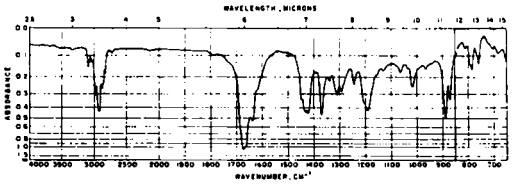

Fig. 1.—Infrared spectrum of isopiperitenone. 4000–850 cm.$^{-1}$ CCl_4 solution; 850–650 cm.$^{-1}$ contact film.

for *M. pulegium* 0.12 to 0.30. The somewhat higher linalyl acetate content of the European oil may likewise be a valuable indicator of botanical identity.

None of the essential oils was fractionated or pretreated prior to analysis. All samples were injected directly as the crude natural products obtained by conventional steam distillation. Coupled gas liquid–thin layer chromatography was used successfully for the detection of trace constituents. Similar analyses of fractions isolated by rectification, column chromatography, and/or other separation techniques would undoubtedly establish the presence in these oils of many more terpene compounds still unaccounted for as shown in Table I.

The experimental data presented summarize results of a series of compositional studies on essential oils of the genus *Mentha*. They serve to illustrate further that distinct biochemical relationships exist between constituents synthesized by its different species and that such data, obtained exclusively by physiochemical analysis, provide important criteria for species characterization and classification *via* qualitative and quantitative chemotaxonomy.

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Quantitative Evaluation of Infiltration Anesthetics in Albino Mice

By W. R. JONES, T. L. KERLEY, and L. C. WEAVER

Infiltration anesthetics were quantitatively evaluated using a test procedure based on the vocalization of mice in response to electrical stimulation.

MOST LABORATORIES today prefer to use the mouse as a laboratory animal for preliminary toxicity work and primary pharmacodynamic evaluation of chemical compounds. The use of this species for the evaluation of local anesthetic activity is advantageous because of economy and the opportunity to compare more tests in the same species. Weidmann and Petersen (1) first used the mouse to study surface anesthesia, and a slight modification of this method has been used in our laboratories (2). In addition, mice have been used for the evaluation of anesthetics injected directly into the tissues (3, 4). A method using mice for the quantitative assessment of infiltration anesthetics is described in this communication.

EXPERIMENTAL

Male Swiss-Webster albino mice were used in the experiments. A constant volume (0.03 ml.) of drug solution was injected beneath the skin on the plantar surface of one hind foot, and an equal volume of 0.9% sodium chloride solution was injected similarly into the opposite foot. Ten minutes after injection, the control foot of each animal was stimulated rapidly and repeatedly until the animal vocalized, then continued to vocalize in response to 10 successive stimulations. Immediately afterward, the treated foot was stimulated five times, and any animal that failed to vocalize one or more times was classified as anesthetic. An electrical current (100 v. d.c.), delivered by a Grass model S-4 stimulator, was used as the stimulus. Because of tissue damage resulting from the intense stimuli, each animal was used only once. To facilitate conduction, each foot was moistened with 10% sodium chloride solution just prior to contact with the stimulating electrodes. Cocaine hydrochloride, procaine hydrochloride, dibucaine hydrochloride, and dyclonine hydrochloride were administered to groups of 10 animals at a minimum of three different concentrations to establish dose response curves. The anesthetic dose for 50% of mice (AD_{50}) was calculated for each drug and relative potency determined with respect to cocaine hydrochloride (5, 6).

RESULTS

The AD_{50} for each of the drugs was determined on four separate occasions, and the results obtained are presented in Table I. Experiments *A*, *B*, and *C* were each completed during a single day for all of the local anesthetics. For the preliminary experiment, the data (except for cocaine) were obtained over a period of 2 days. The results obtained show that the AD_{50} values were consistent for procaine and dyclonine. There was one AD_{50} value that was slightly low for dibucaine and one high for cocaine.

The potency of these agents relative to cocaine

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TABLE I.—REPRODUCIBILITY OF AD_{50} DETERMINATIONS[a]

	Preliminary Expt.	Expt. *A*	Expt. *B*	Expt. *C*
Cocaine HCl	1.38 (0.67–2.10)	2.22 (1.44–2.99)	3.53 (2.42–4.65)	1.24 (0.90–1.59)
Procaine HCl	19.95 (11.22–28.68)	27.11 (24.02–30.20)	23.36 (16.45–30.07)	19.01 (10.69–27.51)
Dibucaine HCl	0.90 (0.57–1.24)	2.12 (1.45–2.79)	3.10 (2.40–3.90)	2.85 (1.73–3.97)
Dyclonine HCl	4.50 (3.06–5.36)	2.56 (1.48–3.65)	4.52 (2.96–6.09)	2.51 (1.37–3.65)

[a] Dose expressed as milligrams per milliliter. Figures in parentheses are 95% confidence limits.

TABLE II.—REPRODUCIBILITY OF RELATIVE POTENCY DETERMINATIONS[a]

	Expt. *A*	Expt. *B*	Expt. *C*
Procaine HCl	Slopes Not parallel	0.15 (0.10–0.22)	0.06 (0.04–0.10)
Dibucaine HCl	0.95 (0.61–1.50)	1.35 (0.50–3.63)	0.41 (0.17–0.64)
Dyclonine HCl	0.94 (0.55–1.62)	0.72 (0.46–1.14)	0.49 (0.30–0.81)

[a] Cocaine hydrochloride used as standard for comparisons. Figures in parentheses are 95% confidence limits.

TABLE III.—LOCAL ANESTHETIC ACTIVITY BASED ON THE COMBINATION OF RESULTS OF REPEATED ASSAYS

Drug	Mice, No.	AD_{50}, mg./ml.	Activity Ratios (Cocaine = 1)
Cocaine HCl	126	2.03 (0.52–7.91)	1
Procaine HCl	88	26.10 (20.82–32.73)	0.11 (0.02–0.25)
Dibucaine HCl	108	2.45 (1.25–4.79)	0.67 (0.35–1.27)
Dyclonine HCl	87	3.28 (1.98–5.44)	0.69 (0.52–0.91)

TABLE IV.—LOCAL ANESTHETIC ACTIVITY TESTED 15 MINUTES AFTER SUBCUTANEOUS INJECTION OF THE DRUGS TO TAILS OF MICE (4)[a]

Drug	Mice, No.	AD_{50}, mg./ml.	Activity Ratios (Cocaine = 1)
Cocaine HCl	160	0.66 (0.53–0.81)	1
Procaine HCl	120	4.25 (3.40–5.31)	0.15 (0.10–0.20)
Dibucaine HCl	140	0.42 (0.31–0.55)	1.57 (1.20–2.29)

[a] Figures in parentheses are 95% confidence limits. Adrenaline hydrochloride (10 mcg./ml.) was added to all solutions.

is shown in Table II. In experiments *A*, *B*, and *C* the results were reproducible. The data for the preliminary experiment were not included because the drugs were not compared simultaneously. In one case (procaine), the slopes of the lines were not parallel, and a statement of relative potency could not be made. Despite considerable variation in relative potencies, there was an overlap of confidence limits for each anesthetic agent.

DISCUSSION

While it is desirable to obtain absolute reproducibility of AD_{50} and relative potency values, it must be recognized that the standard error of a test can only reflect causes of variation that influence the results of the initial test. There may be other factors that are constant at one time or in one laboratory, but that vary from time to time or from laboratory to laboratory (7). For example, in a collaborative study of lethality data obtained in several laboratories, Swope (8) found that not only was there a significant difference in results between laboratories but also between tests within a single laboratory.

To obtain more reliable estimates of the AD_{50} values and relative potency determinations, the results of the separate tests were combined as described by Miller *et al.* (6) and Stewart and Young (9). The results are presented in Table III. The 95% confidence limits for the relative potency determinations for cocaine hydrochloride, procaine hydrochloride, and dibucaine hydrochloride overlap with those reported by Bianchi (4), who compared the effectiveness of these compounds in producing anesthesia in the mouse's tail (Table IV). This illustrates that relative potency determinations from different laboratories are frequently equivalent, even though dissimilar investigative methods are used. Obviously, this degree of agreement between the AD_{50} values is not to be expected.

Since the precision of assays is generally compared by means of the lambda index, an approximate value for lambda was obtained by averaging the reciprocals of the slopes obtained in the separate AD_{50} determinations in these tests and the antilogs of the slope factors reported by Bianchi (4). The results obtained were 0.35 and 0.41, respectively. Both of these values compare favorably with the 0.38 lambda index reported by Mongar (10) for the intradermal wheal method with guinea pigs.

The results obtained by the method described in this paper exhibited considerable variability. Nevertheless, the accuracy of this test appears comparable to that enjoyed by similar biological tests.

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