STUDIES ON THE VISUAL TOXICITY OF METHANOL*

V. The role of acidosis in experimental methanol poisoning

Anita Peek Gilger, M.D., and Albert M. Potts, M.D.
Cleveland, Ohio

Acidosis is prominent among the clinical signs in human methanol poisoning. Røe[1] postulates that, "severe acidosis is necessary for the development of amblyopia and amaurosis." Benton and Calhoun[2] state, "the acidosis does not appear to be the cause of amblyopia but it does act as an accelerating force. If acidosis can be corrected before permanent ocular damage has resulted, a return of normal visual acuity can be expected." The standard therapy of methanol poisoning today consists of combatting acidosis.

In view of this, we have set ourselves the goal of trying to learn with laboratory animals whether acidosis is the cause, or the companion, of visual loss in methanol poisoning. Immediately the question arises as to whether any animals other than humans develop acidosis from methanol. The literature apparently provides both yes and no answers. The second question is whether methanol causes visual damage in nonhuman species. Again the answers in the literature are conflicting. Our work in attempting to solve the problem of whether methanol causes acidosis or visual damage in nonhuman species comprises the material for this paper.

Literature

I. Acidosis

In 1912, Schmiedeberg[3] first postulated the development of acidosis in methanol poisoning. Citing Pohl[4] and Bongers,[5] who had studied the metabolism of methanol in experimental animals, Schmiedeberg considered that in these animals the resultant formic acid was neutralized either by blood base or by newly formed ammonia. Schmiedeberg was undecided on the role of acidosis in visual loss.

Król (1913),[6] acting upon Schmiedeberg's theories, investigated the ammonia output in the urine of three dogs given nonlethal divided doses of methanol. In all three he found the amounts of urinary ammonia were doubled to quadrupled. In one dog he also determined urine formates, finding that formates neutralized only a quarter of the ammonia. Król did not learn what acid or acids did neutralize the remaining 75 percent of the ammonia, although it was not oxalic acid. However, he believed the increased ammonia formation meant his dogs were acidotic.

Grignolo (1913)[7] determined the hydrogen ion concentration of aqueous and blood serum in three dogs with the concentration apparatus of C. Foa. Although he found a very slightly higher hydrogen-ion concentration after single sublethal doses of methanol, Grignolo concluded that these were "changes which do not depart significantly from the physiological values." In spite of this conclusion, he has been erroneously quoted in the literature[8] as having demonstrated acidosis in dogs and as believing the increased hydrogen ion concentration parallelled morphologic changes in the eye tissues.

Tyson and Schoenberg[8-10] reported that methanol produced acidity of the aqueous in dogs and rabbits and acidosis in dogs. The authors presented no data about normal controls. The statement about acidosis was

* From the Eye Service, Department of Surgery, University Hospitals, and the Laboratory for Research in Ophthalmology, Western Reserve University. This work was aided by a grant from the Office of Naval Research. A preliminary report on this material was given before the East Central Section of the Association for Research in Ophthalmology, Pittsburgh, Pennsylvania, January, 1954.

based on determinations of blood electroconductivity with a resistance bridge.

They stated that an increase in electroconductivity of the blood might result from the breaking up of the corpuscles, from an increase in the hydrogen ion content, or from any increase in the inorganic salts. Of these three possibilities, they decided that the increased electroconductivity of their dogs' blood was due to an increase in the hydrogen ion concentration because blood serum following methanol was acid to phenolphthalein.

These authors did not realize that blood of normal dogs is acid to phenolphthalein.[11] These strictures also apply to their description of an acid aqueous.[12] An additional source of error was that their animals suffered severe anoxic anoxia from unventilated inhalation experiments. Severe and prolonged anoxic anoxia causes acidosis (Van Liere).[13] Koehler, Brunquist and Loevenhart[14] found CO_2-combining capacity dropping to 9.8 vol. percent in pigs with anoxic anoxia. It is evident that the conclusions of Tyson and Schoenberg that methanol poisoning in experimental animals produced acidosis has no justification on the basis of their experimental data.

Haskell, Hileman, and Gardner (1921),[15] using the gasometric apparatus of Van Slyke and Cullen to determine plasma CO_2-combining capacity in dogs, reached these conclusions:

"In dogs poisoned with methanol, the severity of the intoxication is, at times, entirely at variance with the degree of acidosis. . . .

"Alkali, in the form of sodium bicarbonate, has, in itself, little or no influence on the course of poisoning."

Although the authors evidently used large numbers of dogs, they give data on CO_2-combining capacity for only three: two receiving single oral doses of 7.9 gm./kg. had normal CO_2-combining capacities shortly before and after death; one receiving 6.3 gm./kg. survived with the following CO_2-combining capacities: 43.9 vol. percent before methanol, 33.2 vol. percent at 24 hours, and 29.6 vol. percent on the third day. The authors reported seven dogs given 6.3 gm. methanol/kg., orally, plus 1.25 to 5.0 gm. $NaHCO_3$/kg./24 hours given either orally or intravenously. All except one died and at intervals bearing no relationship to the amount of sodium bicarbonate. No CO_2-combining capacities were reported as being done on this treated series.

Loewy and Münzer (1923)[16] disagreed with Król's conclusion that increased urinary ammonia formation meant that the dogs were acidotic. In two rabbits and one dog, they found no decrease in the CO_2-combining capacity or increase in pH. Their use of usually sublethal, and often subtoxic, doses of methanol, their paucity of experimental animals, and their lack of duplicate determinations makes their work inconclusive, however.

Leo (1925)[17] next tackled the problem of experimental acidosis by means of survivorship with and without therapy with sodium bicarbonate. He made no CO_2-combining capacity determinations and used four dogs. Two dogs received 1.45 gm. methanol/kg. every day for six days, by stomach tube, a low dosage. One of these two dogs received an average of 2.5 gm. $NaHCO_3$ every day, seven times. The control dog died on the eighth day, whereas the treated dog was completely well at that time. No experimental data were given for the second pair of dogs except that the control dog died on the sixth day and the treated dog on the 10th day. Leo stated that $NaHCO_3$ was without effect in the therapy of methanol-poisoned mice, rats, and rabbits, but gave no experimental data. This is not conclusive evidence, nor does Leo make such a claim. The German dog shortage unfortunately prevented his doing further work. He believed that these experiments indicated a species difference in reaction to methanol, and that sodium bicarbonate successfully combats methanol-induced acidosis in dogs.

Von Oettingen[18] believed that
sumptions were supported by th
Rewiger (1922).[19] Rewiger gav
single, subtoxic, oral doses of
(1.04 to 1.7 gm./kg.) and then
their urinary nitrogen output by
dahl method. Following methanol
maximal increases in the daily uri
gen of from 1.3 to 1.7 times the
output. He likewise gave two
methanol at comparably subtoxic
the species (3.8 and 4.4 gm./kg.)
no effect on the urinary nitrogen
subscribed to the theory that t
parallel between eye damage an
protein metabolism, but reported
examinations in his animals.

Clark and Gibson (1933)[20] repo
in dogs, while "sodium bicarbonat
cient amounts to maintain a normal
balance was ineffective as was the
administration of glucose alone, . .
bined therapy with sodium bicarbon
successful." Experimental details
published in this brief summary of
presented paper, but Dr. Clark ver
made the experimental protocols
to us.[21] Doing CO_2-combining cap
the Van Slyke method, Clark fou
of four dogs fatally poisoned wit
administered, repeated doses of m
three developed severe acidosis.

1. From a normal of 41 vol. percent to
of 20 vol. percent.
2. From a normal of 41 vol. percent to
of 14 vol. percent.
3. From a normal of 45 vol. percent to
of 11 vol. percent.

The fourth dog died before a postm
blood sample was obtained. Only o
was given a single oral dose. This d
vived 7.0 gm. of 20 to 25 percent me
kg. without developing acidosis (f
normal of 36 vol. percent to a low of
percent). Methanol doses and doses of
peutic substances varied in each exper
However, even although dosages can
compared, the increased survival rate

Von Oettingen[18] believed that Leo's assumptions were supported by the work of Rewiger (1922).[19] Rewiger gave six dogs single, subtoxic, oral doses of methanol (1.04 to 1.7 gm./kg.) and then determined their urinary nitrogen output by the Kjeldahl method. Following methanol he found maximal increases in the daily urinary nitrogen of from 1.3 to 1.7 times their normal output. He likewise gave two rats oral methanol at comparably subtoxic levels for the species (3.8 and 4.4 gm./kg.) and found no effect on the urinary nitrogen. Rewiger subscribed to the theory that there is a parallel between eye damage and altered protein metabolism, but reported no eye examinations in his animals.

Clark and Gibson (1933)[20] reported that in dogs, while "sodium bicarbonate in sufficient amounts to maintain a normal acid-base balance was ineffective as was the repeated administration of glucose alone, . . . a combined therapy with sodium bicarbonate seems successful." Experimental details were not published in this brief summary of an orally presented paper, but Dr. Clark very kindly made the experimental protocols available to us.[21] Doing CO_2-combining capacity by the Van Slyke method, Clark found that, of four dogs fatally poisoned with orally administered, repeated doses of methanol, three developed severe acidosis.

1. From a normal of 41 vol. percent to a low of 20 vol. percent.
2. From a normal of 41 vol. percent to a low of 14 vol. percent.
3. From a normal of 45 vol. percent to a low of 11 vol. percent.

The fourth dog died before a postmethanol blood sample was obtained. Only one dog was given a single oral dose. This dog survived 7.0 gm. of 20 to 25 percent methanol/kg. without developing acidosis (from a normal of 36 vol. percent to a low of 31 vol. percent). Methanol doses and doses of therapeutic substances varied in each experiment. However, even although dosages cannot be compared, the increased survival rate of the group treated with sodium bicarbonate and glucose and insulin was not mathematically significant. Survivals were as follows:

1. Methanol only—one out of five survived.
2. Methanol and glucose—one out of three survived.
3. Methanol and $NaHCO_3$—none out of three survived.
4. Methanol and $NaHCO_3$ and insulin and glucose—four out of six survived.

Røe (1948)[1] measured the alkali reserve in rats and rabbits. Nine rats were given single doses of 6.3 gm. methanol/kg. by stomach tube. Our rat LD_{50} per os was 9.5 gm./kg. (50-percent solution) and that of Alder, Buschke, and Gordonoff[22] (LD_{60}) was 8.3 gm./kg. (70-percent solution). Thus Røe's dosage is evidently sublethal for rats. He collected bloods by decapitation on the two following days, finding all CO_2-combining capacities lying between 47 and 60 vol. percent. He also did a series of five rabbits. Two received daily oral doses of methanol varying from 2.4 to 5.5 gm./kg. for three and seven days. Neither showed acidosis. Three received single doses (6.3 and 7.9 gm./kg.). Of these, one of the two 6.3 gm. rabbits showed a drop in CO_2-combining capacity of 20 vol. percent on the second day, returning to normal on the third. This is not unusual for rabbits (see later). Values prior to administration of methanol were not reported for the other two, but second-day CO_2-combining capacities were normal. Røe concluded that "like the rats, the rabbits showed no signs of acidosis."

Because of its bearing on aspects of our experimental work,[23] a description of two early works on human acidosis is indicated. The first clinical use of alkali therapy was made by Harrop and Benedict (1920)[24] who investigated their patient's alkali reserve because of the work of Schmeideberg[3] and Król.[6] Their patient drank about 2.5 to 3.0 gm. methanol/kg. in one evening. About 48 hours later she was almost blind and was acidotic. $NaHCO_3$ therapy corrected the acidosis. On the second day after ingestion

of methanol, the patient was found to have 2,200 cc./liter of N/10 titratable organic acids in her urine. This dropped four days later to a normal 200 to 400 cc. N/10 acid/l. The method used for the determination of titratable organic acids was that described by Van Slyke and Palmer (1919).[25] Later in 1920 Van Slyke and Palmer[26] also described a patient who survived ingestion of methanol and who also showed an increase in titratable organic acids in his urine. Neither Harrop and Benedict nor Van Slyke and Palmer were able to ascertain what specific acid caused the increase following methanol. It was not caused by lactic, formic, or aceto-acetic acid.

II. Ocular effects

The production of clinical ocular damage by methanol in nonhuman animals has been reported by some authors and denied by others. Likewise, there is disagreement about the production of histologic changes in experimental animals. A critical discussion of the much-debated questions relating to histopathologic changes in the peripheral visual apparatus is beyond the scope of the present report; however, for the sake of completeness, we are reporting the authors' conclusions on histologic experiments.

In humans, methanol frequently causes ocular signs and symptoms and blindness, about the appearance of which there is essential agreement in the literature.[2, 27, 28] It is for these effects that we must look in the papers on experimental poisoning. Four common sources of confusion should be considered before discussing the individual papers:

One error is the conclusion that animals in the early stage of intoxication (the first few hours after administration of methanol) who bump or stumble over objects are blind. These are unquestionably ataxic manifestations of alcoholic intoxication and are dependent upon temporary alterations of higher cerebral functions rather than due to any impairment in function of the peripheral visual apparatus. One does not observe blindness at this stage in human cases.

A second error is that visual impairment following exposure keratitis is due to a specific ocular effect of methanol. All authors are agreed that nonprimates shortly after a sufficiently large dose of methanol become comatose. During coma, which can last as long as four days, the eyelids are usually open. A severe exposure keratitis with secondary bacterial invasion occurs in these cases and results in corneal opacification, which undoubtedly diminishes vision. This nonspecific secondary damage is not comparable to the methanol blindness of humans.

The third error is concerned with altered pupillary sizes and reactions. These changes are common in all nonprimates; but neither in the literature nor in our experience do they show correlation with ocular damage as evinced by ophthalmoscopic and histopathologic appearances. They are associated with semicomatose and comatose states. We believe that in nonprimates the mechanism of production of these pupillary changes is one common to all anesthetic agents and is unrelated to the specific ocular damage caused by methanol. Nystagmus is probably developed on a similar basis since higher alcohols are also reported to cause it; however, no authors have claimed loss of vision because the animals developed nystagmus.

A fourth error is that comatose and moribund animals who do not respond to visual stimuli are blind from the toxic amblyopia of methanol. Animals in such states do not respond to *any* type of stimulus.

In 1896 Joffroy and Serveaux,[29] in a study of acute intravenous and intramuscular methanol poisoning, reported nystagmus and pupillary changes (mydriasis and miosis) in dogs and rabbits. In two chronically poisoned dogs no eye changes were noted. These authors did not report having done ophthalmoscopic examinations.

Baer (1898)[30] gave rabbits single oral doses of methanol. He described early pu-

us. One does not observe
is stage in human cases.
ror is that visual impairment
osure keratitis is due to a
effect of methanol. All au-
ed that nonprimates shortly
ntly large dose of methanol
se. During coma, which can
s four days, the eyelids are
A severe exposure keratitis
bacterial invasion occurs in
l results in corneal opacifica-
doubtedly diminishes vision.
fic secondary damage is not
the methanol blindness of

ror is concerned with altered
and reactions. These changes
all nonprimates; but neither
re nor in our experience do
relation with ocular damage
ophthalmoscopic and histo-
earances. They are associated
tose and comatose states. We
nonprimates the mechanism
of these pupillary changes is
o all anesthetic agents and is
the specific ocular damage
hanol. Nystagmus is probably
a similar basis since higher
so reported to cause it; how-
rs have claimed loss of vision
nimals developed nystagmus.
error is that comatose and
nals who do not respond to
re blind from the toxic ambly-
nol. Animals in such states
l to *any* type of stimulus.
roy and Serveaux,[29] in a study
ravenous and intramuscular
ning, reported nystagmus and
ges (mydriasis and miosis)
rabbits. In two chronically
no eye changes were noted.
did not report having done
ic examinations.
)[30] gave rabbits single oral
anol. He described early pu-

pillary changes; with lethal doses he often found nystagmus.

Ward Holden (1899)[31] was the first worker to claim that he produced the toxic amblyopia of methanol poisoning in experimental animals. This claim was based upon one dog who received 4.0 gm. methanol/kg. by stomach tube on days one and five of the experiment. Holden described early pupillary changes and blind drunkenness. On day seven he reported ocular irritation as shown by the dog's rubbing his eyes with his paws. On day 15 (10 days after the last methanol) the corneas became diffusely hazy. On day 16 the dog was found dead. The autolyzed eyes were sectioned and were said to show retinal ganglion cell and optic nerve degeneration caused by methanol. An autopsy to determine the cause of death was not reported.

Harry Friedenwald (1902),[32] in an abstract of an oral presentation, reported ganglion cell degeneration in rabbits with chronic methanol poisoning. No experimental details were published about clinical eye examinations.

Birch-Hirschfeld in a series of scholarly papers (1900, 1901, 1902)[33-35] reported his experiments in detail. He poisoned seven rabbits, three hens, four dogs, and three rhesus macaques with repeated oral doses of methanol. He described primary ganglion cell degeneration of the retina occasionally followed by optic-nerve degeneration. His clinical observations are of considerable interest. All of his animals with the exception of the hens received almost daily ophthalmoscopic examinations and tests for objective signs of visual defects. Of these animals only one, a monkey, was considered by Birch-Hirschfeld to have definite evidence of ocular damage. This monkey, given methanol for 15 days (amounts of methanol/body weight were not reported but produced toxic symptoms), showed from the 11th experimental day optic atrophy and dilatation and tortuosity of the retinal veins along with behavior indicating possible visual loss. One dog, poisoned for 35 days, showed on one examination a transient hyperemia of the discs with dilatation of the retinal veins, but had no clinical evidence of visual loss. Only in the monkey did Birch-Hirschfeld consider the clinical presence of a toxic amblyopia a certainty.

Reid Hunt (1902)[36] reported experiments on rabbits and dogs in which he believed one dog became blind. This dog, who was fatally poisoned with repeated doses and who had repeated episodes of unconsciousness, developed a mucopurulent conjunctivitis followed by corneal clouding. When the corneas became blue, blindness was observed. This case has been extensively cited[18] as methanol toxic amblyopia in dogs. The description in the paper is of blindness due to exposure keratitis and not to methanol toxic amblyopia.

Lesieur (1906),[37] giving lethal intravenous doses of methanol and other alcohols to rabbits, reported all alcohols caused nystagmus rarely and mydriasis frequently.

Nicloux and Placet (1912)[38] reported dilated pupils in one dog fatally poisoned with intravenous methanol.

The paper by Igersheimer and Verzár (1913)[39] has been misquoted[18] in that it is cited as showing methanol amblyopia in hens. Igersheimer and Verzár reported that hens showed "diminishing of the light sense with methanol (that is, weaker scratching [for food] with diminishing light)." The authors said, "This raises the question whether these light sense findings are indicative of changes of the retina itself or should they be interpreted as cerebral fatigue symptoms. We cannot venture at this time to give the answer to this question on the grounds of our experiments." They also found no definite histopathologic changes in the eyes of their experimental animals.

Król (1913)[6] stated that none of his three dogs nonfatally poisoned with divided doses showed any ocular damage. No ophthalmoscopic examinations were reported. Król's doses were barely toxic and well below the

approximate oral minimum lethal dose for dogs.

Kasass (1913)[40] gave 40 rabbits toxic oral doses of methanol for periods varying from one to 267 days. On pathologic examination he found "changes in the vascular membrane, in the membranes of the optic nerve, in the retina, beginning with dropsy and degeneration up to albuminuric retinitis, and, in the optic nerve beginning with parenchymatous degenerated neuritis up to axial atrophy."

In 15 of the 40 rabbits, Kasass reported ophthalmoscopic changes, the most frequent of which (14 rabbits) consisted of narrow retinal arteries. Dilated retinal veins occurred in four. Pale discs "which did not have any special significance" occurred in eight. "White discs of suspicious appearance" were seen in three and acute optic atrophy in three. Kasass was unable to devise any test with which he could determine the presence or absence of vision in rabbits.

Evaluation of this paper is difficult. In the early portion, Kasass stated that "numbers 17, 21, and 23 have to be excluded since they died from other causes"; yet he described the pathologic changes in the peripheral visual apparatus of these three rabbits and based his conclusions on findings in these three animals as well as in others. The unique difficulty of a funduscopic diagnosis of optic atrophy in the presence of the myelinated nervehead of the rabbit needs no emphasis.

Langgaard (1913)[41] saw nystagmus in one of a series of rabbits fatally poisoned with oral methanol.

Tyson and Schoenberg[8-10] reported acute and chronic inhalation experiments using five guinea pigs, one rabbit, nine dogs, and one monkey. They reported ophthalmoscopic changes in 100 percent of the examined dogs and the monkey, and retinal ganglion-cell degeneration in all animals. In passing, it should be mentioned that, except in two instances, all pathologic material was obtained from animals dead for an unknown period of time. Fundus changes reported in the dogs consisted, with one exception, of hyperemia and edema of the optic discs, and dilatation and darkening of the retinal veins.

Their dogs were placed in an unventilated box. Calculations show severe anoxia was produced. The authors made ophthalmoscopic examinations immediately after removing the dogs from the box; and eye changes were *never* reported after the few times in which the authors stated that free ventilation had been given.

Anoxia is reported to cause dilatation and darkening of the retinal vessels, especially the veins (Cusick, Benson, and Boothby;[42] Duguet, Dumont, and Bailliart[43]), enlargement of the blind spot (Goldmann and Schubert[44]), and is believed "to play a role in the production of visual defects associated with papilledema as sometimes occurs in hemorrhagic states" (Walsh[45]).

Thus, anoxia alone can cause the ocular findings Tyson and Schoenberg ascribed to methanol. These eye changes are found in uncomplicated anoxia. Tyson and Schoenberg's unventilated box provided increasing carbon dioxide in addition to decreasing oxygen amounts. It is unnecessary to consider toxic effects of carbon dioxide here.

The exception in the fundus changes was in one dog who from eight to 50 days had optic discs which were "paler than in the normal dog examined." The monkey apparently received free ventilation during at least most of the experiments, as was frequently noted by the authors. On day 19, the discs were considered hyperemic as compared with the first examination; this was subsequently not remarked upon, the monkey dying at 22 days. This might represent toxic amblyopia; on the other hand, they report one dog who was killed when the assistant accidentally closed the vents for too long a time.

In 1920, the official protocol of a meeting[46] stated that, in association with the report of human cases of methanol poisoning, "Birch-Hirschfeld presented data of retina and optic nerve pathology in mals." Apparently this w mary of his 1900 to 19 surmise abetted by the f kopf[47] working under B 1922 failed to cite any than 1902.

Schanz (1920)[48] claime the eye to light precipitate opia of methanol poisor three rabbits single subl methanol. One eye of covered, except during o aminations. One rabbit k showed no abnormalities, killed after 19 days, had eye and large exudates ir the retina of the lighted e description of these exud not resemble the human days after methanol pois mental details whatsoeve rabbit were reported. See paper by Schwarzkopf.[47]

Friedenwald and Fel rats, mice, guinea pigs, methanol doses comparab Birch-Hirschfeld. Ophth nations were not done. Birch-Hirschfeld's histol be explained by artefacts niques rather than to m

Bills and Maukin (192 rats to methanol fum systemic effects and eve no decrease in brightne observed.

Schwarzkopf (1922)[4 soned three rabbits and t anol via stomach tube. He glion cell degeneration a nerve degeneration but n ophthalmoscopic evidence His experiments also dea the eyes to light, which without effect on methar

deSchweinitz (1923)[51 three chronically poisone

optic nerve pathology in experimental animals." Apparently this was merely a summary of his 1900 to 1902 experiments, a surmise abetted by the fact that Schwarzkopf[47] working under Birch-Hirschfeld in 1922 failed to cite any work more recent than 1902.

Schanz (1920)[48] claimed that exposure of the eye to light precipitated the toxic amblyopia of methanol poisoning. Schanz gave three rabbits single sublethal oral doses of methanol. One eye of each rabbit was covered, except during ophthalmoscopic examinations. One rabbit killed after 10 days showed no abnormalities, O.U. Rabbit No. 2, killed after 19 days, had a normal unlighted eye and large exudates in the lower half of the retina of the lighted eye. Schanz gave no description of these exudates, but this does not resemble the human toxic amblyopia 19 days after methanol poisoning. No experimental details whatsoever about the third rabbit were reported. See discussion of this paper by Schwarzkopf.[47]

Friedenwald and Felty (1920)[49] gave rats, mice, guinea pigs, rabbits, and dogs methanol doses comparable to those given by Birch-Hirschfeld. Ophthalmoscopic examinations were not done. They found that Birch-Hirschfeld's histologic findings could be explained by artefacts due to fixation techniques rather than to methanol poisoning.

Bills and Maukin (1921)[50] exposed white rats to methanol fumes, getting toxic systemic effects and even death. However, no decrease in brightness sensitivity was observed.

Schwarzkopf (1922)[47] chronically poisoned three rabbits and two dogs with methanol via stomach tube. He found retinal ganglion cell degeneration and occasional optic nerve degeneration but no definite clinical or ophthalmoscopic evidences of ocular damage. His experiments also dealt with exposure of the eyes to light, which he reported to be without effect on methanol poisoning.

deSchweinitz (1923)[51] reported data on three chronically poisoned dogs studied by himself and co-workers. From oral doses given every two to three days for nine to 80 days, the dogs showed marked intoxication but "during life gave no indication, ophthalmoscopic or otherwise, of defective vision" and pathologic examinations failed to show retinal ganglion cell degeneration. In one dog there was very slight veiling of the discs on one day, but deSchweinitz did not consider this significant.

Munch and Schwartze (1925)[52] studied acute oral toxicities in rabbits. Their only reported eye finding was frequently occurring nystagmus.

Rost and Braun (1926)[53] concluded, on the basis of the literature as well as of their own work, that the specific poisoning by methanol of the nervous apparatus of human eyes was also found in animals. Their conclusions were based upon the following evidence. In poisonings of dogs, rabbits, hens, ducks, and a cat by orally administered, divided doses, one dog after five days of deep narcosis was found to have clinical eye changes. This was a blue-white opacity of the cornea. Ganglion cell changes were found in specimens obtained after dogs had been dead for unknown times; but no pathologic changes were found in the eyes of dogs experimentally killed. Rost and Braun also reported nystagmus in rabbits.

Alfred Leo (1927)[54] gave single doses of methanol to four dogs and chronically poisoned two dogs (both orally). In all experiments he found no eye damage or changes except one episode each in two dogs of early pupillary changes. Leo did not do ophthalmoscopic or histopathologic examinations of the eyes.

Weese (1928)[55] used mice in chronic inhalation experiments. Groll, who examined the histologic sections of the eyes, found degenerative changes of the nervous elements of the retina but was of the opinion that these changes were not necessarily the result of an intravital process.

Noè (1929),[56] on the basis of acute intravenous poisoning experiments, claimed that

rabbits sometimes became blind. The evidence upon which this statement of blindness was made consisted of two rabbits who (1) did not raise their heads to a bright light in a dark chamber and (2) did not immediately go to a proffered cabbage leaf. One of these rabbits was moribund at the time of testing, dying less than four hours later. In consideration of the unpredictability of normal rabbit behavior, we do not find this evidence convincing. Noè reported no ophthalmoscopic or other ocular findings.

Keeser (1931, 1931)[57, 58] found formaldehyde in the vitreous of rabbits given repeated sublethal doses of methanol. After incubating surviving calves' vitreous with methanol, he also found formaldehyde, which he considered the toxic agent in methanol poisoning. He reported, without giving the numbers of rabbits used, that "the animals, which for two weeks had daily received 3.0 cc. methanol plus 0.5 gm. ammonium carbonate/kg. body weight in dilute aqueous solution, showed less extensive changes in their organs by macroscopic examination than those animals who were given only methanol." Keeser did not specify what organs or what changes.

McCord (1931)[59] and Scott, Helz, and McCord (1933)[60] gave single and repeated doses of methanol by skin absorption, inhalation, and ingestion to rats, rabbits, and rhesus monkeys. Reported clinical ocular findings were: early pupillary dilatation and slow reaction to light (species unidentified), corneal opacification in some of the rats and rabbits, clinical optic atrophy in rabbits, and blindness (one monkey, other species unidentified). No correlation was given between dosage and clinical ocular findings. They reported the following histopathologic changes: parenchymatous degeneration and focal necrosis of the liver; parenchymatous degeneration of the epithelium lining the convoluted tubules of the kidney; increased blood-forming activity of the spleen; edema, congestion, and desquamation of the alveolar epithelium and pneumonic consolidation of the lungs; edema, granular degeneration, and necrosis of the muscular fibers of the heart; frequent hyperplasia of the lymph nodes; capillary congestion, edema, and patchy degeneration in the neurones of the spinal cord and brain; peripheral neuritis; constant retinal changes consisting of marked congestion of the choroidal vessels, edema, patchy degeneration of the ganglion cells; and rarely, including one monkey blind at death, degeneration of the optic nerve. The report that a monkey was blind at death is significant. Unfortunately protocols giving details of the clinical and histopathologic findings are no longer available.

Sammartino (1933)[61] gave a "series" of one rabbit a single intravenous sublethal dose of methanol. His only abnormal ocular finding was transient "hyperemia of the papillary veins" in the fundi. In six rabbits given formaldehyde and five given formic acid, he found fundi always normal. A minor fundus change of a debatable nature in one of a species with unusual discs does not constitute toxic amblyopia in our opinion.

Harada (1937)[62] studied the antihelminic action of methanol and nine other drugs in mice and dogs. Along with four other drugs in dogs, methanol was reported to cause "sight damage, as was shown by blindness, mydriasis, slow pupillary reaction to light, anisocoria, and often fixed pupils." Harada gave no evidence as to how he determined "blindness"; apparently his conclusion of blindness was based on the early pupillary changes in narcotized animals. Harada described a variety of histologic degenerative changes common to all 10 drugs; no details of techniques were given.

Alder, Buschke, and Gordonoff[22] worked with rats and rabbits giving both oral methanol. White rats were fatally poisoned with single and repeated doses. Their eyes showed no ophthalmoscopic changes, and with respect to histopathologic findings, the authors concluded that any alterations could equally well have been caused by artificial

means. Although
poisoned with rep
ophthalmoscopic
showed ganglion-c
rabbits were given
solution of methan
4. For the rabbit th
toxic; in our hands
gm. of 30-percent
approximate LD_{100}.

Tomita (1939)[63]
doses of 1.4 to 12
methanol mixed wi
occurred. The diag
dogs of clinical vi
upon pupillary char
tion following exp
moscopic examinat
the dogs always sh
except for one dog
and showing engor
the end. Tomita
changes of the gan
degeneration of
softening, endarter
brain. Fixation of
zero to six hours a

Koppanyi and C
blindness in two
after recovering fr
16 gm./kg. of ab
one dog given 8.0
treated with massiv
of one-percent Na
about double the l
ophthalmoscopic e
ported.

Sayers et al. (
methanol mainly by
experiments by sk
were found to be
from normal ophth
seen in any of the d

It is of great i
mologist in this stu
30 normal control
varied findings: n
congestion of the

means. Although white rabbits nonfatally poisoned with repeated doses showed no ophthalmoscopic changes, their retinas showed ganglion-cell degeneration. These rabbits were given 1.4 gm. of a 70-percent solution of methanol/kg. on days 1, 3, and 4. For the rabbit this dosage should be subtoxic; in our hands a single oral dose of 7.0 gm. of 30-percent methanol/kg. was the approximate LD_{100}.

Tomita (1939)[63] fed dogs repeated daily doses of 1.4 to 12 gm./kg. of 30-percent methanol mixed with cow's milk until death occurred. The diagnosis in some of these dogs of clinical visual damage was based upon pupillary changes or corneal opacification following exposure keratitis. Ophthalmoscopic examinations made on some of the dogs always showed normal eyegrounds except for one dog poisoned for 224 days and showing engorged retinal veins toward the end. Tomita reported degenerative changes of the ganglion cells of the retina; degeneration of the optic-nerve fibers; softening, endarteritis, and bleeding in the brain. Fixation of tissues was done from zero to six hours after death.

Koppanyi and Cutting (1941)[64] found no blindness in two dogs for several weeks after recovering from single oral doses of 16 gm./kg. of absolute methanol, and in one dog given 8.0 gm./kg. All three were treated with massive intravenous infusions of one-percent NaCl. The higher dose is about double the lethal dose for dogs. No ophthalmoscopic examinations were reported.

Sayers et al. (1942),[65] (1944)[66] gave methanol mainly by inhalation and in a few experiments by skin absorption. Dosages were found to be subtoxic. No deviations from normal ophthalmoscopic findings were seen in any of the dogs.

It is of great interest that the ophthalmologist in this study, J. G. Linn, examined 30 normal control dogs with the following varied findings: no abnormalities to slight congestion of the discs; slight to marked congestion of the discs and fundi; granular eyegrounds; pallor of fundi; pallor and fuzziness of the discs; one with slight excavation of the disc; and one with an exudate. Linn believed that slight congestion which may have slightly increased after methanol was on a vascular basis due to reactions to fright or struggling.

Fink (1943)[67] concluded that he had demonstrated ganglion-cell degeneration and edema of the nervehead in histopathologic sections of dogs and rabbits poisoned with repeated doses of methanol. He found no apparent visual disturbances. Experimental details were not published, but Dr. Fink most kindly made available to us the protocols of his experiments. Five rabbits were given approximately (taking average rabbit size to be 2.0 kg.) 4.0 gm. of 100-percent methanol/kg. every other day, six doses. Slightly dilated or tortuous vessels were noted in all. Four dogs were given 3.5 gm. of 100-percent methanol/kg. every other day, five doses. On one day, in one dog, dilated retinal vessels were seen. We assume that Fink felt the appearance of the retinal vessels was not significant since he reported negative results in his published papers. Four rabbits received about 1.0 gm. of 100-percent methanol/kg. every third day, 20 doses, and four dogs received 0.66 gm. of 100-percent methanol/kg. every third day, 20 doses, without either series showing ophthalmoscopic changes. Doses in these last two groups were less than those given by authors previously reporting in vivo ocular changes after chronic poisoning.

Røe (1948)[1] found no retinal ganglion-cell changes in rats poisoned with single oral doses or in rabbits poisoned with single and repeated oral doses. As previously noted, his rat doses were low. He made no report of clinical ocular findings.

Fanta and Mayer-Obiditch (1953)[68] reported deposition of an acidophilic material into sheaths and perivascular connective tissue of the optic nerve in an unspecified number of rabbits who were killed apparently

only several hours after eating an unspecified amount of the methanol which was poured into their food. These authors stated, "There occurred again after the shortest time paralyses in the region of the rear extremities and various signs authorized the assumption that the sight of the animals was disturbed." This was their only mention of clinical ocular findings; so from the context we believe the "various signs" were early narcotic effects such as ataxia and pupillary changes, rather than those of toxic amblyopia.

Marconcini (1953)[69] claimed that subconjunctival injections of hydrogen peroxide apparently ameliorated the histopathologic changes in the optic nerves of rabbits after single systemic doses of methanol. He reported "intense hyperemia of the vessels of the ocular fundus" in three out of his series of four rabbits. One rabbit was given 3.0 gm. of 15-percent methanol/kg., intravenously; the other three were given 2.5 gm./kg., intravenously. Noe[56] reported 4.2 gm. of 20-percent methanol/kg. was the intravenous minimum lethal dose for rabbits. Thus Marconcini's doses were low.

III. Summary

A. Much of the experimental work, from which methanol-induced acidosis in nonprimates is claimed, is technically inadequate. The cases shown in Table 1 have had CO_2-combining capacity determinations made before and after methanol poisoning. This is in contrast to the frequent development of severe acidosis in humans following single oral doses. The numbers involved do not constitute adequate proof or disproof of a similar frequent development of acidosis in nonprimates.

B. Although there are many claims in the literature of clinical visual loss in experimental animals, some are erroneously based on four common sources of confusion which are not related to the typical methanol amblyopia seen in humans. Other papers are inconclusive because (1) animals were given doses which were probably subtoxic or (2) insufficient evidence was reported. Some papers reporting negative results did not include ophthalmoscopic examinations.

Five authors[22, 34, 35, 47, 51, 67] reported negative clinical ocular and ophthalmoscopic findings in rats, rabbits, dogs, and two rhesus monkeys. Almost all of these animals were chronically poisoned by oral administration. One paper[50] reported negative findings in brightness discrimination with rats.

One paper[40] reported positive clinical and ophthalmoscopic changes in chronically poisoned rabbits. We mentioned the possibility that these changes could be on the basis of confusion of appearance in view of myelinated nerve fibers, rather than on the basis of a true toxic amblyopia.

One paper[35] reported one rhesus monkey, fully documented and reasonably incontesta-

TABLE 1

CASES IN THE LITERATURE SHOWING CO_2 COMBINING CAPACITY DETERMINATIONS BEFORE AND AFTER METHANOL POISONING

Species	Number of Animals	Dosage	Result	Acidosis	Reference No.
Dog	3	Repeated	Fatally poisoned	Severe	21
Dog	2	Single	Fatally poisoned	None	15
Dog	1	Single	Nonfatally poisoned	Moderate	15
Dog	1	Single	Nonfatally poisoned	None	21
Rabbit	3	Repeated	Exper. killed but probably fatal doses	None	1
Rabbit	2	Single	Exper. killed but probably fatal doses	None	1
Rat	9	Single	Exper. killed but probably sublethal doses	None	1

ble, with clinical and ophthalmoscopic evidence of toxic amblyopia caused by methanol.

C. Authors' summaries of histopathologic findings in experimental animals following methanol poisoning were listed. Some reported positive findings, mainly of retinal ganglion-cell and optic-nerve degeneration; others were of the opinion that these changes could all be accounted for by autolysis and fixation techniques. We did not make a critical evaluation of histopathologic experiments.

Material and Methods

I. Animals

Rats were male albinos of the Sprague-Dawley strain.

Rabbits were male albinos.

Dogs were mongrel males obtained from the city pound.

Monkeys were wild male rhesus macques (macacus malata), all apparently in good health at the beginning of experiments. Twelve days was the minimum stay in the laboratory prior to being used in experiments. During experiments they were lodged in an air-conditioned room and were given daily multivitamin supplements.

II. Solutions

A. Methanol, Merck, 99.5-percent reagent methanol, acetone free, was used throughout. Concentrations were chosen after consideration of two factors:

1. Probable stomach capacity, since a volume exceeding this would cause regurgitation.

2. Tendency of high concentrations to cause vomiting. Since toxic doses of methanol are so high in nonprimates, choosing a successful procedure was sometimes difficult. Most rodents got 50-percent methanol (weight); whereas, dogs got 16 to 26-percent, and monkeys got five to 22-percent.

B. Sucrose. Methanol solutions for dogs and monkeys contained up to 20 gm. of sucrose. The purpose in adding the surcose was prevention of methanol irritation to the gastric mucosa, which is conducive to vomiting. Since the monkeys showed no signs of nausea or vomiting, this could well be discontinued in future monkey experiments. However, most dogs were nauseated. Apparently the surcose, plus rapid return to feet and elevating the mouth at the end of gavage, plus flattery, kept early vomiting from occurring in all but two of the dog experiments. Keeney and Mellinkoff[70] "postulated that glucose may be a valuable adjunct to alkalinization" in the treatment of methanol poisoning, but Clark and Gibson[20] found glucose alone did not lessen toxic effects of methanol in dogs. Since food intake before experiments was unregulated in our animals, we would not expect the amounts of sucrose given to have any significant additional systemic effect.

III. Route of administration

Methanol solutions were given by gavage to unanesthetized animals, except for the first four rabbit experiments in which it was given intravenously. For rats, a curved steel needle with a bulbous tip was introduced into the esophagus; jaws were held open with string. Rubber catheters introduced orally between wooden mouth gags were used in rabbits (size 12, French) and dogs (size 26, French). Size-8 French catheters were introduced nasally in monkeys, with jaws held firmly closed.

IV. Acidosis studies

A. *Apparatus for carbon dioxide combining power determinations*

The Van Slyke manometric apparatus and technique was used for the first five rabbit experiments.

The Lazarow microgasometric apparatus[71] was used for all other CO_2-combining capacity determinations. This method[72] in our hands had the advantage over the Van Slyke

of greater reproducibility, greater rapidity, and the requirement of less blood.

In all listings of CO_2-combining capacities, all single results are averages of the two closest replicate determinations except when these had an average deviation of more than five percent. In that case the closest replicate determinations are also listed.

B. *Blood specimens*

1. Rats were killed by decapitation with sheep emasculating shears with the sharp edge toward the body. Mixed arterial and venous blood was then collected from drippings from the neck vessels.

2. Rabbits. In the early experiments in which the Van Slyke was used, blood was withdrawn after cut downs from the jugular and by heart puncture. In later experiments bloods were obtained when possible from razor cuts of the ear artery; on a few occasions they were gotten by heart puncture.

3. Dogs. Bloods came from the external jugular or the leg veins.

4. Monkeys. Bloods were obtained from leg veins when possible; otherwise from the femoral.

Any possible variations in the CO_2-combining capacities due to varying sources of blood would be expected to be insignificant beside the 20 to 30 vol. percent drop which occurs in methanol acidosis.

Bloods were collected with a drop of aqueous solution of U.S.P. Heparin (sodium salt) as anticoagulant and centrifuged on the day they were drawn. The separated plasma was kept frozen until the CO_2-combining capacity determinations were made.

Results

Our experiments dealt with acute methanol poisoning by single oral doses in rodents, dogs, and the rhesus macaque. In a few rabbits the intravenous route was used. Four types of observations, contrasting the differences between primates and nonprimates, are presented: (1) Levels of toxic doses, (2) clinical symptoms, (3) eye findings, and (4) acidosis studies. Complete data are reported in Tables 2 through 7.

I. Lethal doses

Lethal doses for rodents and dogs were six to 10 times those for humans. Monkeys had lethal doses in the same range as humans. All toxicity data now being considered are for single oral doses. Our primary interest in this study was to give lethal doses which would permit the animals to survive for 24 hours. In order to achieve this we necessarily accumulated our own toxicity data. Data from the literature are listed in Table 8.

A. *Rodents*

1. Rats. In a series of 23 rats, 9.5 gm. of 50-percent methanol per kg. was the approximate LD_{70}.

2. *Rabbits.* While we did not run a toxicity series on rabbits, we found that out of three rabbits, all died between 24 hours and three days when given 7.0 gm. of 30-percent methanol per kg. orally. One rabbit given 7.0 gm. of a 50-percent solution died in less than 24 hours.

B. *Dogs*

We had nine dog experiments, using 16-percent to 26-percent by weight methanol, with these results:

Oral Dose (gm./kg.)	Results
2.5	Survived
3.5	Survived
4.0	Died 29-46 hr.
4.5	Survived
5.5	Survived
6.4*	Survived
7.0	Survived
8.0	Survived
9.0+*	Died 28-42 hr.

C. *Monkeys*

Acute oral toxicity studies on the rhesus macaque have not previously been reported.

* Estimated because of early vomiting. See Table 6.

Our results using single oral doses of methanol were:

Oral Dose (gm./kg.)	Results
1.0	Survived
2.0	Survived
3.0	Died 32-38 hr.
4.0	Died 29-36 hr.
6.0	Died at 29 hr.
8.0	Died 6-23 hr.

This series is of course too small to give more than approximate lethal doses, especially since, as with humans, nonhuman animals in a given species probably show considerable individual variation to methanol poisoning.

II. General clinical picture

Previous reports on the clinical picture of methanol poisoning have not emphasized the difference between primates and nonprimates—a difference as great as two separate diseases. The first 24 hours after poisoning provided the sharpest contrast in that rodents and dogs getting much less methanol than the minimum lethal dose showed severe symptoms; whereas, a monkey receiving a lethal dose might have no symptoms during the first day except questionable mild intoxication.

The general clinical picture seen in various rodents and dogs was essentially the same. In addition to the animals reported in this paper, our report of clinical findings is also drawn from previous work[73] using hundreds of mice given methanol intraperitoneally.

In nonprimates, as might be expected from the higher lethal doses, narcosis was a predominant symptom. Usually in about a half hour to an hour after oral administration of methanol (less when given intravenously or intraperitoneally) intoxication occurred, as evidenced by varying degrees of ataxia and hypermotility, plus always a change in mental status toward marked happiness and amiability. Previously vicious individuals could be extensively handled without gloves.

From about an hour to several hours after administration of methanol (dependent upon the dose as well as the route of administration), the animals became semicomatose or comatose. Semicoma was considered to be present when no spontaneous motion occurred. Coma consisted of progression to absence of response to pain. Coma lasted from several hours up to four days. Eyelids were kept open and survivors of lengthy comatose states developed exposure keratitis. Most deaths occurred without recovery from semicoma or coma. In general, the picture was one of early onset of severe symptoms continuing unabated (no latent period) until death.

In contrast, rhesus macaques reacted clinically to methanol just like humans. Below a lethal dose, and occasionally with one, they did not get very intoxicated or show any other symptoms. Usually occurring with a lethal dose, monkey inebriation showed almost every type of individual variation which can be seen in intoxicated humans, although increased amiability was not obvious.

Semicoma appeared on the first day only in the monkey getting over two and one-half times the minimum lethal dose and dying probably around 12 hours after methanol. The morning following the administration of methanol usually found the monkeys apparently normal. This was identical with the latent period in humans.

Later on in the second day, monkey pull on chain, which had been slightly diminished during inebriation but had recovered the morning after, would again weaken. The resistance exerted by a monkey to a pull on his chain was a surprisingly good index of his physical well-being. Pull on chain progressively weakened in fatal cases. Eventually, the monkey became sick enough to lie down during daylight in the presence of humans. Semicoma was seen only shortly prior to death. Deaths occurred from respiratory failure.

According to the literature, humans react

to methanol in the same way as monkeys.

III. Eye examinations

Pupillary changes and exposure keratitis were mentioned in our review of the literature. With the onset of narcosis in nonprimates, occurring an hour or so after poisoning, pupillary changes sometimes occurred: mydriasis or miosis, with or without sluggish and sometimes absent reactions to light. During coma eyelids were almost always open; and, when coma lasted about a day or longer, exposure keratitis, secondary infection, and corneal opacification frequently occurred. Two monkeys showed dilated, unreacting pupils a few hours prior to death. They had slight ophthalmoscopic changes, but they were also semicomatose. Consequently, there can be no certainty as to whether pupillary changes in these monkeys were due to toxic amblyopia or to a narcotic effect.

Positional horizontal nystagmus was observed in nonprimates. This was unfortunately not studied in our monkeys; however the literature contains reports of positional nystagmus, similar in character to that seen in our nonprimates, observed in humans poisoned by methanol and ethanol (Menne,[74] Gorman,[75] Meyer zum Gottesberge[76]).

Some authors briefly mentioned the observation of nystagmus in nonprimates after poisoning with methanol and with higher alcohols.[29, 30, 37, 41, 52, 53] The nystagmus developed usually about a half hour after administration of methanol during the intoxicated stage. It was usually present only with the animal's head in the lateral position. When occasionally present in other positions, it was more severe in the lateral position. At first the quick component of the horizontal nystagmus was always down with the animal's head in the lateral position. Duration was from several hours to two days. When it persisted for a day, after the first day in a few instances the quick component of the horizontal nystagmus was up when the animal's head was in the lateral position. No monkeys showed nystagmus in the erect position, but none were examined early in the lateral position.

Repeated ophthalmoscopic examinations were made only on monkeys, dogs, and the rabbits receiving methanol by gavage. One moribund rat was examined; but the observer believed that, although fundi seemed normal, magnification was insufficient for certainty.

Myelinization of retinal fibers in the rabbit makes difficult the appraisal of minor changes involving the discs. However, under our experimental conditions, no rabbits showed any fundus changes.

None of our dogs showed any ophthalmoscopic changes. The disc margins of many dogs normally appeared fuzzy because of tiny irregularities in bordering pigmentation.

Two out of six monkeys, both receiving lethal doses, showed eyeground changes. One developed a small monocular retinal hemorrhage one-half disc diameter temporal to the disc just prior to death and 29 hours after administration of 6.0 gm. methanol/kg. The other, given 3.0 gm./kg., showed at 25½ hours, slight but definite blurring of the temporal disc margins and questionable retinal venous engorgement, O.U. At 31½ hours disc margins were blurred everywhere except nasally, there was possible hyperemia of the discs, and veins near the disc had a diameter estimated to be triple that of the accompanying arteries. Death occurred between 31¾ and 37½ hours, with no examinations made after 31½ hours. At the time eyeground changes were seen, both of these monkeys were too weak to resist handling; thus, there was no question of vascular changes induced by neck stricture.

IV. Acidosis studies

A. *Rats*

Nine white male rats of similar ages were given 9.0 gm. of 50-percent methanol/kg. —approximately the rat oral LD_{10}. Bloods were obtained, each time in three animals, at 4½, 27, and 47 hours after administration

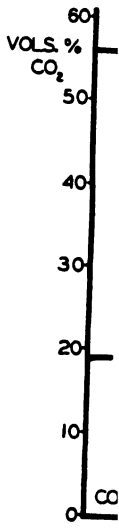

Fig. 1 (Gilger

of methanol. Their C(
ranged from 47 to 80

B. *Rabbits*

Rabbits were found
jects for acidosis stu
mendous variation in
capacity, not only betv
but also in the same
of control periods last
Our rabbits had a nor
to 56 vol. percent.* (
using anesthetized rab
peated 5.0 to 10 cc. b
ported only in the
these experiments laste
which probably did no
for the development o

* Fröhlich[77] found a n
from 26 to 58 vol. percent
loss of 6.0 cc., three times i
decrease in rabbit CO_2-co
reported that ether caused d
combining capacity of ra
finding of considerable indiv
combining capacity obtaine
from unanesthetized rabbits
period, the conclusions of
open to question.

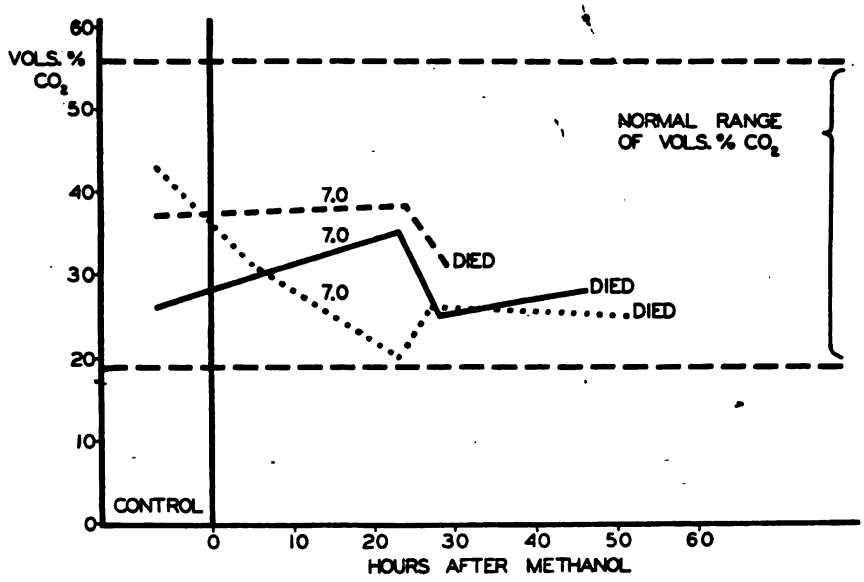

Fig. 1 (Gilger and Potts). Time course of plasma CO_2-combining acapacity in rabbits after 7.0 gm./kg. oral methanol.

of methanol. Their CO_2-combining capacities ranged from 47 to 80 vol. percent.

B. *Rabbits*

Rabbits were found to be very poor subjects for acidosis studies. There was a tremendous variation in normal CO_2-combining capacity, not only between different animals, but also in the same rabbit over the course of control periods lasting up to three weeks. Our rabbits had a normal variation from 19 to 56 vol. percent.* Our five experiments using anesthetized rabbits and drawing repeated 5.0 to 10 cc. blood samples are reported only in the tables. In addition, these experiments lasted less than eight hours which probably did not give sufficient time for the development of acidosis. Suffice it to say here that none showed severe acidosis following methanol.

In four rabbit experiments, 7.0 gm. methanol/kg. was given by gavage to unanesthetized animals, and 1.0 to 2.0 cc. blood samples were drawn. One of these rabbits, receiving a higher concentration than the others, died in less than 20 hours which might not have allowed him sufficient time to develop acidosis.

The CO_2-combining capacity determinations obtained on the three remaining rabbits are shown in Figure 1. The lines begin in the control period at the mean normal CO_2-combining capacity for each animal. Methanol produced no values less than the normal rabbit range. The dotted line rabbit showed a greatest postmethanol drop from his last normal value immediately preceding administration of methanol of 16 vol. percent; whereas, he showed a normal variability of 20 vol. percent.

C. *Dogs*

Figure 2 shows our experience with dogs. It is the same type chart as the preceding one, except that our dogs showed a normal range of only 12 vol. percent among differ-

* Fröhlich[77] found a normal range in rabbits from 26 to 58 vol. percent; he also reported blood loss of 6.0 cc., three times inside of 24 hours caused decrease in rabbit CO_2-combining capacity. Pitt[78] reported that ether caused drops in the plasma CO_2-combining capacity of rabbits. In view of our finding of considerable individual variability in CO_2-combining capacity obtained from 1.0 cc. samples from unanesthetized rabbits inside of an eight-hour period, the conclusions of these authors may be open to question.

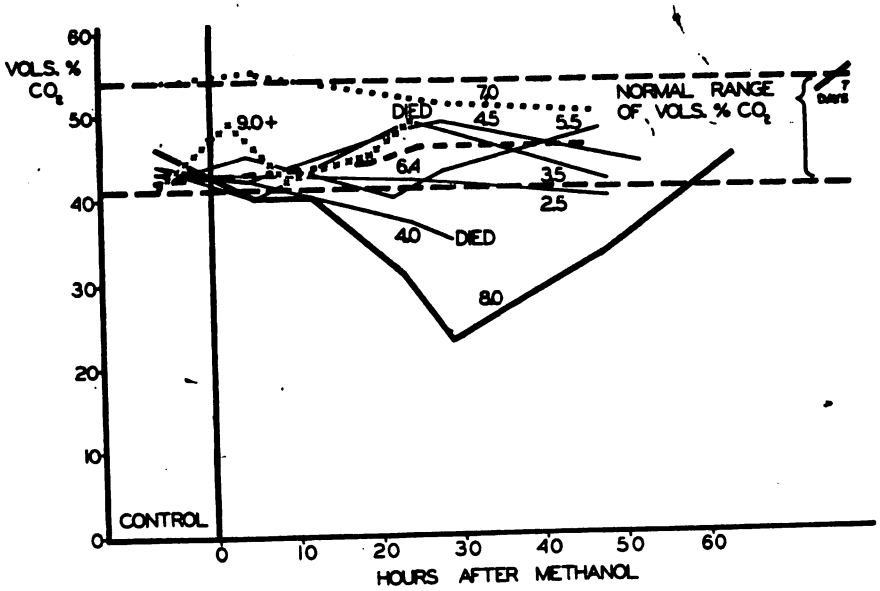

Fig. 2 (Gilger and Potts). Time course of plasma CO_2-combining capacity in dogs after oral methanol. Methanol dose (gm./kg.) indicated adjacent to each curve.

ent animals, and the variation of an individual dog over a period of weeks was insignificant. Control values, unlike those in the rabbit chart, are each from single blood samples drawn shortly before the administration of methanol. Our dogs receiving doses lower than 6.3 gm./kg. are charted with narrow unbroken lines.* All of these dogs showed toxic symptoms, but their lack of acidosis should not be given as much weight as in the others. The slight drop shown by the 4.0-gm. dog who died is insignificant when compared with monkeys or people. The dogs represented by heavier lines all received doses which, according to the literature, should have killed them. Only the dog (line of dashes) who received 8.0 gm./kg. and survived, developed acidosis. His lowest CO_2-combining capacity was 23 vol. percent. The thick solid-line dog, who received over 9.0 gm./kg. and died after 24 hours, maintained a normal acid-base balance.

The possibility occurred to us that dogs as a species might be resistant to the development of acidosis. Therefore, one of our surviving dogs was given dilute HCl orally. Inside of three hours his CO_2-combining capacity had dropped over 20 vol. percent.

D. *Monkeys*

Monkeys again reacted like humans (fig. 3). Our first monkey receiving 8.0 gm./kg. is not charted because we got only one blood sample due to our inexperience with the clinical course of methanol poisoning in primates at that time. Survival occurred only with 1.0 and 2.0 gm./kg. With 1.0 gm.—the dot-dash line—the alkali reserve was unchanged. With 2.0 gm—the solid line—the CO_2-combining capacity dropped by 24 hours to 16 vol. percent and by 48 hours had begun to rise. This 2.0-gm. monkey made quite a contrast with our 7.0 gm./kg. dog.

The dog, after getting horribly drunk, was comatose for 24 hours. He was unable to stand for three days. His CO_2-combining capacities varied during this time between 50 and 54 vol. percent.

The 2.0 gm./kg. monkey was not intoxi-

* In the only sizeable series[28] of dogs given single oral doses of methanol, 6.3 gm. of 100-percent methanol/kg. was found to be the LD_m.

cated. The only clini
able impairment in g
The monkeys gett
4.0, and 6.0 gm./k
capacities decreased
15 vol. percent; an
three cases without r
acidosis.

Conc

I. Lethal doses

We found the a
lethal doses to be:

Animal	Times
1. Rats	.....
2. Rabbits	.....
3. Dogs	.....
4. Monkeys	.....

These doses in ra
comparable to the fig
erature.

Harnack[79] in 191
fact that with singl
was the least toxic
in experimental anim
little methanol could
death. From this he

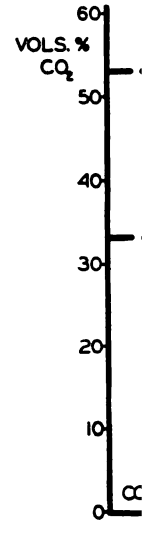

Fig. 3 (Gilger and
meth

cated. The only clinical finding was questionable impairment in grasp.

The monkeys getting lethal doses of 3.0, 4.0, and 6.0 gm./kg. had CO_2-combining capacities decreased by 24 hours to less than 15 vol. percent; and death followed in all three cases without recovery from the severe acidosis.

Conclusions

I. Lethal doses

We found the approximate single oral lethal doses to be:

Animal	Times mean human lethal dose
1. Rats	9
2. Rabbits	7
3. Dogs	9
4. Monkeys	3

These doses in rats, rabbits, and dogs are comparable to the figures available in the literature.

Harnack[79] in 1912 was impressed by the fact that with single lethal doses methanol was the least toxic of the aliphatic alcohols in experimental animals, whereas in man so little methanol could cause blindness and/or death. From this he concluded that methanol reacted differently in different animals. We are in entire agreement with Harnack but would differentiate mainly between primates and nonprimates. Nonprimates required seven or more times the average human lethal dose. Death occurred in our monkeys at 3.0 gm./kg.; whereas humans have been reported as having survived 2.9 gm./kg.[27] Our series of monkey experiments was too small to get accurate toxicity figures but obviously monkey single oral lethal doses are of the same order of magnitude as those for humans.

II. General clinical picture

The general clinical picture of methanol poisoning in primates and nonprimates was that of two different diseases. Nonprimates showed severe early intoxication and narcosis; narcosis lasted until death. Primates showed much less intoxication than nonprimates and much less than primates affected by ethyl alcohol. They then had a symptomless latent period followed by sickness and death. Narcosis appeared only as a terminal manifestation.

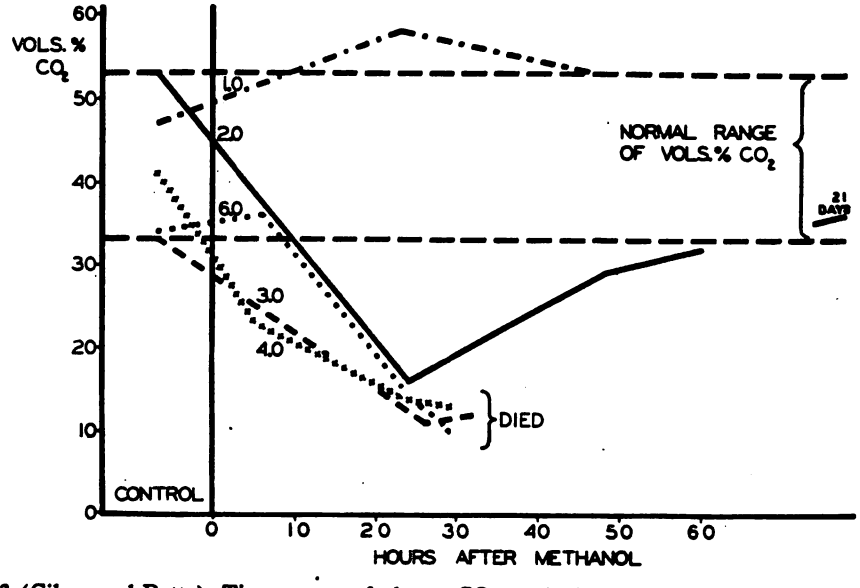

Fig. 3 (Gilger and Potts). Time course of plasma CO_2-combining capacity in monkeys after oral methanol. Methanol dose (gm./kg.) indicated adjacent to each curve.

III. Eye examinations

Early pupillary changes and corneal opacification following exposure keratitis in nonprimates are considered to be due to the central narcotic effects of methanol and not to toxic amblyopia.

The positional horizontal nystagmus produced by methanol has only been of passing interest to most investigators, many undoubtedly overlooking it. Our observations have been inadequate to illuminate the mechanism whereby this is caused.

Eyeground changes were not seen in nonprimates following methanol poisoning but were seen in primates.

IV. Acidosis studies

Our rats showed no acidosis from methanol. Wide normal variability in rabbit CO_2-combining capacities makes them unsuitable for acidosis studies. Under our experimental conditions, methanol did not cause acidosis in rabbits. Only one dog out of nine experiments developed acidosis. The rhesus macaque usually developed severe acidosis.

Thus again we found a contrast in that in nonprimates acidosis occurred rarely whereas in primates severe acidosis developed frequently.

Summary

Both the literature and our experiments indicate that only in primates is there close similarity in response to methanol poisoning with respect to: (1) Levels of toxic doses, (2) generalized clinical symptoms, (3) clinical ocular pathology, and (4) frequent development of acidosis.

University Hospitals (6).

We wish to acknowledge the assistance of Mrs. C. Stuart in locating many of the papers discussed and of Mr. S. Rehmar for translation from the Russian of reference 40 and of Mr. K. Kurahashi for translation from the Japanese of reference 63.

TABLE 2

I. Rats: A. Acute oral toxicity experiments

Experiment No.	Rat Weight (gm.)	Dosage (gm. methanol/kg. rat)	Results
23	478	3.0	Survived
	86	3.0	Survived
	523	5.5	Survived
	87	5.5	Survived
	538	8.0	Survived
	70	8.0	Survived
29	386	9.0	Survived
	347	9.0	Survived
	202	9.0	Survived
27	362	9.5	Survived
	324	9.5	Died 22–23 hr.
	388	9.5	Died at 6 da.
	346	9.5	Died 22–25 hr.
	316	9.5	Survived
	370	9.5	Died 49–68 hr.
26	391	11.0	Died 23–25 hr.
	305	11.0	Died 3½ da.
	411	11.0	Died 43–47 hr.
	391	11.0	Died 2½ da.
	393	11.0	Died 25–43 hr.
	375	11.0	Died 47–49 hr.
25	550	11.0	Died 29 hr.
	523	14.0	Died 3 hr.

Methanol was given in a 50-percent (by weight) solution.

At 3.0 gm./kg. no clinical symptoms. From 5.5–9.0 gm./kg. increasing severity of ataxia, no coma. At 9.5 gm./kg. all showed severe ataxia and two were comatose while under observation. At 11.0 gm./kg. all had severe ataxia; 6 out of 7 were comatose. At 14.0 gm./kg. the rat showed severe ataxia and coma. Nystagmus was present in many.

TABLE 3

I. Rats: B. Acidosis study

Rat No.	Rat Weight (gm.)	Decapitated at (hr. after methanol)	Plasma CO_2 Combining Capacity (vol. %)
1	291	4½	64.4
2	265	4½	46.8
3	237	4½	60.8
4	291	27	80.4
5	286	27	59.1
6	182	27	64.8
7	323	47	77.6
8	294	47	54.7
9	230	47	55.4

Each rat was given a single oral dose of 50 percent methanol/kg. body weight. All were severely ataxic and had nystagmus; two were semicomatose.

II. Rabbi

(Methanol was gi

Experiment No.	Rabbit Weight (kg.)	Anesthetic Given
3		Nembutal
4	3.3	None
5	3.4	Nembutal
6	3.6	Drop ether
7	3.5	Drop ethe

* Insufficient plasma for dupl

II. Rabbits: I

Experiment No.	Weight (kg.)	Dosage MeOH (gm./kg.)
41	2.7	7.0 (30%)
42 & 44	1.9	
		7.0 (30%)
45	2.8	
		7.0 (30%)

TABLE 4

II. Rabbits: A. Plasma CO_2-combining capacity of rabbits under anesthesia with and without methanol

(Methanol was given in 50-per cent solution intravenously. The Van Slyke manometric apparatus was used for CO_2 determinations.)

Experiment No.	Rabbit Weight (kg.)	Anesthetic Given	Time after Start of Experiment (min.)	MeOH (gm./kg.)	Total MeOH (gm./kg.)	CO_2 capacity (vol. %)	Remarks
3		Nembutal	10	0		50.3*	Died 65 min. after start of experiment
			20	0		51.2	
			50	0	0	25.7 (27.2, 24.2)	
4	3.3	None	0	4.2			Died 25 min. after start of experiment
			20	0		47.4	
			25	0	4.2	45.1	
5	3.4	Nembutal	25	0		52.2	Apnea for 5 min. after MeOH; recovery with artificial respiration. Died 40 min. after start of experiment
			30	1.5			
			40	0.7	2.2	31.7	
6	3.6	Drop ether	35	0		23.2	Died 150 min. after start of experiment
			40	0.7			
			50	0.7			
			70	0		36.1	
			80	2.6			
			140	0		46.6	
			150	1.4	5.4	53.9	
7	3.5	Drop ether	45	0		19.0	Killed 380 min. after start of experiment
			70	4.0			
			140	0		17.9 (19.0, 16.8)	
			195	1.0			
			210	1.0			
			225	1.0			
			240	1.0			
			255	1.0			
			270	1.0			
			285	1.6		34.7*	
			345	0		21.8*	
			380	0	11.6	21.8*	

* Insufficient plasma for duplicate determinations.

TABLE 5

II. Rabbits: B. Experiments giving methanol by gavage without anesthesia and using Lazarow apparatus

Experiment No.	Weight (kg.)	Dosage MeOH (gm./kg.)	Time Normal Bloods Drawn (days)	Time Drawn after MeOH (hr.)	CO_2 capacity (Vol. %)	Eye-grounds	Died (hr. after MeOH)	Remarks
41	2.7		1		25.8	Normal		Ataxia. Comatose from 2–22 hr.; semi-comatose thereafter until death. Nystagmus. At 3 hr. pupils unreactive; from 23 hr. on pupils reactive. Died while sample 4 was being drawn. Immediate heart puncture. 46 hr. point on Figure 1 is average of samples 4 and 5.
		7.0 (30%)		23	35.0	Normal		
				28	25.2	Normal		
				46	26.4 (28.0, 24.8)	Normal	46	
				46	30.5			
42 & 44	1.9		1		39.0	Normal OS		Ataxia. Comatose from 1–5 hr.; semi-comatose thereafter until death. Nystagmus. Had previous surgery, O.D.
			2		40.3			
			15		56.2			
			21		36.5			
		7.0 (30%)		5	31.4	Normal		
				23	20.4	Normal		
				27	25.7	Normal	Between	
				51	24.6	Normal	52 and 69	
45	2.8		1 (a.m.)		41.4	Normal		Ataxia; semicomatose from ½ hr. until death. Nystagmus.
			1 (p.m.)		28.6			
			6		41.1			
			13		39.9			
			20		35.2	Normal		
		7.0 (30%)		24	37.5		Between	Pupils moderately dilated.
				29	30.8	Normal	29 and 48	

TABLE 6

III. CO_2-COMBINING CAPACITY OF DOGS GIVEN SINGLE ORAL DOSES OF METHANOL

Experiment No.	Weight (kg.)	Dosage MeOH (gm./kg.)	Percent MeOH (by weight)	Time after MeOH (hr.)	CO_2 capacity (vol. %)	Hyperpnea	Remarks
22	19.9	2.5	17	0	45.2	Normal	Slight ataxia from 1–5 hr. Nystagmus.
				24	41.7	Normal	Somnolence. Recovery by 48 hr.
				48	40.0		
24	16.7	3.5	23	0	44.3	Normal except disc O.D. pink disc O.S. gray	Severe ataxia ½–4½ hr. Somnolence. Recovery by 24 hr.
				5	40.3		
				24	48.2	No change, O.U.	
				48	42.1	No change, O.U.	
18	8.2	4.0	16	0	43.7	Normal	Marked ataxia after 15 min. Somnolence. Nystagmus. By 24 hr. severe hangover (weakness, ataxia, somnolence, "dry heaves," the "shakes"). Because of coughing was examined by veterinarian who reported normal temperature and lungs clear to percussion and auscultation. Died between 29 and 46 hr. No autopsy.
				5	41.6	Normal	
				24	37.2	Normal	
				29	35.3		
28	17.3	4.5	24	0	42.5	No change	Used 30 days previously in Experiment 24. Marked ataxia ½–23 hr. Marked euphoria. Somnolence. Recovery by 28 hr.
				4	42.4		
				23	48.3		
				28	49.4		
				52	43.9	No change	
31	13.5	5.5	25	0	42.4	Normal	Euphoria and ataxia at ½ hr. Comatose with opisthotonos 3–22 hr. Nystagmus. At 22 hr. could not walk. At 28 hr. could walk but not navigate stairs. Recovery by 47 hr.
				4	44.5		
				22	40.2		
				28	42.9	Normal	
				47	41.8		
32	13.5	Given (O) 200 cc. of 6% (by vol.) HCl.		After HCl 0	47.0		Dog used 34 days previously in Exper. 31. About 100 cc. vomited in first half hr. About 30 cc. bright red vomitus at 2½ hr. Diarrheal stools from 4–6 hr. Recovery by 24 hr.
				3	26.7		
				6	30.5		
34	13.4	6.4	24	0	41.8	Normal	Dog used 7 days previously in Exper. 32. Given 6.5 gm. MeOH/kg. Immediate regurgitation of an estimated 30 cc. Dosage thus 6.4 gm./kg. Ataxia and euphoria beginning at 45 min. Unable to stand. Nystagmus. Hangover at 24 hr. Recovery by 44 hr.
				19	43.5		
				25	46.3	Normal	
				45	45.8		
46	11.2	7.0	26	0	54.1	Normal	½ hr.—ataxia and euphoria
				5	54.8		1 hr.—semicomatose
				23	51.7	Normal	3–25 hr.—comatose
				29	51.4	Normal	25–27 hr.—semicomatose—pupils 1–2 mm.
				46	49.5	Normal	diameter. 27–46 hr.—comatose
				3 da.	49.3	Normal	3–5 hr.—convulsive running movements of
				8 da.	53.7	Normal	legs. Positional nystagmus early. 96 hr.—able to stand. Recovery by 7 da.
47	12.4	8.0	27	0	46.2	Normal	10 min.—ataxia and hyperactivity
				5	39.9	Normal	50 min.—unable to stand. Nystagmus. 1½ hr.—4th day—comatose, lids open, ex-
				12	40.3		posure keratitis. 1½–2½ hr.—conjugate de-
				23	30.7	Normal	viation of eyes to left; running leg move-
				29	23.3	Normal	ments. 4–5 hr.—pupillary reactions slight
				47	32.9	Normal	or absent; 24 hr.—quick component of ny-
				7 da.	55.4	Normal O.D. Cornea O.S. opaque	stagmus reversed in direction; peripheral shock. 47 hr.—nystagmus continued. 7th day—tried to sit; too weak to walk 15th day—could walk; left foreleg 3 × size of right. Both corneas opaque obscuring fundi.
49	11.4	9.0+	26	0	41.1	Normal	1 hr.—had vomited a vol. estimated to be 100 cc. greater than gavage. Moderate ataxia. Re-gavaged with identical solution (9.0 gm./kg.) at 70 min. No vomiting after 2nd gavage =9.0+gm./kg. 10–15 min. after 2nd gavage—comatose; nystagmus. 19 hr.—still comatose—exposure keratitis—2 mm. pupils. Death occurred between 28 and 42 hr. without recovery from coma.
				2	48.8	Normal	
				9	42.2		
				19	44.6	Normal posterior pole	
				24	48.6	Normal	

TABLE 7

IV. CO_2-COMBINING CAPACITY OF MONKEYS GIVEN SINGLE ORAL DOSES OF METHANOL

Experiment No.	Weight (kg.)	Dosage MeOH (gm./kg.)	Time after MeOH (hr.)	CO_2-capacity (vol. %)	Eyegrounds	Remarks
19	2.72	1	0	46.7	Normal throughout	No general clinical symptoms
			23	58.1		
			48	53.3		
21	2.19	2	0	52.7 (ave.)	Normal throughout	Monkey used 15 days previously in Exper. 19. No detected clinical symptoms with the following possible exception: At 24 hr. he got partly loose and failed to bite the observer. Subsequently became a pet, showing no evidence of impairment of vision. Died 6 mo. later from acute miliary tuberculosis
			24	15.9		
			48	28.7		
			21 da.	36.3		
20	3.45	3	0	32.6	Normal	No definite signs of intoxication. At 25½ hr. sick, weak, had to lie down after struggling. Pupils dilated but reacted to light. At 33½ hr. sicker; lying down; made only rare spontaneous movements; pupuls dilated and unreactive; hippus present; did not blink at threatening gestures but was semicomatose. Died 34–39½ hr. Rigor mortis present at 39½ hr.
			8	23.3	Normal	
			26	10.6	Blurring temporal disc margins, O.U. Questionable venous engorgement, O.U.	
			34	12.4	O.U.:—disc margins blurred except nasally. Retinal veins had diameter 3 × that of arteries; possible hyperemia of discs	
17	2.88	4	0	41.5	Normal throughout	Ataxia marked by 75 min. At first quiet but by 3 hr. was combative. Apparently mentally alert, although pull on chain weaker. At 22½ hr. rested head against side of cage. At 29 hr. lying down; conscious. Death occurred between 29 and 36 hr.
			5	23.0		
			23	13.9		
			29	12.7		
16	3.32	6	0	33.7	Normal	Ataxia marked by 80 min.; pull on chain weak
			6	35.6	Normal	At 23 hr. stood and was apparently normal
			24	14.4	Normal	At 24 hr. lying down and very weak
			28	11.3 (10.2, 12.1)	Normal	At 27 hr. became semicomatose, remaining so until death
			29	9.8 (10.8, 8.7)	Pinpoint retinal hemorrhage temporal to disc in one eye	At 29 hr. pupils were dilated and unreactive
			29½	14.4		Death at 29½ hr. following respiratory failure
15	2.76	8	5	39.2	Normal throughout	Ataxia marked by 70 min.; increasingly weak
						At 2 hr. lying down; very sick
						At 3½ hr. semicomatose, remaining so
						Death occurred between 6 and 23 hr.: at 23 hr. rigor mortis was pronounced

TABLE 8

ORAL SINGLE LETHAL DOSES OF METHANOL IN NONPRIMATES

Species	Dose gm./kg.	% Methanol	Result	Number of Animals	Source
Rat	8.3	70	LD_{50}	48	Alder, et al.[28]
Rabbit	7.2	?	MLD		Baer[30]
Rabbit	14.2	?	LD_{100} in less than 24 hr.		Munch and Schwartze[53]
Dog	6.3	100	LD_{50}	15	Haskell, et al.[15]
Dog	6.3	50	Approx. MLD	4	A. Leo[54]

Human lethal doses are often difficult to compute accurately. In the literature[27] there are reports that death has resulted from as little as 0.34 gm./kg. and survival has occurred after as much as 2.9 gm./kg. The dose generally accepted as lethal is about 0.85 to 1.4 gm./kg.

REFERENCES

1. Røe, O.: The ganglion cells of the retina in cases of methanol poisoning in human beings and experimental animals. Acta. Ophth., **26**:169-182, 1948.

2. Benton, C. D., Jr., and Calhoun, F. P., Jr.: The ocular effects of methyl alcohol poisoning: Report of a catastrophe involving 320 persons. Am. J. Ophth., **36**:1677-1685, 1953.

3. Schmiedeberg, O.: Ueber Methylalkoholvergiftung. Therap. Monatsh., **26**:329-331, 1912.

4. Pohl, J.: Ueber die Oxydation des Methyl und Aethylalkohols im Thierkörper. Arch. exper. Path. u. Pharmakol., **31**:281-302, 1893.

5. Bongers, P.: Ueber die Ausscheidung körperfremder Stoffe in den Magen. Arch. exper. Path. u. Pharmakol., **35**:415-436, 1895.
6. Król, J.: Ueber das Wesen der Methylalkoholvergiftung. Arch. exper. Path. u. Pharmakol., **72**:444-456, 1913.
7. Grignolo, F.: Biochemische Veränderungen im Kammerwasser bei akutem Intoxikationen durch Methylalkohol und durch Toxipeptide. Klin. Monatsbl. f. Augenh., **15**:157-163, 1913.
8. Tyson, H. H., and Schoenberg, M. J.: Experimental researches in methyl alcohol inhalation. J.A.M.A., **63**:915-922, 1914.
9. ———: Experimental researches in methyl alcohol inhalations. Tr. Sect. Ophth. A.M.A., 1914, pp. 354-382.
10. ———:Changes in the blood and aqueous humor in methyl alcohol inhalation. Arch. Ophth., **44**:275-280, 1915.
11. Berg, M., Mayne, A., and Peterson, W. F.: Variability of blood pH and its association with meteorlogical factors. Am. J. Physiol., **130**:9-21, 1940.
12. Duke-Elder, W. S.: Textbook of Ophthalmology. London, Kimpton, 1942, v. 1, p. 438.
13. Van Liere, E. J.: Anoxia: Its Effect on the Body. Chicago, Univ. Chicago Press, 1942, pp. 61-62.
14. Koehler, A. E., Brunquist, E. H., and Loevenhart, A S.: The production of acidosis by anoxemia. J. Biol. Chem., **64**:313-323, 1925.
15. Haskell, C. C., Hileman, S. P., and Gardner, W. R.: The significance of the acidosis of methyl alcohol poisoning. Arch. Int. Med., **27**:71-82, 1921.
16. Loewy, A., and Münzer, E.: Beiträge zur Lehre von der experimentellen Säuervergiftung. III Mitteilung. Führt Methylalkoholvergiftung zu Acidose? Biochem. Ztschr., **134**:442-446, 1923.
17. Leo, H.: Ueber das Wesen der Methylalkoholvergiftung. Deutsche med. Wchnschr., **51**:1062-1064, 1925.
18. von Oettingen, W. F.: The aliphatic alcohols: Their toxicity and potential dangers in relation to their chemical constitution and their fate in metabolism. Public Health Bulletin No. 281. Washington, D.C., U. S. Public Health Service, 1943.
19. Rewiger, K.: Ueber den Einfluss kleiner Mengen Methylalkohols auf den Stickstoffstoffwechsel. Ztschr. ges. exper. Med., **28**:368-377, 1922.
20. Clark, B. B., and Gibson, R. B.: Treatment of experimental alcohol poisoning in dogs. Am. J. Physiol., **105**:19, 1933.
21. Clark, B. B.: Treatment of methyl alcohol poisoning with sodium bicarbonate, insulin and gluc Thesis, Univ. Iowa, 1932.
22. Alder, P., Buschke, W., and Gordonoff, T.: Experimentelle Untersuchungen über die Toxizität a Methylalkohols. Arch. internat. Pharmacodyn., **59**:416-430, 1938.
23. Potts, A. M.: Studies on the visual toxicity of methanol: VI. The clinical picture of methar poisoning in monkeys treated with base. Am. J. Ophth., **39**:86-92 (Feb., Pt. II) 1955.
24. Harrop, G. A., Jr., and Benedict, E. M.: Acute methyl alcohol poisoning associated with acidosis: Report of a case. J.A.M.A., **74**:25-27, 1920.
25. Van Slyke, D. D., and Palmer, W. W.: Titration of organic acids in urine. Proc. Soc. Exper. Biol. & Med., **16**:140-141, 1919.
26. ———: Studies of acidosis. XVI: The titration of organic acids in urine. J. Biol. Chem., **41**:567-585, 1920.
27. Røe, O.: Methanol poisoning, its clinical course, pathogenesis and treatment. Acta med. Scandinav., Supp. 182, 1946.
28. Wood, C. A., and Buller, F.: Poisoning by wood alcohol. Cases of death and blindness from Columbian Spirits and other methylated preparations. J.A.M.A., **43**:972-977; 1058-1062; 1117-1123; 1213-1221; 1289-1296, 1904.
29. Joffroy, A., and Serveaux, R.: Mensuration de la toxicité experimentale et de la toxicité vraie de l'alcool méthylique; symptomes de l'intoxication aigue et de l'intoxication chronique par l'alcool méthylique. Arch. de méd. expér. et d'anat. path., **8**:473-509, 1896.
30. Baer, G.: Beitrag zur Kenntniss der akuten Vergiftung mit verschiedenen Alkoholen. Arch. f. Anat. u. Physiol. Arch. f. Physiol., 1898, pp. 283-296
31. Holden, W. A.: The pathology of the amblyopia following profuse hemorrhage and of that following the ingestion of methyl alcohol, with remarks on the pathogenesis of optic-nerve atrophy in general. Arch. Ophth., **28**:125-134, 1899.
32. Friedenwald, H.: The toxic effect of alcohol on the ganglion cells of the retina. Bull. Johns Hopkins Hosp., **13**:52, 1902.
33. Birch-Hirschfeld, A.: Beitrag zur Kenntniss der Netzhautganglienzellen unter physiologischen und pathologischen Verhältnissen. Arch. f. Ophth., **50**:166-246, 1900.
34. ———: Experimentelle Untersuchungen über die Pathogenese der Methylalkoholamblyopie. Arch. f. Ophth., **52**:358-383, 1901.
35. ———: Weiterer Beitrag zur Pathogenese der Alkoholamblyopie. Arch. f. Ophth., **54**:68-98, 1902.

36. Hunt, R.: The toxicity of meth
37. Lesieur, C.: Nouvelles reche Physiol. et Path. gen., **8**:427-441, 1906
38. Nicloux, M., and Placet, A.: dans l'organisme de l'alcool méthyli **14**:916-931, 1912.
39. Igersheimer, and Verzár, F.: Versuche zur Pathologie des Lichtsinn
40. Kasass, I. I.: The pathology of
41. Langgaard, A.: Die Giftigkeit
42. Cusick, P. L., Benson, O. O., J f oxygen on the retinal vessels: Pr
43. Duguet, J., Dumont, P., and arterial pressure. J. Aviation Med., 1
44. Goldmann, H., and Schubert, erstoffdruck der Atmungsluft. Arch.
45. Walsh, F. B.: Clinical Neuro-
46. Birch-Hirschfeld, A.: Zwei F Deutsche med. Wchnschr., **46**:311, 1
47. Schwarzkopf, G.: Kritisches u Augenh., **48**:317-342, 1922.
48. Schanz, F.: Wirkungen des L
49. Friedenwald, J. S., and Felty, A tion.)
50. Bills, M. A., and Maukin, O.: discrimination in the white rat. J. Co
51. deSchweinitz, G. E.: Concerni of the usual central and peripheral U. Kingdom., **43**:12-109, 1923.
52. Munch, J. C., and Schwartze, Lab. & Clin. Med., **10**:985-996, 192
Rost, E., and Braun, A.: Zu hole. Arb. Reichsgesundhamt., 5
54. Leo, A.: Ueber chronische Me
55. Weese, H.: Vergleichende derer aliphatischer Alkohole. Ar
56. Noè, M.: Tossicità lontana c 366, 1929.
57. Keeser, E.: Ätiologie und the Methylalkohols. Arch. exper. Path.
58. ———: Ueber die Ursache 399, 1931.
59. McCord, C. P.: Toxicity of **23**:931-936, 1931.
60. Scott, E., Helz, M. K., and Clin. Path., **3**:311-319, 1933.
61. Sammartino, U.: Ricerche endovenosa dell'alcool metilico, de farm. sper., **56**:351-363, 1933.
62. Harada, M.: Pharmakologisc durch Farnkraut Präparate. Japa meet. Japanese Pharm. Soc., 1937, p
63. Tomita, Y.: Histopathologic methyl alcohol poisoning. Psychiat.
64. Koppanyi, T., and Cutting, in narcotic poisoning. J. Lab. & Clin
65. Sayers, R. R., Yant, W. P. J. G.: Methanol Poisoning: I. Ex Rept. of Investigations 3617, 1942.
66. ———: Methanol poisoning concentrations of methanol vapor in
67. Fink, W. H.: The ocular p 815, 1943.