Brit. J. Pharmacol. (1958), **13**, 364.

THE DISTRIBUTION OF METHYLPENTYNOL AND OF METHYLPENTYNOL CARBAMATE IN TISSUES AND BODY FLUIDS OF CATS

BY

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(RECEIVED JULY 2, 1958)

A method is described for the estimation of methylpentynol and methylpentynol carbamate in body fluids and tissues. After intravenous administration, the distribution of these drugs throughout the body was found to be similar to that of ethanol. They entered cells, and crossed the blood-brain barrier and the placenta. They were excreted or secreted into the saliva, milk, gastric secretion, aqueous and vitreous humour, amniotic liquor, bile and urine. Methylpentynol was found in the expired air. Calculation of the volume of distribution and measurement of concentration in the tissues showed that both drugs were only slowly metabolized or excreted, suggesting the possibility that blood concentrations might rise cumulatively over a period of days.

Methylpentynol (3-methyl-1-pentyn-3-ol, Oblivon) is an unsaturated, tertiary alcohol introduced into therapeutics by Margolin, Perlman, Villani, and McGavack (1951), who also studied some aspects of its pharmacology as did Gialdroni and Grassi (1952). Perlman and Johnson (1952) and Perlman, Sutter, and Johnson (1953) investigated the metabolism, distribution, and excretion of methylpentynol. They suggested that the drug was eliminated rapidly from the blood and not stored in the tissues, implying rapid metabolism. For instance, they found that, if dogs were given methylpentynol (200 mg./kg. intravenously), only 20% of the total dose could be recovered from venous blood 10 min. later and 12% after 25 min.; after 2 hr. none could be detected. In rats given methylpentynol (800 mg./kg. orally) and killed at the time of maximum sedation, less than 7% of the total dose could be recovered from the tissues. When the sedative effect had worn off, no methylpentynol could be recovered from the tissues. Moreover, although methylpentynol is volatile, no trace could be detected in the expired air collected for 7 hr. after the administration of 200 mg./kg. of the drug orally to a dog. It has therefore been contended that, though methylpentynol is an alcohol, the body treats it differently from the lower alcohols such as ethanol; its metabolism is considerably more rapid and it is not stored in the tissues. In contradistinction are the clinical accounts of the use of methylpentynol (May and Ebaugh, 1953; Marley and Chambers, 1956) and the more recent derivative methylpentynol carbamate (Bartholomew, Chappell, Marley and Chambers, 1958) from which it would appear that an accumulation of both these substances may occur in humans, leading to toxic phenomena.

Because of the discrepancy between the animal results and those seen clinically in man, we have re-examined the distribution and excretion of methylpentynol in animals. Although the results of experiments on the metabolism and fate of methylpentynol carbamate have not been published, its activity is said to depend on the conversion of the substance to methylpentynol in the body. We have therefore also studied the distribution of methylpentynol carbamate in animals.

METHODS

Reagents

All reagents used were of Analar standard.

Alkaline Silver Reagent.—250 ml. 0.1 M-$AgNO_3$ and 30 ml. 6 N-NaOH were mixed in a 500 ml. volumetric flask. Sufficient concentrated NH_4OH was added to dissolve the precipitate and a further 20 ml. was added in excess. The solution was made up to 500 ml. with distilled water and stored in a dark bottle. Alkaline silver reagent (0.025 M) was prepared by adding an equal volume of distilled water to this solution.

Potassium Thiocyanate.—KCNS (10 g.) was dissolved in water and made up to 1 l., to give an

approximately 0.1 M solution which could then be diluted to 0.05 or 0.025 M as required.

Ferric Ammonium Alum Indicator.—To a cold saturated solution of ferric alum enough nitric acid was added for the brown colour to disappear (approximately 3 g. of the alum, 10 ml. of water, and 2 ml. of 6 N-HNO_3). The same amount of indicator was used for all titrations, namely 0.2 ml.

Estimation of Methylpentynol

The method used for estimating methylpentynol was based on that of Perlman and Johnson (1952) as modified by Perlman *et al.* (1953). They distilled the methylpentynol from a sample into the alkaline silver reagent. The acetylene group of the methylpentynol reacted quantitatively with the alkaline silver reagent to form a precipitate. This precipitate was collected, washed, redissolved in nitric acid, and its silver content determined colorimetrically using Rhodamine reagent after a 500-fold dilution. In our hands this method proved unreliable, recoveries being very widely scattered (50 to 250%). We therefore developed the following technique. The minced tissue or fluid sample was placed in a boiling tube ("sample tube" in Fig. 1) and the volume made up to approximately 15 ml. with distilled water. A trace of silicone antifoam agent was added. The sample tube was heated in the water bath at a temperature of 80 to 85° and a stream of air bubbled through. This

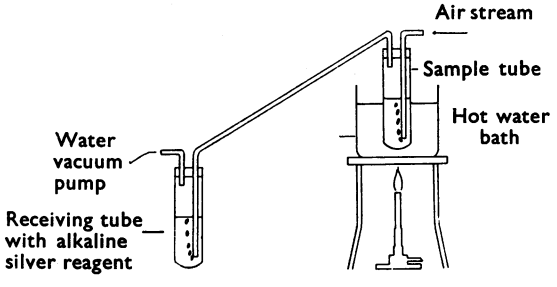

FIG. 1.—Apparatus for distillation of methylpentynol and methylpentynol carbamate from minced tissue samples or body fluids.

volatilized the methylpentynol which was carried in the airstream into 5 ml. of the alkaline silver reagent diluted with 10 ml. of distilled water, where it precipitated with the silver as in the method of Perlman *et al.* (1953). In preliminary experiments, the sample tubes were heated for 30 min., 60 min., and 2 hr. It was found that all the methylpentynol distilled over within 30 min., so this period was used. Six samples were treated at the same time, the air being drawn through both the sample and the receiving tube by suction from a water vacuum pump attached to a manifold. The alkaline silver reagent was then centrifuged at 3,000 rev./min. for 15 min., in order to pack the precipitate at the bottom of the tube. The supernatant layer was carefully decanted, the precipitate washed, and the combined supernatants acidified with 2 ml. concentrated HNO_3. Iron alum indicator (0.2 ml.) was added and the silver estimated by titration with KCNS. A standard curve was obtained by adding 0.25, 0.5, 1.0, 2.0, 4.0 mg. of methylpentynol respectively to 5 ml. of the silver reagent, centrifuging and then back titrating the excess silver present in the decanted supernatant. The amount of thiocyanate required was plotted against the amount of added methylpentynol (Fig. 2). This curve was used for

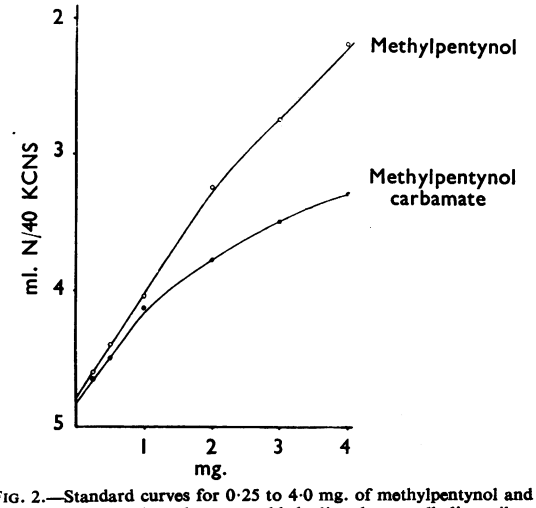

FIG. 2.—Standard curves for 0·25 to 4·0 mg. of methylpentynol and methylpentynol carbamate added directly to alkaline silver reagent (see text).

estimating the amount of methylpentynol present in the samples of body fluids or tissues. New recovery curves were plotted each time fresh solutions were prepared. In this way the need for an exact standardization of a silver or thiocyanate solution was eliminated.

Estimation of Methylpentynol Carbamate

Hydrolysis with 0.2 N-H_2SO_4 was essential for the liberation of methylpentynol from the carbamate. This acid was chosen because of its low volatility. The procedure was identical with that for methylpentynol, except that before the distillation procedure began 2.0 to 3.0 ml. of 3 N-H_2SO_4 was added to the sample tube. Standard curves were obtained for the same amounts of added drug as with methylpentynol (Fig. 2). Alkaline hydrolysis of the carbamate provided better recoveries than acid hydrolysis from distilled water but very variable recoveries from blood.

Recovery from Tissue Samples

Control tissue samples usually of 5 to 20 g. from cats anaesthetized with chloralose or pentobarbitone sodium were minced and extracted by the distillation procedure. No precipitation of the alkaline silver reagent occurred except with distillates of minced foetal tissue samples of about 70 g. These gave a small amount of black precipitate and at the same time a sulphurous smell could be detected in the stream of air from the sample tube. To minimize

liberation of gaseous sulphides, lead nitrate was added to the large foetal tissue samples. Propylene glycol was also added to blank tissue samples before distillation, since it was sometimes used as an injection vehicle for the carbamate. No precipitation of the silver reagent occurred. Having shown that tissue blank values were satisfactory, the next step was to estimate the recovery of known amounts of methylpentynol and carbamate. Between 0.25 and 2 mg. of methylpentynol or the carbamate were added to 10 ml. water, 10 ml. stored cat blood or minced cat tissue (liver, thigh muscle, diaphragm, lung, kidney, brain, and heart: the samples varied between 8 and 17 g.). The samples were then extracted by the distillation procedure. The mean recoveries and standard deviations are shown in Table I. It will be noted that the mean recovery for methylpentynol from blood over a range of 0.25 to 2 mg. of the drug was 98%. This compares favourably with a mean recovery of 73% of added material found by Perlman and Johnson (1952) using their method. The 65% recovery of methylpentynol carbamate was disappointing, and the reason for this has not yet been found. Concentrations of drugs in tissues quoted below have not been corrected for this low recovery of methylpentynol carbamate, and are therefore minimum values.

Recovery from Fresh Blood Samples

To determine whether either methylpentynol or the carbamate was metabolized in fresh blood, samples of blood were taken from an anaesthetized cat and methylpentynol or carbamate was added. After incubation for periods of up to 1 hr. in capped centrifuge tubes at 37° and centrifugation for 15 min. the plasma and red cells were analysed. There was no loss of either methylpentynol or methylpentynol carbamate from blood under these conditions. It was therefore presumed that drug concentrations in blood samples did not change after the samples were taken.

Perlman *et al.* (1953) suggested that the ammoniacal silver reagent may lose ammonia during the estimation and allow precipitation of silver carbonate. To counteract this they sometimes added 2 ml. of concentrated NH_4OH to the reagent during the distillation. We repeated this procedure with solutions which were bubbled for 30 min., and also by adding NH_4OH to the sample tube to charge the airstream with ammonia vapour. In both instances the addition of ammonia reduced the recovery of methylpentynol to between 0 and 20%. Thus the addition of more ammonia to the silver reagent changes its properties and invalidates the method. To test this further the methylpentynol and the carbamate were added directly to the silver reagent together with 1 ml. of concentrated NH_4OH. The reaction of the methylpentynol with the silver reagent was inhibited so much that only about 30% of the expected precipitate was formed. It was also possible to redissolve the precipitate once it had formed by adding more NH_4OH. Tissue and blood blank experiments also showed that the addition of excess NH_4OH was unnecessary, for had any silver carbonate been precipitated it would have shown as a positive reaction.

As a further test of the specificity of the method, a collection of 80 specimens obtained from human tissues was analysed for methylpentynol or methylpentynol carbamate. At the time of analysis it was not known which, if any, of these samples contained methylpentynol or the carbamate. Of the 80 samples 15 were correctly found to contain methylpentynol or its carbamate, one gave a false positive and the other 64 were correctly found to contain no drug.

Animal Experiments

The cats used were anaesthetized either with chloralose (80 mg./kg.) after ether induction, or with intraperitoneal pentobarbitone sodium (30 mg./kg.). In two or three instances infusion of methylpentynol or methylpentynol carbamate was started during ether anaesthesia and anaesthesia then maintained by this infusion. Spinal cats prepared by the method of Kosterlitz, Krayer, and Mattalana (1955) were used in some experiments. Rectal temperature was maintained at 38°. Methylpentynol or the carbamate was given either by infusion at the rate specified or as a single intravenous injection. Arterial blood samples were taken from a polythene cannula in the femoral artery. Tissue samples were obtained by dissection of the specimen between the ligatures. In the pregnant animals 3 to 5 foetuses were usually present. These were removed one by one to coincide with the withdrawal of the tissue or fluid samples. A uterine horn was opened, the amnion incised, the contained

TABLE I

RECOVERY OF METHYLPENTYNOL OR METHYLPENTYNOL CARBAMATE FROM DISTILLED WATER, CAT WHOLE BLOOD AND CAT TISSUES

The values are expressed as % ± s.d. The number of estimations made are given in brackets.

	Methylpentynol				Methylpentynol Carbamate			
Amount added (mg.) ..	0·25	0·5	1·0	2·0	0·25	0·5	1·0	2·0
Recovered from:								
Distilled water	90±0·0 (4)	96±4·0 (3)	90±0·0 (3)	93±5·3 (3)	68±9·3 (3)	60±8·3 (5)	50±0·0 (3)	60±7·1 (3)
Cat whole blood ..	89±7·7 (5)	100±2·0 (5)	102±3·4 (5)	102±1·6 (3)	68±9·2 (6)	64±12·0 (5)	66±10·4 (3)	60±0·0 (3)
Cat tissues	105±8·6 (4)	97±4·3 (4)	98±8·9 (4)	97±8·7 (4)	57±5·9 (3)	58± 6·5 (3)	75±15·2 (5)	76±18·0 (5)

fluid collected, and the foetus and placenta abstracted. The horn was then closed with a tissue clamp, as was the abdomen, until the next specimen was required. In four experiments, the expired air was collected through a respiratory valve in large polythene bags. The contents of the bag were then pumped slowly through the alkaline silver reagent in order to measure the methylpentynol content. The same procedure applied to the contents of a bag filled with London air caused no precipitation of the silver reagent.

Treatment of Samples

Blood samples of 4 to 5 ml. were collected in glassware after intravenous injection of heparin (10 mg./kg.). The final blood sample was obtained by maximal exsanguination. Because methylpentynol is volatile, all the blood samples were kept in closed tubes from the time of withdrawal to the time of estimation. When both blood and plasma values were required, 10 ml. samples were collected and 4 ml. of the blood was immediately lysed with water and used later for whole blood estimation. The rest was centrifuged at 3,000 rev./min. for 15 min., in order to obtain plasma and cell samples. Both whole blood and plasma samples were stored in the deep freeze. Tissue samples were minced with scissors and stored in sealed bottles in the deep freeze until the estimation was performed. They were then warmed to room-temperature, mixed with water and transferred to the boiling tube (or to a conical flask if the sample was large).

The sensitivity of the method depended upon the total quantity of fluid or tissues taken for estimation. Thus, the method can detect as little as 0.1 mg. methylpentynol and this could be in a small amount of tissue, in which instance the sensitivity would be low, or in, say, 20 g. of tissue, allowing the detection of 0.005 mg./g. For the work described in this paper, concentrations of both methylpentynol and the carbamate were measured in samples usually greater than 4 ml. fluid or 5 g. tissue, allowing the detection of 0.02 mg./g. The accuracy of the method depends upon titration technique and the recovery figures. The excess silver was titrated to the nearest drop (0.03 ml.) of thiocyanate, equivalent in the lower range of the titration curve to 0.03 mg. methylpentynol. For samples of 5 g., this means that the accuracy of the method is within 0.01 mg. methylpentynol. The recovery figures for the carbamate were less satisfactory, and in consequence the accuracy is more difficult to assess. Because of this, the concentrations of the carbamate have been given to the nearest 0.05 mg./g. or ml. The concentrations of methylpentynol have been expressed to the nearest 0.01 mg./ml.

For injection and infusion, pure methylpentynol was dissolved in 0.9% saline (w/v). The methylpentynol carbamate was also dissolved in saline for infusion experiments, but for single injections it had to be dissolved in 60% (v/v) aqueous propylene glycol in order to reduce the volume injected.

RESULTS

Fluid and tissue concentrations of both drugs were measured in cats following intravenous injection (up to 200 mg./kg.) and slow intravenous infusion (up to 350 mg./kg.). The blood concentrations are given in Table II. In Experiment

TABLE II

BLOOD CONCENTRATIONS OF METHYLPENTYNOL AND METHYLPENTYNOL CARBAMATE IN THE CAT IN RELATION TO TIME OF SAMPLING AFTER ADMINISTRATION

All cats used were females except those in Expt. Nos. 2, 5, and 7, which were male. Values obtained from exsanguination samples (Expt. Nos. 5, 6, 8, 9, 10 and 13) are given in bold type. The concentrations of methylpentynol or methylpentynol carbamate in the blood at time of sampling are expressed as mg./ml. of whole blood.

Expt. No.	Preparation	Weight (kg.)	Dose Administered (mg./g.)	Blood Concentrations at Time (in min.) After End of Drug Administration								Other Final Samples
				1	2	5	10	20	40	60	100	
Methylpentynol												
1	Chloralose (80 mg./kg.)	2·8	0·1	—	0·35	—	0·15	0·13	0·10	—	—	0·11 (160 min.)
2	,, ,,	4·8	0·1	0·66	0·40	0·34	0·18	0·16	0·09	—	—	—
17	,, ,,	3·8	0·2	0·56	0·49	0·34	0·26	0·20	0·19	—	—	—
3	,, ,,	3·0	0·25	—	0·74	0·49	0·40	0·33	—	—	—	—
6	,, ,,	2·6	0·35 in 45 min.	0·58	—	—	0·36	0·32	0·32	**0·36**	—	—
8	,, ,,	4·1	0·32 in 20 min.	0·25	—	—	0·17	—	0·15	**0·20**	—	—
9	Methylpentynol + 20 mg./kg. chloralose	2·5	0·3 in 15 min.	—	—	0·35	—	—	0·24	—	0·23	0·19 (160 min.) **0·27 (180 min.)**
10	,, ,,	3·0	0·2	—	—	—	—	—	—	—	—	0·29 (240 min.) **0·30 (280 min.)**
5	Spinal	3·4	0·2	—	1·45	0·88	0·41	0·28	0·19	0·15	—	**0·18 (120 min.)**
7	,,	3·1	0·2	—	—	—	—	0·14	—	—	—	0·05 (120 min.)
4	Pentobarbitone sodium (60 mg./kg.)	2·3	0·2	—	0·82	0·60	0·47	0·29	—	—	—	—
Methylpentynol carbamate												
12	Chloralose (80 mg./kg.)	2·6	0·2	—	—	—	0·45	—	0·15	—	0·10	—
13	,, ,,	3·1	0·2	—	—	—	0·35	—	0·10	—	—	0·05 (120 min.) **0·10 (180 min.)**
14	,, ,,	2·6	0·35 in 90 min.	—	0·20	—	—	—	—	0·15	—	—
18	Methylpentynol carbamate	4·1	0·2	—	—	—	—	0·25	—	0·15	—	—
11	Spinal	2·0	0·2	1·30	0·25	0·20	0·20	0·15	0·15	—	—	—

Nos. 1, 2, and 11, both plasma and blood concentrations were measured. They were found to be equal within 2 min. for methylpentynol and within 10 min. for the carbamate. For the other experiments the concentration in whole blood was assumed to be equal to the plasma concentration. The blood concentrations of both methylpentynol and the carbamate declined rapidly within the first 10 min., and then more slowly (Table II and Fig. 3).

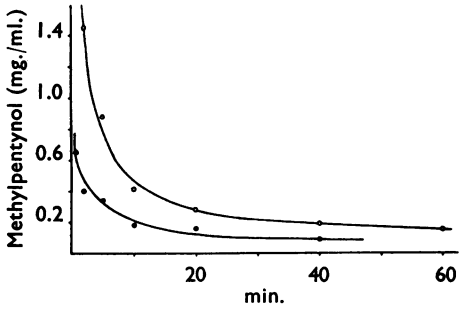

FIG. 3.—Blood concentrations of methylpentynol after single intravenous injections of 200 mg./kg. (O, Expt. No. 5); 100 mg./kg. (●, Expt. No. 2) plotted against time in min. after injection.

Table III shows the volumes of distribution at various times and it will be seen that methylpentynol was distributed throughout the total extracellular fluids within 2 min. of injection and had entered the cells within 10 min. At 40 min., the volume of distribution was about 100%, suggesting that the drug was either being concentrated by cells or being metabolized or excreted. The values for the carbamate show a similar rate of diffusion throughout the body compartments.

TABLE III

VOLUME OF DISTRIBUTION

The values represent the % of body weight through which the drug is distributed as calculated from blood concentrations at specified intervals after injection. The carbamate results are calculated on the assumption that 65% of the actual drug concentrations were measured.

Expt. No.	Time (min.)				
	1	2	10	20	40
Methylpentynol					
1	—	29	67	77	100
2	15	25	56	63	110
3	—	34	63	76	—
4	—	25	43	69	—
5	—	14	49	74	105
7	—	—	—	140	—
Methylpentynol carbamate					
11	10	38	65	86	—
12	—	—	30	—	86
13	—	—	40	—	130

TABLE IV

CONCENTRATION IN TISSUES AND BODY FLUIDS OF CATS AT VARIOUS TIMES AFTER SINGLE INJECTIONS OR INFUSIONS OF METHYLPENTYNOL AND METHYLPENTYNOL CARBAMATE

Single injections were given in Expt. Nos. 7, 12, 13, 17 and 18, and infusions in Expt. Nos. 6, 8, 9, 10, 14, 15 and 16. Concentrations and doses are given as mg./g. or mg./ml., except with gastric contents where the weight (in mg.) of the drug in lumen of stomach at end of the experiment is recorded and with expired air where the value denotes the weight (in mg.) of the drug collected in first hour. In Expt. No. 6, 60 min. after infusion of 0·35 mg./g. of methylpentynol, the concentration in blood was 0·32 mg./ml., and in milk 0·40 mg./ml. In Expt. No. 14, 2 min. after infusion of 0·35 mg./g. of methylpentynol carbamate, the concentration in blood was 0·20 mg./ml. and in milk 0·20 mg./ml. In Expt. No. 15, 2 min. after infusion of 0·07 mg./g. of methylpentynol, the concentration in blood was 0·15 mg./ml. and in saliva 0·16 mg./ml. In Expt. No. 16, 90 min. after infusion of 0·25 mg./g. of methylpentynol, the concentration in the gastric secretion was 0·50 mg./ml.

	Methylpentynol					Methylpentynol Carbamate		
Expt. No.	7	8	9	10	17	12	13	18
Total dose (mg./g.)	0·2	0·18	0·32	0·30	0·20	0·20	0·20	0·20
Time after administration (min.)	120	2	180	280	10	120	180	50
Tissue								
Final blood concentration	0·05	0·15	0·19	0·29	0·20	0·10	0·05	0·15
Brain	—	—	0·35	0·08	—	0·30	0·05	—
Heart	—	—	—	0·28	—	0·15	0·20	—
Diaphragm	0·14	—	0·24	0·26	—	0·05	0·15	—
Thigh muscle	—	—	—	0·12	—	0·10	0·05	—
Mesenteric fat	0·19	—	0·45	0·22	—	0·10	0·10	—
Lung	0·19	—	0·30	0·15	—	0·25	0·15	—
Liver	0·20	—	—	0·17	—	0·10	0·25	—
Kidney	0·16	—	0·33	0·13	—	0·25	0·20	—
Spleen	0·08	—	—	0·17	—	—	—	—
Intestine	0·17	—	—	—	—	—	—	—
Skin	—	—	—	0·10	—	—	0·05	—
Bone	—	—	—	0·06	—	—	0·05	—
Foetus	—	—	—	0·18	—	0·15	—	—
Placenta	—	—	—	0·06	—	0·15	—	—
Bile	0·0	—	0·0	Trace	—	0·0	—	0·15
Urine	0·16	—	—	0·33	—	—	0·10	—
Aqueous humour	—	—	0·10	—	0·19	0·0	0·0	0·10
Vitreous humour	—	—	—	—	0·15	0·10	0·10	—
Amniotic liquor	—	0·14	—	0·29	—	0·10	—	—
Gastric contents	0·8	—	4·5	—	—	—	5·0	—
Expired air	2·5	0 85	—	—	—	0·0	0·0	—

To determine whether the methylpentynol and carbamate were being metabolized, concentrated, or excreted, fluid and tissue concentrations were measured and the results are shown in Table IV. Both substances were found in saliva, gastric secretion, milk, aqueous and vitreous humour, amniotic liquor, bile, and urine. Entry into the lumen of the stomach took place during secretion of acid gastric juice (induced by histamine infusion) and also when the stomach was not secreting. The intestinal contents, after ligation of the pylorus, were also assayed for methylpentynol and the carbamate, but only traces were found.

The high concentration of methylpentynol in the milk (0.4 mg./ml.) at the end of Experiment No. 6 suggested that the drug diffused into preformed milk. This was confirmed in Experiment No. 14 where immediately after ending the infusion of the drug 2 mg. methylpentynol carbamate

was found in 10 ml. (0.2 mg./ml.), a volume of milk which must have been largely preformed.

The tissue concentrations shown in Table IV indicate that both drugs reach all tissues examined in comparable amounts. As these were obtained from 2 to 4 hr. after injection of the drug when, in some experiments at least, the blood concentrations had declined, it is probable that the methylpentynol and carbamate were not being metabolized to any great extent. For instance, in Experiment No. 7 the blood concentration was 0.05 mg./ml., but the concentrations of methylpentynol in the tissues varied between 0.08 and 0.20 mg./ml., the highest being in the liver, fat, and lungs. If the average tissue concentration in this cat was 0.15 to 0.20 mg./g., then of the 200 mg./kg. injected 2 hr. beforehand 150 to 200 mg./kg. (75 to 100%) was still left. The results of the other experiments also indicate that 70 to 100% of the initial dose remained in the tissues 200 to 300 min. later; similar results were obtained with the carbamate.

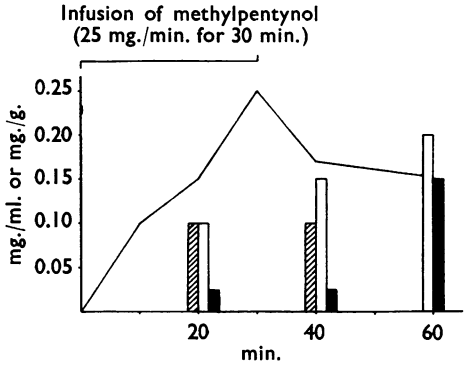

FIG. 4.—Expt. No. 8. Concentrations of methylpentynol in body fluids or tissue plotted against time following infusion of the drug. The line represents the concentration (in mg./ml.) in blood; the solid columns, the concentration (in mg./ml.) in amniotic liquor; the open columns, the concentration (in mg./g.) in the foetus; the crosshatched columns, the concentration (in mg./g.) in placental tissue.

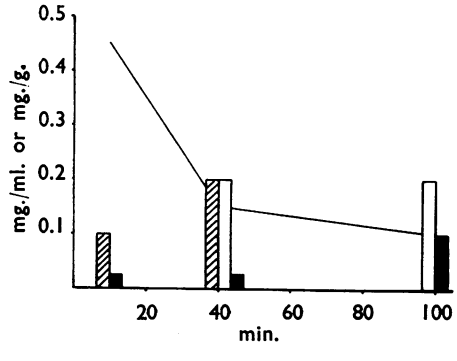

FIG. 5.—Expt. No. 12. Concentrations of methylpentynol carbamate plotted against time following a single intravenous injection of the drug. For explanation of the line and columns see Fig. 4.

Figs. 4 and 5 show that both methylpentynol and methylpentynol carbamate have general access not only to all body tissues examined but also to foetal tissues and eventually to amniotic fluid. Although most foetal tissues did not provide large enough samples to detect either drug, foetal liver and foetal heart were found to contain about the same concentrations of both drugs as the whole foetus. Thus we can say that there is no barrier to the general distribution of both drugs throughout the body.

In two experiments the expired air from the animal was collected in polythene bags and was later pumped through the alkaline silver reagent. Up to 0.4% of the total dose of methylpentynol was excreted into the expired air within 1 hr. of intravenous administration. In two similar experiments following the injection of methylpentynol carbamate, no methylpentynol was recovered from the expired air. Furthermore, although we always looked for methylpentynol in fluid and tissue samples after the administration of carbamate, none was detected, except once when a trace was found in a kidney tissue sample.

All these results indicated that neither methylpentynol nor the carbamate was rapidly metabolized.

DISCUSSION

The method described for estimating methylpentynol and the carbamate was based on that described by Perlman *et al.* (1953). The titration of silver left uncombined with methylpentynol gave more consistent results than colorimetric estimation of the amount of silver precipitated. The other advantage of this modified method was that as little as 0.1 mg. of methylpentynol could be estimated, a tenfold increase of sensitivity over the method of Perlman and Johnson (1952). The addition of further ammonia to the silver reagent during the distillation, a procedure recommended in the original method, was omitted when it was found to inhibit the formation of the precipitate and make the method completely unreliable. This may in part explain why Perlman *et al.* (1953) were unable to detect methylpentynol in the expired air of the dog.

There was no difference between the plasma concentration and the whole blood concentrations

of methylpentynol and the carbamate within 10 min. of intravenous injection. This does not necessarily mean that the alcohol and the carbamate were evenly distributed between blood cells and plasma within 10 min., for further equilibration may have taken place during the 15 min. centrifugation. If this time is taken into account the results show that both drugs were evenly distributed throughout blood cells and plasma within 25 min. of injection and possibly before this.

The main conclusions that can be drawn are first that both methylpentynol and methylpentynol carbamate have free access to all compartments of the body including the uterine contents, and second that excretion or inactivation of the substance in cats is slow. That general anaesthesia does not depress the metabolism or excretion of methylpentynol can be seen from Table II, for although the blood levels at various times were similar in the different cats, some were either spinal or were anaesthetized only with methylpentynol and some had chloralose or pentobarbitone sodium. These conclusions differ from those of Perlman *et al.* (1953), who used rats and dogs.

The general distribution of methylpentynol and its carbamate closely followed that of the lower alcohols. They readily crossed the placenta, both being recovered from the foetus, the amniotic liquor and the placenta within 10 to 20 min. of intravenous injection. The concentration in the amniotic liquor was initially lower than in the foetus but gradually increased as the experiment progressed. Nicloux (1899) demonstrated that alcohol passed from the mother to the foetus and that concentrations in the maternal and foetal blood were of the same order. Olow (1922) found appreciable quantities of alcohol in human foetal blood within 12 min. of injection; maternal and foetal blood reached similar concentrations 40 min. after ingestion, the subsequent rate of disappearance of both being identical. Although we were unable to procure sufficient cerebrospinal fluid to find either the speed of entry or the concentration of the drugs at this site, we did find concentrations in the brain similar to those in other tissues. The fact that both drugs were recoverable from the aqueous humour implies that they had entered the cerebrospinal fluid. Davson (1957) calls attention to the similarity between the blood/aqueous barrier and the blood/cerebrospinal fluid barrier. Even the passage of both drugs into the stomach lumen following intravenous injection is similar to that of ethanol; Grehant (1903) demonstrated alcohol excretion into the stomach lumen after intravenous injection as did Lukas (1930) following rectal administration. The fact that methylpentynol and the carbamate cross the stomach wall but not the duodenal wall implies that absorption of the drug takes place from the stomach following oral administration, as does the absorption of ethanol.

Ethanol is excreted in the expired air and we have found that methylpentynol is also excreted in the breath in amounts up to 0.4% of the total dose within 1 hr. of administration.

The volume of distribution of both drugs after single intravenous injections indicates that they are rapidly distributed throughout the total extracellular fluids and enter cells within 20 min. This is also shown by the tissue concentrations following the administration of both drugs. The fact that the tissue concentrations of both methylpentynol and the carbamate are sometimes higher than the blood concentrations after a period of 60 to 120 min. indicates that, after entry into the cells, there is more difficulty in getting out again. This difficulty seems to be overcome if too much blood is taken from the animal, for terminal values obtained by gross exsanguination are always higher than the penultimate values. This is reminiscent of the behaviour of potassium which is normally concentrated to a high degree by the cells. Anoxia, severe haemorrhage or other traumatic procedures lead to leakage of potassium from the cells into the blood (Scudder, Smith and Drew, 1939; Winkler and Hoff, 1943).

From these results in the cat it might be expected that methylpentynol and the carbamate would accumulate if given over a long period of time to man, and Bartholomew, Bourne, and Marley (1958) have shown that daily doses of methylpentynol and carbamate lead to rising blood concentrations in man.

Except for traces in the kidney, methylpentynol was never found in blood following administration of the carbamate, nor was there any respiratory excretion of the alcohol. This seems to be good evidence that the carbamate is acting as such and not through breakdown into methylpentynol as has been suggested. Thus the relationship between methylpentynol and methylpentynol carbamate seems to be similar to that between ethyl alcohol and ethyl carbamate (urethane). Furthermore, the general distribution of methylpentynol and its carbamate are similar to that of ethyl alcohol.

We should like to thank Professor W. D. M. Paton, F.R.S., for his stimulating interest throughout this work, also British Schering Ltd. for a generous supply

of methylpentynol and methylpentynol carbamate. We are indebted to Mr. D. A. Green and staff for technical assistance. The work was carried out during the tenure by one of us (E. M.) of a Medical Research Council Clinical Research Fellowship.

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