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A New Experimental Approach to the Evaluation of the Effect of Narcotic Analgesics Upon Respiratory Function *

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ABSTRACT — Experimental models often used to study the effects of drugs upon respiratory function employ anesthetized animals. The present study eliminated the possible interference of anesthetics by using dogs altered only by a permanent tracheostomy. Respiratory function was evaluated by determining end-expiratory (alveolar) CO_2 tension. The experimental design, an extension of the cross-over type, permitted the estimation of possible residual effects of the drugs applied in sequence to the same animals. Morphine Sulfate increasingly caused on elevation in olveolar pCO_2 while Meperidine HCl had no such effect. An antitussive meperidine derivative, WIN 13187, had respiratory effects similar to meperidine.

The methods commonly used to study altered respiratory function, following narcotic administration, include the measurement of minute volume, the respiratory response to exogenous carbon dioxide, and the measurement of end-expiratory (alveolar) CO_2 tensions both before and after the administration of the drugs under study. Most such experimental studies employ anesthetized animals (Severinghaus et al, 1957, Greisheimer et al; 1960, Lambertsen and Wendel, 1960).

The study reported here proposed to avoid the use of anesthetized animals because of the complicating actions of anesthetic agents upon the effects of experimental drugs. Using conscious animals, however, ordinarily prevents measuring minute volume and respiratory response to endogenous hypercapnia as paramenters because of the animals' inability to cooperate. In this study, therefore, changes in respiratory function were evaluated by following end-expiratory CO_2 tensions in conscious animals with fractional to-and-fro sampling through a permanent tracheostomy using the Liston-Becker continuous infrared CO_2 analyzer. This reliable monitoring apparatus has been quite adequately validated for measuring alveolar pCO_2 by Collier et al (1955). Eckenhoff et al (1956), Saxton (1953) and Robin et al (1958). Also, fractional to-and-fro sampling approaches the ideal because the function it measures is in no way interfered with by mask, mouth piece or endotracheal tube.

By thus eliminating the effects of anesthetic agents and developing a technique by which experimental animals can be used repeatedly, it was also deemed advisable to apply a type of randomized statistical design which would permit the unbiased use of animals receiving the series of treatments. The procedures followed eliminated inter-animal variability and readily allowed for the detection of and adjustment for residual effects if present.

The total experiment was designed to eliminate as many complicating factors as possible in the comparison of drugs that have analgesic or respiratory depressant effects, with morphine as a standard.

Materials and Methods

Side to side tracheostomies were performed on six adult mongrel dogs (see front cover). A midline incision was made over the trachea and the anterior one-third of 2 to 4 tracheal rings was resected just below the cricoid cartilage. The incision was closed by sewing the skin to the free edge of the cartilage with a continuous running suture. The superior and inferior margins of the original incision were left unsutured for drainage.

A local chemotherapeutic drug, nitrofurazone, was applied to the wound with gauze squares. After sufficient healing the animals were again anesthetized and the sutures removed. During recovery (6 to 10 days postoperatively), the dogs were familiarized with the handler and experimental procedure.

Once the actual experiment was started subsequent runs for each animal were made only after 4-day rests to decrease the possibility of residual effects. On the day of a procedure the animal was placed in a Pavlov type framework 30 to 60 minutes before the experiment was begun. A tracheotomy tube was tied into place (see Figure 1) and the pliable probe of an electronic thermometer was inserted into the rectum and taped in place.

Carbon dioxide was measured by the Liston-Becker (L-B) continuous rapid infrared CO_2 analyzer with

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FIGURE 1. Sampling set-up in place in animal V.

"microcatheter" cell. The signal was recorded on the Esterline Angus Model AW recording milliameter. A vacuum pump, sump and flowmeter maintained a continuous flow of air through the sampling cell.

A length of Clay-Adams #90 polyethylene tubing was attached to a blunt needle that was, in turn, attached to the L-B "pick-up." The other end of the polyethylene tubing was attached to a small hollow tracheotomy trochar that could then either be fitted into the tracheotomy tube previously inserted into the animal's tracheostomy (see Figure 1), or placed in tubes that were flushed with known concentrations of CO_2. The CO_2 came from tanks previously analyzed by the micro-Scholander method (Scholander, 1947) and was used to calibrate the L-B just prior to each sampling (for details see Pierce, 1961). The concentration of alveolar CO_2 in mm. Hg was calculated by the following formula:

$$P_ACO_2 = F_ACO_2 (P_B - P_{H_2O_T})$$

Where

P_ACO_2 = Pressure of alveolar CO_2 (pCO_2) in mm. Hg.

F_ACO_2 = Fraction of alveolar CO_2 = % $CO_2 \div 100$.

P_B = Ambient barometric pressure corrected for room temperature (maintained between 20° and 24° C).

$P_{H_2O_T}$ = Partial pressure of water vapor corrected for core body temperature.

Samples for analysis were taken 4 times at regular intervals during the 30-minute pretreatment period and 13 times over the 4 hours of post-injection observation. All collected and calculated data were recorded on a standard form (Pierce, 1961).

After studying the effects of several dosage levels of morphine SO_4 in preliminary studies, 2 mg./Kg. i.v. was found to produce consistent depression of respiratory function with a minimum of "trauma" and panting. "Traumatic" effects of concern were convulsions, excitement, nausea with retching, spontaneous defecation, urination and severe prostration. Meperidine HCl was given in a dose of 12 mg./Kg. i.v. as this was the largest reported ratio (Morphine to Meperidine) of equal analgesia and equal respiratory depression effects (Thorp et al., 1947). Since the new experimental drug (Grumbach, 1959), here designated as WIN 13187, is a meperidine derivative, and preliminary experiments revealed that its dosage effects were comparable to those of meperidine, the dosage was set in a range approximating the meperidine dosage. Those dosages studied were 5 mg./Kg., 10 mg./Kg., 20 mg./Kg. and 30 mg./Kg. All drugs were given intravenously immediately after the 30-minute pretreatment sampling.

A "crossover" experimental design (Cochran & Cox, 1957) possessing a special property of balance that allowed adjustment for residual effects from the preceding drug, if these existed, was used. The analysis was then based upon intra-dog comparisons of respiratory function after elimination of variance between periods.

The actual statistical design utilized a 6 x 6 Latin square particularly devised for experimental arrangements such as the present one in which treatments (letters) are applied in sequence to animals (roman numerals).

Time Period	Subject No. I	II	III	IV	V	VI
	Drug Sequence for each animal					
1	A	B	C	D	E	F
2	C	D	E	F	A	B
3	B	C	D	E	F	A
4	E	F	A	B	C	D
5	F	A	B	C	D	E
6	D	E	F	A	B	C

The *time period* was defined as the time required to utilize each of the experimental subjects, once. For example, running one dog a day, it took six working days to complete one period.

The *drug sequence* was the order in which a given animal received the treatments being studied.

Randomization of this design proceeded in two steps leading to an experimental master sheet that *governed* the duration of the experiment.

Step 1: Treatment ($T_1 - T_6$) were assigned to letters (A-F) at random. Thus, if $T_1 - T_6$ were as follows:

T_1 = WIN 13187 – 5 mg./Kg.
T_2 = WIN 13187 – 10 mg./Kg.
T_3 = WIN 13187 – 20 mg./Kg.

T_4 = WIN 13187 – 30 mg./Kg.
T_5 = Morphine SO_4 – 2 mg./Kg.
T_6 = Meperidine HCl – 12 mg./Kg.

and the letters A, B, C, D, E, F were assigned to 1, 5, 4, 6, 2, 3, a sequence obtained from a table of random numbers, the following randomization of drugs resulted:

A = T_1 = WIN 13187 – 5 mg./Kg. = WIN 5
B = T_5 = Morphine SO_4 – 2 mg./Kg. = MS-2
C = T_4 = WIN 13187 – 30 mg./Kg. = WIN-30
D = T_6 = Meperidine HCl – 12 mg./Kg. = Dem-12
E = T_2 = WIN 13187 – 10 mg./Kg. = WIN-10
F = T_3 = WIN 13187 – 20 mg./Kg. = WIN-20

Step 2: The sequence or order in which the dogs were used was randomized. From the table of random numbers the following random requence was obtained: 4, 2, 5, 6, 3, 1. This led to the following experimental master.

	Dog IV	Dog II	Dog V	Dog VI	Dog III	Dog I
1	Dem-12	MS-2	WIN-10	WIN-20	WIN-30	WIN-5
2	WIN-20	Dem-12	WIN-5	MS-2	WIN-10	WIN-30
3	WIN-10	WIN-30	WIN-20	WIN-5	Dem-12	MS-2
4	MS-2	WIN-20	WIN-30	Dem-12	WIN-5	WIN-10
5	WIN-30	WIN-5	Dem-12	WIN-10	MS-2	WIN-20
6	WIN-5	WIN-10	MS-2	WIN-30	WIN-20	Dem-12

Assessment of the overall experimental response was facilitated through the fitting, by least squares, of a straight line to the P_ACO_2 response data of each animal (Acton, 1959). The slope of the line then furnished a relevant measure of trend, with the intercept reflecting the general level of the animal's P_ACO_2 response to the drug employed.

Analysis of variance for direct and residual effects of the slopes and over-all means of the P_ACO_2 determinations as well as standard errors and significance tests were computed according to Cochran and Cox (1957). The procedure was based upon the analysis originally outlined by Williams (1949).

Consistency of the experimental procedure was demonstrated by the following variance analysis of results. As might be anticipated, variability among dogs was highly significant ($p < 0.01$), for both slopes and intercepts. On the other hand, no significant differences appeared among the six observation periods ($p > 0.05$). Residual effects for the above variables were negligible, $p > 0.05$.

Figure 2 presents mean P_ACO_2 tension both for the pre-injection control and also for the post-injection experimental response of the animals to the six drugs under test. Table 1 contains the data in tabular form.

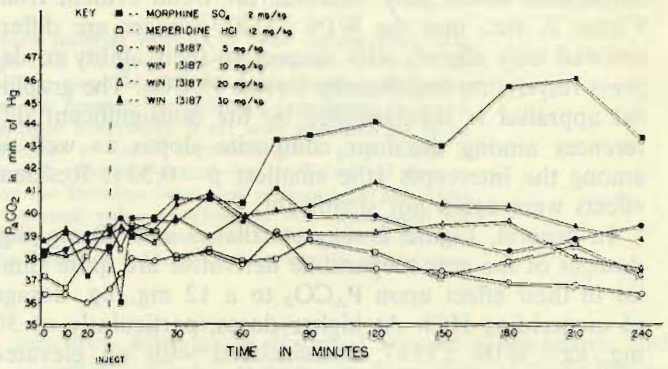

FIGURE 2. Mean P_ACO_2 (in mm. Hg) before and after injection of 2 mg./kg. morphine SO_4, 12 mg./kg. meperidine HCL and 5, 10, 20 and 30 mg./kg. of WIN 13187.

Focusing first upon Morphine SO_4 and Meperidine HCl, it will be seen that during the pre-injection control phase, the fluctuation in the P_ACO_2 values is random. However, during the post-injection phase, there is evident a steady increase in P_ACO_2 with morphine SO_4, whereas the response to Meperidine HCl does not exhibit this positive trend.

A statistical test of the difference between the average slopes of these two groups revealed a significant difference in the two linear trends. Specifically, the difference in slopes was 0.0287, the standard error was 0.0055, so that $t = 5.2$, with 15 degrees of freedom and a significance probability (p) value of < 0.001. The residual trend effect was, as hoped, not significant ($p = 0.53$).

Similarly, subjecting the intercepts to analysis revealed the direct P_ACO_2 response to morphine sulfate to be significantly higher than that to Meperidine HCl, ($p < 0.001$). The residual intercept effect, however, was again not significant ($p > 0.80$).

Turning next to the inter-dosage contrast of the four

TABLE 1. Average P_ACO_2 in mm. Hg of Six Animals

Pre-treatment (time in min.)				Post-treatment (time in min.)												
0	10	20	30	5	10	20	30	45	60	75	90	120	150	180	210	240
Morphine sulfate—2 mg./kg.																
38.3	39.2	37.2	38.5	36.2*	39.8*	39.8*	41.7	41.7	41.5	43.3	43.5	44.1	43.0	45.7	46.0	43.4
Meperidine HCl—12 mg./kg.																
37.2	36.7	38.1	39.4	38.4	39.6	39.2*	38.0	38.4	37.9	38.0	38.8	38.3	37.2	37.9	38.6	37.3
WIN 13187—5 mg./kg.																
36.4	36.4	36.0	36.6	37.3	38.0	37.9	38.1	38.3	37.7	39.2	37.6	37.5	37.4	36.9	36.8	36.4
WIN 13187—10 mg./kg.																
38.1	38.4	39.1	39.3	39.8	39.3	39.7	39.7	40.8	39.7	41.1	39.9	39.9	39.0	38.3	38.9	39.5
WIN 13187—20 mg./kg.																
38.3	38.1	38.5	38.3	39.3	39.2	38.7	39.8	39.0	39.9	39.0	39.1	39.1	39.5	39.6	38.0	37.5
WIN 13187—30 mg./kg.																
38.8	39.3	38.8	39.1	39.4	39.0	39.2	40.4	40.9	39.9	40.1	40.3	41.4	40.4	40.3	39.4	38.8

* Mean from less than 6 determinations.

levels of WIN 13187, it will be noted from Figure 2 that here, also, the pre-injection control values fluctuate nonsystematically, with the exception of those associated with the 5 mg./kg. group for which no assignable cause was detected. However, even if the level of response to 5 mg./kg. of WIN 13187 was somewhat underestimated, adjustment would only reinforce the trend evident from Figure 2, viz., that the WIN 13187 dosages are differentiated only slightly with respect to their ability to depress respiration and thereby elevate P_ACO_2. The graphical appraisal is substantiated by the nonsignificant differences among the four composite slopes as well as among the intercepts (the smallest $p = 0.33$). Residual effects were again not significant.

In general, Figure 2 suggests that 5 and 10 mg./kg. dosages of the new meperidine derivative are quite similar in their effect upon P_ACO_2 to a 12 mg./kg. dosage of meperidine HCl. At higher doses, particularly at 30 mg./kg., WIN 13187 is associated with an elevated P_ACO_2, not differing significantly in slope and intercept from Morphine SO_4. Once again no significant residual effects occurred.

Conclusion

The experimental approach presented here possessed several features critical to the valid assay of the effects of drugs upon respiratory function:

1. Respiratory function as measured by alveolar pCO_2 was determined when the animals were in an unanesthetized state. It was demonstrated that eliminating the possibly confounding effects of anesthetic drugs was most desirable.
2. The sampling technique employed was unusually simple and reproducible. The procedure was well tolerated by the animals, and they can be used indefinitely with only normal laboratory care.
3. The experimental design, by virtue of its balanced crossover structure, allowed for the detection of residual effects of drugs applied in random sequence to the same animals. Analysis indicated that a 4-day interval between succeeding treatments was ample for the dissipation of any residual respiratory effects from the drugs involved.
4. In addition, the six periods of observation did not differ significantly in mean response, showing that a substantial consistency of experimental procedure was attained.

As anticipated, there was a significant variation in average response among dogs – an indication that more than a few animals are necessary in the evaluation of physiological parameters after the administration of exogenous agents.

The effects of morphine and meperidine upon alveolar pCO_2 showed that, at the 6:1 ratio studied, meperidine is considerably less of a respiratory depressant in the dog than morphine. Comparable data for the dog are not to be found in the literature.

The antitussive meperidine derivative (WIN 13187), reported herein, affected respiration in a manner similar to meperidine. Analysis showed no significant difference among the dosages studied. Thus, even with relatively large dosages of WIN 13187 little effect upon respiratory function may be expected. Thirty mg./kg. of WIN 13187 did have effects upon P_ACO_2, but they were considerably less than those produced by 2 mg./kg. of morphine and were not significantly different from those produced by 12 mg./kg. of meperidine.

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