ANESTHESIA. XXXVIII: QUANTITATIVE MEASUREMENT OF MUCOSAL IRRITATION OF VOLATILE ANESTHETICS * †

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The irritating properties of a volatile anesthetic agent are of importance in determining its clinical usefulness. This is notably true when two different anesthetics are similar in anesthetic potency, anesthetic syndromes and side actions. Then the difference in their irritating properties may determine which one would be preferred by the anesthesiologists.

Although a number of papers have been published in the last few decades dealing with the determination of irritating properties of various substances, for the most part they have not been suitable for evaluating the irritating properties of volatile anesthetic agents. This evaluation has had to depend largely upon personal observation either on the subjective feelings of patients or on the behavior of animals respiring the anesthetic vapors. Obviously, the results thus obtained are more or less associated with subjective phenomena. However, in the absence of a more accurate objective method, information obtained in such a manner may be of much value. Thus, the irritating properties of certain volatile ethers have been estimated and compared by adopting this principle (1–5).

Many methods have been devised for the evaluation of the irritating properties of chemicals, depending upon the production of hyperemia, edema and so forth, on local application of the irritants to the skin or mucous membranes of animals (6–10) or man (7, 11, 12, 13). The determination of the degree of irritation depended entirely upon the assignment of scores. Here again, unavoidable subjective factors enter into the methods. However, the methods with weighted scores, used by Friedenwald, Hughes and Herrmann (8), Draize, Woodard and Calvery (6) and Hoppe, Alexander and Miller (10), for the measurements of injuries to rabbit eye lend a certain degree of objectivity.

The well-known trypan blue test for the evaluation of irritation has been used by a number of investigators (10, 14–18). Here again, the

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intensity of dye staining was estimated on a score basis. However, the refinement in assigning scores and the suggestion of using T.I.C. (threshold irritant concentration), as indicated in Hoppe's paper, have further improved this method.

Finnegan, Fordham, Larson and Haag (19) developed a more precise quantitative method for evaluating smoke irritation. They found that the upper palpebral conjunctival membrane of rabbit showed a graded increase in water content to increasing exposure to tobacco smoke. By using this same principle, Küchle and Loeser (20) reported a simple device to measure the thickness of the upper lid of rabbit eyes. The thickness thus measured was claimed to reflect very nicely the degree of edema which developed following instillation of various chemical solutions. Their results are claimed to be comparable to those obtained by Finnegan *et al.*

In view of the fact that irritation is a complicated phenomenon, and probably no single index can include all of the manifestations produced by an irritant, it is difficult to design a method which would represent the entire process of irritation. However, with certain limitations and accepting certain assumptions, a scientific objective method for evaluating a certain irritating property of any chemical seems to possess merit that other subjective methods do not. Since such a method has not appeared to be available for general anesthetic agents, we devised the following procedure.

PRINCIPLE OF THE METHOD

The method consists essentially of a quantitative measurement of edema-producing properties of volatile anesthetics on mucous membranes. The assumption is made that their irritating properties are intimately associated with production of edema. The degree of edema can be estimated by measuring the water content in the excised membrane upon drying. The upper palpebral conjunctival membrane of rabbit was found most suitable for this purpose, since it is readily exposed and easily excised. The mucous membrane along the respiratory route, although it would represent more adequately the conditions of passage of the anesthetic vapor, was observed to be less satisfactory in this regard.

In order to simulate the natural clinical application, a mixture of movable air and anesthetic vapors was allowed to pass across the membrane instead of instillation in an aqueous solution. In accomplishing this, a certain amount of volatile anesthetic to be tested was placed in a specially devised bottle which was kept at a constant temperature. The bottle was connected by rubber tubing to an eyecup as used by Finnegan *et al.* (19). The eyecup was attached to a pulling device as originally described by Bradford, Harlan and Hanmer (21), with some modification. As shown in figure 1, when the clamp (x) was released the falling of the water column in the long vertical glass tubing created

a partial vacuum which pulled the air through the volatile anesthetic in the bottle. (The bottle R in figure 1 should be placed below the glass tubing at this time.) Consequently, a certain amount of vapor mixed with air passed through the whole system when the water column was falling. At constant temperature, with a constant height of water column as the starting point and within a constant period of time to allow the water column to fall, a constant amount of anesthetic vapor was delivered. The quantity was determined by comparing the weight of the bottle containing the volatile anesthetic before and after the procedure. Further, it is easily changed according to the demand by varying only the temperature of the bath and keeping all other conditions constant. In order to assure an even bubbling of air, thus facilitating its uniform mixing with anesthetic vapors, the orifice of the opening (a) of the bottle was intentionally narrowed and glass beads were placed in the bottle. Since the dead space between the opening (b) of the bottle and the eyecup is constant and comparatively small, the amount of the anesthetic vapor passed through the eyecup or across the conjunctival membrane should bear a direct relationship to the amount measured by comparing the difference in weight of the bottle before and after the procedure. The amount thus measured serves as a direct representation for the molarity of anesthetic vapors mixed with air to which the conjunctival membrane was exposed within a constant period. Thus, the relationship between the dosage and the production of edema can be found. By using this method we were able to demonstrate that the upper palpebral membrane of rabbit eye showed a graded response in the form of edema to increasing concentrations of anesthetic vapor. This response then may be utilized as the basis for comparison of irritating properties of various anesthetics to be tested.

EXPERIMENTAL

Male albino rabbits, weighing between 1.2 and 2.6 kg., were employed. They were injected with morphine sulfate, 20 mg. per kilogram subcutaneously, in order to minimize struggling. Twenty-five minutes later two drops of physiologic saline solution were instilled into the conjunctival sacs of each eye to assure a uniform moistening of the tissues subject to exposure. Five minutes later the upper lid of the right eye was everted and the eyecup was placed over the lid and surrounding tissues. The cup was held firmly in place. The system was connected to the bottle, containing no anesthetic, immersed in the water bath. The whole system was opened by releasing the clamp (x), simultaneously turning the cap of the bottle so that the air could be drawn across the eye. The cup was held in place exactly one minute after the opening of the system. Then it was closed again by turning the cap of the bottle and simultaneously clamping at (c). This right eye served as the control.

The same procedure was repeated on the left eye except that the amount (usually 15 cc.) of volatile anesthetic to be tested was put into the bottle and the bottle containing the anesthetic was weighed before and after the exposure of the eye. The detailed procedure is described as follows:

The bottle containing volatile anesthetic was immersed in the water bath for two minutes while keeping the orifice (b) open. Then, by turning the cap of the bottle, the bottle was closed and weighed as soon as possible to the second decimal place. The bottle was quickly returned to the water bath and connected to the system for exposure. As soon as the exposure procedure was completed it was closed again by turning the cap of the bottle, and again weighed. The difference between the two weighings represents the amount of volatile anesthetic escaping out of the bottle.

After the procedure of exposure, the entire system is cleaned of anesthetic vapors by flushing with air. Before doing this the bottle (R) should be lifted as high as possible so that the ascending of the water column in the vertical glass tubing can expel the anesthetic vapors. In order to facilitate the ascent of the water column, the clamp (e) over the by-pass (d) is released. The by-pass (d) containing a piece of glass tubing with large caliber was made especially for this purpose. It was clamped off during the exposure, whereas the by-pass (c) made of constricted glass tubing remained open.

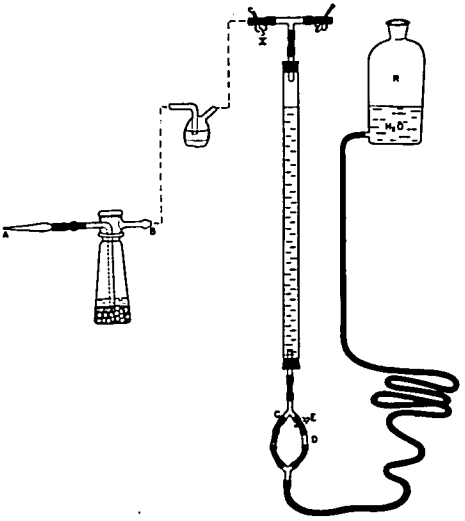

FIG. 1. Schematic illustrations of the device for the determination of mucosal irritation of volatile anesthetic vapors. (Figure not drawn to scale.)

A period of one hour from the completion of the exposure of the eye to anesthetic vapors was allowed to elapse for the development of edema. At the end of this period the animal was killed by injecting approximately 5 cc. of saturated magnesium sulfate solution into the marginal ear vein. The membranous lining of the upper lids of both eyes was retracted and excised. The excised tissues were immediately placed in tared vials and weighed. They were then placed in an oven at 104 C. for twenty-two to twenty-four hours, at which time they were again weighed to obtain the dry weights. It was determined that a constant dry weight can be obtained upon two successive weighings by this procedure.

From the wet and dry weights of the exposed and control tissues the moisture content of each is calculated. The ratio of moisture content to dry weight for each tissue is then calculated. The percentage change in water content between the two tissues may then be obtained. In a series of determinations the changes in percentages in treated eyes serve as a basis for statistical evaluation.

RESULTS

The Reliability of the Method.—In 6 normal rabbits not subjected to exposure to anesthetic vapors or any other procedures, the amounts of water content in the membranous linings of the upper lids of both eyes were found to be remarkably close. The mean percentage difference was 0.2 ±2.8 (standard deviation). Further, in 4 other animals the right eyes were subjected to manipulations necessary for exposing procedures by drawing air across the eye from the bottle immersed in the water bath at room temperature for one minute, whereas no such procedure was performed on the left eyes. The mean percentage difference in water content of membranous linings of upper lids of both eyes in the same animal was found to be 2.3 ±4. This indicates that the manipulations do not exert any significant influence upon the water contents of these tissues.

By using diethyl ether as an example, the upper lids of rabbit eyes were found to exhibit a graded response in the production of edema to increasing concentrations of vapor of the ether. As illustrated in figure 2, a linear relationship was obtained by plotting the -log mole of diethyl ether against the percentage increase in water content of upper lid membranes in treated eyes as compared with those in control eyes. It was also shown that the test object exhibited a high sensitivity in the production of edema to the anesthetic vapors.

Official ether was employed throughout these experiments with the idea that its use would be closer to clinical conditions. As ether U.S.P. contains 2 to 4 per cent water and alcohol, the results thus obtained are affected by these constituents. For the same reason, vinyl ether U.S.P. (which contains 4 per cent alcohol and 0.025 per cent preservative) was used.

Based upon the foregoing experiments it seems that this method is suitable for our purpose by accepting the assumption that the production of edema is at least one of the characteristics of irritating properties of volatile anesthetics.

Comparison of the Irritating Properties of Volatile Anesthetics. As stated in the description of the method, the amount of volatile anesthetic passing across the upper palpebral membrane can easily be varied by changing the temperature of the water bath in which the bottle containing the anesthetic agent is immersed. Since many volatile anesthetics have different boiling points, their vapor pressures differ considerably from one another at a constant temperature. By varying the

FIG. 2. The relationship between the production of edema of upper palpebral conjunctival membrane of rabbit eye and the different amounts of ether U.S.P. The upper and low points represent the average values obtained from 4 rabbits each. The middle point represents the average values obtained from 10 rabbits.

temperature in the water bath their vapor pressures can be well adjusted to close proximity so that, under otherwise identical conditions, the amounts of different anesthetics emerging from the bottle can be made similar in molarity at each exposure. On such equimolar basis, the irritating properties of the four ethers were compared.

As the concentration of ether vapor required to produce a measurable edema is quite high (see fig. 2), the temperature of the environment should be slightly higher than that in the water bath in order to assure that no condensation occurs at the mucosal membrane when the vapors pass across the eye. Since the highest environmental temperature which can be reached is limited by mucosal temperature of the eye, the temperature for comparison in a series of experiments was arbitrarily chosen at a lower range. Therefore, none of the corresponding environmental temperatures for any anesthetic thus tested could exceed the

TABLE 1

COMPARISON OF THE PRODUCTION OF EDEMA OF THE UPPER PALPEBRAL CONJUNCTIVAL MEMBRANES OF RABBITS EXPOSED TO THE EQUIVOMOLAR AMOUNTS OF THE FOUR ETHERS*

Ether (U.S.P.)				Vinyl Ether (U.S.P.)				n-Propyl Methyl Ether				Ethyl Vinyl Ether			
Rabbit No.	Theoretical Yield, Gm.	Actual Yield, Gm.	Difference in Upper Conjunctival Water Content of Both Eyes, per cent	Rabbit No.	Theoretical Yield, Gm.	Actual Yield, Gm.	Difference in Upper Conjunctival Water Content of Both Eyes, per cent	Rabbit No.	Theoretical Yield, Gm.	Actual Yield, Gm.	Difference in Upper Conjunctival Water Content of Both Eyes, per cent	Rabbit No.	Theoretical Yield, Gm.	Actual Yield, Gm.	Difference in Upper Conjunctival Water Content of Both Eyes, per cent
68	0.836	0.83	+27.8	90	0.791	0.80	+12.3	78	0.836	0.83	+123.2	100	0.813	0.81	+ 63.0
69	0.836	0.82	+78.0	91	0.791	0.78	+30.2	80	0.836	0.84	+136.5	101	0.813	0.80	+ 99.0
70	0.836	0.85	+03.0	92	0.791	0.79	+17.8	81	0.836	0.86	+119.8	102	0.813	0.79	+ 60.0
71	0.836	0.83	+59.0	93	0.791	0.79	+20.0	82	0.836	0.83	+134.5	103	0.813	0.82	+ 74.5
72	0.836	0.84	+21.6	94	0.791	0.80	+11.3	83	0.836	0.82	+130.0	104	0.813	0.82	+ 54.3
73	0.836	0.85	+13.8	95	0.791	0.79	+38.8	84	0.836	0.83	+101.2	105	0.813	0.83	+114.4
74	0.836	0.84	+20.6	96	0.791	0.78	+20.8	85	0.836	0.83	+ 76.0	106	0.813	0.81	+109.2
75	0.836	0.83	+49.4	97	0.791	0.78	− 3.0	86	0.836	0.83	+113.2	107	0.813	0.80	+126.0
76	0.836	0.84	+58.2	98	0.791	0.80	+12.1	87	0.836	0.83	+ 39.5	108	0.813	0.80	+109.6
111	0.836	0.83	+44.8	99	0.791	0.77	+ 3.0	89	0.836	0.82	+164.3	110	0.813	0.79	+ 91.0
Mean	0.836	0.836	+43.6		0.791	0.788	+16.9		0.836	0.832	+113.8		0.813	0.807	+ 90.1

* The conditions for the four ethers during exposure were identical except for the temperatures, which were as follows:

	Ether U.S.P.	Vinyl Ether U.S.P.	n-Propyl Methyl Ether	Ethyl Vinyl Ether
Bath Temp., C.	24.5°	19.2°	27.3°	25.1°
Room Temp., C.	26.0°	25.0°	29.0°	27.0°

usual skin temperature of the face and head of the rabbit, assuming that the temperature of the upper palpebral membrane is similar or close to that of the face and head.

Based upon these theoretical considerations, it was found that the amount of anesthetic emerging from the bottle each time for a specific ether was remarkably close to that theoretically calculated. The differences of both values lay within the experimental error of the balance. The results are summarized in table 1. Analysis of variance of the difference between values in edema production among four ethers yields P values (< 0.01 for vinyl ether U.S.P. and ether U.S.P.; < 0.001 for ether U.S.P. and ethyl vinyl ether; <0.001 for ether U.S.P. and n-propyl methyl ether, and a little greater than 0.1 for ethyl vinyl ether and n-propyl methyl ether). Therefore, the differences are highly significant except for ethyl vinyl ether and n-propyl methyl ether. Thus, the irritating effect, as reflected by the edema-producing property, of the four ethers tested should be arranged in the following order: ethyl vinyl ether >ether U.S.P. > vinyl ether U.S.P.; *n*-propyl methyl ether > ether U.S.P. > vinyl ether U.S.P.

DISCUSSION

In transferring qualitative or semiquantitative observations into quantitative data which can be expressed by numerical values, the method described has advantage owing to the objectivity of measurement. As shown by the wide separation of the degree of edemas produced by different concentrations of diethyl ether as well as by the same concentrations of the four ethers tested, the method reveals a high degree of sensitivity.

It is recognized, however, that the method has certain limitations. Only those irritating volatile anesthetics that produce edema may be measured. Obviously, subjective phenomena associated with irritation are not measured. Further, the production of edema has generally been considered as a phase or a type of manifestation of irritating properties. Diethyl ether in higher concentrations was actually demonstrated to be capable of increasing capillary and cellular permeabilities in plants (22–24) as well as in animal tissue (25). There has been no direct evidence to prove that the edema produced by anesthetic vapors is proportionally related to their irritating properties. The acceptance of such an assumption to form the basis of this method is, therefore, purely arbitrary. The phenomena of irritation probably are very complex. As pointed out by Heubner (26), different irritants could affect different parts of tissues simultaneously. On the other hand, our results agree with the statements mentioned in Heffter's Handbook (1) that the order of irritation is: ethyl vinyl ether > diethyl ether > divinyl oxide. This suggests the validity of the foregoing measurements as compared with subjective observations. The statement was made by

referring to the data which were obtained from observations upon the behavior of mice toward the anesthetic vapors.

Our results are contrary to the clinical reports (27) that *n*-propyl methyl ether is less irritating than diethyl ether. It has a more bland odor. We cannot at present explain these disagreements. However, it might be possible that, owing to the higher potency and higher boiling of *n*-propyl methyl ether than diethyl ether, the concentration for producing anesthetic syndromes in the respiratory tract would be lower than that of the latter, resulting in less irritation. Another possibility is that *n*-propyl methyl ether would less powerfully stimulate the sensory nerve endings of the olfactory tracts than would diethyl ether, and this property does not necessarily bear a parallel relationship to the property of production of edema.

Obviously, this method can also be used for higher boiling volatile anesthetics. With these agents it is desirable to set the temperature of the standard anesthetic (we used diethyl ether) lower for comparison than that described in this communication, since such temperature was chosen purely arbitrarily. Thus, the temperatures for all anesthetics to be compared can be set correspondingly lower so that the corresponding environmental temperature will never exceed the rabbit skin temperature of the face. These conditions are considered essential in order to avoid the possibility of condensation. Under such circumstances the time period for exposure would have to be lengthened somewhat to assure the production of measurable edema.

With certain adaptation the method appears suitable also for evaluating the irritating properties of anesthetic gases.

SUMMARY

Based upon the production of edema in upper palpebral conjunctival membrane of the rabbit by volatile anesthetic vapors, a quantitative and objective method suitable for evaluation of their irritating properties is presented.

The quantitative data thus obtained can be expressed in numerical values and the comparison of the irritating properties among different volatile anesthetics can be statistically made on a molar basis.

By the application of this method the irritating properties of the following four ethers have been compared and found to be in descending order: ethyl vinyl ether > ether U.S.P. > vinyl ether U.S.P.; *n*-propyl methyl ether > ether U.S.P. > vinyl ether U.S.P.

n-Propyl methyl ether was found to be more irritating than ethyl vinyl ether, but with a P value little greater than 0.1

The advantages, disadvantages and limitations of the method are discussed.

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