MEASUREMENT OF CHANGES IN OXYGEN CONSUMPTION AND RESPIRATORY RATE IN THE RAT TO DETERMINE THE ACTIVITY OF BARBITURATES AND ANALGESICS WHEN COMBINED WITH CHLORPROMAZINE AND HYDERGINE(R)

by

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INTRODUCTION

The wide application and large number of central nervous system depressant drugs now utilized in clinical medicine warrants an extensive and continued study of their various effects upon both human and animal subjects. Numerous new compounds are being released for general clinical use with great frequency and often before adequate and sufficient fundamental experimental and clinical knowledge as to their various effects has been obtained. Therefore it is always expedient to compare their therapeutic activity and untoward actions with the older well-established drugs by as many suitable tests as can be practicably used. Before a new drug can be accepted and released for general clinical use, its toxic effects and therapeutic index of safety must be evaluated in comparison with the tried and tested drugs with which it is to compete as a safe and meritorious therapeutic agent.

The narcotic analgesics and the barbiturates, as well as the more recently introduced so-called tranquilizing or ataractic drugs are among the most widely employed of those agents possessing potent central nervous system depressant effects. Because of their extensive clinical usage and since their depressant action may cause severe toxic effects or even death, it is extremely important that accurate and valid methods of pre-clinical study precede their release for therapeutic trial.

For the most part appraisal of the effect and activity of such drugs as the barbiturates and ataractics in small animals has been a matter of subjective measurement, rather than by more accurate, carefully recorded and gauged objective experiments. Such methods as

determinations of sleeping time [1,2,3,4,5,6], of the righting time [7,8,9,10], the loss of postural reflexes [11], measurements of the performance time [7,12] and of hypnotic action [13,14,15,16] are, at best, approximate and certainly do not provide an objective record for evaluation and the comparison of different drugs and dosages. Winter [17] and other workers have devised different types of apparatuses employing such devices as photoelectric counters or suspended balanced cages [18,19] for quantitatively studying the changes in the activity of small animals following administration of depressant drugs. Observation of the electroencephalographic change in cerebral activity resulting from increasing dosages of barbiturates also has been employed as a method for measuring the depressant effect [20].

However, the majority of these methods reflect only indirectly the actual physiologic changes which such drugs induce. Furthermore, they fail to demonstrate the toxic effects, short of death, which may simultaneously occur. Since these hypnotic, ataractic or analgesic drugs are widely used in debilitated patients and for intravenous anesthesia or as adjuncts to general anesthesia, it would seem desirable to evaluate their useful therapeutic effects and their toxic qualities at the same time.

A. Pharmacologic Aspects of Central Nervous System Depressant Drugs:

Central nervous system depressant drugs in general depress all types of body activity. In particular they depress respiratory activity, this being the most dangerous untoward action since it directly threatens the patient's life. Both the opiates and the barbiturates are primary respiratory depressants by virtue of their direct effect on the

respiratory center [21]. Courvoiser [15], in his excellent review of recent experiments with some of the new ataractic drugs, has shown that those drugs also depress metabolism and decrease respiratory activity. With all of these drug groups, the depression of respiration and metabolism is proportional to the dose administered [21]. Further, it has been shown that there is a direct correlation between oxygen uptake by tissue cultures proportional to the tissue content of the depressant drug [22,23] and also that the arterial-venous oxygen difference decreases regularly with increasing blood levels of these drugs [22,24,25].

B. New Methods for Measuring Effects of Central Nervous System Depressant Drugs:

It would seem that more accurate methods for determining the effects of these depressant drugs than those previously mentioned could be devised. One such method which appears to possess certain advantages is the measurement of changes in the basal metabolism to provide objective measurements rather than observations of merely sedative effects or subjective changes in performance. Recent experiments by Labort [25] indicate that the depressant drugs depress metabolic activity by peripheral as well as by central action and that changes in the respiration are more physiologic than heretofore believed. If a drug's primary action is to depress activity, it follows that it should proportionately depress caloric requirements and, thereby, the oxygen consumption. A method employing the measurement of basal metabolism should then be applicable to a study of these drugs.

Many different oxygen consumption methods have been used by workers in several fields for the measurement of such functions as thyroid activity [26,27], bioenergetics [28], the ventilatory function [29,30,31] and the effects of a multitude of drugs. There are numerous references to the change in respiratory rate [32,33,34], the respiratory quotient [28,35], the caloric requirement [28], the tissue oxygen uptake [22,23] and the plasma, oxygen and carbon dioxide levels [24,25] as a result of the administration of central nervous system depressant drugs. However, to this writer's knowledge, there have been no attempts to establish a dose-response curve in relation to the above experiments and to compare other drugs to the results so obtained.

It is desirable to have an accurate and reasonably rapid method of measuring these drugs alone as to their particular qualities. Also needed is an objective method by which the new and experimental sedatives, ataractics and analgesics could be compared with the older, well-established representatives of that particular field. A dose-response curve based upon the effects on metabolism, which at the same time would illustrate the respiratory toxic effects of the drug, would appear to be of great value. Furthermore, such a method would be applicable to the evaluation and study of the potentiating effects of various agents on the hypnosis produced by the barbiturates and the analgesic action of the narcotic or synthetic analgesic drugs.

C. Drug Potentiation:

Interest in the potentiating* action of various drugs has recently reached an all-time high with the discovery of the antihistamines and phenothiazine derivatives such as chlorpromazine and promazine. It now seems to be evident that certain of these drugs, although depressant in themselves, exert more than an additive effect in reducing the metabolic and respiratory activity while at the same time enhancing other desirable effects of the barbiturate hypnotics and the narcotic analgesics.

Various writers have reported results of the potentiating effect of disulfuram (Antabuse(R)) (36,37,38), alcohol(39), Hydergine(R) (40,41,42, 43,44), certain phenothiazine derivatives(16,45,46,47,48), the antihistamines(6,49,50,51), brain metabolites(52) and many other agents(53,54, 55,56) upon the effect of the barbiturates and analgesic drugs. Most of these experiments were concerned with studies of the hypnotic effect, changes in the sleeping time, the degree of anesthesia, or differences in the pain threshold, i.e., the major desirable therapeutic effects. While these observations are not to be questioned as to their validity or accuracy, they fail to reveal evidence of the "potentiation" of possible undesirable side-effects, the most serious again being respiratory depression.

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* "There are instances in which the combined action of two drugs is greater than that which can be anticipated from the sum of their individual actions. For example, the administration of acid-forming saline diuretics prior to a mercurial diuretic results in a greater volume of urine than is predictable on the basis of individual effects"- quoted from THE PHARMACOLOGIC BASIS FOR THERAPEUTICS, Second Edition, by Goodman, L. S. and Gilman, A., The Macmillan Co., New York, 1955.

With respect to the analgesic drugs, although the obvious desired effect is an increase in pain threshold and most determinations of the activity of these agents are based on this effect, there is a direct correlation between the degree of respiratory depression and the degree of analgesia (21). Therefore, analgetic action could be indirectly measured by oxygen consumption methods. As for the barbiturates and the new tranquilizing drugs, a direct correlation has been demonstrated between the dosage used and such effects as peripheral tissue metabolism (25), oxygen consumption (16,43), respiratory rate (21) and the degree of central depression (21,47).

Measurement of the effects of combinations of these drugs as regards the changes they produce in basal metabolism and respiration would, therefore, be of great importance clinically. Such studies would also provide an accurate index as to the presence and degree of any "potentiation" which may occur.

PURPOSE

In other studies in this department, a number of investigations dealing with the effect of various drugs on the oxygen consumption have been carried out (5,26,43). Since we were familiar with the use of the Peoples' apparatus and methods for measuring oxygen consumption in small animals, we decided to extend these studies to the investigation of other drugs. In the past, considerable work has been done in the department dealing with the pharmacological and clinical aspects of new analgetic drugs. In one of these investigations, certain interesting and clinically applicable effects of the dihydrogenated ergot compound called Hydergine (R) on enhancing the analgesic action, modifying the constipative effect and forestalling the development of tolerance and addiction to the narcotic opiates and synthetic analgesics were explored (40,41,42,44,57,58,59). It was of interest, therefore, to extend these studies to an investigation of the effects of the analgesic drugs on oxygen consumption and respiration in the rat. We were particularly interested in determining whether or not it would be possible to quantitatively measure these effects of the analgesic drugs by the Peoples' method. It was felt that it would be of additional value to attempt to correlate analgetic activity with changes in oxygen consumption and respiration since at the same time we could observe the toxic qualities of these drugs. We therefore selected a new synthetic analgesic drug (SKF #5137) - not yet released for general usage - to compare, as regards effects on oxygen consumption and respiration, with the changes induced by morphine.

Because of the intense interest in the action of the new tranquilizing compounds such as chlorpromazine (Thorazine(R)) when used in conjunction with analgesics, an attempt was made to quantitate this "potentiating" activity through measurement of central nervous system depressant activity. Many clinicians have expressed the belief that, although the analgetic effects of morphine-like drugs are certainly enhanced in patients also receiving chlorpromazine[16,25,45,46,60,61,62], the toxic qualities imparted by the analgesic compound are also greatly enhanced. Since respiratory depression becomes a serious side-effect in clinical procedures such as anesthesia, obstetrics and in the elderly patient suffering a variety of pulmonary difficulties, it was felt that a method of determining activity of these drug combinations by measurement of respiratory effects would be of great value and clinical importance.

METHODS AND MATERIALS

Since the purpose of this investigation was to measure and compare the depressant effect of several analgesic or hypnotic drugs when used alone or in combination with other drugs such as Hydergine (R) or the ataractic drugs to study potentiating effects, it was decided to employ two procedures. The first procedure utilised a modification of the Peoples' apparatus for photographically recording the changes in the oxygen consumption of small animals. The second procedure was employed later on during this study when it was decided to make direct observations of the changes in the respiratory rate of the animal by counting the fluctuations of the water meniscus in the manometer of the Peoples' apparatus since these fluctuations were due to the respirations of the animal. Part A describes in detail the Peoples' apparatus and its use in measuring the oxygen consumption of rats while Part B deals with the procedures used to count the respiratory rate simultaneously with the measurement of the oxygen consumption.

A. Measurement of Oxygen Consumption:

Most methods used for determining the rate of oxygen uptake in animals consist essentially of methods for measuring the volume of oxygen utilized over known periods of time (27,32,63,64,65). These measurements are expressed as average values only for the time interval observed so that any rapid changes which may have occurred are obscured. More recently, several methods of measuring oxygen consumption by means of a recording spirometer have been developed (66,67,68,69). Employment of the later apparatus, although providing data similar to the Peoples'

device used in this study, was felt to be cumbersome and the results somewhat difficult to interpret.

1. Description of the Peoples' Apparatus:

The apparatus, first described by Peoples (70) in 1941, was designed for use in experiments where changes in metabolism occur rapidly and where a prolonged continuous record is desired which allows the estimation of instantaneous values for the rate of oxygen utilization. This apparatus was further modified by Phatak and Saxey (33) in 1947 and used by them to illustrate the effects of barbiturates upon oxygen consumption in rats. Vidgoff and Stempher(26), working in the Pharmacology Laboratory of the University of Oregon Medical School, used this apparatus and method for determining the effects of thyroid when fed over long periods to rabbits. The writer of this report, in conjunction with other workers, has previously reported on a study of the potentiating effects of Hydergine (R) on certain barbiturates, using data obtained from this method.(71) During these investigations, further modifications of Peoples' apparatus, as originally described, have been devised.

Since this apparatus had been found satisfactory for measurement of the respiratory effects of drugs in rats and rabbits in previous work done in this laboratory, and since it made possible long range determinations of oxygen consumption - taking into account rapid changes in metabolism, it was selected for use in the experiments reported here.

a. The Peoples' Apparatus:

Figure 1 is a diagram of this apparatus as modified for use in our experiments. In using this apparatus to measure oxygen consumption,

FIGURE 1

Peoples' Apparatus as Modified for Use in the Experiments for Determinations of Oxygen Consumption and Respiratory Rate in the Rat.

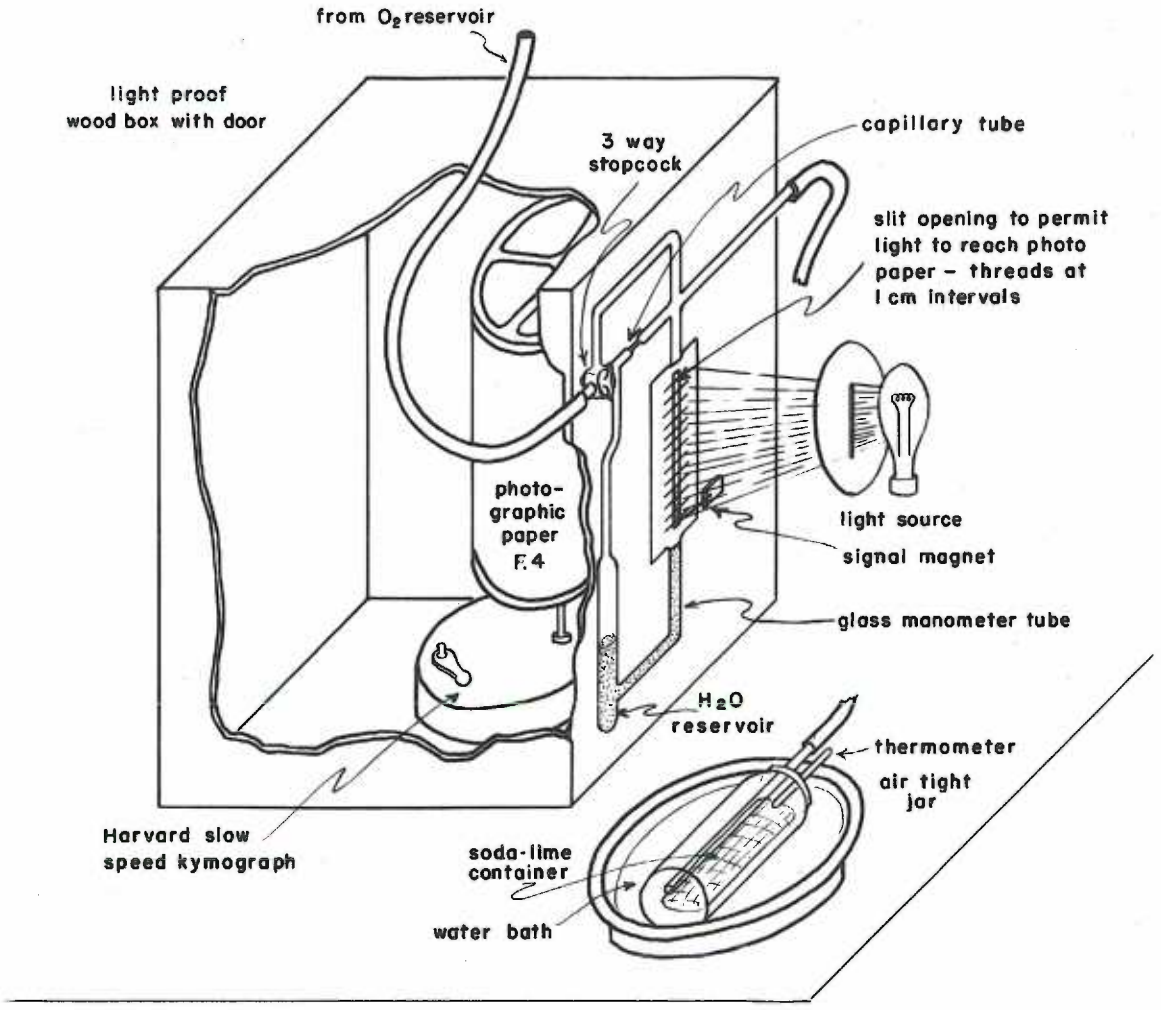

from O_2 reservoir
light proof
wood box with door
3 way
stopcock
capillary tube
slit opening to permit
light to reach photo
paper – threads at
1 cm intervals
photo-
graphic
paper
F.4
light source
signal magnet
glass manometer tube
H_2O
reservoir
thermometer
air tight
jar
Harvard slow
speed kymograph
soda-lime
container
water bath

Fig. 1

the animal is placed in an air-tight glass chamber such as a Mason Jar. Oxygen, contained in a flexible plastic balloon reservoir filled to less than the capacity of two liters, passes to the animal. Expired carbon dioxide and water vapor within the chamber are absorbed by soda lime held in several small copper screen containers. Care was taken to see that the animals used were of such size and weight that activity within the chamber was minimal and limited. The temperature within the chamber, as measured by an indwelling thermometer, was maintained at 28.4 ± 0.4 degrees centigrade by immersing the jar in a large water bath and maintaining constant circulation of air within the room.

Oxygen utilized by the animal first passes from the balloon container through a capillary tube and over a glass manometer mounted directly in front of a slit-like opening in the light-proof box. The water in the manometer reservoir contains a small amount of quaternary ammonium compound as a wetting agent to decrease surface tension. The stopcock distal to the oxygen reservoir is opened to bypass the capillary tube while the apparatus and the contained oxygen flow come to equilibrium. The stopcock is then closed. As the oxygen is taken up by the animal and carbon dioxide is absorbed, the pressure in the animal chamber falls. This causes a difference of pressure across the capillary tube resulting in a flow of oxygen into the chamber. When this flow balances the uptake of the animal, the pressure remains stationary and the rate of flow, as registered by the manometer, gives the true rate of oxygen utilization by the animal. For this reason the manometer record is only exact where the rate is constant since it will lag slightly behind a rapidly increasing rate and give high readings

while the rate is actually falling. However, this lag is very slight so that changes occurring over a period of a few mimutes are fairly accurate and the direction and magnitude of change can be determined within a few seconds. Following Bernoulli's theorem (72), the rate of oxygen flow determines the pressure upon the water in the manometer and the column fluctuates in accordance with utilization of oxygen by the animal.

A vertical beam of light is focused upon the manometer tube and through the slit in the front of the apparatus onto the photographic paper pasted to a kymograph drum. The kymograph revolves slowly and is adjusted so that the paper just clears the vertical slit in the box. The light is of such intensity that it only slightly exposes the slow speed (AZO F-4 single weight) photographic paper. Water in the manometer tube, however, acts as a lens to concentrate the light rays and fully expose the paper, except where the centimeter marking threads cross the slit and where the signal marker lines break the light beam at the bottom of the slit.

b. Standardization of the Peoples' Apparatus:

In this investigation a battery of four apparatuses was employed. Each device was calibrated so that the relationship to the height of the water column and the rate of oxygen flow was known. Calibration was carried out as follows: oxygen was allowed to flow at a constant rate from the balloon and through the capillary tube so as to maintain a constant height of the water column in the manometer tube. The oxygen passing through the rubber tube supplying the animal container jar was collected in a volumetric flask by the displacement of

oxygen saturated water. This calibration was carried out for at least five different manometer heights and, for each, the time necessary to collect a known amount of oxygen was determined.

The results obtained by these procedures were graphed by plotting the oxygen flow, measured in cubic centimeters per minute, against the height of the water level registered by the manometer, as shown in figure 2. These graphs show the relationship to be almost perfectly linear. By measuring the slope of the graph, it is possible to compute the oxygen flow per centimeter height of the water column when it is photographed directly by exposing the photographic paper. Barometric readings were taken and the temperature was maintained, as previously mentioned, at 28.4 ± 0.4 degrees centigrade. All records obtained were computed to standard conditions.

2. Calculation of the Oxygen Consumption from the Photographic Record:

The measurement of the animal's oxygen consumption at the different periods while the animal was kept in the glass container utilizing oxygen, is made from the developed photographic records. The vertical axis of the exposed part of the record is measured in centimeters and represents the volume of oxygen consumed at any one time. The relationship of oxygen volume utilized to the height of the exposed portion of the record is linear and therefore the pre-injection and post-injection heights of the record are proportional. The horizontal axis represents time, each three centimeters being equal to approximately one hour with a fifteen minute interval, shown by vertical lines at the base of the record, between each signal magnet exposure. The average oxygen

FIGURE 2

Graphic Representation of Calibration of Oxygen Consumption Apparatus to Determine Relationship of Manometer Levels to Oxygen Flow.

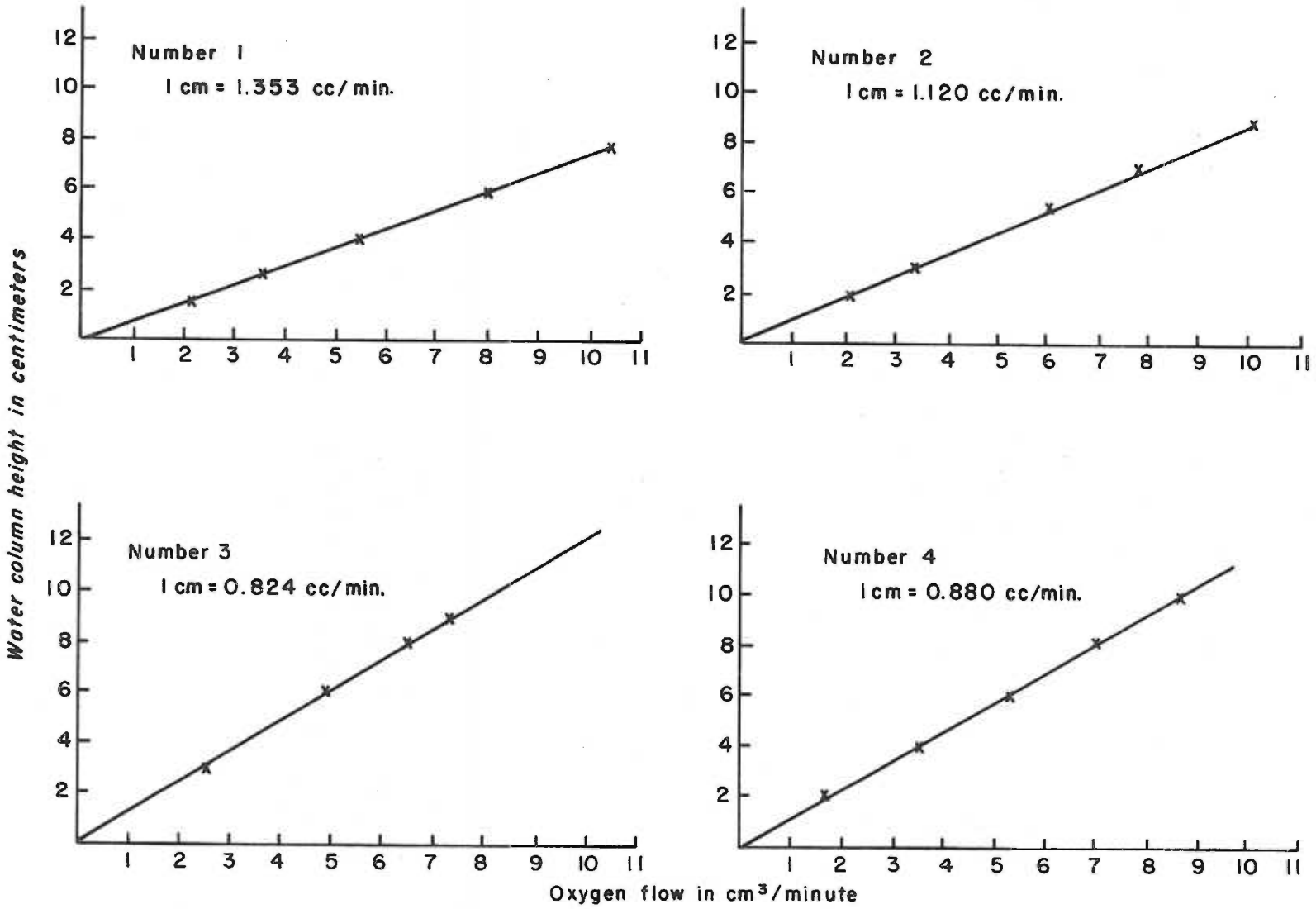

Number 1
1 cm = 1.353 cc/min.
Number 2
1 cm = 1.120 cc/min.
Number 3
1 cm = 0.824 cc/min.
Number 4
1 cm = 0.880 cc/min.
Water column height in centimeters
Oxygen flow in cm³/minute

Fig.2

consumption for any period can be calculated from the photographic record by measuring the exposed area on the film by means of a compensating planimeter and thus determining the average height of the water column in the manometer during the period. Figure 3 is shown as an example of this photographic record.

3. Method of Reporting Results and Data:

a. Measurements made from Photographic Records:

Each determination of oxygen consumption in the rat was performed in several steps. Figure 3 is a representative photographic record of one of these tests made on a rat subjected to two injections and taken over a period of several hours. Each experimental step or period is designated in the record and further described and explained below:

The Four Periods of an Experiment to Determine Effects of Drugs on Oxygen Consumption in the Rat

1) The Pre-injection Control Period:

Normally, after the rat is placed in the glass container, the animal shows a ten to fifteen minute period of acclimitization during which time it moves about in the container seeking escape. This increased activity is reflected by an increase in oxygen consumption. This phase is then followed by a reasonably stable utilization of oxygen as shown by the nearly "smooth" level of the record. The last forty-five to sixty minutes of this initial period is measured and reported as the control value for oxygen consumption.

2) First Injection Period:

After the rat has been removed from the jar for injection and then replaced, the animal's oxygen consumption shows another sharp,

FIGURE 3

Sample Photographic Recording of Oxygen Consumption Changes in the Rat Showing Time Intervals and Portions of Record which were Measured.

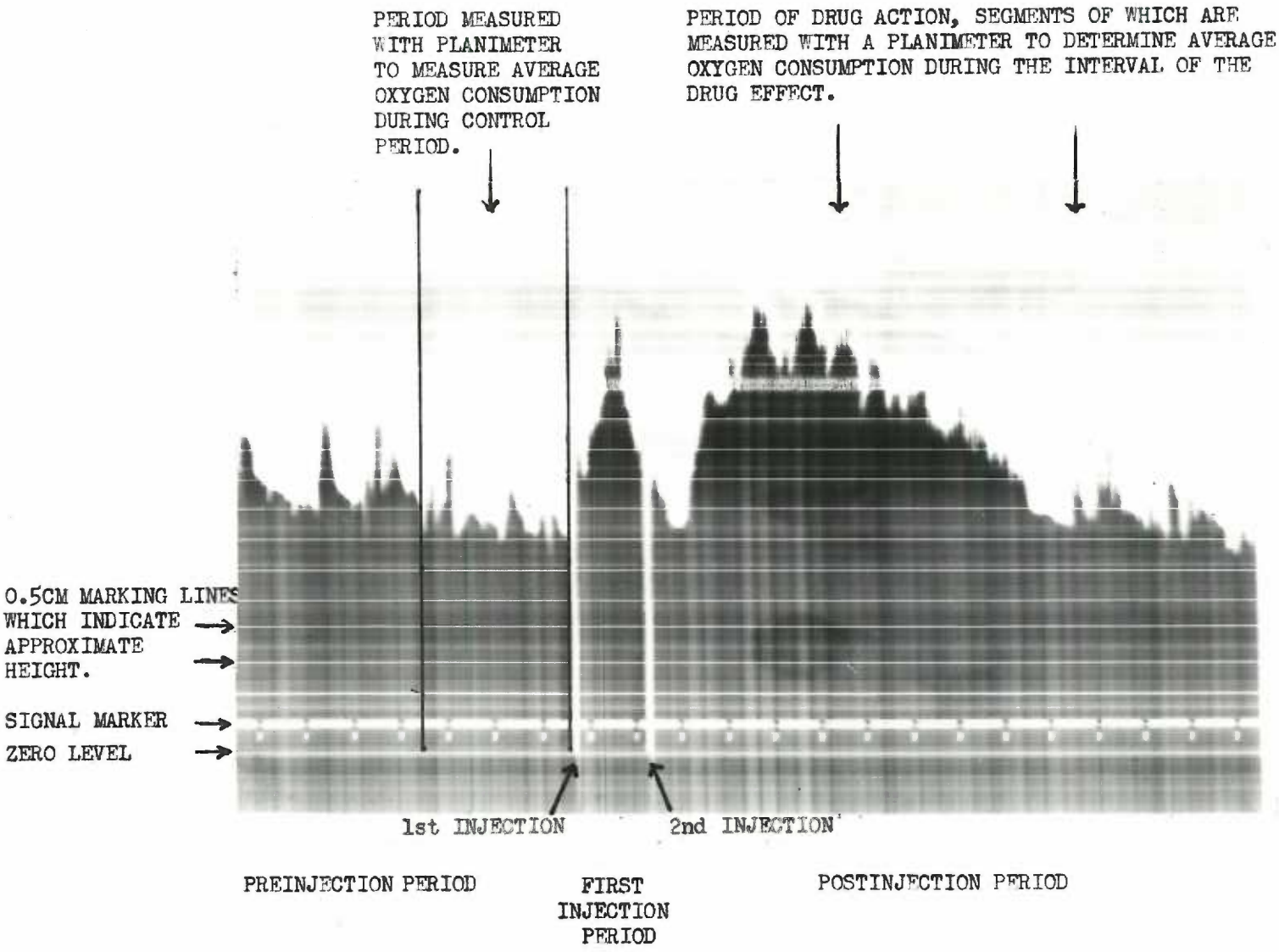


FIGURE 3. SAMPLE PHOTOGRAPHIC RECORD FOR OXYGEN CONSUMPTION DETERMINATIONS IN THE RAT.

transitory increase due to the effects of handling and the injection. The time of injection is indicated on the photographic record by the unexposed vertical line.

3) Second Injection Period:

The second drug used was injected during this time which is represented on the photograph by the second white vertical line. The record obtained immediately after this period when control (saline) injections were given shows another transitory increase in oxygen consumption but of slightly less intensity and duration than seen after the first injection. Depending upon the depressant drug and dosage given, other records show varying changes in oxygen consumption. Often the brief period of excitation is entirely eliminated when rapidly-acting drugs are used.

4) Post-injection Period:

Depending upon the drug or drug combinations used, the change in the oxygen consumption over an extended period of time is carefully measured and compared with that of the control period. That portion of the record which shows the maximum drug effects, or the entire record, whichever is desired, is measured by means of the planimeter. From several such planimeter readings made during this period, the average oxygen consumption values for any time period can be calculated.

The accuracy of the photographic method for recording and calculating changes in the oxygen consumption of a small animal was determined in the following way. This special experiment was devised and carried out to answer two questions: First, does the photographic record register only the maximum values of oxygen consumption during the

period under observation and fail to record transitory minimum values? Considering the fact that the kymograph drum speed is only 3.2 centimeters per hour or 0.53 millimeters per minute and the respiratory rate for a rat under normal conditions is approximately one hundred and twenty respirations per minute, it could be that only maximum values would be recorded. Second, since the water column of the manometer acts as a lens to expose the paper to light, is it possible for an occasional high reading to become superimposed over preceding low values?

b. Special Experiments to Determine Accuracy of Photographic Records used for Measurements of Oxygen Consumption:

Two sets of experiments were carried out for the express purpose of testing the accuracy of the photographic records obtained and used for measuring the oxygen consumption at any one period and under varying conditions.

1) Manually Produced Changes in Meniscus Height of Manometer:

Oxygen from the supply tank was permitted to flow filling the rubber balloon to full capacity and from this point allowed to pass through the capillary tube at such speed as to maintain a constant height of 3.2 centimeters of water in the manometer tube. By manually squeezing the balloon reservoir and forcing an increased volume of oxygen to flow through the capillary tube, the water level was raised to the desired level in the manometer and was held there for a certain length of time. The kymograph drum was allowed to rotate at the usual slow speed in order to expose the photographic record. Various direct observations were made and a photographic

record obtained for each test made. The varying conditions and results obtained are tabulated in table 1.

From the results of this special experiment it can be seen that the water level in the manometer must reach a given height at a minimum rate of somewhere between twelve and forty-five fluctuations per minute (experiments 3 and 4, table 1). Sudden increases in height for less than two seconds do not focus sufficient light to expose the photographic record (experiment 6, table 1). Water column levels fluctuating at the rate of seventy-five times per minute to a given height for eight seconds do allow, however, sufficient time to expose the record (experiment 5, table 1). Levels rising to a given height for a brief period at the rate of twelve per minute are not recorded (experiment 6, table 1).

2) Direct Measurements of Respiratory Rate:

Other observations providing information as to the sensitivity and accuracy of the photographic method for measuring the changes in oxygen consumption were made later. The results of these observations are mentioned here as additional support to the findings cited above. Over fifteen hundred one to two minute observations of the fluctuating meniscus were made while counting the respiratory rate. During this time the effects of many different drug and dosage combinations on oxygen consumption were studied. This study showed that during approximately 90 per cent of the observations, the amount of change in the height of the water column level was barely perceptible, i.e., approximately 0.5 to 1.0 millimeters excursion. When the rats were severely depressed by large doses of drugs, the meniscus fluctuations attained their maximum values. Even then, only

TABLE 1

Effect of the Height and Rapidity of Change of Water Column Fluctuations in the Manometer Tube upon the Accuracy of the Photographic Records

Change in Manometer Water Level Produced Manually	Change in Water Level	Times Water Level Changes	Results (Recorded on Photographic Paper)
1. Raised suddenly one time only	3.2 to 6.5 cm.	1 raise	No record obtained (the "slow" F.4 photographic paper was not sensitive enough to record sudden single fluctuations in water level).
2. Rapid, alternate raises and lowerings	3.2 to 3.7 cm.	80 changes per min.	A comparison of experiment 2 and 3 shows that no difference in recording was obtained since the drum speed is too slow to allow recording of separate peaks for each fluctuation.
3. Rapid, alternate raises and lowerings	3.2 to 3.7 cm.	45 changes per min.	
4. Rapid, alternate raises and lowerings to two heights	3.5-4.3 cm. every 5th to 5.5 cm.	75 changes per min.	A constant level of about 4.3 cm. was recorded. Fluctuations attaining 5.5 cm. at a rate of 15/sec. did not expose the film.
5. Alternately raised to two different levels, each 10 times.	4 to 5 cm. and 4 to 6 cm.	75 changes per min.	Entire record exposed to 6 cm. level. The higher fluctuations to the 6 cm. level were superimposed upon the lower readings.
6. Rapid, alternate raises and lowerings	3.2 to 3.8 cm.	12 changes per min.	No record obtained — rate too slow to expose film.
7. Rapid, alternate raises and lowerings	3.2 to 3.8 Every 20th to 5.2 cm.	68 changes per min.	Only those regular fluctuations up to 3.8 cm. were recorded.
8. Occasional raises and lowerings 2 times in rapid succession	3.2 to 5.7 cm.	4 changes per min.	No record obtained — two high raises in succession were not sufficient to expose the film.

occasionally did the water level fluctuate as much as 2 to 3 millimeters. Also, following stabilization after drug administration, there were no extreme, sudden changes of the meniscus height and at no time did the rat appear to be breathing periodically.

Probably the most important factor concerned in limiting the extent of the changes in the manometer water level is the construction of the Peoples' apparatus itself. The experimental oxygen system of this apparatus embodies a large volume and the changes in the rat's respiration causing oxygen to flow over the manometer tube are at the extreme distal end of the circuit so that the degree of these changes, as shown by fluctuations of the water column, are greatly diminished. Moreover, due to the compressibility of air and oxygen, the change in the level of the meniscus of the water column in the manometer tube brought about by a normal tidal volume exchange of the rat is barely perceptible. Sudden changes in the rat's respiratory volume are, therefore, not recorded by this apparatus. Since this experiment is designed to compare drug effects upon the oxygen consumption over a long range period, and since the animal's oxygen consumption after drug administration is being compared to that of his own control period, it was felt that the accuracy of the records was within the range of experimental error for this study.

B. Measurement of Respiratory Rate:

A random sample of seventy three rats was observed while subjected to the usual oxygen consumption experiment using all varieties of drug combinations, as well as saline controls. Their respiratory rates, as reflected by fluctuations of the meniscus in the manometer

tube, were observed and counted for one to two minute periods at 60-90 and 120 minutes during the control period; 5-10-15-20 minutes after the first injection; and 5-10-15-20-25-30-40-60-80-100-120-150-180-210 minutes after the second injection. In all, almost 1500 respiratory rates were counted during all conditions of the experiment. Results of these experiments showed:

1. The mean control period respiratory rate per minute for all rats was 120.10 $\pm$ 3.93 respirations per minute (95 per cent confidence level).

2. The extreme average control values for any one particular drug group were 72 to 146 respirations per minute, while the average range was 54 respirations per minute.

3. About 50 per cent of the rats which had received large doses of depressant drugs and thus had slow respiratory rates, i.e., from 80 to 100 respirations per minute, occasionally showed irregular fluctuations of the meniscus, ranging from 2 to 3 millimeters during the period of maximum drug action.

4. It was observed that during no single period did the animals breathe periodically. During the three control period observations, repeated counts gave very similar respiratory rates.

C. Statistical Analysis of Records and Method of Reporting Results:

1. Oxygen Consumption:

a. Control Records:

Table 2 shows the change from "basal" oxygen consumption for rats after receiving two saline injections 30 minutes apart. The average change in oxygen consumption following the second injection

TABLE 2

Control Records for Oxygen Consumption in the Rat
Showing Effect of Two 1 cc./kg. Saline Injections

Rat. No.	Per cent of Pre-injection Period "Basal" Oxygen Consumption during Post-injection Interval Minutes after Second Saline Injection 0-10 %	10-20 %	20-30 %	30-40 %	40-60 %	60-80 %	80-100 %	100-120 %	120-150 %	150-180 %
1	98	108	106	96	94	98	100	101	96	86
2	144	134	136	128	126	126	123	117	109	105
3	158	131	126	124	121	115	115	111	108	105
4	116	130	99	99	109	118	108	104	99	96
5	123	113	113	108	113	118	108	108	108	102
6	113	108	104	109	116	115	103	95	109	105
7	145	120	126	134	126	108	113	130	119	114
Average	125	121	116	114	115	114	110	109	106	102

falls gradually from an initial peak increase of 125 per cent of the "basal" value to 115 per cent at the end of the first hour, to 109 per cent by the end of the second hour and to only 102 per cent at the end of the third hour. It should be noted that this fall is steady and gradual, exhibiting no reversals or sudden changes. The average oxygen consumption for the entire post-injection period was exactly 110 per cent of the control period value. From this table it can be seen that only in rare instances did the rats ever consume less oxygen during the post-injection period than during the control period. This fact adds additional significance to the low values of oxygen consumption seen after administration of central nervous system depressant drugs.

b. Relationship between Weight and Oxygen Consumption:

Table 3 shows the average "basal" oxygen consumption values for all rats tested. The mean values of each weight group differed less than one standard deviation from the population mean of 5.50 cubic centimeters of oxygen per minute; however, it was shown (table 4) that this difference was significant ($p = 0.0001$). Further illustration of the expected correlation between body weight and basal oxygen consumption values is shown in figure 4. It is of interest to note that the methods which were used to determine oxygen consumption values in the rat were sensitive enough to show differences in animals which differed in weight by only 10 grams.

An analysis of basal oxygen consumption values for the same animal which had been tested at two different weights showed that the mean difference in oxygen consumption values was only 0.06 cubic

TABLE 3

Statistical Analysis of Control Records for Oxygen Consumption and Respiratory Rate in Rats of Different Weights

	Oxygen Consumption			Respiratory Rate		
Wt. Range Grams	No. Rats	Mean Oxygen Consumption cc./min.	95% Confidence Limits on Mean cc./min.	No. Rats	Mean Resp. Rate per min.	95% Confidence Limits on Mean per min.
190-199	22	4.95	± 0.38	7	105.72	± 20.4
200-209	25	5.05	± 0.30	6	119.66	± 10.0
210-219	25	5.18	± 0.28	7	122.86	± 7.8
220-229	36	5.46	± 0.29	8	110.24	± 19.8
230-239	30	5.61	± 0.31	7	113.42	± 13.2
240-249	24	5.56	± 0.45	8	129.24	± 11.0
250-259	14	6.13	± 0.25	5	118.80	± 30.0
260-269	15	5.65	± 0.26	8	117.50	± 14.1
270-279	15	6.39	± 0.45	11	118.36	± 12.9
280-289	7	6.05	± 0.67	6	133.46	± 19.5
Total	213			73		
Average		5.50	± 0.11		120.10	± 3.92

TABLE 4

Statistical Analysis of the Relationship of Oxygen Consumption and Respiratory Rate to Rat Weight

Application of Rank Correlation Test* to Group Means Shown in Table 3 to Test the Hypothesis: "The Two Variables (Weight and Oxygen Consumption or Respiratory Rate) are Independent and the Difference between the Population Means is Zero."

Formula: $$r_s = 1 - \frac{6\Sigma d_i^2}{N(N^2-1)}$$

Relationship of Oxygen Consumption to Rat Weight	Relationship of Respiratory Rate to Rat Weight
$d_n^2 = 12$	$d_n^2 = 100$
$N = 10$	$N = 10$
$r_s = 0.93$	$r_s = 0.39$
$p = <0.001$	$p = >0.10$
Conclusion: There is a significant difference between the means; therefore, there is a direct correlation between oxygen consumption and animal weight for rats between 190 and 290 grams.	Conclusion: There is no significant difference between the means; therefore, respiratory rate is not directly correlated with difference in weight for rats weighing between 190 and 290 grams.

* Dixon, W. J. and Massey, F. J., Jr., Introduction to Statistical Analysis, 2nd. Ed., McGraw-Hill Book Co., Inc., New York, 1957.

FIGURE 4

Histogram Showing Relationship between Weight and "Basal" Oxygen Consumption of Rats Used in the Experiments.

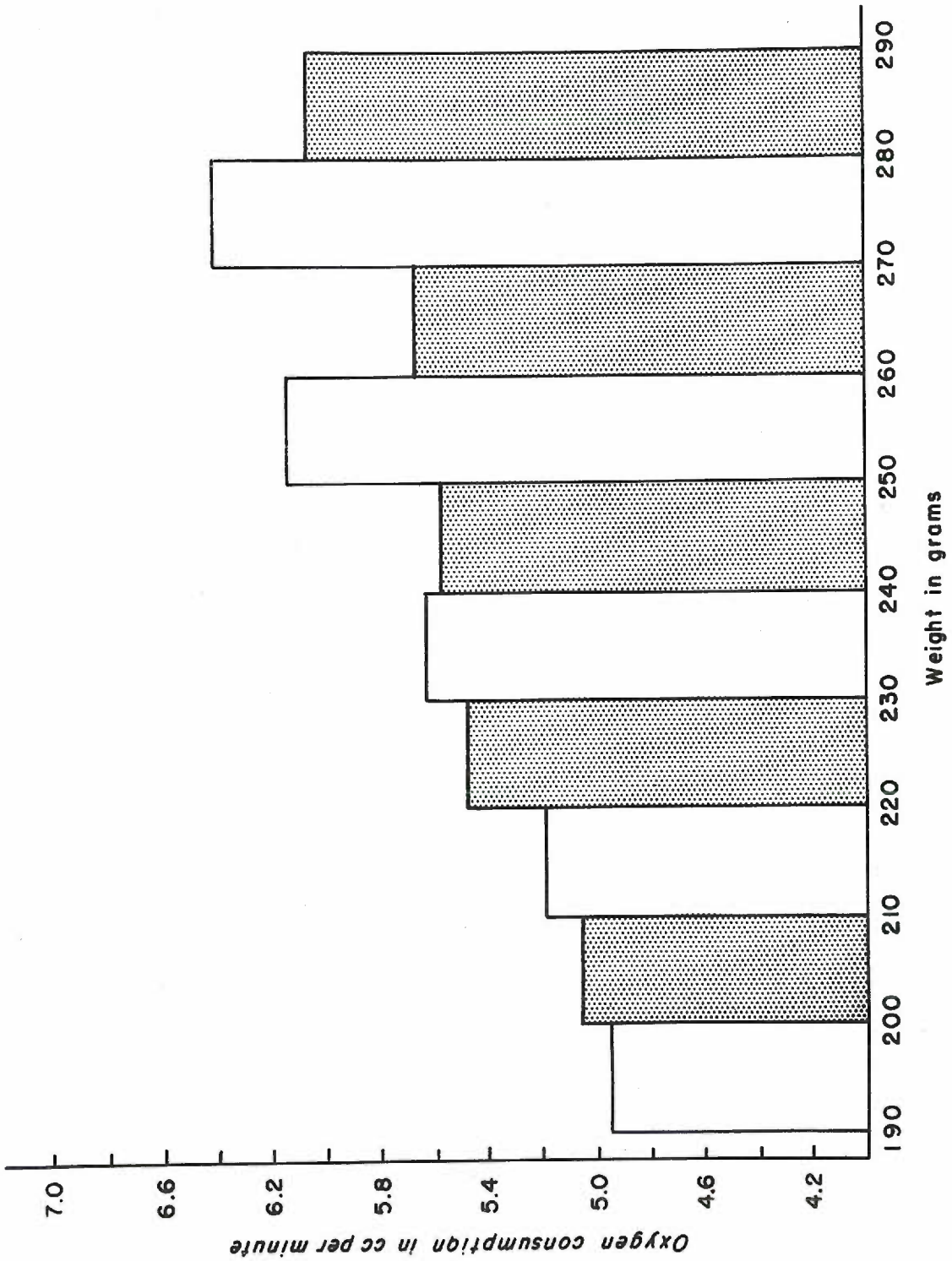

Oxygen consumption in cc per minute
7.0
6.6
6.2
5.8
5.4
5.0
4.6
4.2
190
200
210
220
230
240
250
260
270
280
290
Weight in grams

Fig. 4

centimeters per minute although the average weight increase had been 20 grams. The expected difference in mean oxygen consumption values for rats selected at random at these two different weights is 0.45 cubic centimeters per minute. It would appear, therefore, that the individual variation among the random rats was greater than the variation with respect to difference in weight alone. While statistical analysis of these results did not confirm this hypothesis, they indicate that if the sample size was larger, or if the difference in weights of the same rats tested twice had been greater, this hypothesis would probably hold true.

c. Method of Reporting Results:

Each rat served as its own control and all of the drugs were administered on a milligram per kilogram basis. Therefore, the slight differences in oxygen consumption with respect to weight need not be considered in reporting the results. Although exact oxygen consumption values can be easily calculated, they were not felt necessary since the drug effect could be readily demonstrated by comparing the post-injection and control period values. The difference in post-injection oxygen consumption values from the control value as a result of administration of various drugs is reported as the per cent change.

A direct indication of drug effect upon the animal is thus shown; not merely a relative difference between drug effects, which is all one could evaluate if each animal did not serve as his own control.

2. Respiratory Rate:

a. Control Records:

Three or more observations of the respiratory rate were made at from 15 to 30 minute intervals during the last ninety minutes of the pre-injection "control" period. The variations in rate during the control period were very minor and the average rate for all animals observed was 120.1 respirations per minute. Table 3 shows the average respiratory rates for animals of different weights; this relationship of weight to respiratory rate is more clearly illustrated in figure 5. Further analysis of these results showed that there was no direct correlation between difference in animal weights and their respiratory rates as was shown to be true for oxygen consumption (table 4).

b. Method of Reporting Results:

Again, each animal has served as its own control; furthermore, the control respiratory rate values showed that all animals observed came from the same statistical population. The changes in respiratory rate as a result of drug administration, therefore, are reported as per cent change from the control respiratory rate. As with the results for changes in oxygen consumption, this allows not only comparison of effects of different drugs, but in addition, shows the direct effect of the drug upon the animal tested.

D. Materials:

1. Animals and Injections:

Sprague-Dawley albino male rats weighing between 190 and 290 grams were used. Each animal was used not more than three times, and then after at least a one week interval, so that drug tolerance would

FIGURE 5

Histogram Showing Relationship between Weight and "Basal" Respiratory Rate of Rats Used in the Experiments.

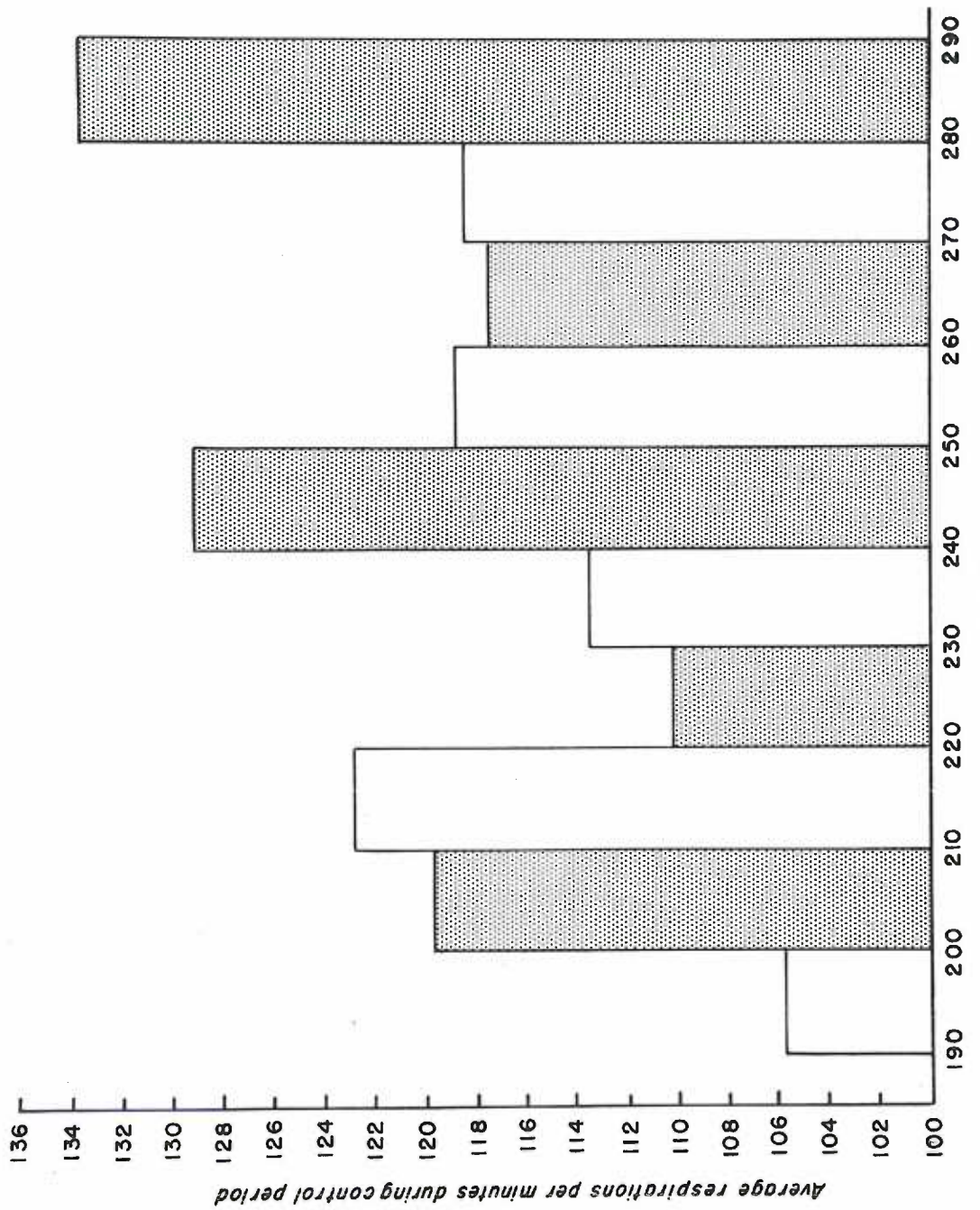

Average respirations per minutes during control period
100
102
104
106
108
110
112
114
116
118
120
122
124
126
128
130
132
134
136
190
200
210
220
230
240
250
260
270
280
290
Weight in grams

Fig.5

not develop. Animals were so selected that for each drug group there was a representative sample of weights and of animals which had been subjected to one, two or three previous experiments. It was shown earlier in this paper that the animal's response to injection was not significantly altered as a result of previous experiments. The experiments were carried out at the same time each day and all animals were allowed a free diet of water and Purina Laboratory Chow.

All solutions were made in such concentration that each animal would receive one cubic centimeter per kilogram of body weight (cc./kg.) so that all injections would be of the same volume. The injections were administered subcutaneously into the back of the neck with opposite sides being employed for each injection. It was felt that intraperitoneal injections would be less desirable since they cause more initial excitation of the animal, thereby leading to unnecessarily high levels of oxygen consumption which would mask minor changes caused by small doses of depressant drugs.

The period between injections was thirty minutes. In our own, and in other workers' experience, this interval proves most satisfactory; furthermore, most of the comparative experiments have used this time interval. Although maximal potentiation is produced within a period of sixty minutes. The difference between thirty and sixty minutes is only slight. There is, however, considerable difference between the effects noted at fifteen minutes and thirty minutes after drug injection(6).

2. Drugs:

Since this investigation comprised two different problems, the drugs used in each study will be considered separately.

a. Barbiturates and Potentiation of their Activity by Hydergine(R):

1) Barbiturates:

According to Goodman and Gilman(21), the barbiturates produce a rather uniform depression of the respiratory center roughly proportional to the dosage administered. The depth of respiration is characteristically reduced whereas the rate is more related to blood carbon dioxide tension and may be increased or decreased. Others, working with the rat, have shown that the barbiturates significantly depress oxygen consumption(37, 73, 74). Lee(74) in his experiments on rats has further shown that increasing dosages of Amytal(R), administered both subcutaneously and intraperitoneally, cause corresponding decrease in the basal metabolic rate. Guedel(75) states that the "reflex irritability" of an individual under general anesthesia parallels the metabolic rate, which in turn reflects the oxygen demand of the body cells. This is in general agreement with People's(76) feeling that the effects of barbiturates on oxygen consumption are no more than would be expected from a similar degree of relaxation in sleep.

Since previous work has shown that there is a direct relationship between dosage of barbiturate administered to the rat and the resultant change in oxygen consumption, it was felt that initial studies using these drugs would serve to help establish the validity of our particular methods and apparatus.

Seconal(R) (secobarbital sodium) and Nembutal(R)

(pentobarbital sodium) were selected for use in these studies because of their frequent usage in general practice and because they are employed as pre-anesthetic adjuvants. Since the chemical structure (figure 6), as well as the duration and degree of action of these two barbiturates is very similar, it was felt that a study of both these compounds might serve to illustrate common dose-response curves for changes in oxygen consumption in the rat.

2) Hydergine(R):

This drug is a mixture of equal parts of the dihydrogenated ergot alkaloids: dihydroergocornine, dihydroergocristine and dihydroergokryptine (figure 6). It has been shown to potentiate the analgesic(42) and constipative(40, 41) actions of morphine and synthetic analgesics when given subcutaneously in doses of 0.06 milligrams per kilogram to rats. However, the respiratory depressant effect of l-isomethadone, a synthetic analgesic, was not changed in rabbits pre-treated with Hydergine(R)(25). With respect to the alteration of barbiturate action by Hydergine(R), little is known so far. Rothlin and Cerletti(77) have suggested that Hydergine(R) exerts an inhibitory effect "on the function of the vegetative centers of the brain stem." Previously, both Rothlin(78) and Baer(79) presented experimental and clinical evidence that the ergot alkaloids ergotamine, ergocornine, ergocristine and ergokryptine intensify the sedative effect of the barbiturates through a central action. Since the dihydrogenated components of Hydergine(R) possess the same lysergic acid basic nucleus as the natural ergot alkaloids, it would be expected that it would have a similar potentiating action on the barbiturates.

FIGURE 6

Drugs Used in Barbiturate and Hydergine(R) Study.

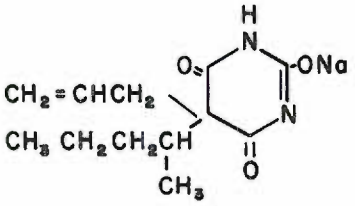
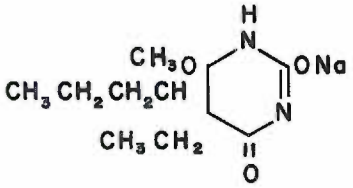
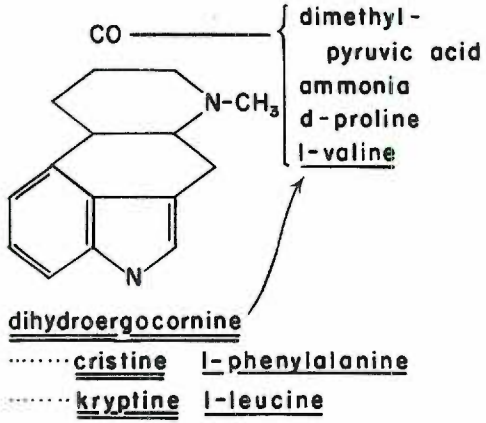
STRUCTURAL FORMULA	GENERIC NAME	TRADE NAME
 5-allyl-5-(1-methyl-butyl) barbiturate	Secobarbital Sodium	Seconal(R)
 5-ethyl-5-(1-methyl-butyl) barbiturate	Pentobarbital Sodium	Nembutal(R)
 dihydroergocornine .....cristine l-phenylalanine .....kryptine l-leucine	A mixture of equal parts dihydroergocornine,....cristine, and.... kryptine	Hydergine(R)

Fig.6

We undertook to explore the possibility that Hydergine(R), used in the small doses of 0.06 milligrams per kilogram which were previously found effective in intensifying some of the actions of morphine, could enhance the action of the barbiturates. An attempt was made to show modification, by pre-treatment with Hydergine(R), of the depressant effects of the barbiturates as illustrated by changes in oxygen consumption. In addition, a toxicity study involving forty to fifty determinations upon combinations of Hydergine(R) and secobarbital was made to find out if Hydergine(R) protected against the lethal effects when large doses of the barbiturates were used.

b. Analgesics and Potentiation of Their Activity by Chlorpromazine:

1) Morphine Sulfate:

Morphine sulphate, because of its frequent use and its general acceptance as a model example of a narcotic analgesic, was chosen as the standard to determine, if possible, a dose-response curve for changes in respiration and oxygen consumption in the rat. Furthermore, as previously mentioned, a great deal of experimentation to determine the physiological effects of morphine, alone and in combination with "potentiating" agents, was already available for comparison.

The effect of morphine and related compounds upon respiration has been shown to vary greatly depending on the experimental animal, dosage schedule, route of administration and concomittant drugs used. In barbiturate anesthetized humans Bodman(80) found that 50 milligrams of Demerol(R) predominately produced a decrease in rate of respiration and response to inhaled carbon dioxide. Landmesser(34) has demonstrated

the effect of 10-12 milligrams of morphine on anesthetized humans to be primarily a slowing in respiratory rate. Goodman and Gilman[21] state that all phases of respiration are depressed. The results of the extensive experimental work of Breckenridge and Hoff[81] clearly demonstrate the effects of morphine in dogs. They used both normal dogs and animals with mid-collicular decerebration, pontine section and vagotomy in an attempt to localize the action of morphine. Their results show that 10 to 40 milligrams of morphine given intravenously to normal dogs produces an initial polypnea followed by deep sighing respiration superimposed on a pattern of slowed eupnea. Miller, et al.[31], working with rabbits, observed that a 50 per cent decrease in both respiratory minute volume and rate was produced by 4 milligrams per kilogram of morphine given intravenously. In contrast, the cat is uniformly stimulated by morphine, the end results being an exhausted convulsive death[21]. In a cursory search of the literature no information as to changes in oxygen consumption in the rat after receiving morphine was found.

2) SKF #5137:

This new synthetic analgesic has only recently been available for clinical and laboratory study. So far, it is designated only by the Smith, Kline and French Laboratories' new drug investigational number. SKF #5137 was selected in order to compare its effects upon oxygen consumption and respiration with those of morphine. It was hoped that there might be a correlation between its analgesic activity as compared to morphine and with its effects upon oxygen consumption and respiration as compared to morphine, thereby showing a correlation between analgesic activity, respiration and metabolism.

As can be seen in figure 7, SKF #5137 is an amidone-like compound somewhat similar to alpha-acetylmethadol. All reports and data on effects of this drug, except those previously demonstrated in our laboratory, were received in a confidential report from Smith, Kline and French Laboratories[82]. It has been reported to have a rapid onset of action similar to that of meperidine and to be more effective on a milligram per kilogram basis than morphine. The drug was supplied to us by courtesy of Smith, Kline and French Laboratories as the base dissolved in equal molecular amounts of hydrochloric acid in water. It has been shown by other workers that SKF #5137 is approximately five to ten times as active as morphine and twenty times more active than meperidine when given subcutaneously[82,83]. At the same time, SKF #5137 has a higher therapeutic index of safety[82]. Reports regarding its relative respiratory depressant effect have been contradictory and unrevealing; but, for the most part, it has been claimed that SKF #5137 has relatively innocuous side effects[82].

3. Chlorpromazine:

Chemically, chlorpromazine is 10-(γ-dimethylaminopropyl)-2-chlorphenothiazine hydrochloride. This compound has been extensively tested both experimentally and clinically in Europe, particularly in France under the name Largactil(R) and in Germany under the name Megaphen(R). Smith, Kline and French Company have American patents for this drug under the proprietary name Thorazine(R).

The most interesting pharmacologic aspect of chlorpromazine is its effect on the central nervous system. It causes reduction in motor activity, quietness and drowsiness without true hypnosis or anesthesia

FIGURE 7

Analgesic Drugs Showing Chemical Relationship between SKF #5137, Morphine Sulphate, Alpha-acetylmethadol and Meperidine.

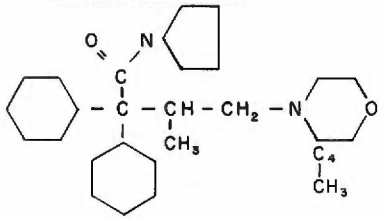
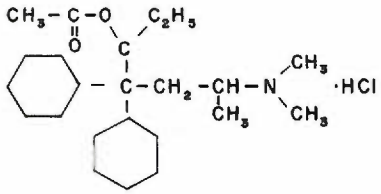
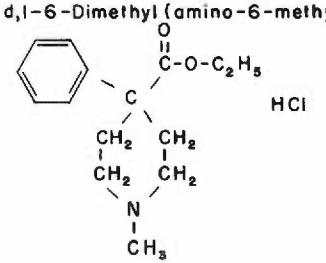
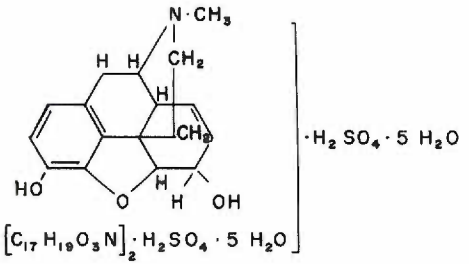
STRUCTURAL FORMULA	GENERIC NAME	TRADE NAME
 dl-N-[2:2-diphenyl-3-methyl-4-(N-morpholino) -butryl]-pyrrolidine (base)	None yet given	SKF #5137 (company designation)
 d,l-6-Dimethyl (amino-6-methyl-4-4-diphenyl-3-acetyl-hexane hydrochloride	Alpha-Acetylmethadol	None
 N-Methyl-4-phenyl-4-carbethethoxypiperidine	Meperidine, USP	Demerol(R)
 $[C_{17}H_{19}O_3N]_2 \cdot H_2SO_4 \cdot 5\,H_2O$	Morphine Sulphate, USP	Morphine(R)

Fig. 7

(84,85,86,87). Electroencephalographic records of animals given chlorpromazine resemble those observed in a normal state of drowsiness or sleep with rapid and normal appearing arousal in response to stimuli (88,89,90). The drug blocks conditioned responses, but does not interfere with responses to unconditioned stimuli such as electric shock(16,47,86,91,92).

Chlorpromazine has a multitude of actions, some stimulatory to, and others depressant upon the central nervous system. It is strongly antiemetic, being effective in clinical states complicated by emesis and in counteracting most emetic drugs(16,93,94,95,96,97). Also it lowers body temperature in various laboratory animals, the extent depending upon the dosage and the room temperature(16,98,99,100).

Rats given single subcutaneous or oral doses of chlorpromazine from 10-50 milligrams per kilogram usually showed little change or a slight decrease in oxygen consumption(16,47,101,102,103), which appeared to be in proportion to the decrease in body temperature. No antagonism to the metabolic effects of thyroxine was found(47,103,104) when the differences due to change in body temperature were taken into consideration, nor were the effects of dinitrophenol(103,104,105) or adrenaline(105) antagonized even at low body temperatures. Further evidence that the changes in oxygen consumption were due to changes in body temperature is found in coldroom studies(16,101,105). Dogs have shown similar effects in that little change in metabolic rate was noted at normal room temperatures, while there was an increase equal to that of untreated animals when they were exposed to cold(95,101). In human subjects, the basal metabolic rate changed but little after chlor-

promazine in doses up to 2 milligrams per kilogram[106, 107]. Cerebral oxygen consumption was not changed after intravenous or intramuscular doses of 50-300 milligrams, unless blood pressure dropped considerably [108, 109]. Anesthetized dogs also showed no change in cerebral oxygen consumption after 2-10 milligrams of chlorpromazine parenterally.

One of its many other actions, now becoming very important in modern therapeutics, is the ability of chlorpromazine to potentiate the effects of analgesic and anesthetic medications, although in itself it has little or no analgesic action[47, 110]. This potentiation is accomplished, usually, without change in the toxicity of the primary drug[47].

The potentiating action of chlorpromazine resembles, but is stronger than, the potentiating effect previously ascribed to many antihistaminic compounds. Much experimental work has been done to show that the antihistamines, particularly Phenergan(R), have a potentiating action on the effects of anesthetics, hypnotics and analgesics[1, 7, 12, 16, 45, 46, 49, 111, 112]. Phenergan(R), contrasted to other antihistamines, is a phenothiazine derivative and is very closely related to chlorpromazine (figure 8). The potentiating action of chlorpromazine appears to extend to all barbiturates[16, 47, 92, 113] and to some of the general anesthetics[16, 47]. The time of onset of anesthesia is shortened and the duration is considerably increased in mice, rats and guinea pigs after a 1.25 to 20 milligrams per kilogram dose of chlorpromazine subcutaneously. Marked increases in the analgesic effects of morphine, meperidine and salicylates were observed in mice pretreated with chlorpromazine[16]. Furthermore, chlorpromazine

FIGURE 8

Comparison of Chemical Structure of Chlorpromazine, Phenergan(R) and Benadryl(R).

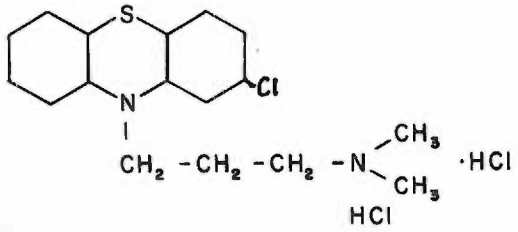
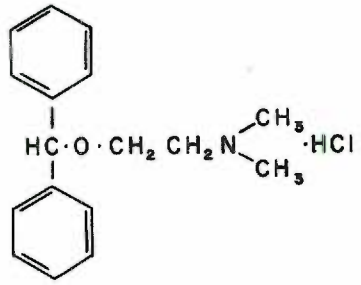
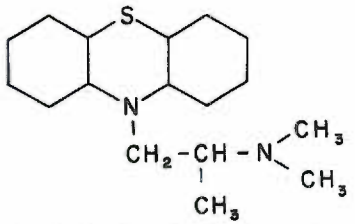
STRUCTURAL FORMULA	GENERIC NAME	TRADE NAME
 10-(γ-dimethylaminopropyl)-2-chlorphenothiazine hydrochloride	Chlorpromazine Hydrochloride	Thorazine(R)
 2-(Benzhydryloxy)-N,N-dimethylethylamine hydrochloride	Diphenhydramine Hydrochloride	Benadryl(R)
 10-(2-Dimethylamino-1-propyl) phenothiazine	Promethazine	Phenergan(R)

Fig. 8

significantly reduces the requirement for narcotics in patients with chronic pain. This valuable clinical action is apparently through chlorpromazine's ability to alter the patient's reaction to pain, producing a more relaxed and cheerful subject[62].

Since potentiation of a multitude of desired therapeutic effects of many drugs has been well demonstrated, this study was directed at determining if it were possible to quantitate this potentiating action of chlorpromazine through measurement of changes in oxygen consumption and respiratory rate; thus, simultaneously demonstrating any increases in central nervous system and respiratory depression which might accompany other desired effects. It seemed possible that there might also be a correlation between the potentiation of analgesia as measured by other workers and the potentiation of central nervous system depression as measured by this method.

RESULTS

A. Barbiturate Study:

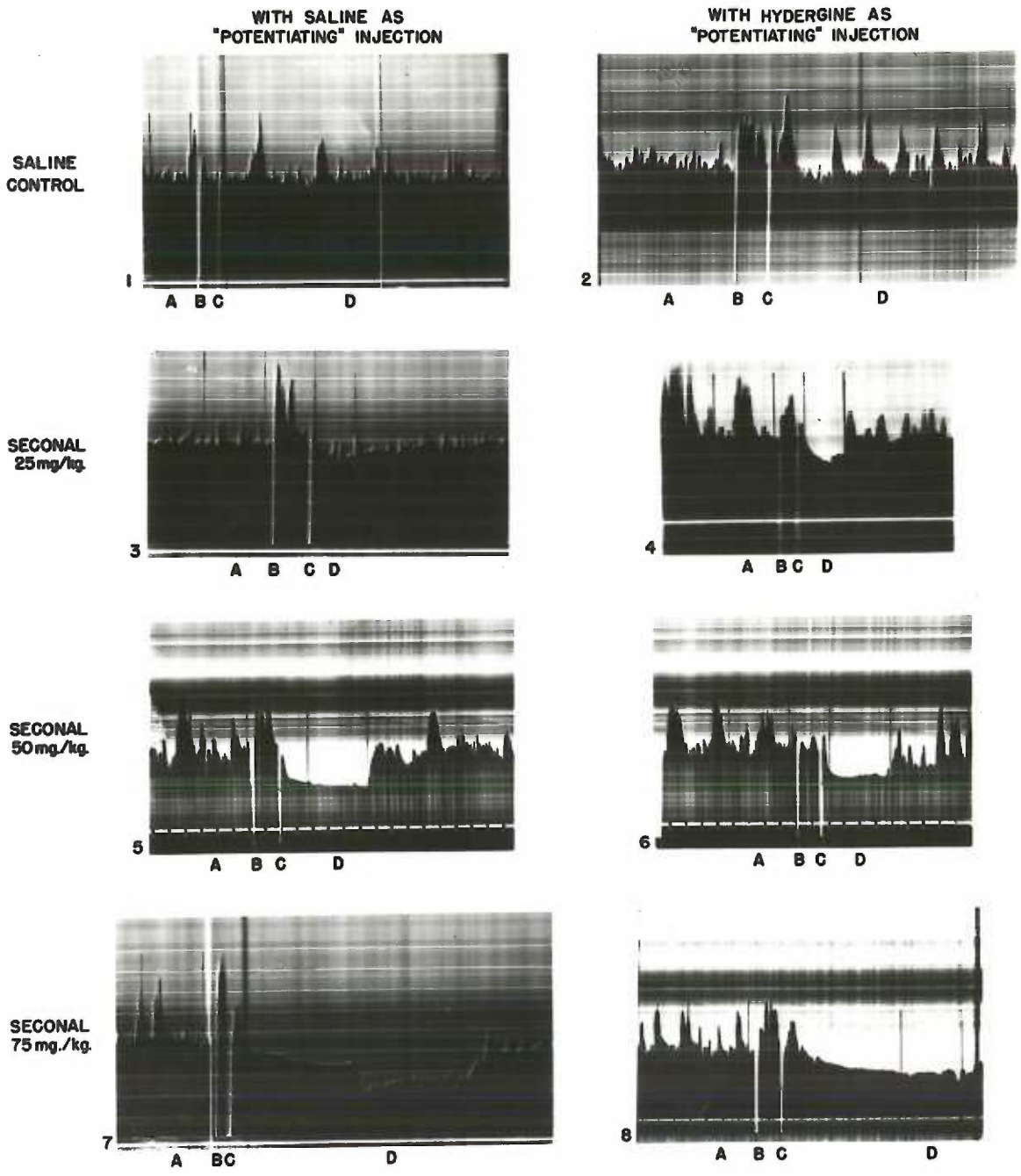
Changes in oxygen consumption in rats injected with the several drugs used are shown in figure 9. The calculations for oxygen consumption obtained from these recordings appear in table 5. In all experiments when saline alone was used for control studies, the oxygen consumption was measured for the entire period of three to four hours in order to provide a base line of effect for the rat being subjected to the experimental conditions of the apparatus. When drugs were used, the post-injection period varied in length depending on the dose of barbiturate used. When small doses of from 10 to 25 milligrams per kilogram of body weight were given the post-injection period, measured from the photographic record, was 2 centimeters (about thirty minutes). With larger doses the period measured was 3 centimeters (about forty-five minutes), since the duration of maximum drug action was lengthened.

Figure 10 shows the effects on oxygen consumption when increasing doses of sodium secobarbital were given either after saline or after Hydergine(R). When these results are expressed as per cent of change in oxygen consumption from control value, it is noted that administration of Hydergine(R) prior to administration of secobarbital in dosages up to 40 milligrams per kilogram further decreases oxygen consumption. If, however, more than 40 milligrams of secobarbital per kilogram of body weight was used Hydergine(R) lessened the decrease in oxygen consumption caused by secobarbital alone. This suggested a protective

FIGURE 9

Composite Photograph of Oxygen Consumption Records Obtained from Rats Receiving Different Dosages of Saline-Secobarbital and Hydergine(R)-Secobarbital Drug Combinations.

SAMPLE RECORDS OF OXYGEN CONSUMTION IN THE RAT
SUBJECT TO VARIOUS DOSES OF SECOBARBITAL SODIUM



A = PRE-INJECTION (CONTROL) PERIOD
B = AFTER SALINE OR HYDERGINE
C = CONTROL OR SECOBARBITAL
D = POST-INJECTION PERIOD

Fig. 9

TABLE 5

Changes in Oxygen Consumption in Rats Injected with Saline-Secobarbital or Hydergine-Secobarbital as Calculated from Photographic Records

Photo Record No.	Wt. Gm.	Pre-treatment Drug*	Seco-barbital mg./kg.	Oxygen Consumption Pre-inject. cc./min.	Post-inject. cc./min.	Per cent Change %
1	250	Saline	(Saline)	6.27	5.98	— 4.80
2	210	Hydergine	(Saline)	4.80	4.73	— 1.47
3	250	Saline	25	5.72	4.98	—12.70
4	220	Hydergine	25	6.02	4.36	—27.60
5	220	Saline	50	6.46	3.62	—44.10
6	215	Hydergine	50	6.46	3.92	—38.60
7	230	Saline	75	5.72	2.77	—51.60
8	238	Hydergine	75	5.73	3.76	—34.60

* Saline used in 1 cc./kg. of body weight; hydergine in 0.06 mg./kg. of body weight, both given 25 minutes prior to injection of secobarbital.

FIGURE 10

Dose-response Curve Showing Effect of Increasing Doses of Saline-Secobarbital and Hydergine(R)-Secobarbital Drug Combinations on Oxygen Consumption in the Rat.

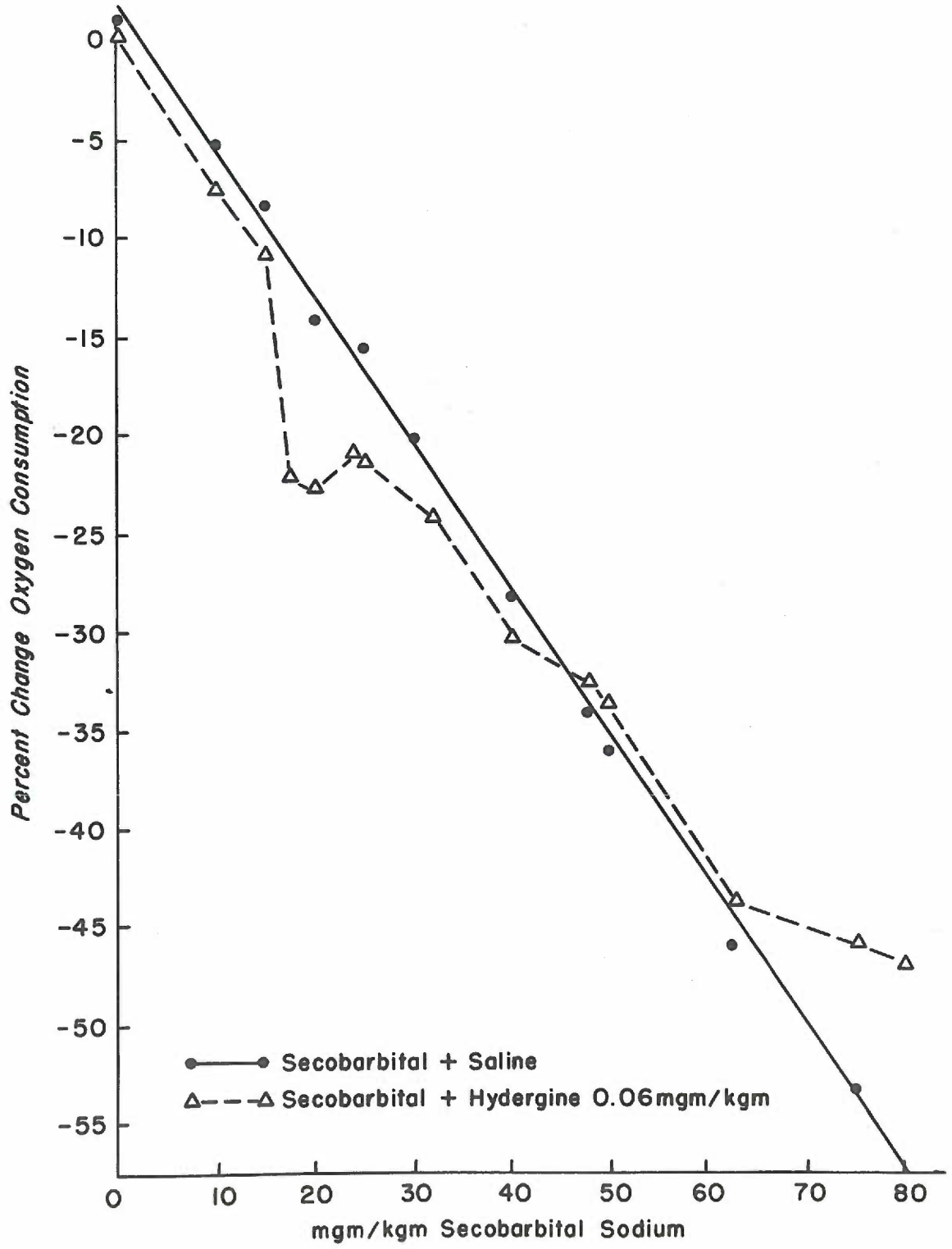

Percent Change Oxygen Consumption
0
-5
-10
-15
-20
-25
-30
-35
-40
-45
-50
-55
0
10
20
30
40
50
60
70
80
mgm/kgm Secobarbital Sodium
Secobarbital + Saline
Secobarbital + Hydergine 0.06mgm/kgm

Fig 10

action against the lethal effects of the barbiturate.

In addition, figure 10 shows a dose-response curve constructed from the data in table 6. The graph for administration of secobarbital preceded by saline is nearly a straight line up to the maximal tolerated dose under the imposed experimental conditions) of 75 milligrams per kilogram of body weight. When Hydergine(R) was administered before secobarbital, however, some deviation of the dose-response curve was noted in the range from 15 to 30 milligrams of secobarbital. The dosage range for secobarbital of 15 to 30 milligrams per kilogram of body weight is considered as the M. H. D. (minimal hypnotic dose) for the rat(114). Comparison of the saline and Hydergine(R) pretreatment values in this range was found significant (P = 0.05). Also, when pentobarbital, in concentrations within this M. H. D. range, was preceded by Hydergine(R), more than a twofold decrease in the amount of oxygen consumption, compared to that for pentobarbital alone, resulted as shown in table 6.

The curve for the combination of Hydergine(R) and secobarbital crosses that for secobarbital alone (figure 10) at the dosage of 45 milligrams per kilogram, but with increasing dosage of the barbiturate, in the toxic range, less decrease in oxygen consumption is noted. This suggests a pro ective action upon toxicity caused by large doses of secobarbital. Therefore experiments were performed to determine whether pretreatment with Hydergine(R) caused any change in the LD_{50} (dose at which fifty per cent of the animals tested do not survive) of secobarbital.

Table 7 presents the results of the toxicity studies for combinations of Hydergine(R) and various doses of secobarbital. The

TABLE 6

Changes in Oxygen Consumption in the Rat after Injections of Hydergine and Sodium Secobarbital or Sodium Pentobarbital Combinations

No. of Rats	Average Wt.	Pre-treatment Drug*	Secobarbital or Pentobarbital	Per cent Change in Oxygen Consumption		
				Average	Range Min.	Max.
	Gms.		mg./kg.	%	%	%
6	230	Saline	Saline	+ 0.83	- 4.8	+ 6.2
7	227	Hydergine	Saline	+ 0.66	- 6.8	+ 5.0
7	219	Saline	10 Secobarb.	- 5.08	+ 0.5	-13
9	210	Hydergine	10 "	- 7.37	0	-11
9	259	Saline	15 "	- 8.73	- 2	-16
7	267	Hydergine	15 "	-10.59	- 5	-19
7	249	Hydergine	17.5 "	-22.00	-10	-29
5	240	Saline	20 "	-14.74	- 5	-25
14	227	Hydergine	20 "	-22.48	-14	-39
15	225	Saline	25 "	-15.47	- 6	-26.5
15	227	Hydergine	25 "	-21.03	-13.1	-38.4
8	244	Hydergine	24 "	-20.83	-15	-29
8	227	Saline	30 "	-20.10	-13	-35
6	228	Hydergine	32 "	-23.93	-19.5	-29.7
8	222	Saline	40 "	-28.40	-21	-36
5	226	Hydergine	40 "	-30.14	-21	-35
5	244	Saline	48 "	-34.56	-31.5	-38.8
6	233	Hydergine	48 "	-32.50	-23	-42.5
11	219	Saline	50 "	-36.30	-29	-44
10	204	Hydergine	50 "	-33.30	-19	-42
6	234	Hydergine	60 "	-43.15	-24	-56
4	240	Saline	62.5 "	-46.32	-42	-48
10	222	Hydergine	62.5 "	-43.70	-30	-52
7	208	Saline	75 "	-53.10	-35	-64
8	226	Hydergine	75 "	-45.70	-33	-70
3	240	Saline	80 "	(Animals Did Not Survive)		
3	242	Hydergine	80 "	-46.70	-39	-56
6	248	Saline	20 Pentobarb.	- 9.05	- 4.2	-12.2
5	255	Hydergine	20 "	-24.48	-17.6	-32.6
3	234	Saline	25 "	-14.03	-12.0	-16.4
3	229	Hydergine	25 "	-29.03	-26.3	-33.9

* Hydergine, in dosages of 0.06 mg./kg. of body weight or saline, 1 cc./kg. was given 25 minutes before secobarbital or pentobarbital.

TABLE 7

Toxicity of Subcutaneous Injections of Saline-Secobarbital and Hydergine-Secobarbital in the Rat

No. Rats	Average Wt.(gm.)	Sod. Secobarbital (mg./kg.)	Pre-injection Drug*	No. Died	Per Cent Died
8	286	100	Saline	0	0
8	287	100	Hydergine	0	0
7	269	125	Saline	1	14.3
13	274	125	Hydergine	0	0
6	260	150	Saline	1	16.7
9	262	150	Hydergine	2	22.2
6	257	175	Saline	3	50 (LD_{50})
7	255	175	Hydergine	2	28.6
15	277	200	Saline	8	53.3
8	263	200	Hydergine	2	25
9	273	225	Saline	7	77.7
6	282	225	Hydergine	3	50 (LD_{50})
5	264	250	Saline	5	100
6	272	250	Hydergine	4	66.7
4	272	275	Saline	4	100
4	264	275	Hydergine	4	100
-	-	300	Saline	-	-
4	284	300	Hydergine	4	100

* Saline 1 cc./kg. or Hydergine(R) 0.06 mg./kg. were given 25 minutes before injection of Secobarbital.

LD_{50} for secobarbital given subcutaneously was between 175 and 200 milligrams per kilogram of body weight while that for Hydergine(R)-secobarbital was 225 to 250 milligrams per kilogram or some 30 per cent greater. Also, prior administration of Hydergine(R) served in each instance to reduce the toxicity of secobarbital when given in doses of 125, 225 and 250 milligrams per kilogram in that fewer deaths occurred with the combination of drugs (figure 11).

B. Analgesic Study:

1. Comparison of SKF #5137 and Morphine:

Changes in oxygen consumption in rats receiving either saline alone or graded doses of SKF #5137 preceded by an injection of saline, are shown in figure 12. Values were calculated for the time intervals indicated (0-5, 5-10, 10-15, etc. minutes) and reported as per cent of the pre-injection control level of oxygen consumption (table 8). It is to be noted that the curves for increasing dosages of SKF #5137 show a definite dose-response pattern. Depression of oxygen consumption from the control valve uniformly becomes of greater duration and magnitude; and, also, shows a more rapid onset of depression as the analgesic dosage is increased. Changes in oxygen consumption following administration of morphine are shown in figure 13. Again, for the major portion of the period observed, the changes in oxygen consumption can be seen to correlate with the concentration of morphine administered. Both of these drugs cause initial increase in oxygen consumption following small doses, the increase being roughly proportional to the dosage of drug. However, in large doses there is initial depression of oxygen consumption with SKF #5137. This effect is exhibited much later when morphine

FIGURE 11

Dose-response Curve Showing Toxicity of Subcutaneous Injections of Saline-Secobarbital and Hydergine(R)-Secobarbital Combinations in the Rat.

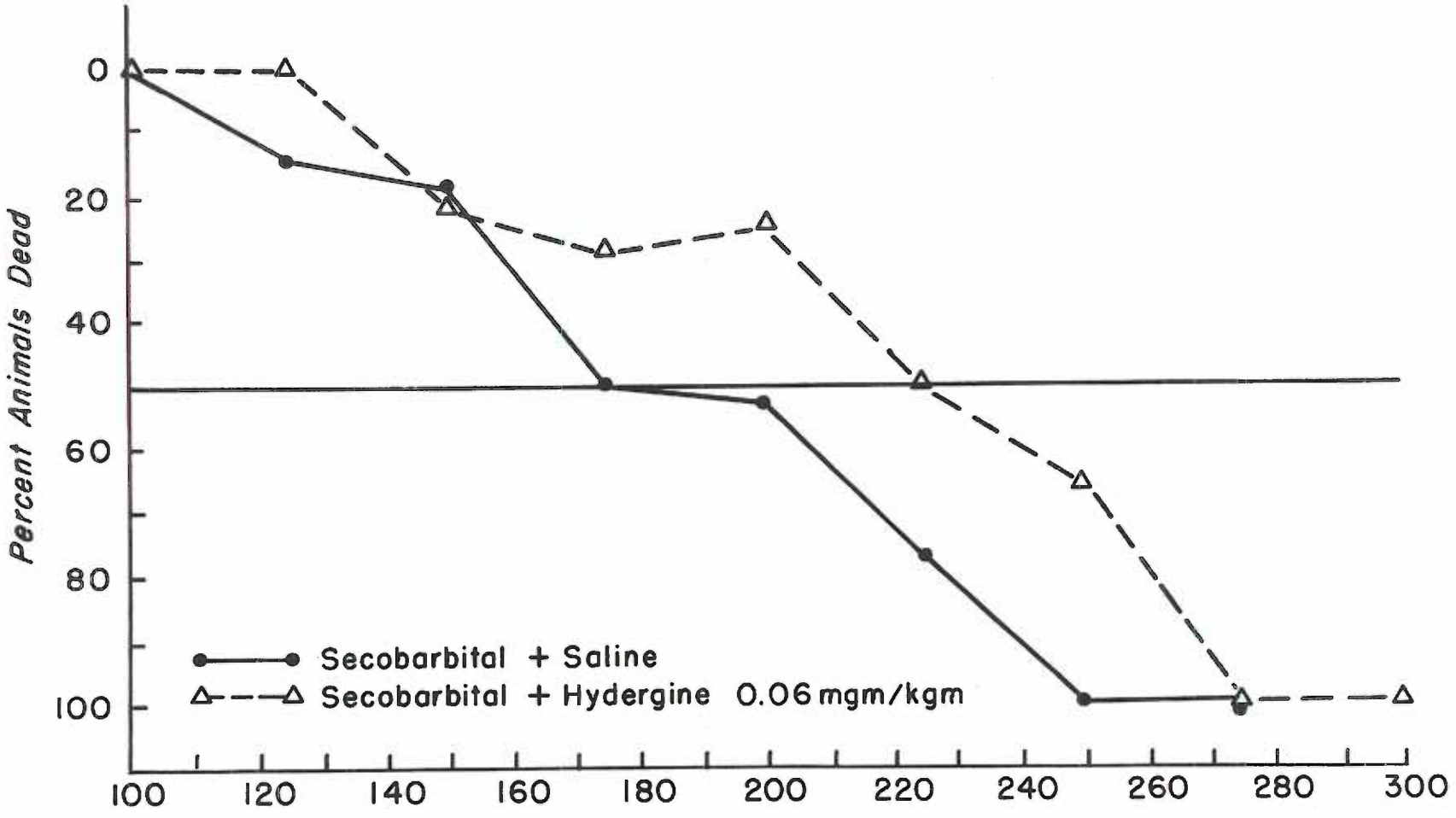

Percent Animals Dead
0
20
40
60
80
100
Secobarbital + Saline
Secobarbital + Hydergine 0.06 mgm/kgm
100
120
140
160
180
200
220
240
260
280
300
mgm/kgm Secobarbital Sodium

Fig. 11

FIGURE 12

Dose-response Curve Showing Effect of Increasing Doses of SKF #5137 on Oxygen Consumption in the Rat.

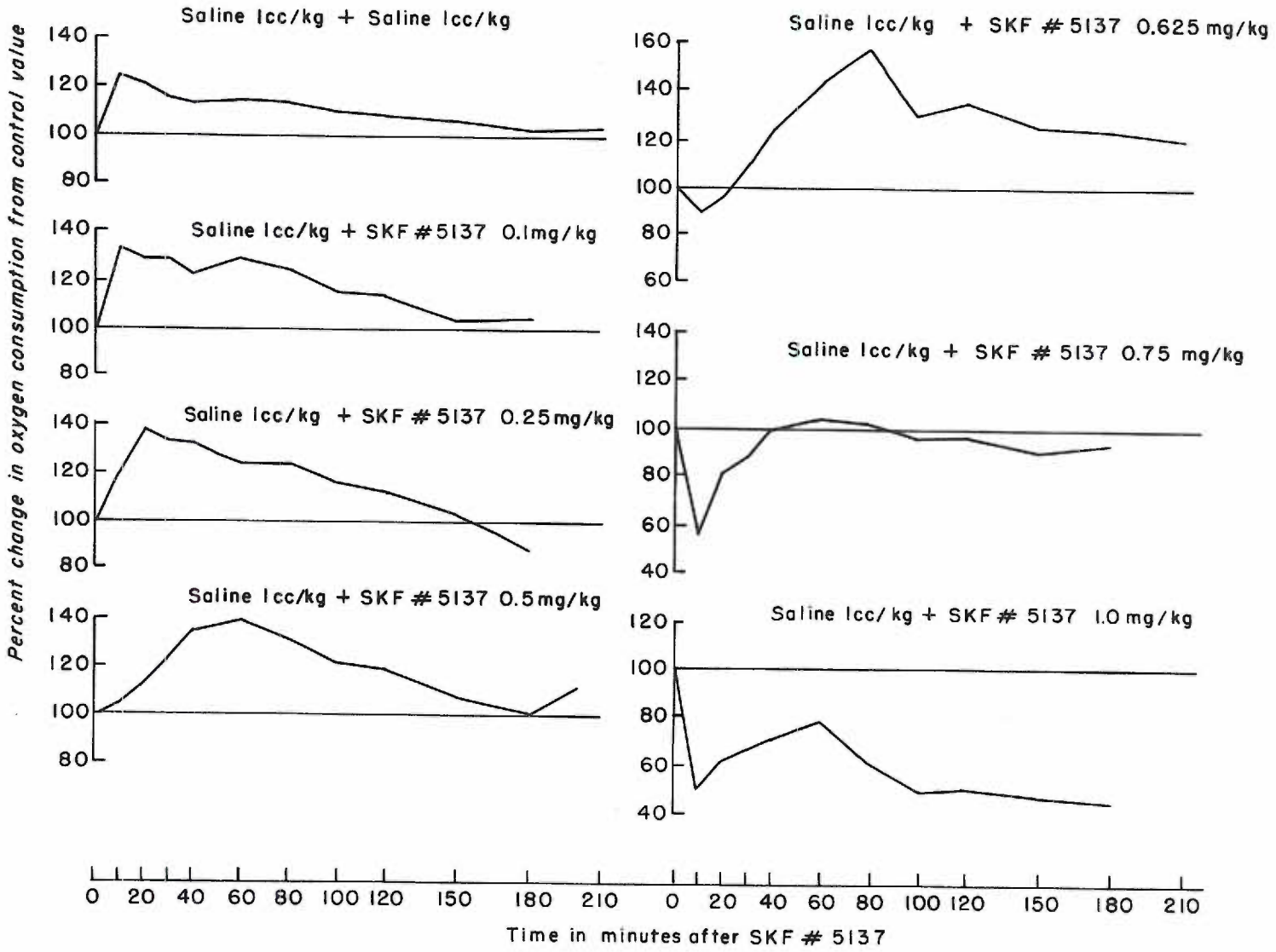

Saline 1cc/kg + Saline 1cc/kg
Saline 1cc/kg + SKF #5137 0.1mg/kg
Saline 1cc/kg + SKF #5137 0.25mg/kg
Saline 1cc/kg + SKF #5137 0.5mg/kg
Saline 1cc/kg + SKF #5137 0.625 mg/kg
Saline 1cc/kg + SKF #5137 0.75 mg/kg
Saline 1cc/kg + SKF #5137 1.0 mg/kg
Percent change in oxygen consumption from control value
Time in minutes after SKF #5137

Fig.12

TABLE 8

Change in Oxygen Consumption in Rats after Receiving Various Drug Combinations - Reported as Per cent of Control Value

No. of Rats	Ave. Wt.	Control Oxygen Consump.	First Injection		Second Injection		Time in minutes after second injection										
			Drug	Dose	Drug	Dose	0 to 10	10 to 20	20 to 30	30 to 40	40 to 60	60 to 80	80 to 100	100 to 120	120 to 150	150 to 180	180 to 200
	Gms.	cc./min.		mg/kg		mg/kg	%	%	%	%	%	%	%	%	%	%	%
8	267	6.23	Saline	*	Saline	*	125	121	116	114	115	114	110	109	106	102	103
18	229	5.16	Chlor.	5	Saline	*	121	120	116	120	108	103	98	96	96	93	84
8	222	5.06	Saline	*	SKF 5137	0.1	134	129	129	122	129	124	114	113	103	104	-
10	225	5.92	Saline	*	SKF 5137	0.25	120	138	134	133	125	124	116	112	103	88	-
6	228	6.93	Chlor.	5	SKF 5137	0.25	120	126	128	122	115	111	101	95	85	85	72
14	234	5.55	Saline	*	SKF 5137	0.5	104	113	124	135	139	131	122	120	107	101	112
14	241	5.47	Chlor.	5	SKF 5137	0.5	112	121	136	141	135	127	115	113	104	99	91
7	232	5.48	Saline	*	SKF 5137	0.625	90	97	109	123	143	156	130	135	125	123	121
4	258	6.04	Chlor.	5	SKF 5137	0.625	99	100	115	129	128	121	99	92	88	85	81
8	234	6.60	Saline	*	SKF 5137	0.75	56	81	88	100	104	102	97	97	91	94	-
8	221	5.21	Chlor.	5	SKF 5137	0.75	74	74	76	81	88	97	91	87	73	68	70
4	235	5.23	Saline	*	SKF 5137	1.0	51	63	67	72	79	61	50	50	47	45	-
7	234	5.14	Chlor.	5	SKF 5137	1.0	70	64	72	79	105	128	123	109	106	105	88
10	236	5.84	Saline	*	Morphine	3.0	102	123	138	146	146	134	124	111	99	98	93
8	219	5.95	Chlor.	5	Morphine	3.0	85	84	98	109	113	110	98	89	81	77	71
9	225	5.01	Saline	*	Morphine	6.0	104	120	130	130	134	129	133	125	118	109	99
4	207	4.57	Chlor.	5	Morphine	6.0	86	93	95	97	104	119	125	128	118	102	87
6	227	5.50	Saline	*	Morphine	9.0	109	114	111	118	116	110	100	111	99	81	82
4	240	4.93	Chlor.	5	Morphine	9.0	81	79	83	80	68	76	83	72	63	55	49

* Saline given 1 cc./kg.; second injections followed 30 minutes after first injection.

FIGURE 13

Dose-response Curves Showing Effect of Increasing Doses of Morphine Sulfate on Oxygen Consumption in the Rat.

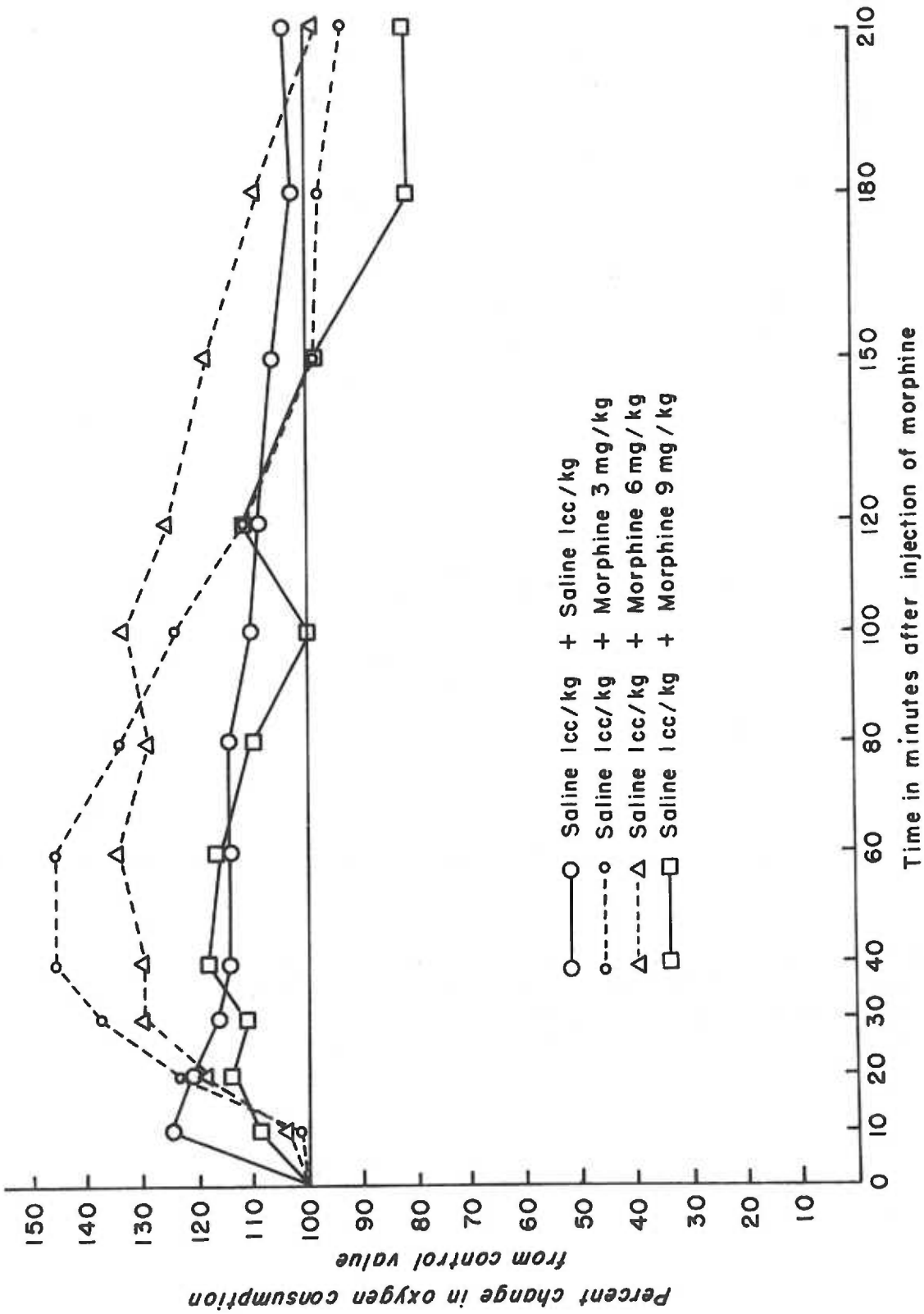

Percent change in oxygen consumption
from control value
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
10
20
30
40
60
80
100
120
150
180
210
Time in minutes after injection of morphine
Saline 1cc/kg + Saline 1cc/kg
Saline 1cc/kg + Morphine 3 mg/kg
Saline 1cc/kg + Morphine 6 mg/kg
Saline 1cc/kg + Morphine 9 mg/kg

Fig.13

is used.

Since the major differences in the curves for the two drugs occurred during the first portion of the record, comparison of the average per cent change in oxygen consumption for the first hour after drug administration is shown in figure 14.

According to literature received from Smith, Kline and French Laboratories, SKF #5137 is from five to ten times more active than morphine on a milligram per kilogram basis. We have arbitrarily selected a ratio of 8.35 : 1 for comparison of these two drugs. A comparison of the dose-response curves for SKF #5137 and morphine, as regards change in oxygen consumption, is illustrated in figure 15. From examination of these graphs it can be seen that the changes in oxygen consumption for both the drugs in equal analgesic doses are practically identical, except with the larger dosage. Here it can be seen that oxygen consumption was markedly depressed after administration of 0.75 milligrams per kilogram of SKF #5137, an effect not observed with a comparable dose of morphine. It should be mentioned that observation of the rats showed that those which had received this concentration of SKF #5137 were subjectively much more severely depressed during the first thirty to forty-five minute period than were those which had received morphine.

Of interest at this point is the fact that the first hour change in oxygen consumption for 0.625 milligrams per kilogram of SKF #5137 was exactly the same as that for 9 milligrams per kilogram of morphine. Furthermore, the entire dose-response curves for 0.625 milligrams per kilogram of SKF #5137 resembles more closely that for 9 milligrams per

FIGURE 14

Dose-response Curve Comparing Effect of Increasing Doses of both SKF #5137 and Morphine Sulfate during the First Hour after Injection on Oxygen Consumption in the Rat.

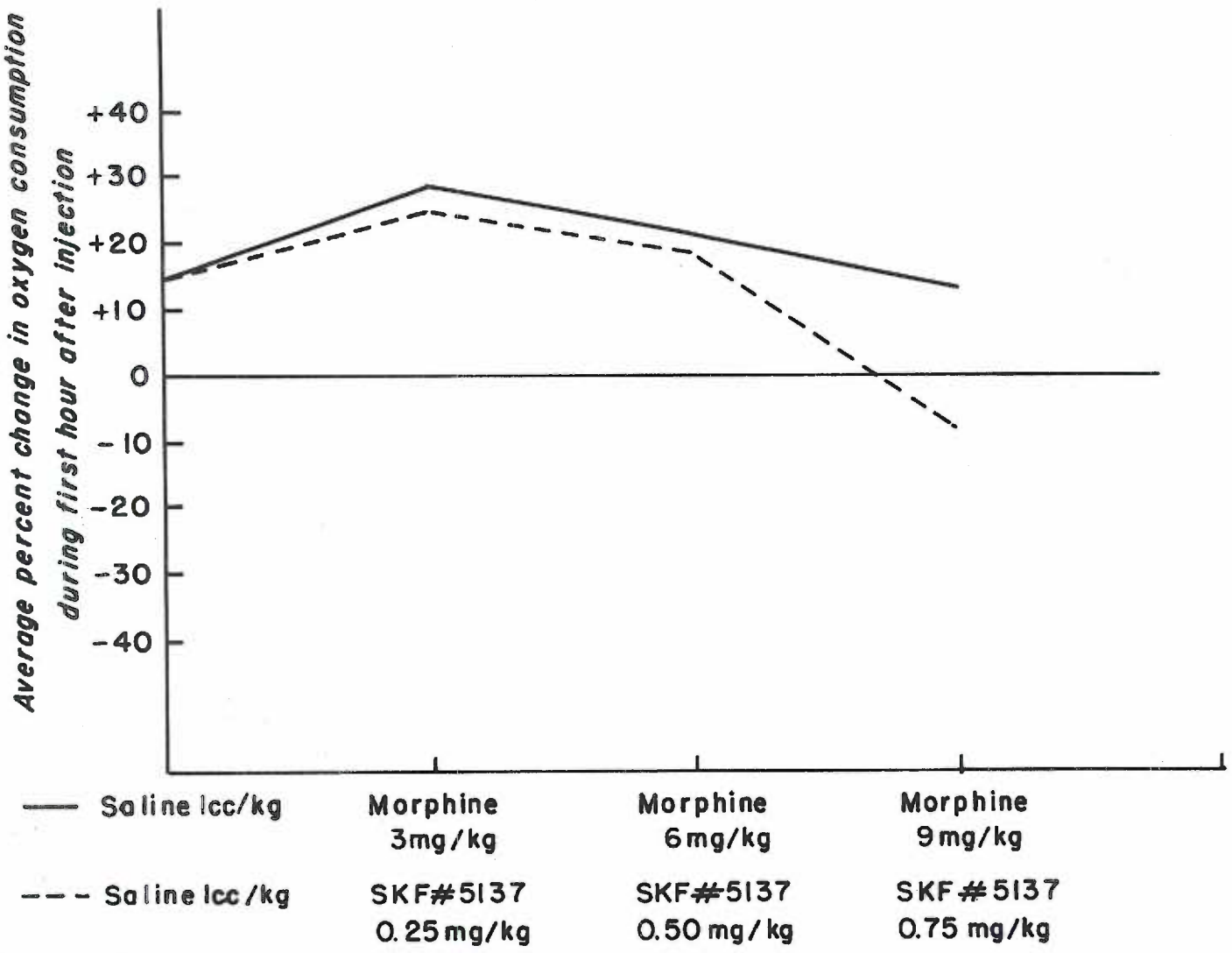


Fig.14 Concentrations of morphine and SKF # 5137 mg/kg preceeded by saline 1cc/kg

FIGURE 15

Dose-response Curves Comparing Effects of Similar Analgesic Doses of SKF #5137 and Morphine Sulfate on Oxygen Consumption in the Rat.

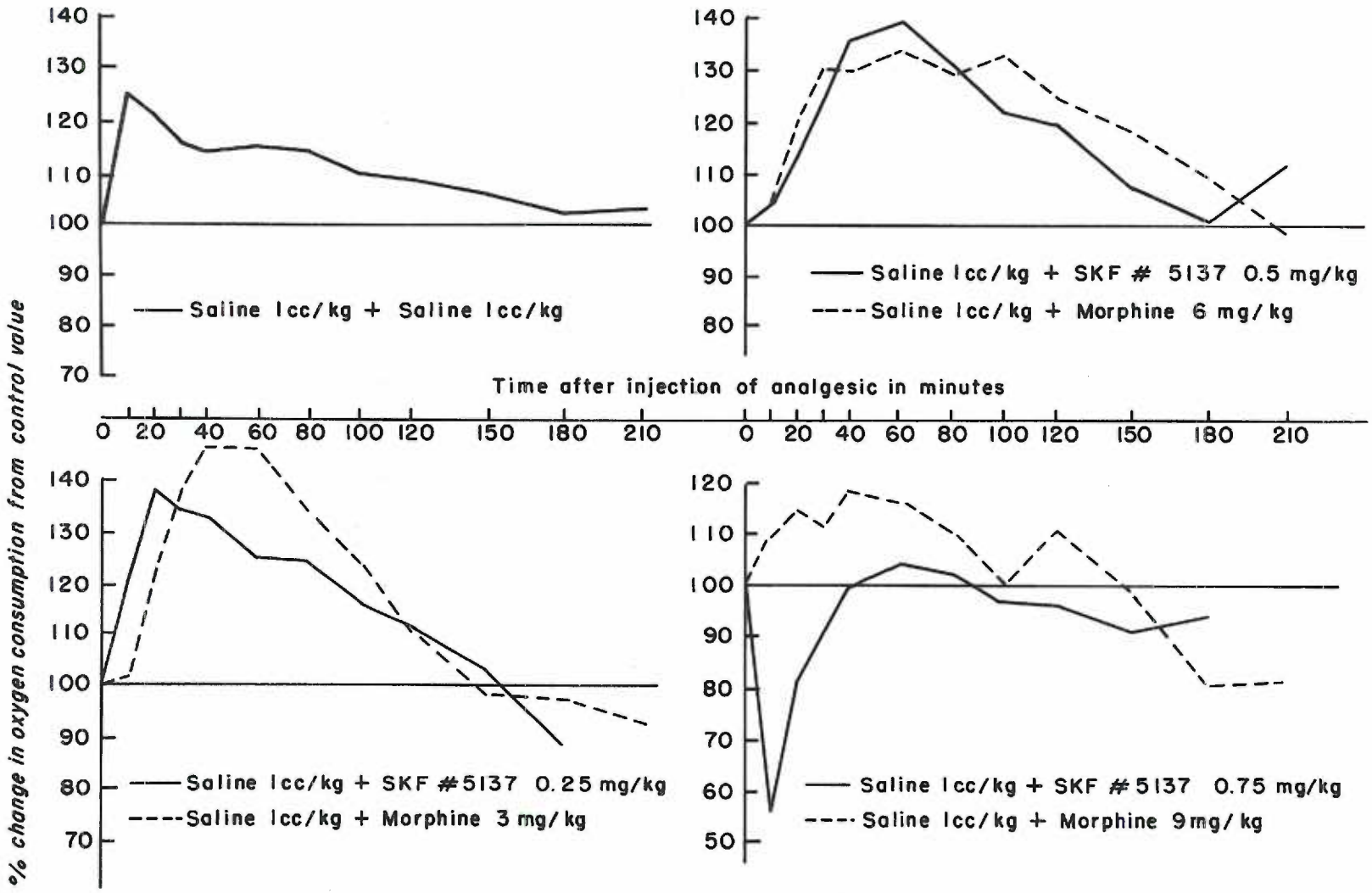

% change in oxygen consumption from control value
Time after injection of analgesic in minutes
Saline 1cc/kg + Saline 1cc/kg
Saline 1cc/kg + SKF # 5137 0.5 mg/kg
Saline 1cc/kg + Morphine 6 mg/kg
Saline 1cc/kg + SKF #5137 0.25 mg/kg
Saline 1cc/kg + Morphine 3 mg/kg
Saline 1cc/kg + SKF #5137 0.75 mg/kg
Saline 1cc/kg + Morphine 9mg/kg

Fig.15

kilogram of morphine than does the curve for SKF #5137 at 0.75 milligrams per kilogram (figures 12 and 13). Since the change in oxygen consumption caused by the two drugs is of a different ratio at the higher level, it is possible that the analgesic activity of these drugs may parallel this finding. This may account for the differences in opinion as to the relative analgesic activity of these drugs reported by other workers(47).

Changes in respiratory rate following administration of SKF #5137 and morphine are also reported as per cent of the pre-injection control value (table 9). Effects of increasing doses of these drugs on respiratory rate are depicted in figures 16 and 17. Again it is to be noted that these figures show a definite dose-response relation for both the drugs. Depression of the respiratory rate is shown in all curves, except that of the smallest dose of morphine. A comparison of the dose-response curves for approximately equal analgesic doses of the drugs is shown in figure 18. Although there is more depression of respiratory rate with large concentrations of SKF #5137 than with morphine, the curves are quite similar.

Dose-response curves comparing drug effects upon both oxygen consumption and respiratory rate after injection of SKF #5137 and morphine are shown in figures 19 and 20. It can be seen that the simultaneous depression of both respiratory rate and oxygen consumption is evident only with relatively large doses of these analgesic drugs.

2. Chlorpromazine "Potentiation" of Analgesic Central Nervous System Depressant Activity:

After establishing dose-response curves for SKF #5137 and

TABLE 9

Effect of Various Drug Combinations on the Respiratory Rate of the Rat

Combinations of Drugs Used	Dose mg/kg	No. of Rats	Ave. Wt. Gms.	Control Resp. Rate min.	Per cent of Control Respiratory Rate — Time in minutes after second injection: 5	10	15	20	25	30	40	50	60	80	100	120	150	180	210
					%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
Saline & Saline	*	8	267	120	90	89	90	87	85	85	84	85	90	92	92	92	100	100	101
Chlor. & Saline	*	8	251	114	97	97	105	103	100	97	98	93	100	97	97	103	98	97	98
Saline & SKF #5137	0.25	4	217	114	84	76	82	86	90	90	90	91	90	93	102	103	105	109	102
Chlor. & SKF #5137	0.25	4	208	106	74	74	68	72	72	70	76	78	83	79	87	89	91	81	79
Saline & SKF #5137	0.5	7	252	126	76	65	70	72	75	73	73	72	78	89	91	97	97	99	95
Chlor. & SKF #5137	0.5	7	259	124	65	63	63	76	76	79	81	81	87	85	85	89	89	84	89
Saline & SKF #5137	0.75	4	230	126	60	48	56	65	76	86	94	80	81	81	84	84	104	103	95
Chlor. & SKF #5137	0.75	8	221	96	69	65	60	58	58	65	65	65	71	79	81	86	87	90	94
Saline & Morphine	3.0	4	274	100	96	112	120	126	104	100	102	106	118	120	108	114	112	106	-
Chlor. & Morphine	3.0	4	209	112	98	110	116	98	97	98	98	101	110	93	90	104	88	105	88
Saline & Morphine	6.0	4	276	130	76	83	77	74	74	71	65	68	91	97	93	99	109	-	-
Chlor. & Morphine	6.0	4	188	120	80	83	93	98	115	106	98	92	95	97	101	100	111	110	93
Saline & Morphine	9.0	4	242	132	86	71	80	83	80	80	74	73	82	80	89	87	85	85	85#
Chlor. & Morphine	9.0	4	240	100	70	70	76	88	80	76	78	72	66	72	76	92	90	82	40#

* Saline 1 cc./kg. or Chlor. (Chlorpromazine) 5 mg./kg.; # Two rats in group died after 180 minutes.

FIGURE 16

Dose-response Curves Showing the Effect of Increasing Doses of SKF #5137 on the Respiratory Rate in the Rat.

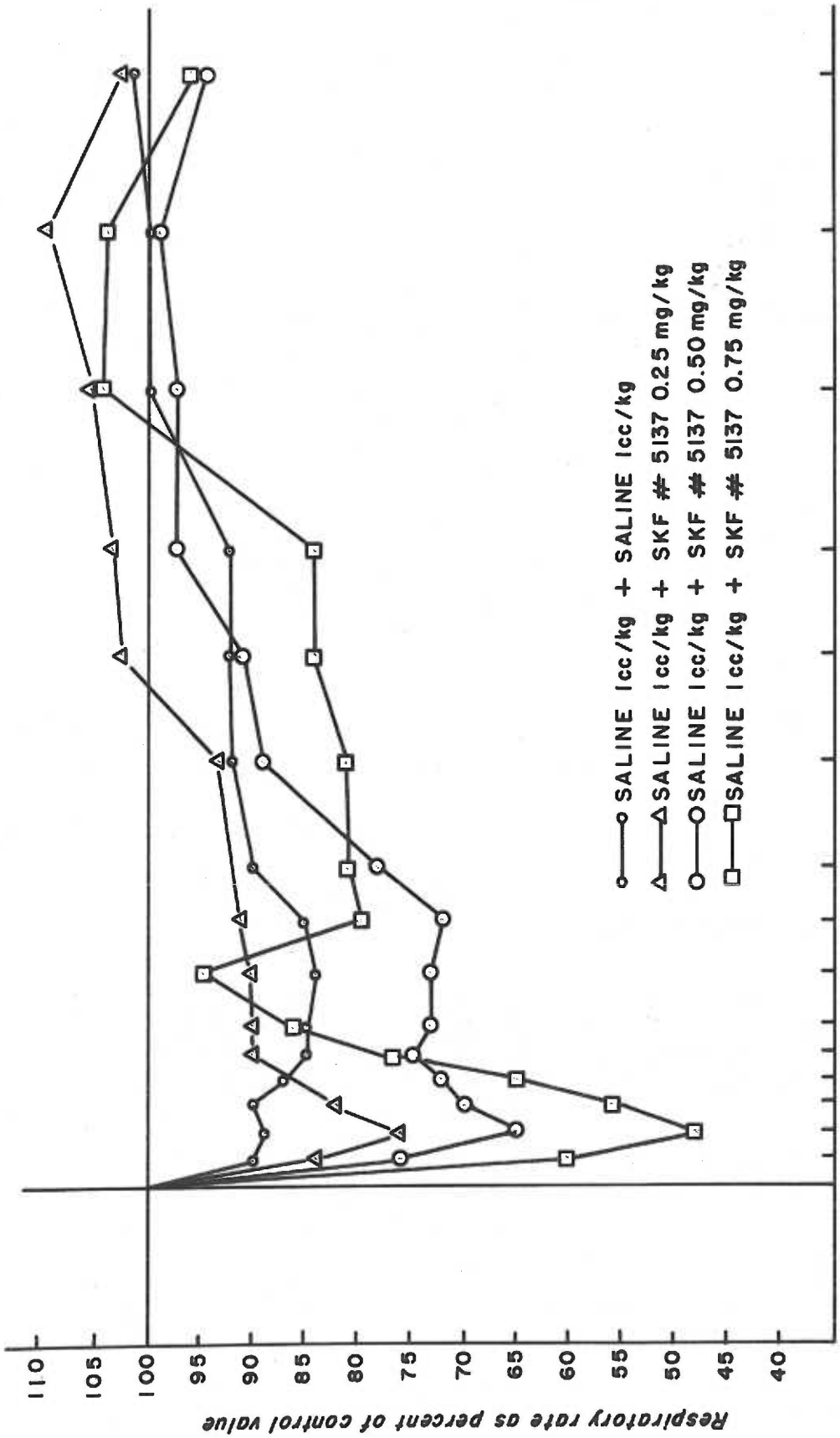

Respiratory rate as percent of control value
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
Control Period
10
20
30
40
50
60
80
100
120
150
180
210
Time in minutes after injection of SKF # 5137
SALINE 1cc/kg + SALINE 1cc/kg
SALINE 1cc/kg + SKF # 5137 0.25 mg/kg
SALINE 1cc/kg + SKF # 5137 0.50 mg/kg
SALINE 1cc/kg + SKF # 5137 0.75 mg/kg

Fig16

FIGURE 17

Dose-response Curves Showing the Effect of Increasing Doses of Morphine Sulfate on the Respiratory Rate in the Rat.

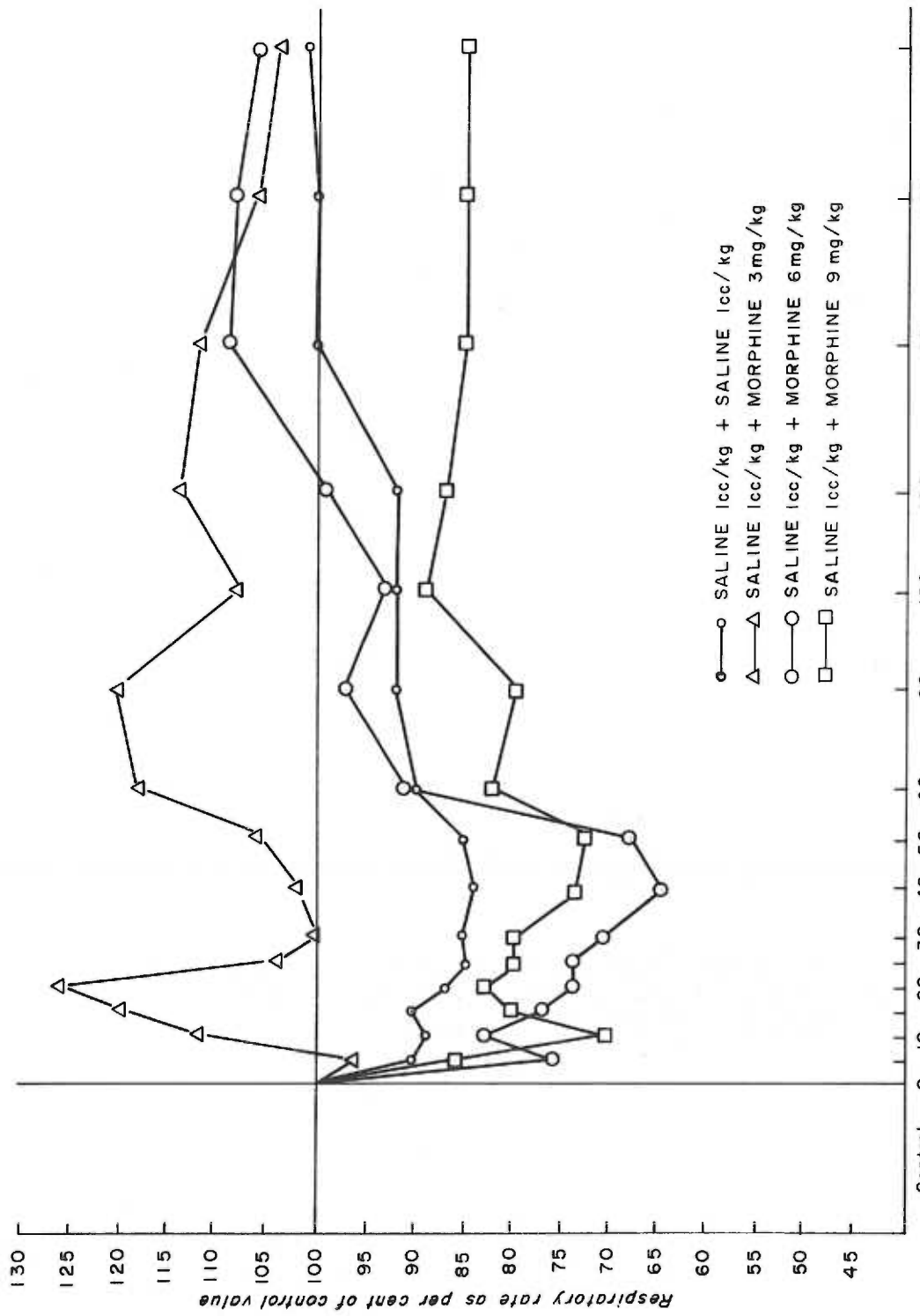

Respiratory rate as per cent of control value
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
Control Period
0
10
20
30
40
50
60
80
100
120
150
180
210
Time in minutes after injection of morphine
SALINE 1cc/kg + SALINE 1cc/kg
SALINE 1cc/kg + MORPHINE 3mg/kg
SALINE 1cc/kg + MORPHINE 6mg/kg
SALINE 1cc/kg + MORPHINE 9 mg/kg

Fig.17

FIGURE 18

Dose-response Curves Comparing the Effects of Similar Analgesic Doses of SKF #5137 and Morphine Sulfate on the Respiratory Rate in the Rat.

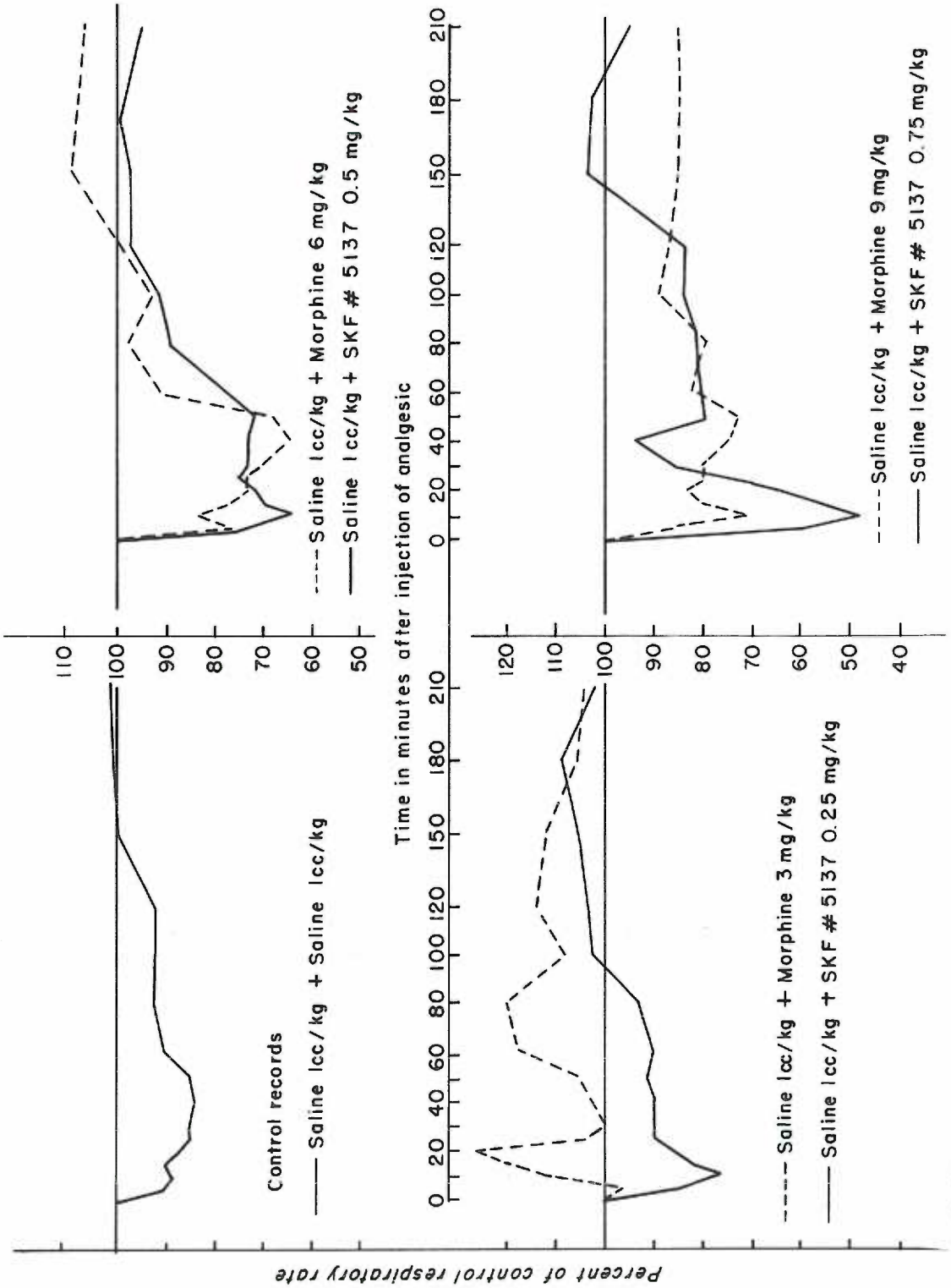

Percent of control respiratory rate
Time in minutes after injection of analgesic
Control records
—— Saline 1cc/kg + Saline 1cc/kg
---- Saline 1cc/kg + Morphine 3 mg/kg
—— Saline 1cc/kg + SKF # 5137 0.25 mg/kg
---- Saline 1cc/kg + Morphine 6 mg/kg
—— Saline 1cc/kg + SKF # 5137 0.5 mg/kg
---- Saline 1cc/kg + Morphine 9 mg/kg
—— Saline 1cc/kg + SKF # 5137 0.75 mg/kg

Fig. 18

FIGURE 19

Composite Dose-response Curves Showing Effect of Increasing Doses of SKF #5137 on Oxygen Consumption and Respiratory Rate in the Rat.

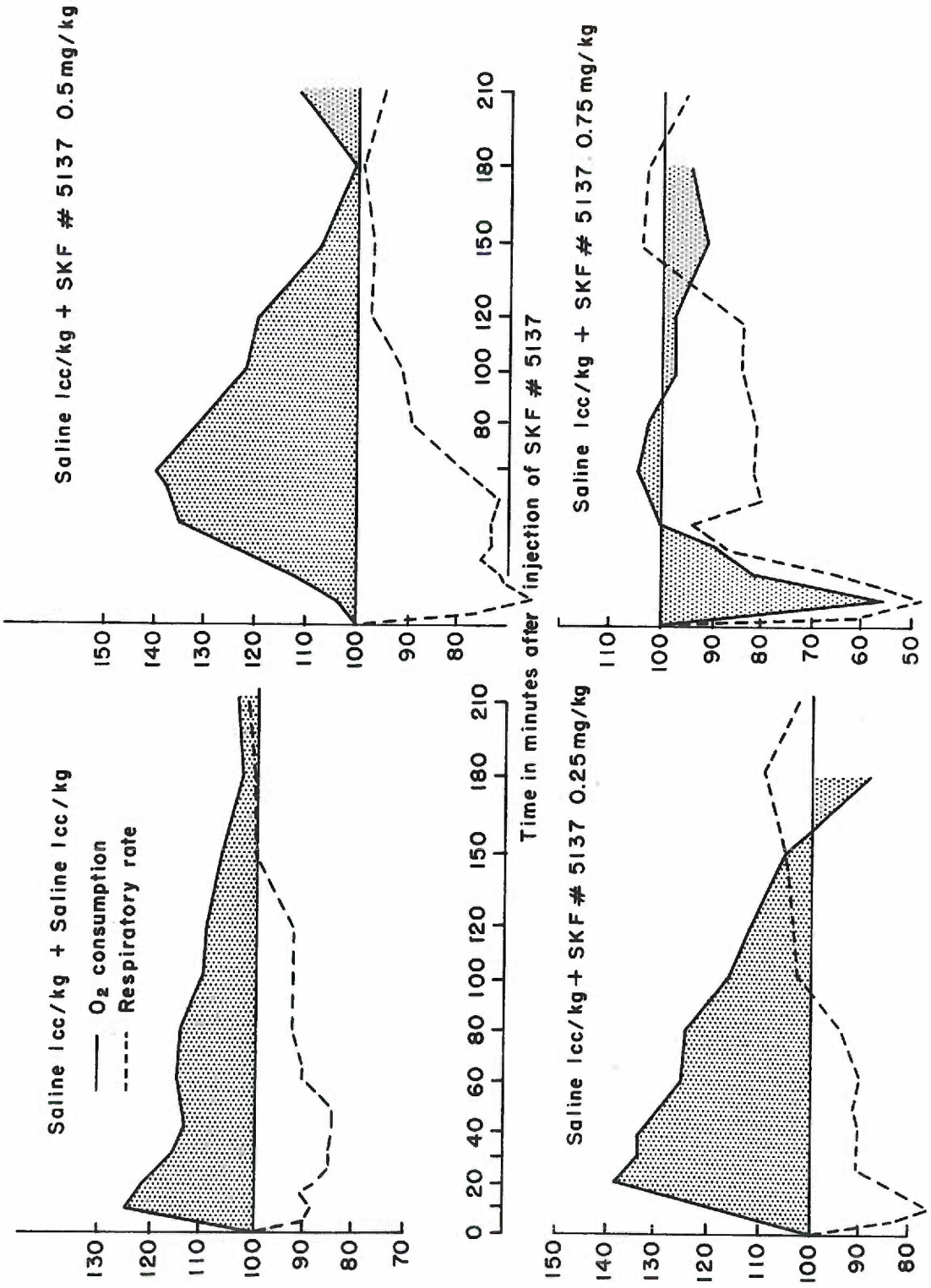

Saline 1cc/kg + Saline 1cc/kg
O2 consumption
Respiratory rate
Saline 1cc/kg + SKF # 5137 0.5 mg/kg
Saline 1cc/kg + SKF # 5137 0.75 mg/kg
Saline 1cc/kg + SKF # 5137 0.25 mg/kg
Time in minutes after injection of SKF # 5137
Percent change in oxygen consumption and respiratory rate from control value

Fig.19

FIGURE 20

Composite Dose-response Curves Showing Effect of Increasing Doses of Morphine Sulfate on Oxygen Consumption and Respiratory Rate in the Rat.

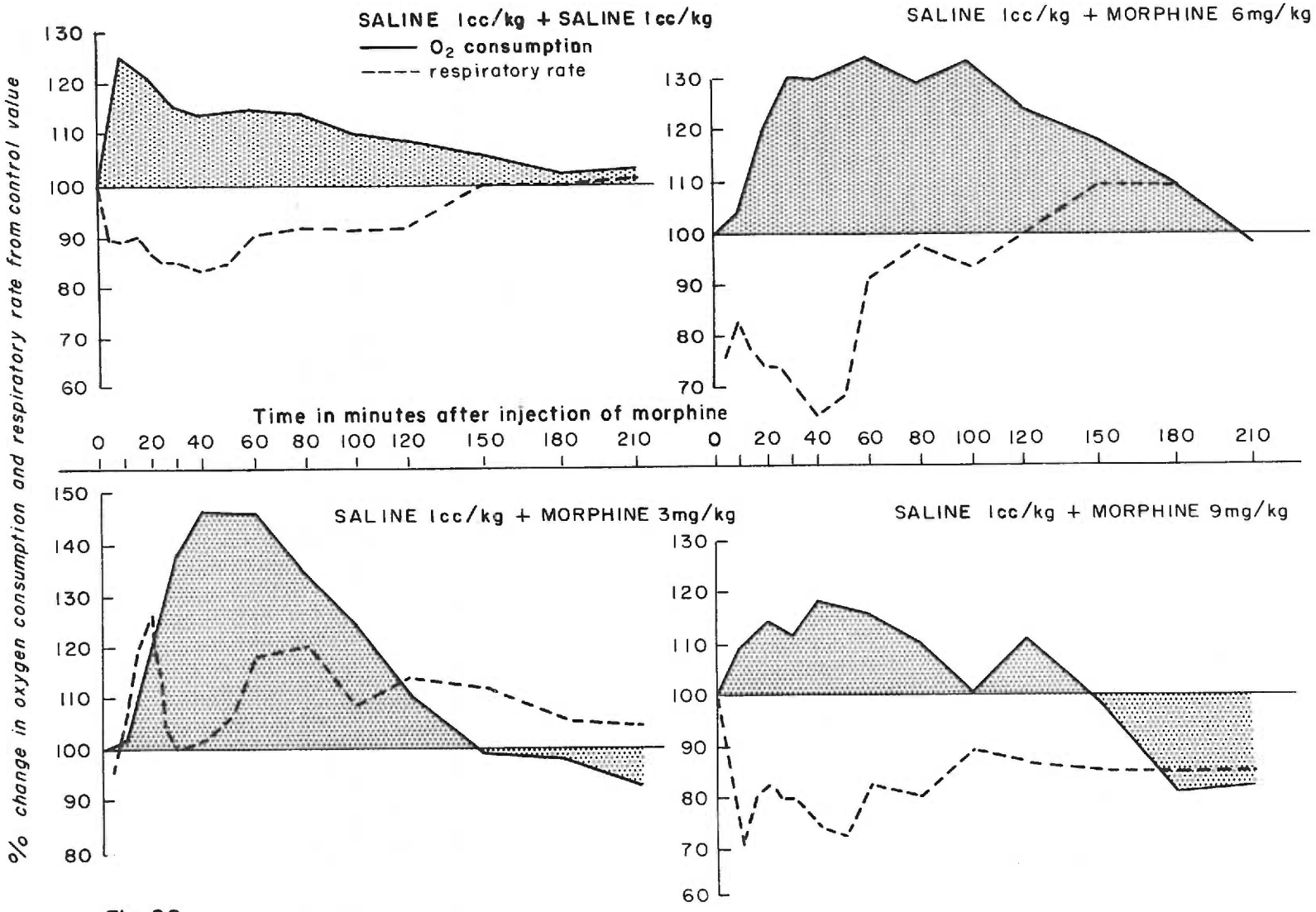

SALINE 1cc/kg + SALINE 1cc/kg
O2 consumption
respiratory rate
SALINE 1cc/kg + MORPHINE 6mg/kg
SALINE 1cc/kg + MORPHINE 3mg/kg
SALINE 1cc/kg + MORPHINE 9mg/kg
Time in minutes after injection of morphine
% change in oxygen consumption and respiratory rate from control value

Fig.20

morphine when preceded by saline injections, the studies were repeated using 5 milligrams per kilogram of chlorpromazine injected subcutaneously thirty minutes prior to the analgesic drug (tables 8 and 9). Figure 21 shows the difference in effect upon oxygen consumption after administration of SKF #5137, preceded by saline and by chlorpromazine. The graph for chlorpromazine alone shows a slight decrease in oxygen consumption as compared to the control. This finding corresponds to the results of other experiments previously cited. These graphs show only slight increase in the depression of oxygen consumption when SKF #5137 is given in concentrations less than 1.0 milligram per kilogram following chlorpromazine injection. Also, the curves obtained for both drug combinations appear very similar. When one takes into consideration the fact that chlorpromazine alone decreases oxygen consumption slightly, it would appear that the effect of chlorpromazine was merely additive. However, when this drug precedes 1.0 milligram per kilogram concentrations of SKF #5137, there is much less depression of oxygen consumption than would be expected. These results are similar to those seen following large doses of secobarbital when the animal had been pretreated with Hydergine$^{(R)}$ (figure 10). Figure 22 shows the effect upon the respiratory rate of saline-SKF #5137 and chlorpromazine-SKF #5137 drug combinations. It should be noted here that chlorpromazine alone caused less depression of respiratory rate than saline, but when it preceded administration of SKF #5137, slightly more depression of the respiratory rate was observed.

The effect of pre-treatment with chlorpromazine on morphine-induced changes in oxygen consumption is shown in figure 23. It can be seen that the depression of oxygen consumption observed when

FIGURE 21

Dose-response Curves Showing "Potentiation" of Effects of Increasing Doses of SKF #5137 on Oxygen Consumption in the Rat when Combined with Chlorpromazine as Compared to Effects of SKF #5137 alone.

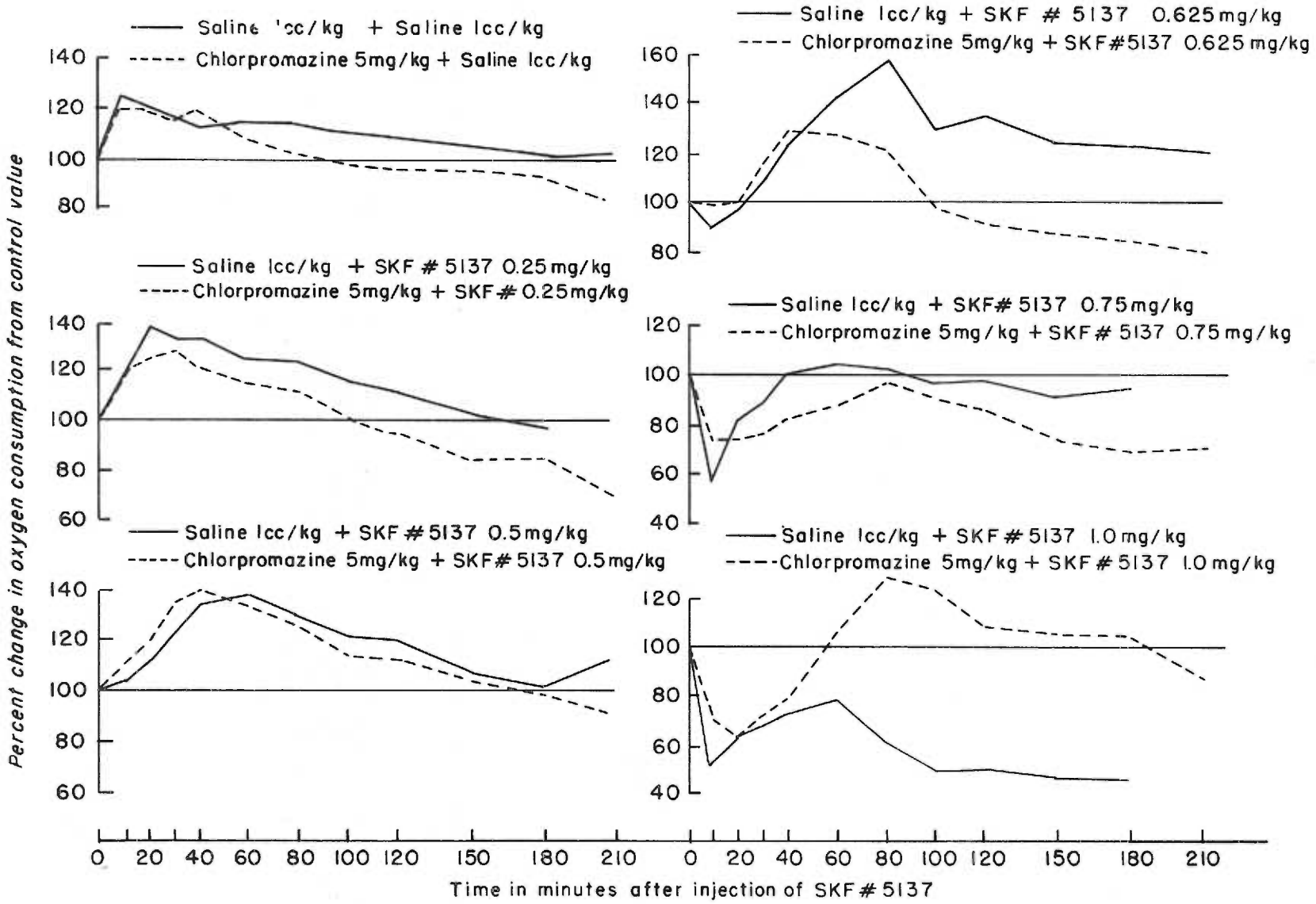

Saline 1cc/kg + Saline 1cc/kg
Chlorpromazine 5mg/kg + Saline 1cc/kg
Saline 1cc/kg + SKF # 5137 0.625mg/kg
Chlorpromazine 5mg/kg + SKF#5137 0.625 mg/kg
Saline 1cc/kg + SKF # 5137 0.25mg/kg
Chlorpromazine 5mg/kg + SKF# 0.25mg/kg
Saline 1cc/kg + SKF# 5137 0.75mg/kg
Chlorpromazine 5mg/kg + SKF # 5137 0.75 mg/kg
Saline 1cc/kg + SKF # 5137 0.5mg/kg
Chlorpromazine 5mg/kg + SKF# 5137 0.5mg/kg
Saline 1cc/kg + SKF # 5137 1.0 mg/kg
Chlorpromazine 5mg/kg + SKF # 5137 1.0 mg/kg
Percent change in oxygen consumption from control value
Time in minutes after injection of SKF # 5137

Fig.21

FIGURE 22

Dose-response Curves Showing "Potentiation" of Effects of Increasing Doses of SKF #5137 on the Respiratory Rate in the Rat when Combined with Chlorpromazine as Compared to Effects of SKF #5137 alone.

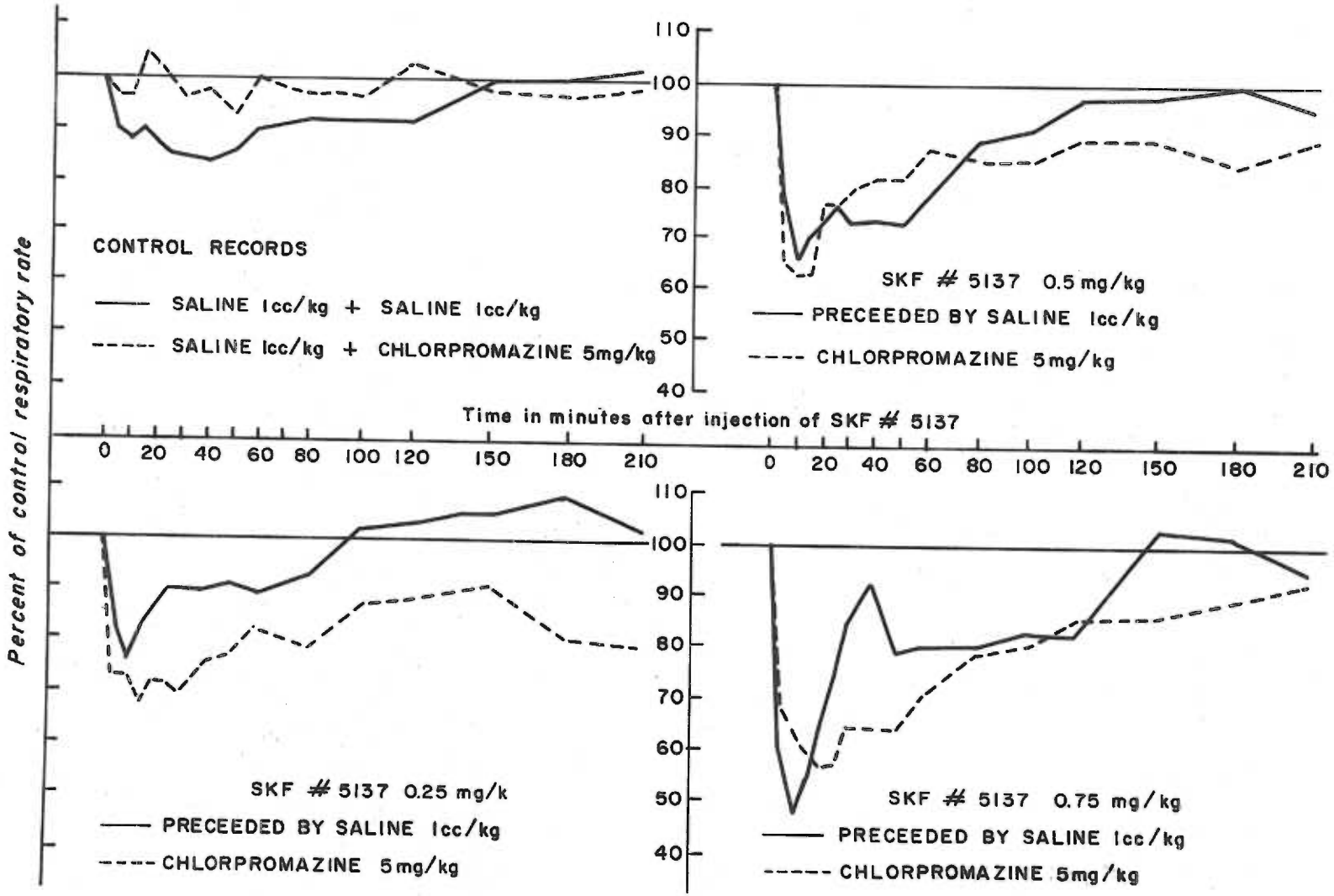

Percent of control respiratory rate
110
100
90
80
70
60
50
40
CONTROL RECORDS
SALINE 1cc/kg + SALINE 1cc/kg
SALINE 1cc/kg + CHLORPROMAZINE 5mg/kg
SKF # 5137 0.5 mg/kg
PRECEEDED BY SALINE 1cc/kg
CHLORPROMAZINE 5mg/kg
Time in minutes after injection of SKF # 5137
0 20 40 60 80 100 120 150 180 210
SKF # 5137 0.25 mg/k
PRECEEDED BY SALINE 1cc/kg
CHLORPROMAZINE 5mg/kg
SKF # 5137 0.75 mg/kg
PRECEEDED BY SALINE 1cc/kg
CHLORPROMAZINE 5mg/kg

Fig.22

FIGURE 23

Dose-response Curves Showing "Potentiation" of Effects of Increasing Doses of Morphine Sulfate on Oxygen Consumption in the Rat when Combined with Chlorpromazine as Compared to Effects of Morphine Sulfate alone.

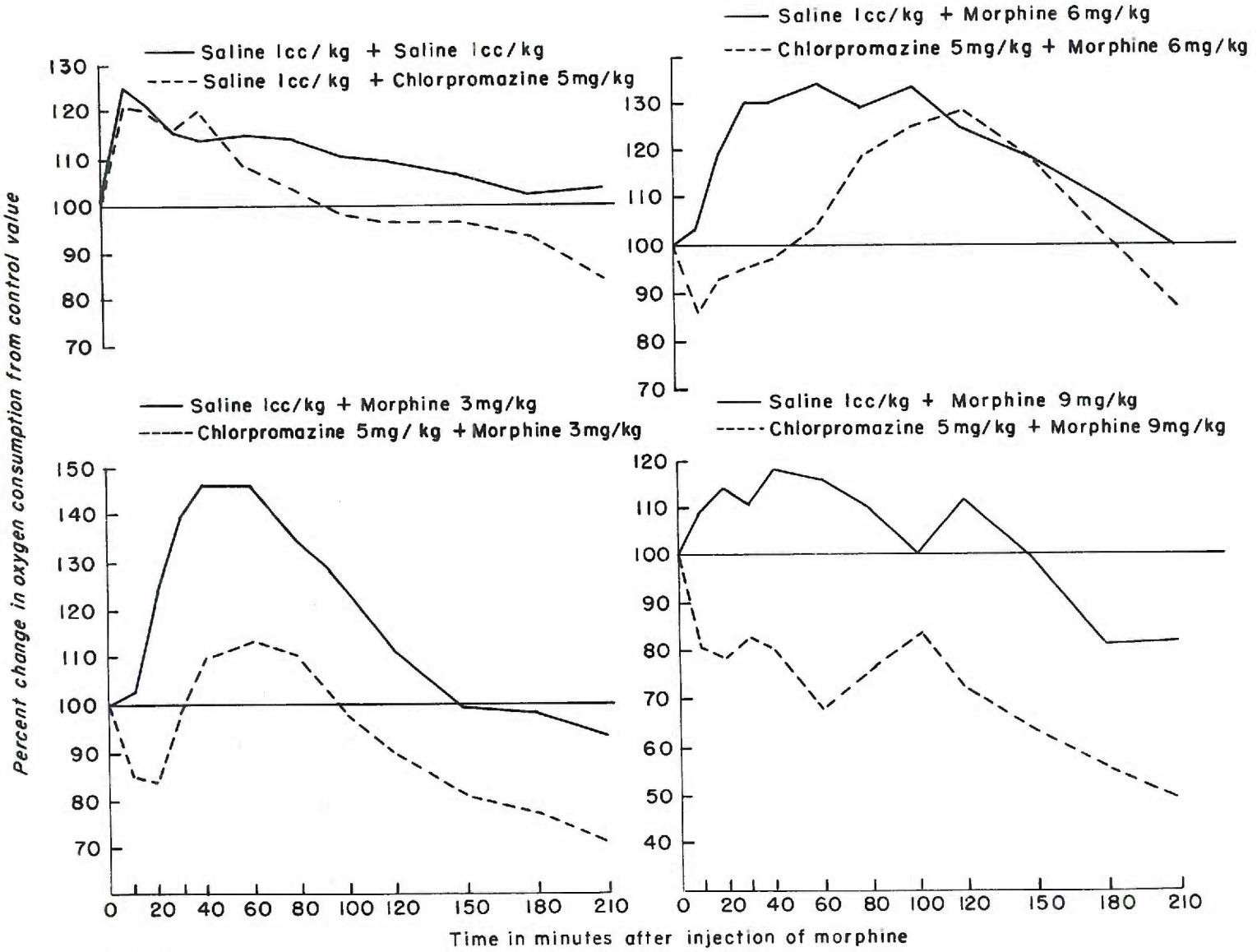

Saline 1cc/kg + Saline 1cc/kg
Saline 1cc/kg + Chlorpromazine 5mg/kg
Saline 1cc/kg + Morphine 6mg/kg
Chlorpromazine 5mg/kg + Morphine 6mg/kg
Saline 1cc/kg + Morphine 3mg/kg
Chlorpromazine 5mg/kg + Morphine 3mg/kg
Saline 1cc/kg + Morphine 9mg/kg
Chlorpromazine 5mg/kg + Morphine 9mg/kg
Percent change in oxygen consumption from control value
Time in minutes after injection of morphine

Fig.23

chlorpromazine is combined with morphine is far more than an additive effect. Furthermore, the curves are strikingly parallel, indicating a direct potentiation of the morphine-induced changes in oxygen consumption. The changes in respiratory rate with chlorpromazine-morphine drug combinations (figure 24) tend to parallel the changes seen with saline-morphine combinations and fail to show much potentiation of the depressant effect of morphine. The small number of animals used in the series receiving 9 milligrams per kilogram of morphine plus chlorpromazine tends to decrease the significance of the results of this group in which fifty per cent (two) of the animals died. However, when this record is compared with that of changes in oxygen consumption for animals receiving this same drug combination (figure 23), it is again seen that the animals receiving chlorpromazine were much more severely depressed.

Since the maximum drug effects upon changes in oxygen consumption and respiratory rate induced by SKF #5137 occurred within the first hour and since the differences in changes caused by the two analgesic drugs were most pronounced during this time, figures 25 through 28 are shown to illustrate dose-response curves for the different drug combinations during the first one hour period. The potentiating effect of pre-treatment with chlorpromazine on morphine-induced changes in oxygen consumption is much more marked than with SKF #5137 (figures 25 and 26). Again note that the curves for both drug combinations with saline and chlorpromazine are parallel. The curves showing changes in respiratory rate for chlorpromazine and SKF #5137 and for chlorpromazine and morphine during the first hour are very similar to those produced when these drugs were combined with saline (figures 27 and 28).

FIGURE 24

Dose-response Curves Showing "Potentiation" of Effects of Increasing Doses of Morphine Sulfate on the Respiratory Rate in the Rat when Combined with Chlorpromazine as Compared to Effects of Morphine Sulfate alone.

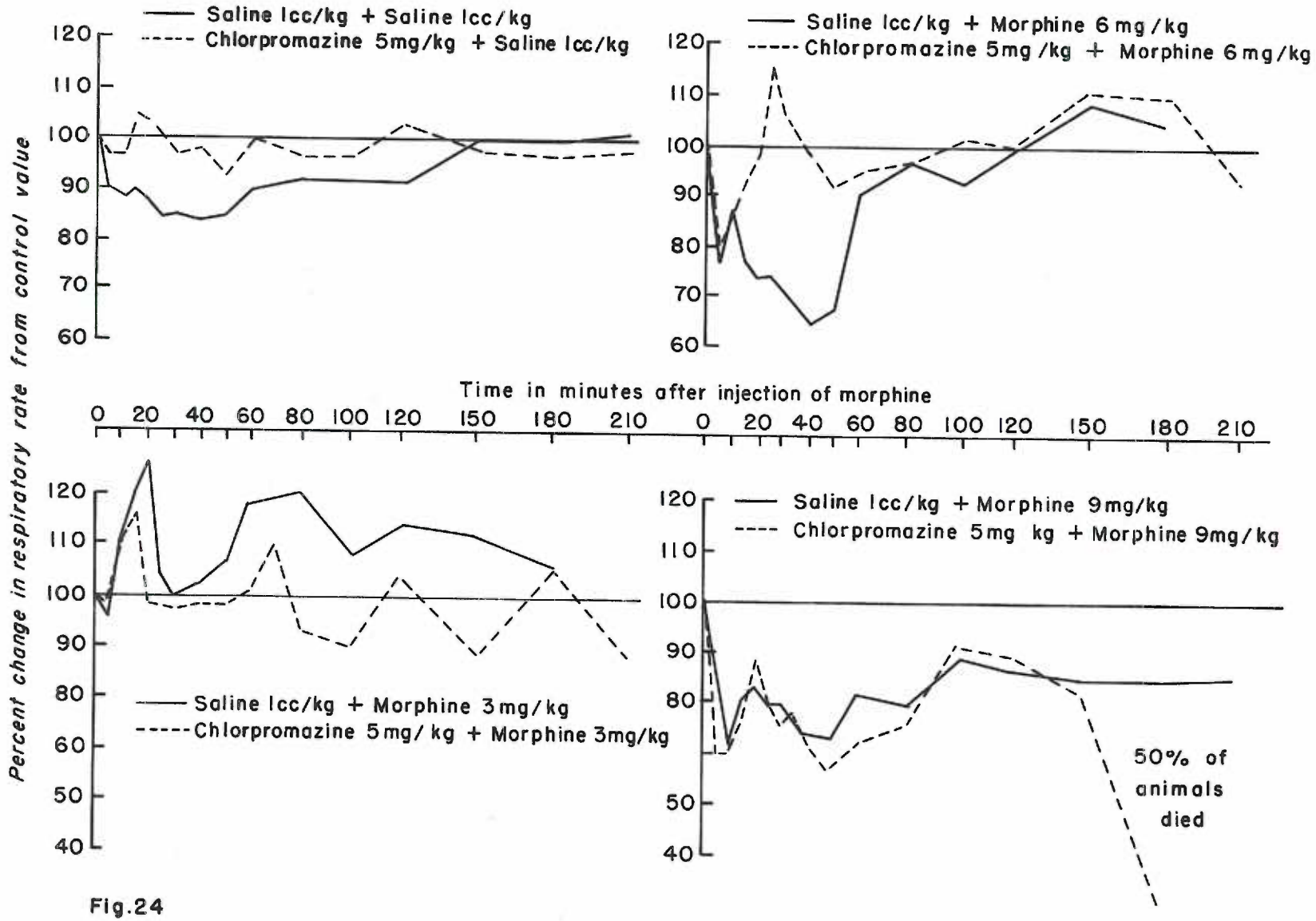

Saline 1cc/kg + Saline 1cc/kg
Chlorpromazine 5mg/kg + Saline 1cc/kg
Saline 1cc/kg + Morphine 6 mg/kg
Chlorpromazine 5mg/kg + Morphine 6 mg/kg
Time in minutes after injection of morphine
Percent change in respiratory rate from control value
Saline 1cc/kg + Morphine 3mg/kg
Chlorpromazine 5mg/kg + Morphine 3mg/kg
Saline 1cc/kg + Morphine 9mg/kg
Chlorpromazine 5mg kg + Morphine 9mg/kg
50% of animals died

Fig.24

FIGURE 25

Dose-response Curves for the First One Hour Period after Injection of Saline-SKF #5137 and Chlorpromazine-SKF #5137 Drug Combinations, Comparing their Effects upon Oxygen Consumption in the Rat.

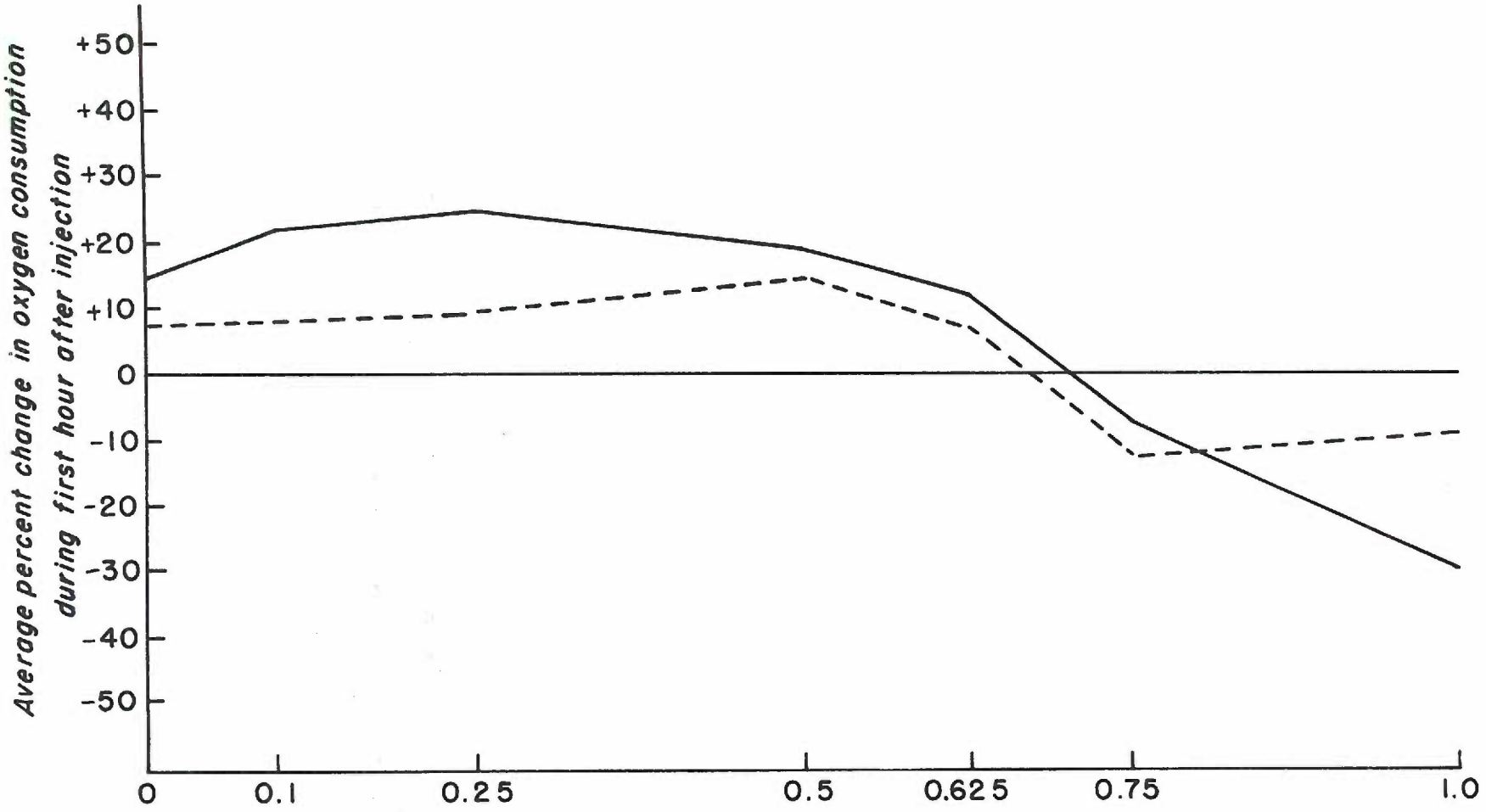

Average percent change in oxygen consumption during first hour after injection
+50
+40
+30
+20
+10
0
-10
-20
-30
-40
-50
0
0.1
0.25
0.5
0.625
0.75
1.0
Concentration of SKF # 5137 in mg/kg preceeded by: —— Saline 1cc/kg
---- Chlorpromazine 5mg/kg

Fig.25

FIGURE 26

Dose-response Curves for the First One Hour Period after Injection of Saline-Morphine Sulfate and Chlorpromazine-Morphine Sulfate Drug Combinations, Comparing their Effects upon Oxygen Consumption in the Rat.

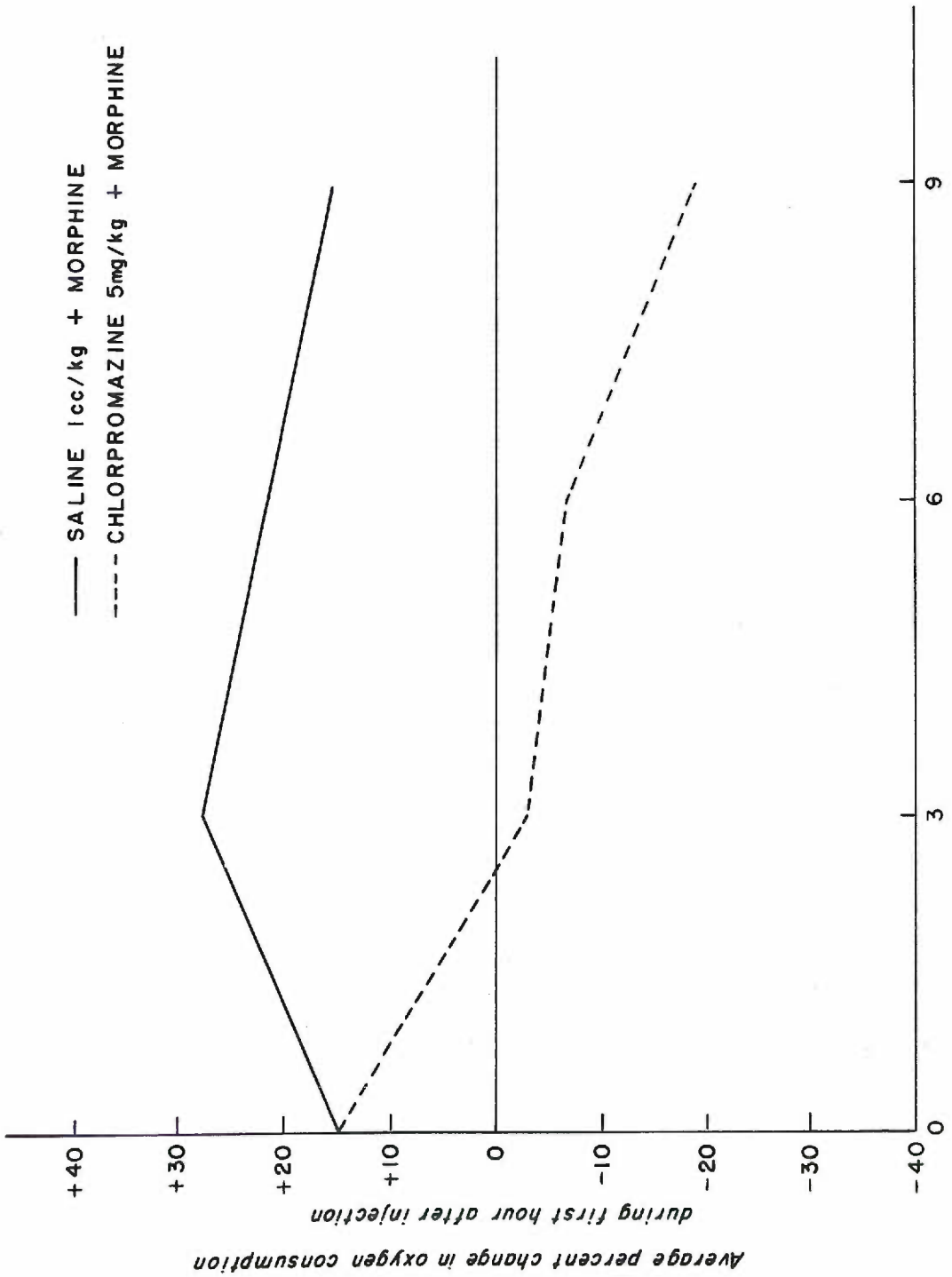

SALINE 1cc/kg + MORPHINE
CHLORPROMAZINE 5mg/kg + MORPHINE
Average percent change in oxygen consumption during first hour after injection
+40
+30
+20
+10
0
-10
-20
-30
-40
0
3
6
9
Concentrations of morphine in mg/kg

Fig.26

FIGURE 27

Dose-response Curves for the First One Hour Period after Injection of Saline-SKF #5137 and Chlorpromazine-SKF #5137 Drug Combinations, Comparing their Effects upon the Respiratory Rate in the Rat.

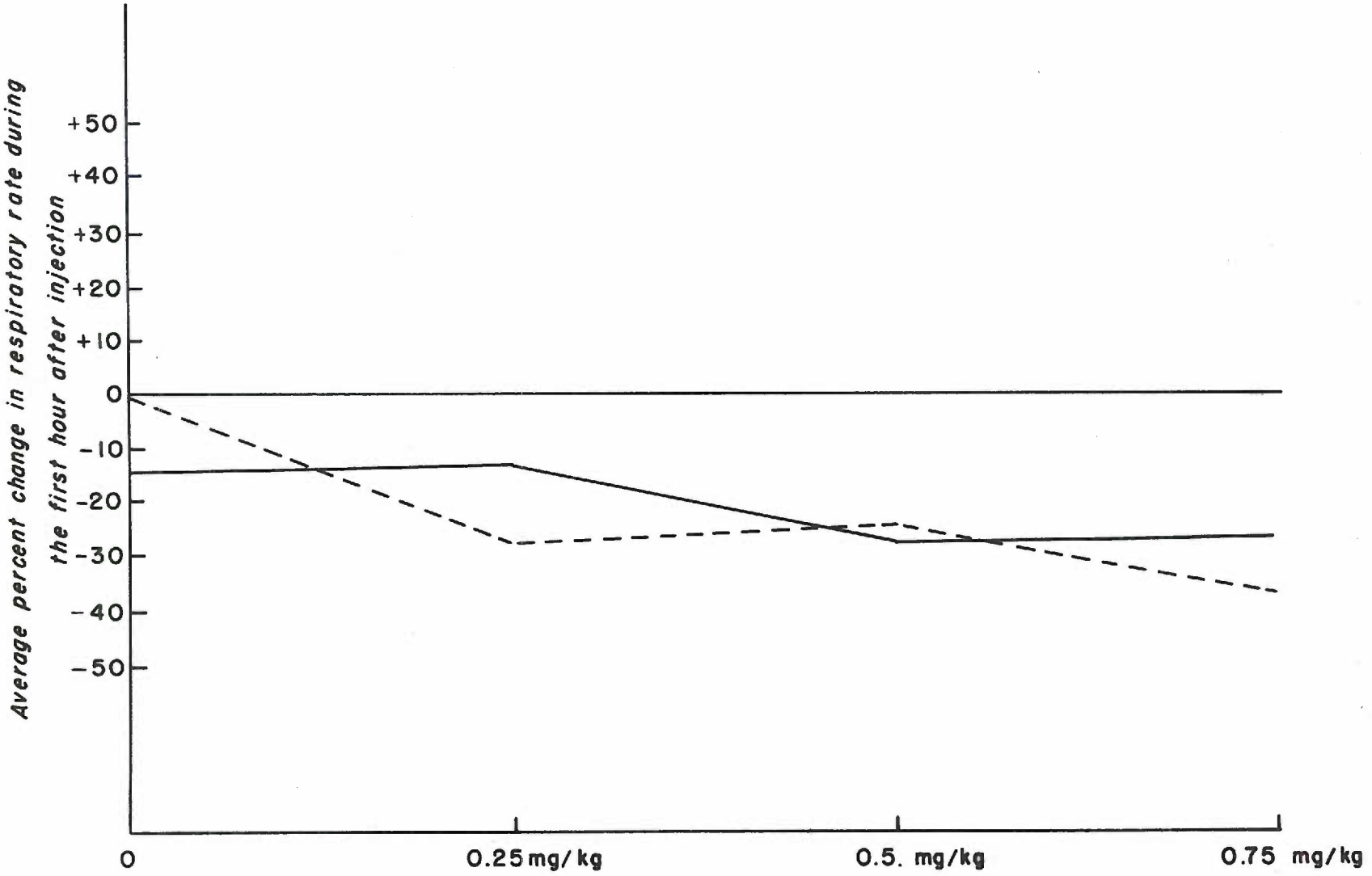


Fig.27 Concentration of SKF # 5137 preceeded by: —— Saline 1cc/kg
----- Chlorpromazine 5 mg/kg

FIGURE 28

Dose-response Curves for the First One Hour Period after Injection of Saline-Morphine Sulfate and Chlorpromazine-Morphine Sulfate Drug Combinations, Comparing their Effects upon the Respiratory Rate in the Rat.

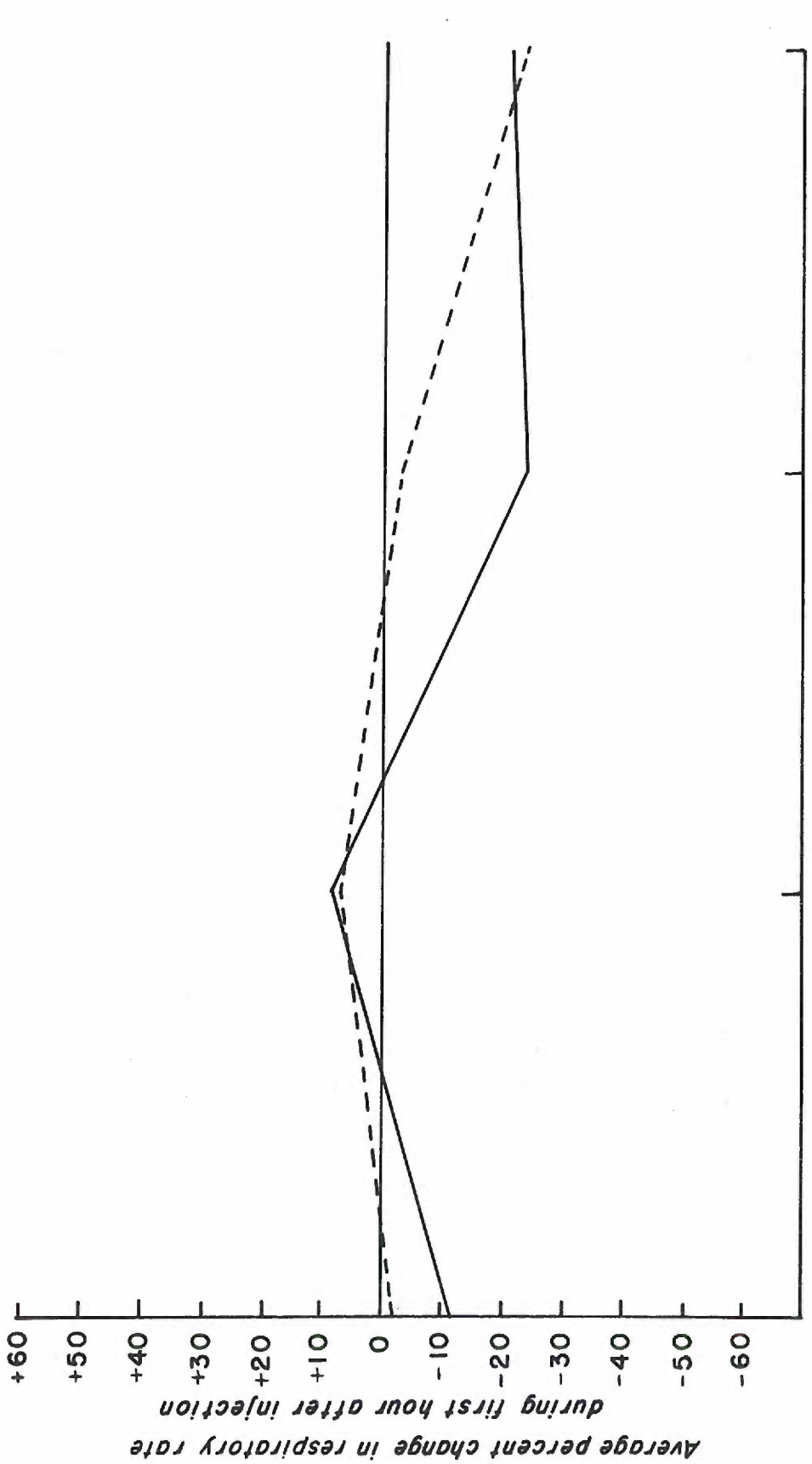

Average percent change in respiratory rate during first hour after injection
+60
+50
+40
+30
+20
+10
0
-10
-20
-30
-40
-50
-60
3 mg/kg
6 mg/kg
9mg/kg
Concentration of morphine preceeded by: —— Saline 1cc/kg
-----Chlorpromazine 5 mg/kg

Fig.28

DISCUSSION

These experiments have been undertaken to show the correlation between the activity of central nervous system drugs, such as the barbiturates, analgesics and some of the newer potentiating drugs; and the changes which they produce in oxygen consumption and respiratory rate.

Of prime importance in a study of this type are the validity and reliability of the investigative procedures. This requires the use of methods and apparatus which can be depended upon to give consistent and accurate measurements. Some of the outstanding advantages of using the Peoples' method for measurement of oxygen consumption and respiratory rate are:

1. Each animal serves as his own control. It has been pointed out earlier in this paper that the individual variation among rats, even though they are of the same strain, sex and age, is considerable. This agrees with the findings of others[49,66] working with the rat as well as with the studies of Orkin and Morales[14] who, in experiments on man, found that individuals of the same age, sex and with the same basal metabolic rates after drug administration showed highly individualized changes in oxygen consumption. In the studies reported in this paper, each animal's response to the various drug combinations administered is reported in relation to this same rat's own control or "basal" oxygen consumption value. In addition, the results obtained from any one drug group are compared to the entire control group of animals for interpretation of the findings; thus a "double control."

2. The changes caused by the drugs are recorded and measured objectively, leaving permanent records which can be evaluated and compared to those of other experiments. Thus few, if any, subjective errors enter into the results. This is of extreme importance. The majority of other methods for evaluating central nervous system depression, anesthesia or hypnosis involve techniques such as estimation of the time for the animal to go to sleep, and the duration and depth of sleep. Such subjective observations are not only difficult to interpret, but lack control observations to which the results can be compared. Furthermore, it would seem difficult enough to accurately judge onset and duration of drug action, let alone quantitate depth or degree of drug activity and thereby compare it to another drug.

3. Dose-response curves obtained through this method illustrate not only the activity of a depressant drug, and quite possibly other drugs, but in addition give an indication of the concomitant undesirable side-effects of depression of oxygen consumption and respiratory rate.

4. The reliability of the methods used in this study was proven in the statistical analysis of the control records. Successive experiments showed that the results were reproducible, the mean values and their standard deviations for both oxygen consumption and respiratory rate were within the limits of expected variation as compared to the studies of other workers (28,115). That the method is valid was also shown. The average oxygen consumption and respiratory rate values obtained were in close agreement with accepted normals and the changes in these values after drug administration were similar to those found by other workers (5,33,35). It was also shown by separate experiments that the accuracy

of the photographic method for recording changes in oxygen consumption in the rat was within the expected experimental error of the experiment.

Before starting this study, it was hypothesized that there should be a direct correlation between the depressant activity of a drug and the changes it caused in oxygen consumption. Furthermore, since the hypnotic effects of the barbiturates and the analgetic effects of morphine and similar compounds usually parallel their depressant effects, it was believed that these effects should also correlate with changes in oxygen consumption and respiratory rate. This was clearly illustrated by the results obtained when secobarbital and pentobarbital alone were studied (table 5). The dose-response curve for secobarbital (figure 10) showed a linear decrease in oxygen consumption for rats receiving increasing doses of this drug. These barbiturates have very similar hypnotic activity and, as would be expected, the values obtained for changes in oxygen consumption were also similar.

An attempt to demonstrate a dose-response curve for changes in oxygen consumption caused by analgesic drugs was also successful. The results of the experiments using a new synthetic analgesic (SKF #5137) show an extremely regular and directional decrease in oxygen consumption with increasing dosage (figure 12). Similar results were also obtained after administration of morphine (figure 13). Although both drugs caused a slight increase in oxygen consumption when given in minimal amounts, with increasing dosages the oxygen consumption was proportionately depressed.

The changes in respiratory rate after administration of the two

analgesic drugs also show a definite dose-response pattern. It was observed (figure 16) that SKF #5137 caused initial depression of respiratory rate in all dosages tested. The degree of depression was directly proportional to the dose, and all of the dose-response curves were of similar shape. Respiratory rate changes after injection of morphine showed similar results although the initial depression was not as marked (figure 17).

The foregoing results would tend to confirm the original hypothesis: changes in oxygen consumption and respiratory rate should correlate with the dosage of depressant drug administered.

A comparison of dose-response curves obtained for equal analgesic doses of SKF #5137 and morphine show the changes both in oxygen consumption and in respiratory rate to be practically identical except there is more initial depression of activity following large amounts of SKF #5137 (figures 15 and 18).

Furthermore, these same dose-response curves show that the action of SKF #5137 is of a more rapid onset and of slightly greater initial intensity but of shorter duration than that of morphine. Experiments of other workers concerning the relative analgesic activity of these two drugs have shown these same differences in activity[82,83].

The aforementioned results would also tend to confirm the hypothesis that a comparison of analgetic and hypnotic activity of drugs can be made through experiments of this type.

The experiments concerning the effect of potentiating agents upon the activity of hypnotics and analgesics as measured by this method are somewhat more difficult to interpret. When Hydergine(R) was combined

with the barbiturates in amounts within the minimal hypnotic dose range, more depression of oxygen consumption was observed than was seen after administration of the barbiturate alone (figure 10). Since the dose-response curve for the barbiturate alone showed the depression of oxygen consumption to be proportional to the dosage, which in turn parallels the degree of anesthesia, it is not unreasonable to conclude that Hydergine(R) significantly increases the hypnotic activity of barbiturates in this dose range. It was further observed that after administering toxic doses of barbiturates, pre-treatment with Hydergine(R) prevented depression of oxygen consumption to the expected value (figure 10). This appears to indicate a protective action of Hydergine(R) when administered prior to toxic doses of barbiturate. This was proven by the results of the LD_{50} studies (figure 11).

These results might be interpreted to indicate that Hydergine(R) in combination with the barbiturates would be of value in decreasing the possible side-effect due to barbiturate overdosage which might occur in anesthesia. Since the concentration of Hydergine(R) used (0.06 milligrams per kilogram) is not known to have undesirable clinical effects in itself, and has been shown to be of value as an antihypertensive, combination of this drug with the barbiturates may have value in treatment of mild hypertension.

Chlorpromazine, when combined with the analgesic drugs tested, caused little alteration in the dose-response curves for changes in respiratory rate observed after the analgesics were given alone (figures 22 and 24). The changes in dose-response curves for oxygen consumption, after pre-treatment with chlorpromazine, were much more dramatic.

When 5 milligrams per kilogram of chlorpromazine preceded administration of SKF #5137, given in dosages of 0.10 to 0.75 milligram per kilogram, the change in the dose-response curves could be explained as merely an additive effect. That is, the dose-response curve for chlorpromazine and SKF #5137 was parallel to the curve for SKF #5137 alone, but at a slightly lower value, which was approximately equal to the depression seen after administration of chlorpromazine alone (figure 21). However, when chlorpromazine preceded injection of 1.0 milligram per kilogram of SKF #5137, there was far less depression of oxygen consumption than seen with this dosage of SKF #5137 alone. These results may be analogous to those seen after injection of the larger concentration of Hydergine(R)-barbiturate combinations indicating a protective action of chlorpromazine upon the toxic effect of SKF #5137. Confirmation of this possibility through LD_{50} studies would, of course, be desirable.

Changes in oxygen consumption observed after chlorpromazine-morphine drug combinations are quite constant. When combined with morphine, chlorpromazine appears to directly potentiate the depression of oxygen consumption seen with morphine alone (figure 23). The dose-response curves for saline-morphine and chlorpromazine-morphine drug combinations are strikingly similar, except that the decrease seen after pre-treatment with chlorpromazine is far greater than can be attributed to an additive effect due to the depression caused by chlorpromazine alone. Again LD_{50} studies would be of considerable interest and would probably show whether this increased depression of oxygen consumption contributed significantly to the toxicity of morphine.

Experiments by other workers studying the analgesic activity of

SKF #5137 and morphine in rats which had received 5 milligrams per kilogram of chlorpromazine subcutaneously thirty minutes prior to injection of the analgesic drug, showed a significant increase in both degree and duration of analgesia. In the present study it was shown that chlorpromazine does not cause a significant increase in the depression of the respiratory rate or oxygen consumption when combined with SKF #5137. In fact, chlorpromazine actually caused a decrease in the expected depression seen with larger doses of SKF #5137 alone. The clinical significance of this is evident; a combination of these drugs should be of more therapeutic value than the analgesic alone. It is not unlikely that other possible side-effects of the analgesic such as nausea or emesis would also be decreased since chlorpromazine is known to have strong antiemetic action. Furthermore, the tranquilizing action of this drug would probably be beneficial to the majority of patients in need of pain relief.

With respect to the clinical use of chlorpromazine-morphine combinations, it would appear that the potentiation of the depression of oxygen consumption was of sufficient degree to contraindicate their use, at least in cases where an additional depression of oxygen consumption would be dangerous. It has been thought that in some obstetrical cases, mothers given combinations of these drugs are often delivered of babies more cyanotic and severely depressed than would be expected after administration of the analgesic or chlorpromazine alone. Our studies show that the respiratory rate is not significantly altered when chlorpromazine precedes the administration of the analgesic drug. Hence, observation only of the mother would fail to reveal the change in oxygen

consumption which quite possibly could be the cause of the cyanotic infant since both of these drugs readily cross the placental barrier. However, in the continued treatment of chronic pain, as in carcinoma, the potentiation of morphine analgesia by chlorpromazine should outweigh any reasonable depression.

SUMMARY AND CONCLUSIONS

1. Evaluation of the activity of barbiturate and analgesic drugs was determined through changes in the oxygen consumption and the respiratory rate in the rat as measured by a modification of the Peoples' method.

2. The Peoples' apparatus was shown to be both reliable and valid for measurements of this type.

3. By the methods used, it is possible to not only compare actions of different drugs through the changes they cause in respiratory rate and oxygen consumption, but, also, to evaluate the direct effect of the drug upon the animals tested.

4. Dosages of pentobarbital and secobarbital were shown to correlate directly with the depression of oxygen consumption observed. Measurement of their relative activity was shown to correlate with the known clinical effects of these drugs.

5. Dosages of a new synthetic analgesic (SKF #5137) and of morphine sulfate were shown to correlate directly with the changes in oxygen consumption and respiratory rate observed in the rat after subcutaneous injection of these drugs. The relative analgesic activity of these compounds, as well as their therapeutic index as measured by other methods, was found to correlate with the results of this study.

6. It was found that depression of oxygen consumption did not parallel depression of respiratory rate except when large dosages of analgesic drugs were administered.

7. It was felt that this method of measuring activity of central nervous

system depressant drugs, such as the barbiturates and some of the analgesics, was more objective than most other methods used. Furthermore, it offered additional information in that the major undesirable toxic effects of respiratory depression were concomitantly shown.

8. Hydergine(R) was shown to potentiate the action of secobarbital and pentobarbital in the rat when given in amounts within the minimal hypnotic dose range, although by itself it caused no change in oxygen consumption. However, when toxic doses of secobarbital were administered, pre-treatment with Hydergine(R) showed a protective action in that less than the expected decrease in oxygen consumption occurred. Further studies showed that pre-treatment with Hydergine(R) caused a 30 per cent increase in the LD_{50} for secobarbital alone.

9. Chlorpromazine, when combined with morphine, directly potentiated the depression of oxygen consumption observed after administration of morphine alone without causing significant change in the respiratory rate.

10. Chlorpromazine administered prior to SKF #5137 caused only slightly more depression of oxygen consumption than did SKF #5137 alone in dosages from 0.1 to 0.75 milligram per kilogram. This effect was thought to be merely additive since chlorpromazine alone caused a slight decrease in oxygen consumption. Chlorpromazine-SKF #5137 drug combinations caused little change in respiratory rate when compared to changes attributed to SKF #5137 alone.

11. Rats which had been pre-treated with chlorpromazine showed far less than the expected depression of oxygen consumption after receiving 1.0 milligram per kilogram of SKF #5137. The possibility that chlorpromazine also decreased the toxicity of large dosages of SKF #5137 was not explored.

12. Some clinical implications of these results were discussed. A consideration of the possible sites and mechanisms of the combined actions of these drugs was felt to be beyond the scope of this study.

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