ganglion cells, whose nuclei are usually situated in the pe-

riphery, most of the tigroid substance is generally to be seen beside the border of the cytoplasm, close to the nucleus. Now and then, most of the tigroid substance is to be seen in the border opposite the nucleus.

No tigrolysis could be seen in the preparations from the poisoned rabbits. Nissl's bodies could be plainly seen in all



Fig. 5 a.

a: Ganglion cell of a normal rabbit.

the ganglion cells. Their nuclei were circular or oval, and were in no way situated more in the periphery than in the normal preparations (fig. 5).

- Most of the observers of experimental investigations have evidently attached most importance to changes in the tigroid substance (*Birch-Hirschfeld, Schwarzkopf, Alder* and his collaborators). In the publication by the last-named on experimental poisoning of rabbits, a drawing is reproduced of the ganglion cells of a normal rabbit's retina (fig. 6 in the central column) of the retina of a rabbit poisoned with synthetic methanol (in the column to the left) and of the retina of a rabbit poisoned with *Merck's* methanol (in the column to the right).

No nuclei are to be seen with the same pathological features which are so constant in the retina of human beings who have died of methanol poisoning. If we compare the cells of the same size in the central column with those of the columns to the left and right, we shall also find no differences in the

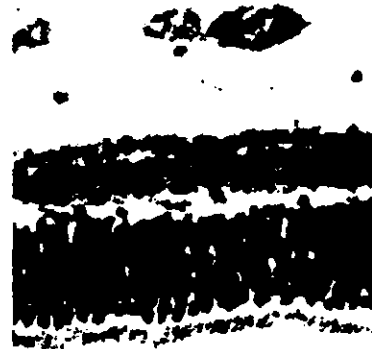


Fig. 5 b.

b: Ganglion cell of poisoned rabbit (nr. 5).  
Thionin ( $\times 600$ ).

quantity of tigroid substance. The lowest cell in the central column evidently does not contain more tigroid substance than the uppermost cell in the column to the left. There is also no difference of importance in the quantity of tigroid substance in the uppermost cell in the central column and the uppermost cell in the column to the right.

#### 6. Summary and Conclusion.

An examination of the ganglion cells of the retina of human beings dying of methanol poisoning has shown severe degenerative changes. The shape of the nucleus, which always

lies in the extreme periphery of the cell, is irregular. It is pressed flat, and its nucleolus is situated in the periphery. Many granules, stained blue, are to be seen, particularly in the nuclei of the small ganglion cells. There is central tigrolysis in the cytoplasm, with a wreath of tigroid granules in the periphery. In preparations impregnated with silver, no fibrillary structure of the ganglion cells could be seen.

In rats and rabbits poisoned with methanol I have failed to find any degenerative changes in the ganglion cells of the retina. As these animals, in contrast to human beings, do not suffer from any reduction of importance of the alkali reserve, my experiments confirm my clinical investigations which have shown that diminution of vision occurs only when there is severe acidosis. The experiments carried out on dogs by Haskell and his collaborators show that it is not only herbivorous animals which fail to develop severe acidosis when poisoned with methanol.

A comparison of the experimental with the clinical findings shows that there is a fundamental difference in the action of methanol on animals and human beings. Undoubtedly the outcome of experiments on animals has contributed to the belief, which has become general, that acidosis does not play any part

No  
metabolic  
Acidosis

Very  
Good!

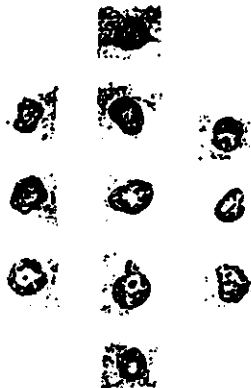


Fig. 6.

In Carnivores & herbivores  
181

of importance to the course and prognosis of methanol poisoning. This teaching has contributed to the neglect of the treatment of the acidosis, and several human beings have on this account lost their lives or their sight.

#### BIBLIOGRAPHY

- Alder, P., Buscke, W., Gordonoff, T.: Experimentelle Untersuchungen über die Toxizität des Methylalkohols. Arch. internat. de pharmacodyn. et de thérap. 1938; 59: 416.
- Birch-Hirschfeld, A.: Beitrag zur Kenntnis der Netzhautganglionzellen unter physiologischen u. pathologischen Verhältnissen. Arch. f. Ophth. 1900; 50: 166.
- Experimentelle Untersuchungen über die Pathogenese der Methylalkoholamblyopie. Arch. f. Ophth. 1901; 52: 358.
- Haskell, G. G., Hillemann, S. P., Gardner, W. G.: The Significance of the Acidosis of Methyl Alcohol Poisoning. Arch. Int. Med. 1921; 27: 71.
- Ingersheimer und Verzar, F.: Zur Pathogenese der Methylalkohol- und Atoxylamblyopie. Arch. f. Augenh. 1913; 75: 27.
- Loewy, A. und Münzer, E.: Führt Methylalkoholvergiftung zu Acidose? Biochem. Ztschr. 1923; 131: 432.
- MacDonald, A. E.: Die Pathologie von Methylalkohol-Amblyopie. Zentralbl. f. d. ges. Ophth. 1930; 22: 791.
- Meane, F. R.: Methyl Alcohol Poisoning. Arch. Path. 1938; 26: 77.
- Nissl, F.: Kritische Fragen der Nervenzellenanatomie. Neurolog. Centralbl. 1906; 15: 98.
- Pick, L. und Bietschowsky, M.: Über histologische Befunde im Auge und im Zentralnervensystem des Menschen bei akuter tödlicher Vergiftung mit Methylalkohol. Berl. Klin. Wschr. 1912; 1: 888.
- Polyak, S. L.: The Retina. The University of Chicago Press, Chicago 1941.
- Boe, O.: Methanol Poisoning. Acta Med. Scandinav. 1916, Suppl. 182.
- Schwarzkopf, G.: Kritisches und Experimentelles über die Methyl- und Optochinamblyopie. Ztschr. f. Augenh. 1922; 48: 317.
- Turner, O. A.: A Manual of Neurohistologic Technique. Mosby, St. Louis 1940.

#### DISCUSSION

Gosta Kurpe (Stockholm): An investigation by means of the electroretinogram of a case of methanol poisoning showed that during the acute stage of intoxication the electroretinogram was of a negative type with an enlarged A-wave and diminished B-potential.

Seven weeks after intoxication, when the visual acuity had improved, the electroretinogram was normal, there was no A-wave, and the B-wave was normal. Five months after the intoxication, when the visual acuity has diminished and there was atrophy of the disks, the electroretinogram was of subnormal type. Another case of less severe methanol poisoning was investigated during the acute stage, and also in this case the electroretinogram was negative. It was not possible to get this patient examined during the later stages.

It is thus possible by means of the electroretinogram to register the changes in the retina during methanol poisoning and the consecutive degenerative processes.

J. Sebbi:

**XERODERMA PIGMENTOSUM WITH AFFECTIONS  
OF THE EYE**

(To be published in Br. J. o. Ophthl.)