Very Important J. Indust. Hyg. & Toxicol. 19:337-345 (1937)

Much faster disappearance (339) from Heart Muscle Brain Stem and Spinal Cord after being removed from source for 48 hours

Lots lower concentration than the blood (340) 339, 340, 342

DISTRIBUTION OF METHANOL IN DOGS AFTER INHALATION AND ADMINISTRATION BY STOMACH TUBE AND SUBCUTANEOUSLY*

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A STUDY of the distribution of methanol in the body fluids and tissues of dogs was made a part of an extensive investigation of the fundamentals of methanol poisoning and the health hazards from breathing methanol vapor. The primary interest in this particular phase of the investigation was to obtain information on the rapidity with which methanol was distributed throughout the body; the quantitative distribution among the various tissues and fluids with special consideration to selective absorption, accumulation, and predilection; and selective retention during elimination. Most of the work dealt with inhalation of methanol vapor in air, although for comparative purposes one experiment was made with methanol administered by stomach tube and another by subcutaneous administration.

TEST PROCEDURE

The procedure followed in experiments with methanol vapor in air consisted of exposing the dogs to known concentrations, dispatching them after predetermined periods, and determining the methanol content of various tissues and fluids. Previous experiments had shown the rate of accumulation and elimination of methanol by the blood; and, also, that for a particular concentration of methanol in the air there tended to be a maximum or equilibrium value for the blood concentration. The results of these previous experiments were used as a basis for choosing methanol-air concentrations that would produce concentrations in the blood that could be determined with satisfactory accuracy; also for choosing durations of exposure that would provide animals dispatched during the period of accumulation of methanol, after equilibrium had been established and after the exposure had been terminated and partial to practically complete elimination had taken place. From 17 to 26 different specimens were taken from each dog.

Methanol Distribution Heart Brain-Stem Spinal-Cord

For comparison one dog each was given methanol by stomach tube and subcutaneously but only a few specimens were taken. The dose given by stomach tube was 2.5 gm. methanol per kgm. of body weight and was diluted with an equal volume of water. The subcutaneous dose was 5 gm. per kgm. and was administered undiluted in the flank region. Both administra-

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Tissues about the same as blood on initial dose when taking into consideration methanol dislike for fat tions were well tolerated by the animals.

Immediately after the animals were dispatched, weighed portions of the tissues or fluids were transferred to bottles that contained enough 5 per cent sulfuric acid solution to cover the specimen. Duplicate specimens of most of the tissues and fluids were taken except for dogs 234, 237 and 240. The well-stoppered bottles were kept in a refrigerator until the distillation procedure was performed. Each specimen was first steam-distilled until a volume of 50 ml. (approximately equal to original volume) had been recovered; the distillate was made alkaline with a drop or two of a concentrated solution of sodium carbonate and then straight distilled, a volume of 20 ml. being collected. This distillate was then analyzed for methanol by the method of Chapin* using a Schiffs reagent prepared according to the method of Elvove.†

Results of Tests

Distribution of Methanol in Dogs Exposed to Methanol Vapor in Air

Eight dogs were used in the study with methanol vapor. Two dogs (M269 and M272) were exposed continuously to about 4000 ppm. methanol vapor in air by volume for 12 hours and dispatched immediately after termination of exposure. This experiment was planned to give information on the distribution at a particular time during accumulation. Previous experience indicated that the concentration of methanol in the blood at the end of 12 hours would be approximately one-third the equilibrium value. Two dogs (M234 and M237) were exposed to 15,000 ppm. methanol vapor for 22 to 23 hours and were dispatched immediately. These represent a condition during accumulation but approaching equilibrium. Two dogs (M270 and M271) were exposed to about 4000 ppm. methanol vapor in air continuously for 5 days and dispatched within an hour after termination of exposure. This group represents an equilibrium condition of the blood. Dog M240 was exposed to 15,000 ppm. methanol for 24 hours and was allowed to eliminate methanol for the succeeding 48 hours which reduced the concentration of methanol in the blood to about one-third the amount present at the end of the exposure, and represents distribution conditions during elimination. Dog M273 was exposed to about 4000 ppm. methanol vapor in air continuously for 5 days and killed 120 hours later. This represents a condition of practically complete elimination.

Table 1 gives the concentration of methanol found in the various tissues and fluids.

The amounts found for each group of 2 animals subjected to the same experimental conditions are, with few exceptions, in good agreement for particular tissues and fluids, but there is a distinct difference in the amounts found in the various tissues and fluids. The finding of practically the same amount in the various organs of dog M240 (which was exposed to 15,000

* Chapin, R. M.: Improved Denige's test for the detection and determination of methanol in the presence of ethyl alcohol. Indust. and Eng. Chem., *13*, 543 (1921).

† Elvove, E.: A note on the detection and estimation of small amounts of methyl alcohol. Indust. and Eng. Chem., *9*, 295 (1917).

p.p.m. for 24 hours and then allowed to eliminate methanol for a 48-hour period after exposure before being dispatched), as was found in M270 and found. However, the finding that the variations among the tissues and fluids of M240 at the particular stage in the elimination process is very similar to

TABLE 1

Methanol in tissues and body fluids of dogs exposed to methanol vapor in air

(Results given in milligrams of methanol in 100 grams of material as sampled)

CONCENTRATION	4000 ppm.		15,000 ppm		4000 ppm.		15,000 ppm.	4000 ppm.
CONTINUOUS EXPOSURE FOR	12 hours		22 hours		5 days		24 hours	5 days
DISPATCHED	Immediately		Immediately		Within 1 hour		48 hours later	120 hours later
DOG NUMBER	M269	M272	M234	M237	M270	M271	M240	M273
Tissue or fluid:								
Cerebral hemispheres	86	83	720	875	360	295	295	0.0
Cerebellum	80	74	[a]	925	329	282	295	0.0
Brain stem	64	64	[a]	1100	272	240	200	0.0
Spinal cord	59	59	[a]	960	265	245	145	0.0
Heart	84	75	1200	1010	332	293	160	0.0
Lungs	81	72	1120	745	315	275	300	0.0
Liver	71	61	1040	1065	305	225	296	0.0
Pancreas	71	61	750	660	270	250	280	0.0
Spleen	73	69	850	1000	285	245	280	0.0
Stomach wall	77	65	1080	880	300	275	280	0.0
Intestinal wall	73	65	800	610	270	255	250	0.0
Kidneys	95	73	1038	800	335	325	240	0.0
Testicles	[a]	[a]	850	1065	[a]	[a]	295	[a]
Muscle	78	76	1000	960	315	275	240	0.0
Thymus	[a]	[a]	[a]	1240	[a]	[a]	[a]	[a]
Eye minus aqueous and vitreous humor	63	51	[a]	590	300	211	355	0.0
Aqueous and vitreous humor	94	100	[a]	780	413	418	450	0.0
Adipose tissue, intestinal	17	7	120	80	28	45	35	0.0
Adrenal	40	2	[a]	[a]	246	166	[a]	0.0
Bone marrow	74	33	490	400	161	103	155	0.0
Blood	100	84	1470	1390	348	317	335	0.0[b]
Urine from bladder	[a]	91	1025	1365	478	390	305	0.0
Spinal fluid	[a]	[a]	[a]	705	[a]	[a]	403	[a]
Bile	96	71	[a]	1230	361	274	490	0.0
Stomach content	103	102	750	1260	439	273	360	0.0
Feces from large intestine	63	[a]	680	800	290	200	240	1.25

[a] Not determined.

[b] Concentration at end of exposure was 570 mgm. methanol in 100 gm. of blood.

M271 (exposed to approximately 4000 ppm. for 5 days) is only a coincidence which resulted from a decrease by elimination, from a value similar to that of M234 and 237, to the amount that for the other animals exposed to different conditions and before elimination had begun, is significant.

Table 2 gives values which represent the relative distribution of methanol

TABLE 2

Relative distribution of methanol in tissues and fluids of dogs exposed to methanol vapor in air—presented on the basis of 100 chosen to represent the amount found in the blood

Concentration	4000 ppm.		15,000 ppm.		4000 ppm.		15,000 ppm.	Average of all animals
Continuous exposure for	12 hours		22 hours		5 days		24 hours	
Dispatched	Immediately		Immediately		Within one hour		48 hours later	
Dog number	M269	M272	M234	M237	M270	M271	M240	
Tissue or fluid:								
Blood, from heart	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Aqueous and vitreous humor	93.9	119.0	[a]	56.2[b]	118.6	132.0	134.2	119.5
Urine from bladder	[a]	108.2	69.7	98.2	137.3	123.0	91.0	104.6
Bile	96.0	84.4	[a]	88.4	103.8	86.6	146.2	100.9
Stomach content	103.0	121.3	51.0	90.6	126.0	86.2	125.8	100.6
Heart muscle	84.0	89.2	81.6	72.6	95.4	92.5	47.7[b]	85.9
Cerebellum	79.9	88.0	[a]	66.6	94.5	82.4	88.0	83.2
Cerebral hemispheres	86.0	98.8	49.0	63.0	103.4	93.2	88.0	83.1
Kidney	95.0	86.9	70.6	57.6	96.2	102.5	71.6	82.9
Lungs	80.9	85.7	76.2	53.6	90.5	86.8	89.5	80.5
Muscle, from the leg	78.0	90.4	68.0	69.1	90.5	86.8	71.6	79.2
Stomach wall	76.9	77.3	73.5	63.3	86.3	86.8	83.6	78.2
Liver	71.0	72.6	70.8	76.6	87.6	71.8	88.3	76.9
Spleen	72.9	82.0	57.8	71.9	81.9	77.3	88.6	75.3
Brain stem	64.0	76.2	[a]	79.1	78.2	75.7	59.6	72.1
Testicles	[a]	[a]	57.8	76.6	[a]	[a]	88.0	71.8
Eye minus aqueous and vitreous humor	62.9	60.7	[a]	42.5	86.3	66.6	105.8	70.8
Pancreas	71.0	72.6	51.0	47.5	77.6	78.9	83.6	68.9
Intestinal wall	72.9	77.3	54.4	43.9	77.6	80.5	74.6	68.7
Spinal cord	59.0	70.2	[a]	69.1	76.1	77.3	43.3	65.8
Feces from large intestine	62.9	[a]	46.3	57.6	83.3	63.0	71.6	64.1
Adrenal	40.0	2.4[b]	[a]	[a]	70.7	52.4	[a]	54.4
Bone marrow	74.0[b]	39.2	33.3	28.8	46.3	32.5	46.2	37.7
Adipose tissue, intestinal	17.0	8.4	8.2	5.8	8.1	14.2	11.2	10.4

[a] No specimen taken for analysis.

[b] Values deviate markedly from the general findings and are not used in obtaining averages or ratios.

in tissues and fluids. For convenience in presenting this relation the values given in table 1 have been converted:

$$\text{Value given} = \left(\frac{\text{concentration in tissue or fluid} \times 100}{\text{concentration in blood}} \right)$$

to values which represent the relative amounts of methanol in the tissues and fluids on the basis that the concentration in the blood is 100. The tissues and fluids are listed in decreasing order in accordance with the average for all dogs, as given in the eighth column of data. As the methanol had been practically eliminated by dog M273, table 2 does not present data for this animal.

The values in table 2 show clearly a variation in the relative amounts of methanol for different tissues and fluids from the individual animals and that the aqueous fluids or semiaqueous materials have the highest, the tissues lower, and the bone marrow and adipose tissue the lowest concentrations. There are some differences in the order of the values for the various tissues and fluids from the individual animals as compared with the average group, but with few exceptions the differences which affect the change in order might readily be incurred by errors in sampling and analysis and/or differences in water content of the material from one dog as compared with another, considering the edema that accompanies methanol poisoning and especially materials such as bile, stomach content and feces. The significance of water content will be discussed later. The values for methanol in the urine are in good agreement considering that the urine in the bladder represents a collection over a period when in some experiments the concentration in the animal was changing, as by absorption during exposure or elimination following exposure, and for these reasons would not necessarily represent a condition closely associated with the concentration in the blood or tissues at a particular instant. The exceptional variations that are believed to be unexplainable errors, are the values for aqueous humor, dog M237; heart, dog M240; bone marrow, dog M269; and adrenal, dog M272. These values are not included in the average for all dogs or in the calculation of methanol concentration-water ratios.

The agreement between all the values in table 2 for a particular tissue or fluid regardless of whether the body concentration was low or high, table 1, or whether the animal was in a state of comparatively rapid accumulation of methanol (M269 and M272), comparatively slow accumulation (M234 and M237, a state of equilibrium between absorption and elimination M270 and M271 or eliminating methanol (M240) indicates that there is apparently no particular selectivity of methanol by any tissue or fluid either during accumulation, a state of equilibrium, or elimination except on the basis of its water content.

It is regretted that determinations of water content were not made on the same specimens that were used for methanol determinations, but at the time of performing this study the interest was solely that of the methanol content of the material examined. A casual observation of the relative concentration values given in table 2 indicates a relation between the concentrations of methanol and the water content of the substances. In order to study this relation the values for the water content of various kinds of tissues and fluids were obtained from several literature sources. It was not possible to obtain a great deal of data for dogs as the readily available literature dealt mainly with humans, mammalians, or the average for a group of humans, monkeys, dogs and cats. Also, it is obvious that the water content would vary somewhat for individuals, but in the main the data, regardless of its source, were in good agreement.

Table 3 gives the average water content of the tissues and fluids and the relative methanol concentration-water content ratio. The first numerical column of table 3 gives the average of the values found in the literature for the water content of a considerable number of the tissues and fluids analyzed for methanol content. The average is for data found in the literature for humans (male and female), mammalians and humans, monkeys, dogs and cats as a group. This procedure was followed owing to a lack of specific data for dogs in the literature available to the writers. Unfortunately no reliable data could be obtained for bone marrow and adipose tissue but, on the other hand, it was stated that these tissues contained about 80 to 95 per cent fat. By difference these tissues would contain a relatively small amount of water, which is in general agreement with the relation of methanol concentration to water content.

The succeeding columns of table 3 give the methanol-water ratio as obtained by dividing the relative methanol concentrations as given in table 2 by the water content of the tissue or fluid. If a direct relation exists the results for all tissues and fluids should be constant. It will be noted in the column that represents the ratios for the average of all animals, that the values obtained range from 0.95 to 1.25 and that the variations are without definite trend over the range from high to low methanol concentrations. In calculating the averages the blood, urine and bile were excluded for the reasons that the blood is the basis of calculation for the values, and the concentrations in the urine and bile do not necessarily have a direct relation to the blood concentration. Owing to lack of data of sufficient accuracy for bone marrow and adipose tissue, the ratio cannot be obtained for one extreme of the table. The previously mentioned estimations by difference

TABLE 3

Relation of methanol in tissues and body fluids to water content as shown by relative methanol concentration-water content ratios

	WATER CONTENT	RELATIVE METHANOL CONCENTRATION-WATER CONTENT RATIO				
		Dogs M269 and M272	Dogs M234 and M237	Dogs M270 and M271	Dog M 240	Average of all animals
	%					
Tissues or fluid:						
Blood, from heart	80.0	1.25	1.25	1.25	1.25	1.25
Aqueous and vitreous humor	98.7	1.08	—	1.27	1.36	1.21
Urine from bladder	95.0	1.14	0.88	1.37	0.96	1.10
Bile	86.0	1.05	1.03	1.11	1.70	1.17
Heart muscle	74.8	1.16	1.03	1.26	—	1.15
Cerebellum	79.6	1.05	0.85	1.10	1.10	1.04
Cerebral hemispheres	77.9	1.19	0.72	1.26	1.13	1.07
Kidney	76.8	1.18	0.83	1.29	0.93	1.08
Lungs	80.8	1.03	0.80	1.10	1.11	1.00
Muscle, from the leg	74.0	1.21	0.93	1.20	0.97	1.07
Liver	70.9	1.01	1.04	1.12	1.25	1.08
Spleen	75.1	1.03	0.86	1.06	1.10	1.00
Brain stem	73.3	0.96	1.08	1.05	0.81	0.98
Pancreas	72.2	1.01	0.68	1.08	1.16	0.95
Mean ratio exclusive of results for blood, bile, and urine	—	1.08	0.88	1.17	1.14	1.06

cannot be used because an error of but a few percent when dealing with the small amount of water would markedly alter the ratio, whereas the same error would not be of great significance when dealing with an organ that contained 70 percent.

The ratios obtained for the dogs exposed under a particular condition also have an apparent significance. Those obtained for dogs exposed for 12 hours to 4000 p.p.m. methanol are in general slightly lower than those obtained for two dogs that were exposed to this same concentration for 5 days. This indicates a small though rather insignificant lag of the tissues and fluids when compared to blood concentrations. The lowest ratios obtained are for animals that were exposed to 15,000 ppm. methanol for 22 to 23 hours and dispatched immediately. The approximately 16-percent lag as compared with dogs (M269 and M272) that were also in a state of accumulating methanol, is believed to be due to impaired circulation, as they were in a moribund condition at the termination of exposure. The ratios for the animal that was allowed to eliminate 60 to 70 percent of the previously acquired methanol are in general agreement with the animals exposed for 12 hours and those exposed for 5 days to 4,000 ppm. methanol. There are wider variations from the mean for this animal (dog M240), but the results for this dog represent only single specimens whereas for the other ratios the average of the results of at least single specimens taken from two dogs were used, and in some cases the average of duplicate specimens from each of two dogs was used.

It is believed that the data obtained substantiate very well the relation of water content of the tissue or body fluid to the methanol concentration; also that a knowledge of the concentration in the blood will permit a computation within practical limits of the amount in various organs. It is, of course, not intended to state that no constituents in the tissues and fluids except water influence the amount of methanol found, but from a practical viewpoint the influence of the other constituents is not significant except perhaps in the case of such tissue as bone marrow and adipose tissue.

In commenting on the lower methanol content of the bone marrow and adipose tissue and the probable solubility of methanol in the fat content, it is believed that water content is again the most important influence. The partition of methanol between water and fats is greatly in favor of water.

It is interesting to note (table 1) that the amount of methanol found in the stomach content of dogs exposed to methanol vapor and deprived of means of injestion of methanol except swallowing saliva is practically the same and in some instances even exceeds the concentration in the blood. This is good evidence that methanol is secreted into the stomach. The methanol found in the feces may have its origin in the stomach or be secreted into the intestines. The bile also contains practically the same amount of methanol as the blood.

The methanol content of the urine is practically the same as that found in the blood, bile and stomach content and confirms the findings of other work with animals and in man for both methanol and ethyl alcohol.

Distribution of Methanol Administered by Stomach Tube and Subcutaneously

In order to study briefly the effect of different modes of entrance of methanol into the blood, two additional experiments were conducted. One dog was given 2.5 gm. of methanol per kgm. as a 50-percent aqueous solution by stomach tube, and the other was given 5 gm. of methanol per kgm. subcutaneously in the flank region. The dogs were dispatched 5 hours after administration of the methanol, and duplicate samples of blood, muscle, liver and brain were analyzed for their methanol content. The results are given in table 4 as well as the relative methanol content on the basis of blood being 100. The relative methanol concentration-water content ratio, obtained by dividing the relative methanol value by the water content, is also given.

TABLE 4

Methanol in body tissues and blood after administration by stomach tube and subcutaneously

	Methanol mgm./100 gm.		Relative methanol concentration blood = 100		Relative methanol concentration-water content ratio	
	M283[a]	M284[b]	M283	M284	M283	M284
Tissue or fluid:						
Blood	365	390	100	100	1.25	1.25
Muscle	340	373	93.1	95.6	1.26	1.29
Liver	310	357	84.9	91.5	1.20	1.29
Brain	351	383	96.2	98.2	1.22	1.25

[a] Administered by stomach tube.
[b] Administered subcutaneously.

The results show a very close agreement between the relative methanol concentration-water content ratio for all tissues and the blood. These findings are in agreement with those obtained when the methanol was obtained by inhalation, and indicate further the significance of the water content of the tissues and fluids in relation to the distribution of methanol.

Summary

The rapidity with which methanol is distributed throughout the body, the quantitative distribution of methanol among the various kinds of tissues and body fluids and the possibility of selective retention during elimination of methanol was studied in experiments with dogs. Methanol was administered by inhalation, by mouth or stomach tube, or by subcutaneous injection. A maximum of 26 kinds of tissue was examined. The results show that regardless of mode of administration the methanol is distributed very rapidly to all tissues and fluids and the amount found in any particular tissue or fluid is closely related to the amount of water in the tissue or fluid. There is practically no lag in the methanol-water concentration of the blood behind that found in any tissue at a particular instant regardless of whether the animal was accumulating methanol, was in a

steady state or was eliminating methanol following exposure. Consequently all kinds of tissue or body cells are exposed to practically the same methanol-water concentration, there being no selective accumulation, retention or predilections. The results also show that the amount of methanol in the body or in any particular tissue can be estimated from a determination of the methanol in any tissue or fluid, preferably the blood.