same animal that the negative response which is presumably obtained from a small-dose effect is followed on additional doses by the low positive response.

This low positive response of approximately 50 microvolts could be distinctly measurable, perhaps, with other equipment; and with absence of noise might show a more detailed picture.

Finally, in the case of the Dithizone experiment, we have had similar experiences in the past. This is presumably based on the insolubility of Dithizone, even in a solvent such as alcohol, and the tendency to form small, highly toxic emboli in the capillary circulation.

DR. DAVID G. COGAN (Boston, Massachusetts): If Dr. Davis told us what the histology was, I missed it. Could he tell us what the sections showed in the eye of that alloxan rabbit with obliteration of the electroretinogram?

DR. ROBERT J. DAVIS (Iowa City, Iowa): I did not mention the histologic changes, Dr. Cogan, because we found no pathologic change in our sections. We attributed this fact to the time factor. We did not feel that our animals had received the drugs over a long enough period of time to bring about a histologic change, although there was a physiologic change.

That is essentially all we have to say about it. We realize there are some unanswered questions.

Our experience with Dithizone was as Dr. Potts pointed out; we were unable to get it into solution. It still remains a mystery to me how the Italian workers who initially performed this experiment were able to do it.

Potts Methanol Eye Acidosis Chronic WOW

STUDIES ON THE VISUAL TOXICITY OF METHANOL*

VIII. ADDITIONAL OBSERVATIONS ON METHANOL POISONING IN THE PRIMATE TEST OBJECT

ALBERT M. POTTS, M.D., JULIUS PRAGLIN, M.D., IRENE FARKAS, M.S., LOWELL ORBISON, M.D., AND DONALD CHICKERING, M.D.

Cleveland, Ohio

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In a previous report from this laboratory,[1] the complete parallelism between symptoms of methyl-alcohol poisoning in the human and in the rhesus monkey was described. In a subsequent report[2] we told how the typical retinal edema, pupillary dilatation, and, in many cases, death occurred in methanol-poisoned monkeys even though the usual metabolic acidosis was completely prevented by administration of base.

There are additional aspects of methanol poisoning in which the primate test object can be used advantageously to help clear up points still in some doubt. One of these areas of confusion deals with the histologic findings in the retina of methanol-poisoned animals. In the first paper, a review was presented of histologic findings in experimental animals without attempting a critical evaluation of the findings presented. Suffice it to say that there are equally vocal adherents of no retinal findings and of marked histologic changes in lower animals poisoned with methanol. Since these animals do not show the typical signs of human poisoning, and since the dose employed is excessive in comparison to the human or the monkey toxic dose, the point is perhaps an academic one. In the case of the single monkey reported by Birch-Hirchfeld[3] and those of Scott, Helz, and McCord,[4] there is no discrimination between findings in the monkeys reported and in the lower animals.

In the cases of human methanol poisoning where eyes were studied histologically, there are similar discrepancies. Roe[5] claims marked changes in the ganglion cells of the 12 patients examined by him; and in the case of one eye fixed rapidly 45 minutes after death, he claims to have controlled post-mortem changes. However, McGregor[6] and Orthner[7] assert with equal positiveness that

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no typical changes are found in the retinas of individuals dying of methanol poisoning. Thus, it seems that histologic studies on eyes of valid experimental animals where supply is not a limiting factor and where ophthalmoscopic, clinical, and electric findings would all be available should prove of direct interest.

Another aspect of the methanol problem was emphasized by the finding[2] that despite combating of acidosis, a number of monkeys died in what appeared to be central nervous-system collapse. This once more opens the question of the cause of methanol death and makes advisable studies on the rest of the animal tissues and particularly the central nervous system. This consideration is underlined by the reports of Dozauer[8] and of Orthner[7] of symmetrical lesions in the putamen of patients dying of methyl-alcohol poisoning. Here again, ample supplies of tissues from susceptible animals receiving methanol under controlled conditions would be highly desirable subjects for study.

Finally, in order to bring to the study additional evidence on visual function, the use of electroretinography on the monkeys given methanol and its oxidation products would be highly desirable. It had been shown previously[9] that cats and rabbits receiving minute doses of formaldehyde exhibited marked accentuation of the negative a-wave and obliteration of the positive b-wave of the electroretinogram. For evident reasons it was desirable to investigate the reaction of the primate test object to methanol and its oxidation products.

This report concerns the histologic findings in the eye, brain, and other tissues of monkeys receiving methanol and its degradation products, and electroretinograms on such animals.

Experimental

Methods

Young adult rhesus monkeys were treated with methanol and with base as described previously.[2] In addition several animals were made acidotic by administration of ammonium chloride by stomach tube, and a number of other monkeys were given formate by the same route. Several animals were given formaldehyde by intravenous drip.

At death eyes, whole brain, and samples of lung, heart, spleen, liver, kidney, intestinal tract, and muscle were removed and fixed in formalin. Eyes were imbedded in celloidin, other tissues in paraffin, and all were stained routinely with hematoxylin and eosin. Where indicated, sections were stained with Weigert's myelin sheath stain.

Electroretinograms were recorded as described in Paper IV of this series.[9]

Results

The eyes of six animals which had received 6.0 gm./kg., that is 188 mM of methyl alcohol per kg., were examined histologically. In all of these eyes cystoid degeneration of the external nuclear layer was a constant finding. There is much question whether significance can be attributed to this particular phenomenon. Whereas this may be a histologic manifestation of the observed retinal edema, it may well be a post-mortem artefact. Only a more extended series of eyes with precise control of post-mortem times can answer this point. With one exception there were no observable changes in the ganglion cell layer of the retina despite the reports referred to previously. This exception was monkey No. 3 which alone of all the series lived as long as nine days. This animal had shown severe retinal edema, fixed and dilated pupils, and apparent blindness, but retinal edema had disappeared by the time of death. Histologically, this animal showed patchy demyelinization of the optic nerve and some questionable loss in numbers of ganglion cells in comparison to the other eyes examined. Photomicrographs of the retina and optic nerve of this animal are shown in Figures 1 and 2. These findings may be compared with those of monkey No. 40, which died in 23 hours of methanol poisoning,

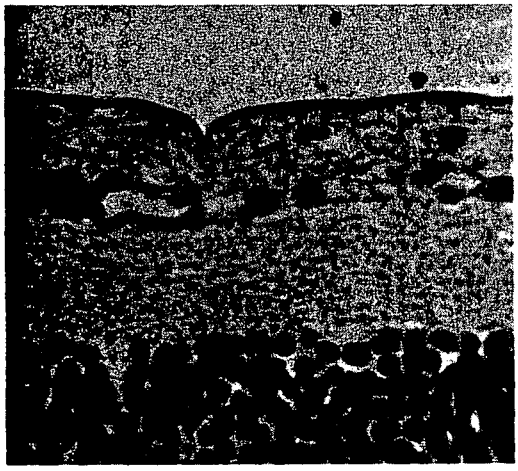

Fig. 1 (Potts, Praglin, et al.) Retina of monkey No. 3. Ganglion cell and inner nuclear layers. (Severe ocular symptoms and nine-day survival.)

showing marked retinal edema and positive electroretinographic changes.

The whole brain of three monkeys dying of 6.0 gm./kg. methyl alcohol was examined and in each of these marked changes were found. In monkey No. 31, the putamen was grossly involved and to a lesser degree the caudate nucleus (fig. 4). In monkeys No. 34 and No. 36, the damage was again confined to the basal ganglia, but here the caudate nucleus seemed to have sustained the major damage, whereas damage to the putamen was

Fig. 2 (Potts, Praglin, et al.). Optic nerve of monkey No. 3, showing demyelinization.

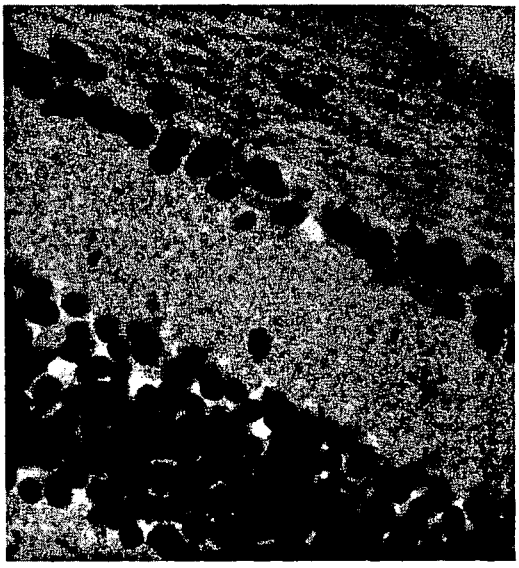

Fig. 3 (Potts, Praglin, et al.). Retina of control monkey. Ganglion cell and inner nuclear layers.

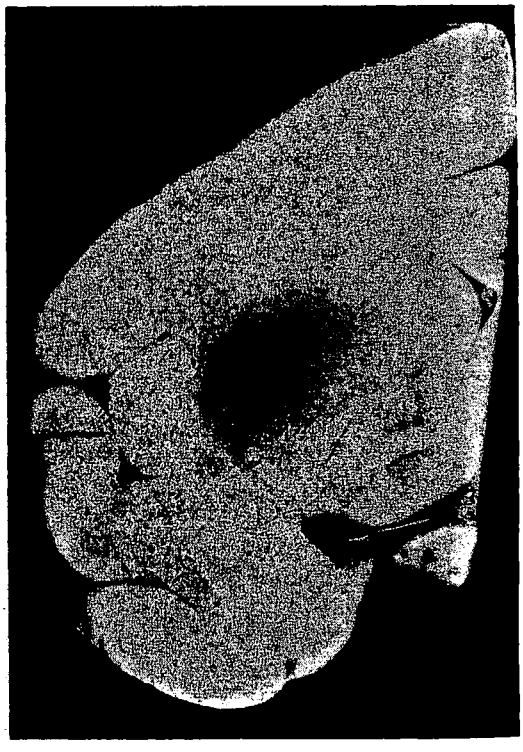

Fig. 4 (Potts, Praglin, et al.). Monkey No. 31, methanol. Gross section through basal ganglia showing necrosis and hemorrhage into putamen.

less significant. Brains of two monkeys dying of intravenous formaldehyde and two monkeys given sodium formate 188 mM/kg. showed no such changes microscopically. The cells in the affected regions show large pericellular spaces. The largest neurons are pale and the nuclei are almost indistinguishable from cytoplasm. The smaller neurons have distorted cytoplasm and eccentric pyknotic dark-staining nuclei and indistinct nucleoli. The intracellular substance of the brain seems coarsened and fibrillar, possibly due to local edema. Comparable sections of the brains of formaldehyde and formate animals showed no such changes. These findings are illustrated in Figures 5 and 6.

Histologic examination of other tissues was performed in five monkeys dying of methyl-alcohol poisoning. In no case were any histologic abnormalities noted in heart, liver, kidney, lung, spleen, skeletal muscle, intestine, or pancreas. In one animal which received ammonium chloride in order to produce acidosis, there were casts found in the collecting tubules of the kidney. In one animal receiving formaldehyde intravenously, a

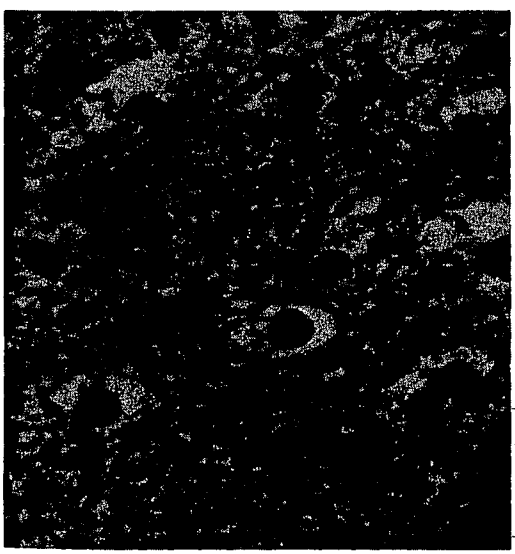

Fig. 5 (Potts, Praglin, et al.) Microscopic section of putamen of monkey No. 31, showing degenerative changes.

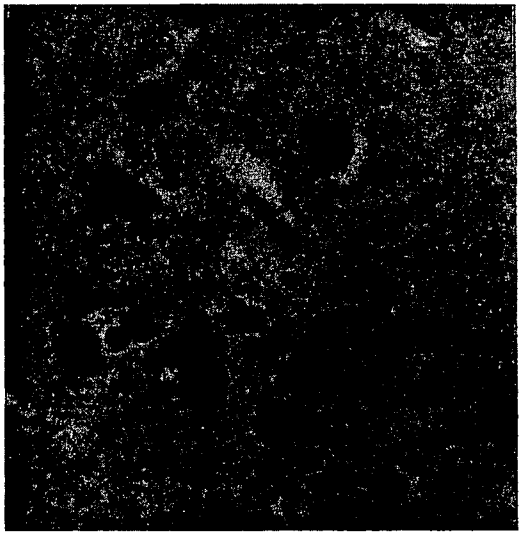

Fig. 6 (Potts, Praglin, et al.). Comparable section of animal killed with formaldehyde. Normal section.

focal chronic interstitial nephritis was observed in the kidney. That these are directly connected with ammonium chloride or formaldehyde administration is certainly not proved although suggested by these findings. In other respects the tissues of formaldehyde animals, formate animals, and ammonium chloride animals were entirely normal.

The electroretinogram was measured in seven monkeys dying of 6.0 gm./kg. of methyl alcohol, in three monkeys receiving formaldehyde by intravenous drip, and in three monkeys receiving doses of formate comparable to the methanol doses. In all three cases, the electroretinograms were similar to those elicited in the lower animals, consisting of an accentuated negative a-wave and an absent b-wave. It should be noted that the effect in the methanol animals did not occur until the second day, that is at 20 to 30 hours after administration at a time when most of the methanol had left the body and when the eyes showed visible retinal edema. The formaldehyde and formate effects were immediate, appearing within one to two hours, and obtainable instantaneously by the proper dosage of formaldehyde, as

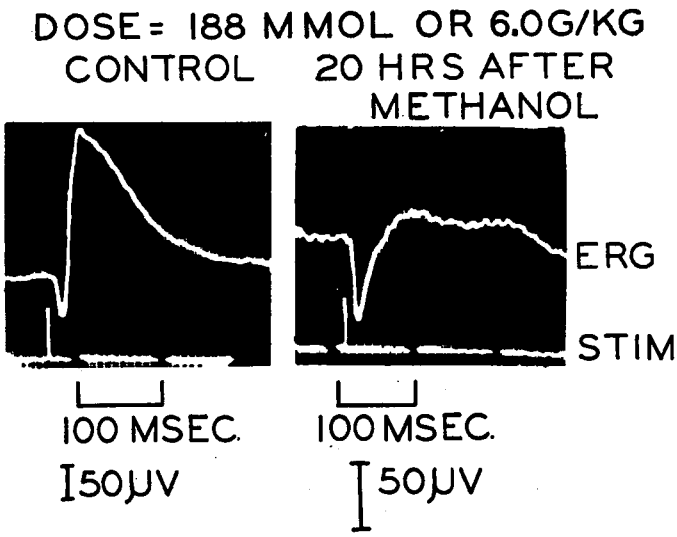

Fig. 7 (Potts, Praglin, et al.). Monkey electroretinogram before and 20 hours after administration of 6.0 gm./kg. methanol.

reported previously. These results are illustrated in Figures 7 and 8.

Discussion

In regard to the histologic eye findings, one fact is outstanding. In our monkeys which showed or had shown severe retinal edema, severe changes in the electroretinogram, pupillary dilatation, and apparent blindness, there were no marked findings. In the one animal which possibly showed changes in the ganglion-cell layer and certainly showed them in the optic nerve survival was an unusual nine days. It is beyond question that one of the late effects in human methanol poisoning is optic atrophy; and

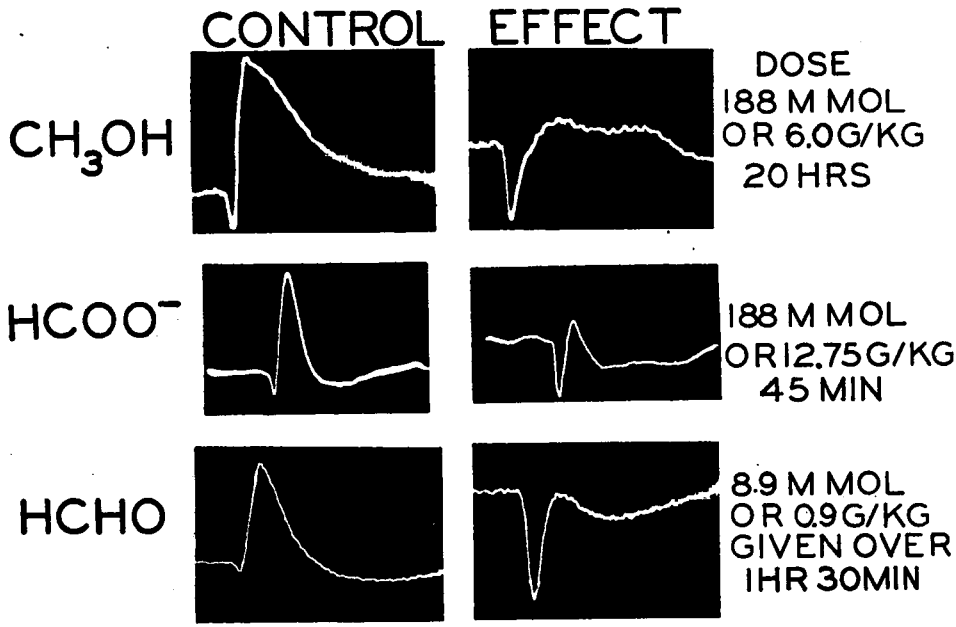

Fig. 8 (Potts, Praglin, et al.). Comparative effects of methanol, formaldehyde, and formate on the monkey electroretinogram.

since the optic nerve represents a collection of axons whose neurons reside in the ganglion-cell layer of the retina, it is reasonable to expect degeneration of the ganglion-cell layer of the retina as a primary finding. However, it is not necessarily reasonable to expect that any such degeneration detected by histologic methods need be visible at the time eye symptoms and ophthalmoscopic findings are at their peak. On the contrary, the lack of change seen in the ganglion cells is ample demonstration of the inadequacy of the histologic method in the face of far-reaching physiologic changes. Cell death does not in itself cause histologically detectable changes since all histologic examinations are done on dead material. It is only when cells are selectively dead long enough to allow degeneration to set in that the histologic method distinguishes these cells from their neighbors and offers a useful contribution.

Such a situation obtained in the case of the brain of methanol-poisoned animals. Here, the time is apparently long enough for damage to the basal ganglia to be evident in stained preparations, and in some cases even in gross specimens. None of the animals in which brain findings were described was allowed to become acidotic. Thus, neither retina change nor brain change can be attributed to the effect of acidosis. One is apparently dealing with yet another specific metabolic effect of methyl-alcohol poisoning. It is important, too, to note that the animals which showed central nervous system lesions, namely the methanol animals, all showed the central nervous-system depression described previously. Several of the animals showed extensor rigidity and tremor, characteristic of basal ganglion lesions. This was particularly true in the case of monkey No. 3.

Here, one has a clue for the first time to the cause of death after acidosis has been combated. Thus, methyl-alcohol poisoning unfolds itself as a complicated phenomenon. First, the narcotic effect of an extremely high dose may cause death of its own accord, and in this respect differs not at all from the other aliphatic alcohols. This is the only phase operative in experimental animals lower than primates. Second, the typical metabolic acidosis, if unrecognized and untreated, may in itself cause death at a later time and after the typical latent period. Finally, even though neither of the first two phenomena may be operative with the dose too low for narcotic death and the acidosis treated with base, still a third cause of death may be the action of a metabolic product on the central nervous system manifested so far principally by the histologic findings in the basal ganglia. One should note once more that the eye effects of methanol poisoning lie in this third phase. Thus, no therapeutic procedure proposed to date can be adequate, since none takes cognizance of this third metabolic poisoning—presumably mediated by a more proximal toxic agent, a metabolic product of methanol itself.

The question presents itself whether the lesions seen histologically in the basal ganglia are an adequate explanation for the late death of the animals. There is little question that such lesions can adequately explain prostration, motor inco-ordination, extensor rigidity, and tremor observed in these monkeys at various times. Whether these of themselves can cause death is another matter and no final answer is possible because of our incomplete knowledge of the physiology of the basal ganglia. It should be noted that the brain stem was examined carefully for histologic changes with negative results. This of course does not exclude other central nervous-system changes—particularly those due to edema and consequent increased intracranial pressure; we can only say no changes were found in our sections.

The electroretinographic findings in the monkeys, as in the nonprimates, show, first, that the electroretinogram may be used as an additional indicator of methyl-alcohol poisoning. This agrees with the report of Karpe (in his discussion of Reference 5) that a similar electroretinogram is found in human methanol poisoning. Retinal edema,

pupillary effects, and electroretinographic changes correlate quite closely. Secondly, when one considers the doses needed to elicit the electroretinographic effect, the formaldehyde is, as before, by far the most potent agent. To date it has not been possible to reproduce the typical central nervous-system findings of methanol poisoning when formaldehyde is given, even by intravenous drip over a period of several hours. This is not too surprising, since here again time for cell death or selective cell death has not been allowed. Further, one still cannot at this stage be certain whether the proximal toxic agent affects the basal ganglia more severely because it is manufactured most readily at that site, or whether the site is most susceptible to its actions. Experiments are now in progress to differentiate these two possible explanations.

Summary

1. The only consistent early change in sections of retinas from methanol-poisoned, bicarbonate-treated monkeys is cyst formation in the external nuclear layer. This is despite marked ophthalmoscopic, pupillary, and electroretinographic changes.

2. The only animal to show possible changes in the ganglion-cell layer of the retina was one which survived nine days. This animal also showed demyelinization of the optic nerve.

3. Methanol-poisoned, bicarbonate-treated monkeys consistently showed edema and nuclear pyknosis in the basal ganglia particularly the putamen and caudate nucleus.

4. When ophthalmoscopic edema and pupillary dilatation set in in these animals, the electroretinogram shows a large a-wave and no b-wave. The same picture may be reproduced with appropriate doses of formaldehyde and formate.

5. The bearing of these findings on our present knowledge of methanol poisoning is discussed.

The authors wish to acknowledge the technical assistance of Mrs. Violet Lima.

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Discussion

Dr. George Wald (Cambridge, Massachusetts): I would like to ask Dr. Potts, though he may have told us, what area of the brain is involved in these lesions?

Dr. Albert M. Potts (Cleveland, Ohio): The area in the basal ganglia is more external than that. The putamen and caudate nucleus are involved. In Orthner's cases, only the putamen was involved; in our cases, there was almost even distribution between the putamen and caudate nucleus.

The whole brain was gone over carefully, and nothing else was found histologically, except in this one area.

The brain was fixed in formaldehyde immediately after removal.

DR. HERMANN M. BURIAN (Iowa City, Iowa): I just want to ask Dr. Potts what light adaptation he used.

DR. POTTS: They were done in dark-adapted animals, ordinarily.

DR. DAVID G. COGAN (Boston, Massachusetts): I would like to ask Dr. Potts if the pupillary reaction would not also have been a satisfactory method of testing their visual function, rather than the electroretinogram, although I realize that, quantitatively, the electroretinogram might have been better.

The other question I would like to ask is: "How does Dr. Potts account for the refractoriness of subprimate animals to methanol blindness?" Maybe he answered that last year.

DR. ALBERT M. POTTS (Cleveland, Ohio): In answer to the first question, the pupillary reaction is a little bit irregular. We have a number of animals which show retinal changes, good retinal edema with a submaximal pupillary response, but with a maximal electroretinogram response. In some cases, we get the so-called cogwheel pupil response rather than fixed dilatation. Last year we talked about this condition, in which the pupil comes up stepwise and comes down stepwise in reaction to darkness or light, respectively.

As far as the explanation for the toxic phenomena is concerned, this is difficult, of course. The obvious hypotheses are available: that local manufacture of the proximal toxic agent—let's say formaldehyde, for the sake of argument—takes place selectively in the retina of the primate or in the liver of the primate, or that the retina of the primate is selectively susceptible to the effect of this toxic agent.

We tend to favor the latter, because we had evidence of good manufacture of formaldehyde in rats when given C_{14} methanol.

DR. WERNER K. NOELL (Buffalo, New York): In our experiments with the previously described poisons, we observe distinct histologic changes, and I am surprised that in the preceding papers, especially in the experiments of Dr. Davis, normal histology was associated with slowly progressing failure of the electroretinogram. It may be that structural changes develop faster in the rabbit than in the monkey or that our conditions are more specific than those in the other rabbit experiments, but damage to the outer limbs is easily overlooked and formalin fixation, for instance, may not reveal early necrosis of the visual cell.

DR. HERMANN M. BURIAN (Iowa City, Iowa): In these experiments with anesthesia, where he got definite changes in the electroretinogram, would you suspect that there was a visible, detectable anatomic effect?

DR. WERNER K. NOELL (Buffalo, New York): Certainly not at all. It depends upon the duration for which the damage is imposed upon the cell. Certainly, one will find nothing histologically if one examines an eye the electroretinogram of which has just been abolished by anesthesia or anoxia.

But the case where the rabbit's electroretinogram has practically disappeared for several days, as shown, makes me wonder why there are no histologic changes. I would expect changes.

DR. ALBERT M. POTTS (in closing): As far as formalin fixation of the brain is concerned (which is the thing we talked about first) the control brains and the experimental brains were fixed in exactly the same way; so this is hardly a fixation artefact.

In connection with the fixation of the eyes, we are doing a set of experiments now, using carotid injection in an animal at the peak of its symptoms. We will have some results from these studies in a while, but do not anticipate marked differences.