Biochemical Pharmacology, 1964, Vol. 13, pp. 1239–1247. Pergamon Press Ltd., Printed in Great Britain.

METABOLISM OF VOLATILE ANASTHETICS—I

CONVERSION *IN VIVO* OF SEVERAL ANESTHETICS TO $^{14}CO_2$ AND CHLORIDE

RUSSELL A. VAN DYKE,* MAYNARD B. CHENOWETH* AND ALAN VAN POZNAK†

Biochemical Research Laboratory, The Dow Chemical Company, Midland, Mich.,* and Department of Anesthesiology, Cornell University Medical College, New York, N.Y., U.S.A.†

(*Received* 23 *December* 1963; *accepted* 6 *April* 1964)

Abstract—The metabolism *in vivo* of several volatile anesthetics has been determined in the rat. The anesthetics studied were ether, chloroform, methoxyflurane, and halothane, all of which have been obtained isotopically labeled with chlorine-36 or carbon-14. The extent of conversion of these anesthetics to $^{14}CO_2$ and urinary metabolities labeled with ^{14}C or ^{36}Cl is presented as a percentage of the amount of anesthetic administered.

THE volatile anesthetics have been placed in a group of materials considered to be, *ad hoc*, biochemically inert. This classification has been based on work which has lacked the proper sensitivity to detect transformations as they occur. The use of radioactive isotopes as tracers, as currently employed, has the proper sensitivity for metabolic studies of this nature and has enabled several investigators to determine the metabolism of various materials previously thought not to be metabolized.

Recent publications have shown that chloroform is metabolized in both *in vitro* and *in vivo* systems.[1–3] Although not a material one would care to use for clinical anesthesia, carbon tetrachloride is an extremely potent anesthetic,[4] and it has been found to be metabolized by several groups of investigators.[2, 3, 5] It was recognized quite early that trichloroethylene is very extensively metabolized. This was originally reported by Barrett and Johnson[6] and more recently by Daniel.[7]

Methyl chloroform, 1,1,1-trichloroethane, was found to be a potent anesthetic in man by Dornette and Jones,[8] and its biotransformation in the rat was reported by Hake *et al.*[9] This paper presents evidence that diethyl ether, chloroform, metoxyflurane,‡ and halothane§ are metabolized.

METHODS

The anesthetic agents were obtained isotopically labeled as follows: ^{14}C-chloroform and diethyl-1-^{14}C-ether purchased from New England Nuclear Corp.; ^{14}C-methoxyflurane labeled in the methyl group prepared for us by Dr. E. Larsen of the Halogens Research Laboratory, The Dow Chemical Co.; ^{36}Cl-methoxyflurane and halothane prepared by New England Nuclear Corp. by the chlorine-36 chlorination of intermediates supplied by the Halogens Laboratory; ^{36}Cl-chloroform purchased from Volk Radiochemical Co. All materials were purified by vapor-phase chromatography.

‡ 2,2-Dichloro-1,1-difluoroethylmethyl ether.

§ 1,1,1-Trifluoro-2-chloro-2-bromoethane.

Vapor phase-chromatography with an electron capture and a hydrogen flame detector on a Wilkins Aerograph Hy-Fi model 600-C showed that detectable impurities in the ^{36}Cl-halothane were 0·50% and the detectable impurities in the ^{14}C and ^{36}Cl-methoxyflurane were 0·18%. There were no detectable impurities in the ^{14}C chloroform and ^{14}C-diethyl ether.

The anesthetics had the following isotopic activity per 0·1 ml: ^{14}C-chloroform, $1{\cdot}06 \times 10^7$ disintegrations/min (dpm); diethyl-1-^{14}C-ether, $1{\cdot}84 \times 10^7$ dpm; ^{14}C-methoxyflurane, $9{\cdot}8 \times 10^6$ dpm; ^{36}Cl-methoxyflurane, $1{\cdot}29 \times 10^7$ dpm; ^{36}Cl-halothane, $2{\cdot}13 \times 10^7$ dpm.

The entire study to date has been carried out in rats not selected on the basis of sex but chosen on a weight basis (200 ± 15 g). Administration of the anesthetics was by intraperitoneal injection of 0·1 ml doses. Immediately after the injection the animals were placed in an all-glass metabolism cage as previously described by Hake *et al.*,[9] which was designed to allow the recovery of the expired gases and separate collection of urine and feces. The animals remained in these containers for periods up to 96 hr and were given access to food and water.

The air entering the system was dry and CO_2-free, and was drawn through the metabolism cage and then through a series of scrubbing towers which allowed the recovery of either the metabolically produced CO_2 or the anesthetic, or both, from the expired air of the animal, depending on the solution in the towers. The CO_2 was collected in 2 N NaOH, and the anesthetic was collected in xylene. All the anesthetics studied were stable in basic solution with the exception of chloroform. To determine this, a sample of each of the anesthetics was placed in the empty metabolism cage. Collections in NaOH were made as they would be if an animal were in the cage. Only in the case of chloroform was any isotopic activity detectable in the NaOH.

The special precaution of removing the chloroform from the expired air prior to the removal of the CO_2 therefore had to be accomplished, or falsely high results were obtained. The chloroform was removed by passing the expired air through xylene before the passage through the sodium hydroxide. This was also how the methoxyflurane and halothane were removed from the expired air of the rats. It was determined, however, that there was no difference in either total or hourly excretion of $^{14}CO_2$ if the xylene was not included in the collection train. Aliquots of the xylene solution were taken for counting in the Packard Tri-Carb scintillation spectrometer.

The CO_2 which was collected in the sodium hydroxide was regenerated in a closed system containing a vial of 5 ml 1 M *p*-(diisobutyl-cresoxy ethoxyethyl)dimethyl benzylammonium hydroxide (hydroxide of Hyamine). The method is described by Possman *et al.*[10] To the 5 ml of hydroxide of Hyamine solution containing the CO_2 was added 15 ml of a toluene–alcohol scintillation fluid prepared with 660 ml toluene, 300 ml absolute ethanol, and 40 ml toluene containing 4 g PPO (2,5-diphenyloxazole) and 50 ml POPOP (*p*-bis(2-[5-phenyloxazolyl])benzene. The mixture was then counted in the scintillation counter. Because of the high volatility and water solubility of diethyl ether the $^{14}CO_2$ collected in NaOH from these animals was precipitated as the barium salt. The CO_2 was then regenerated from the $BaCO_3$ in the above system. This method was also performed as a check on the $^{14}CO_2$ recovery from the other anesthetics.

The urine was collected while the animal was in the metabolism cage and was removed for analysis for isotopic content. The urine samples were taken to dryness

by means of a vacuum and then resuspended in water for counting. This was done to ensure that the isotopic activity in the urine was not a volatile material.

As a rule the animals were narcotized by the anesthetic injection; however, it was difficult to assess the level of anesthesia since even a normal animal was inactive when placed in the metabolism cage.

RESULTS

Diethyl ether

The rats which had been injected i.p. with 0·1 ml of 1-^{14}C-diethyl ether were narcotized for periods up to 2 hr. However, after their initial arousal from anesthesia, they remained almost as inactive in the cage as during anesthesia. The results from five animals of the hourly $^{14}CO_2$ exhalation are presented in Fig. 1. The total radioactivity

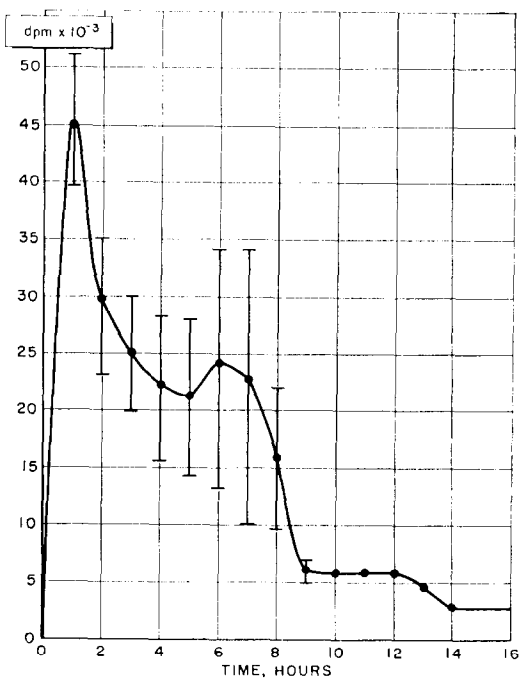

FIG. 1. The pattern of the $^{14}CO_2$ exhalation after a single dose of diethyl-1-^{14}C ether administered to 5 rats.

in the CO_2 collected in a 24 hr period was 4% of the amount injected. The urine was collected over the 24-hr period and contained 2% of the injected radioactivity, none of which was volatile, since the samples were taken to dryness before being counted.

Chloroform

The chloroform used in this study was labeled isotopically with either carbon-14 or chlorine-36. Since chloroform is base unstable, care was taken to exclude the possibility that any of the $C^{14}O_2$ collected had arisen from anything except the biotransformation of chloroform. The total amount of $^{14}CO_2$ collected was 4–5% of

the total ^{14}C-chloroform injected. In this case the anesthesia produced by a 0·1-ml injection of chloroform was variable, since in some cases the animals slept for 4 hr and in other cases the animals were barely anesthetized, sleeping for only a few minutes. The results of the hourly CO_2 collection are presented in Fig. 2. Because of the variation in sleeping times and total conversion to CO_2, the data from 30 animals were used in this figure.

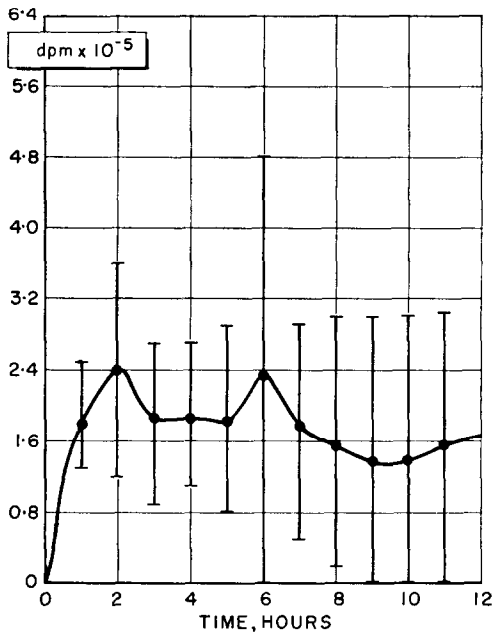

FIG. 2. The pattern of $^{14}CO_2$ exhalation by 30 rats after a single dose of ^{14}C-chloroform.

Because of the variation in appearance of the ^{14}C-labeled metabolites in the urine, no valid average per cent urinary metabolite can be assigned. The range of per cent urinary metabolites varied from zero to as high as 2% of the injected dose.

The possibility that some of the administered anesthetic might be transported into the intestinal tract and metabolized by the intestinal microorganisms was considered. In order to exclude this possibility, rats were treated orally with neomycin (2% of the diet) for one week, and their metabolism of chloroform was then determined. The same amount of metabolism was found with these rats as was found previously, and the same variation in metabolism was noted.

The ^{36}Cl-labeled chloroform was used to assess the total amount of chloride removed from the chloroform molecule and to determine whether the urinary chloride was in the organic form or organically bound. However, the same problem of individual variation was noted with ^{36}Cl-labeled chloroform as was found with the ^{14}C-labeled material. The ^{36}Cl which did appear in the urine was 73% in the inorganic form; the remaining 27% which was not precipitable as $Ag^{36}Cl$ has not been further identified. It is important to keep in mind that the ^{36}Cl removed from the chloroform molecule is not preferentially excreted but mixes with the body chloride pool, and thus

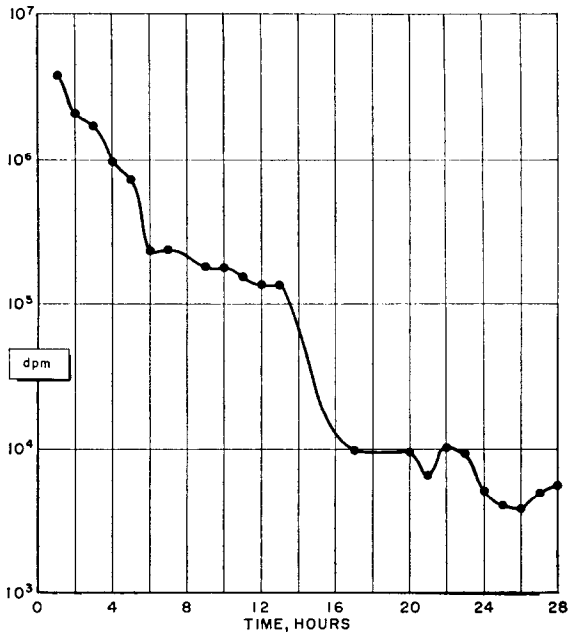

FIG. 3. The pattern of the appearance of ^{36}Cl-halothane in the expired air of 9 rats after the administration of a single dose of ^{36}Cl-halothane.

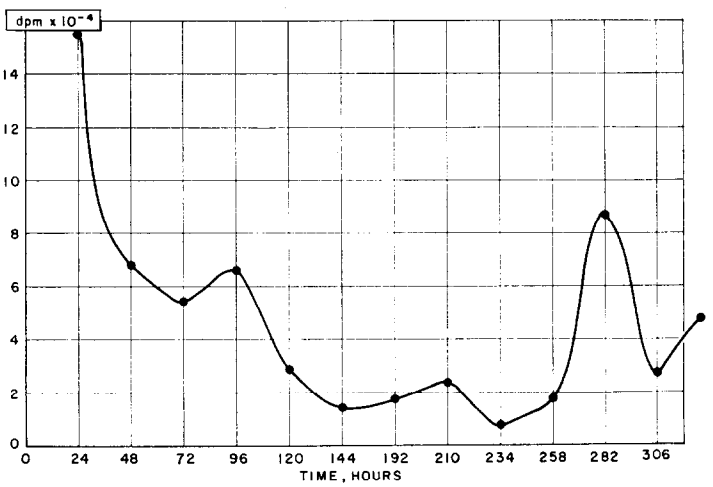

FIG. 4. The pattern of the daily appearance of total ^{36}Cl in the urine of an individual rat after the administration of ^{36}Cl-halothane.

the radioactivity is diluted to the extent of the chloride pool. This leads to low recoveries of ^{36}Cl unless the urine is collected over a period of weeks or the total body chloride is determined and its radioactivity assessed (see Fig. 4).

Halothane ($CF_3CBrClH$)

The only isotopically labeled halothane available to us at this time is ^{36}Cl-halothane. As was the case with several of the other anesthetics, the injection of 0·1 ml of halothane produced anesthesia which lasted in some cases up to 2 hr.

The recovery of halothane from the expired air of nine animals is presented graphically in Fig. 3. It is interesting to note that of the halothane expired in 30 hr, 70% is expired in the first 2 hr. The total ^{36}Cl recovered in 30 hr was 85–90% of the administered dose in four animals.

Figure 4 represents the appearance of ^{36}Cl in the urine. If the halothane molecule were dechlorinated it would be expected that the chloride would appear in the urine for long periods of time. As indicated, this was the case. At the end of 14 days the ^{36}Cl was still appearing in the urine as inorganic chloride and at the time amounted to 2·9% of the injected dose.

Methoxyflurane ($CH_3OCF_2CCl_2H$)

Methoxyflurane was injected in 0·1-ml quantities labeled either with ^{14}C in the methoxyl group or with ^{36}Cl. Having been injected with this amount of methoxyflurane, the animals were anesthetized for periods up to 3 hr.

The total amount of CO_2 arising from a single injection of ^{14}C-methoxyflurane was variable. As a percentage, the values ranged from 0·5% with the majority of the animals yielding a 1–2% conversion. With the same animals, the amount of ^{14}C-metabolites appearing in the urine was 3–5% of that injected.

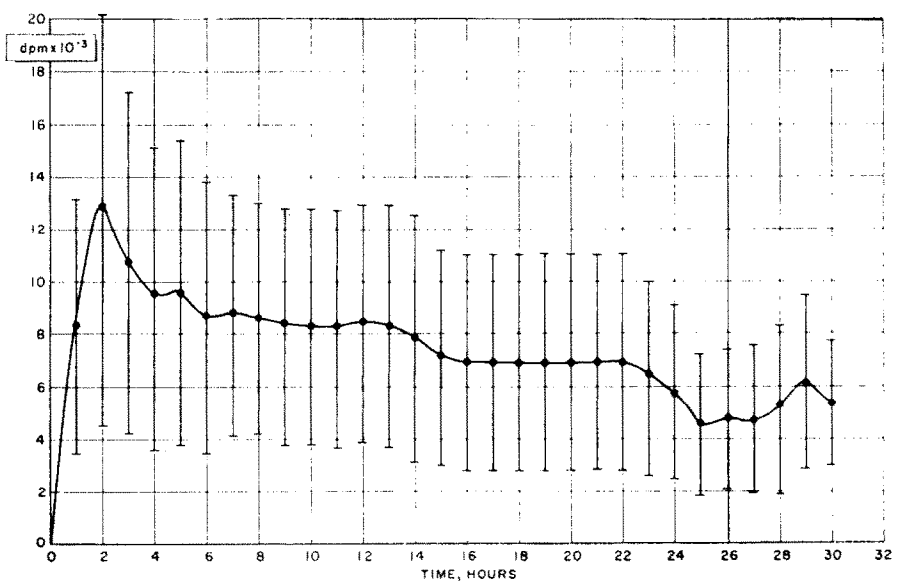

FIG. 5. The pattern of the hourly exhalation of $^{14}CO_2$ from 51 rats after the administration of ^{14}C-methoxyflurane.

The hourly exhalation of $^{14}CO_2$ produced metabolically from methoxyflurane is presented graphically in Fig. 5. This represents the average of 51 animals. The average hourly exhalation of ^{14}C-methoxyflurane from seven rats is presented graphically in Fig. 6. The total amount of unchanged methoxyflurane exhaled in 30 hr was 85–90% of the injected dose. This was repeated in four rats with ^{36}Cl-methoxyflurane and found to be the same. In addition, 1·1% of the injected ^{36}Cl appeared in the urine

in 48 hr. The urine was not collected for an extended period as was the case with ^{36}Cl-halothane, but it is presumed the pattern would be similar.

DISCUSSION

The studies *in vivo* of the conversion of chloroform, ether, and methoxyflurane to CO_2 have revealed that these anesthetics are not so biochemically inert as they were previously considered to be. Halothane was also found to undergo a biotransformation, since the occurrence of urinary inorganic ^{36}Cl indicated dehalogenation.

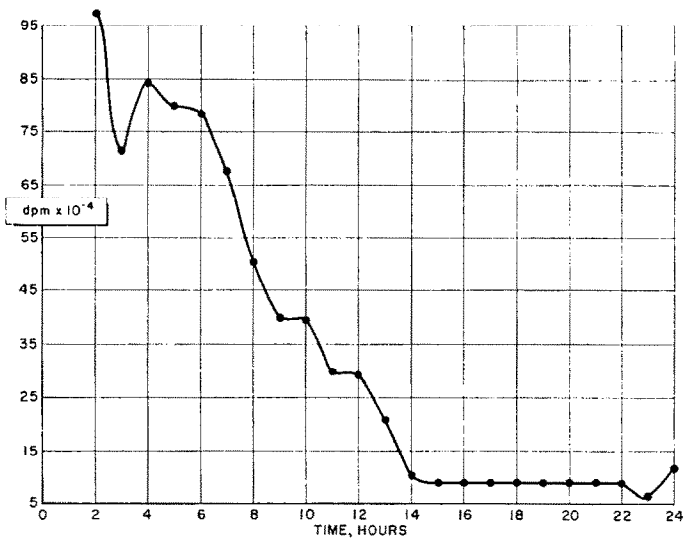

FIG. 6. The pattern of the hourly exhalation of ^{14}C-methoxyflurane after the administration of a single dose of ^{14}C-methoxyflurane.

In the figures depicting the exhalation of $^{14}CO_2$ from animals given ^{14}C-labeled ether, chloroform, or methoxyflurane, it is noticed that there is a bimodal pattern. This point has not as yet been investigated thoroughly. However, it has been noticed that except for the initial peak, which corresponds with the time that the maximal concentration of anesthetic is present in the animal, the other peaks generally corresponded with an increased activity of the animal.

The dose of anesthetic as given in this study yields a variable sleeping time. Some animals sleep for 200 min while others, given the same anesthetic, were anesthetized for 5–10 min. It is possibly this fact which has caused such great hourly variation of $^{14}CO_2$ exhalation between animals.

Diethyl ether has not been studied with techniques employing isotopically labeled material. Haggard,[11] Krantz,[12] and Onchi and Asao[13] have all studied the recovery of diethyl ether from the expired air of animals given a certain dose of the anesthetic and have reported that 87–90% of the absorbed diethyl ether can be accounted for as unchanged diethyl ether. The remaining 10–13% has not been traced to determine whether any has been metabolized to CO_2. That ethers are cleaved is not a new fact. Axelrod[14] and Bray *et al.*[15] have shown that O-dealkylation occurs under the catalysis

of an enzyme found in microsomes. It remains to be seen if this same enzymatic system is responsible for the cleavage of an ether such as diethyl ether.

The evidence for enzymatic dehalogenation occurring in the intact animal has been reviewed by Chenoweth and McCarty.[16] Specific evidence has been presented by Heppel and Porterfield[17] for the existence of a halidase that catalyzes the removal of a halogen from a number of compounds. More recently, Paul and Rubenstein[3] have shown that chloroform and carbon tetrachloride are enzymatically converted to carbon dioxide *in vitro* as well as *in vivo*. Their *in vivo* data with chloroform agree very closely with a previous report from this laboratory.[1] The work presented in this paper provides evidence for the removal of halogens from even more complex molecules and the subsequent oxidation of the remaining carbon skeleton.

Inherent in any metabolism studies *in vivo* are at least two prime factors which control the extent of metabolism of materials foreign to the organism. In the first place, an enzyme must be present to catalyze the transformation of the material. The present study presents no direct evidence that the biotransformation of the anesthetics has occurred under enzymatic control. Indirect evidence, such as the variations noted between individuals, permits the surmise that this biotransformation is, indeed, enzymatic.

The second factor is the necessity for a large enough concentration of material to be available to the enzyme to achieve conversion to a metabolite in sufficient concentration that the metabolite may be detected. The fact is extremely important in the present study, since we are dealing with volatile anesthetics. Chenoweth *et al.*[18] studied the rate of uptake and clearance of the anesthetics from dogs and found that most anesthetics are comparatively rapidly cleared from the body when administration is terminated. This means that the length of time a single injection of the anesthetic is available for biotransformation is relatively short.

A subgroup inder this 'second factor' involves the distribution of the anesthetics throughout the body as reported by Chenoweth *et al.*[19] In these studies we found that the liver contained a relatively low concentration of the anesthetic compared to other organs. Presumably, however, this is the organ that is responsible for the greatest amount of the biotransformation which is noted.[3, 17]

Therefore, on the basis of the distribution studies and the clearance time of these anesthetics, the values reported here for the metabolism probably indicate that there is a considerable capacity for metabolism. It must be recalled that these results are based on a single dose and thus no equilibrium was established except what might occur by the slow release of the anesthetic from the body lipid. A portion of the administered ether, chloroform, or methoxyflurane is stored in the fat and released slowly. This is particularly true of methoxyflurane. Therefore, as this slow release occurs, the amount in the animal is maintained at a constant, although low, level which allows the metabolism of the anesthetics under the conditions of the experiments here reported. Thus, extrapolating this argument further, we predict that during the actual clinical usage of these anesthetics, where a steady state is maintained for a few hours, the level of biotransformation would be greater than that found in this study.

The intermediates arising in the conversion of these anesthetics to CO_2, when identified, should reveal interesting information concerning the pathway of metabolism of these and other similar materials. These intermediates may also be important for

the toxicological differentation of these anesthetics, particularly with respect to the halogenated compounds. In addition, the intolerance of certain individuals may be indicative of their lack of ability to metabolize completely the intermediates formed.

REFERENCES

1. R. A. Van Dyke, A. Van Poznak and M. B. Chenoweth in *Uptake and Distribution of Anesthetic Agents*, E. M. Papper and R. J. Kitz, Eds., p. 187. McGraw-Hill, New York (1963).
2. T. C. Butler, *J. Pharmacol.* **134**, 311 (1961).
3. B. B. Paul and D. Rubenstein, *J. Pharmacol.* **141**, 141 (1963).
4. Unpublished results.
5. D. D. McCollister, W. H. Beamer, G. J. Atchison and H. C. Spencer, *J. Pharmacol.* **102**, 112 (1951).
6. H. M. Barrett and J. Johnston, *J. biol. Chem.* **127**, 765 (1939).
7. J. W. Daniel, *Biochem. Pharmacol.* **12**, 795 (1963).
8. W. H. L. Dornette and J. P. Jones *Anesth. Analg. Curr. Res.* **39**, 249 (1960).
9. C. L. Hake, T. B. Waggoner, D. N. Robertson and V. K. Rowe, *Arch. environm. Hlth.* **1**, 101 (1960).
10. J. M. Possman, N. S. Radin and J. A. D. Cooper, *Analyt. Chem.* **28**, 484 (1956).
11. H. W. Haggard, *J. biol. Chem.* **59**, 745 (1924).
12. J. C. Krantz, Jr., *Curr. Res. Anesth.* **21**, 234 (1942).
13. Y. Onchi and Y. Asao, *Brit. J. Anesth.* **33**, 544 (1961).
14. J. Axelrod, *Biochem. J.* **63**, 634 (1956).
15. H. G. Bray, V. M. Craddock and W. V. Thorpe, *Biochem. J.* **60**, 225 (1955).
16. M. B. Chenoweth and L. P. McCarty, *Pharmacol. Rev.* **15**, 673 (1963).
17. L. A. Heppel and V. T. Porterfield, *J. biol. Chem.* **176**, 763 (1948).
18. M. B. Chenoweth, D. N. Robertson, D. S. Erley and R. Golhke, *Anesthesiology* **1**, 101 (1962).
19. M. B. Chenoweth, R. A. Van Dyke and D. S. Erley, *Nature, Lond.* **194**, 575 (1962).