min B_{12}) failed to counteract the growth retardation of immature mice fed massive doses of desiccated thyroid, thyroxin or iodinated casein while extracted liver residue was as potent as whole liver powder in this regard, it appears that extracted liver residue contains one or more factors other than vitamin B_{12} which are also required in increased amounts by the hyperthyroid mouse. These findings are in agreement with those previously reported for the rat.[4] When animals on purified rations are made hyperthyroid, a multiple dietary deficiency may be precipitated. Whether the first limiting factor will be a deficiency of vitamin B_{12}, of the antithyrotoxic factor(s) of extracted liver residue or of still other nutrients appears to depend on such factors as the pre-test dietary regime, the composition of the diet employed, bacterial synthesis or strain and species differences in nutritional requirements.

Summary. Whole liver powder prolonged survival and counteracted the growth retardation of immature mice fed massive doses of desiccated thyroid, thyroxin or iodinated casein. The protective factor(s) was retained in the water-insoluble fraction of liver.

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An Evaluation of Respiratory Depression by Alveolar Gas Changes During Pentothal Sodium Anesthesia.*

RICHARD AMENT,† MITZI SUSKIND, AND HERMANN RAHN.

From the Department of Physiology and Vital Economics, University of Rochester, School of Medicine and Dentistry, Rochester, N. Y.

Recent studies of intravenous anesthetic agents, notably pentothal sodium, have placed renewed emphasis on quantitative analyses of physiological changes occurring during anesthesia. The work of Gruber,[1] Gruhzit, Dox, Rowe and Dodd,[2] Beecher and Moyer,[3,4] Moyer,[5] and most recently McCann,[6] contain pneumographic tracings which illustrate the characteristic respiratory patterns produced by intravenous anesthetic agents. Beecher and Moyer[4] have correlated these qualitative studies with minute volume determinations and intermittent blood levels of oxygen and carbon dioxide. Etsten and Himwich[7,8] intensively investigated the reflex responses under pentothal anesthesia and have set forth criteria for determining the stages of pentothal anesthesia. Previous studies have employed

* Work done under contract with the Air Materiel Command, Wright Field.

† Present address: Department of Anesthesia, Bellevue Hospital, New York.

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the measurement of total ventilation volume as an index of respiratory depression or stimulation. As will be discussed below such measurements may lead to erroneous conclusions when applied quantitatively. Instead, it is here proposed to describe the degree of the effective ventilation in terms of the alveolar gas concentration only, which makes the consideration of volume measurements unnecessary. This method has the further advantage that the alveolar gas concentrations reflect the gas pressures of the arterial blood if corrected for the alveolar-arterial gradient. Suskind[9] has recently shown that during anesthesia this gradient, at least for CO_2, is nonexistent. Thus changes which occur in the pCO_2 of the alveolar air allow one to calculate changes in the arterial pH if the plasma bicarbonates are known.

Method. Alveolar air was automatically sampled by a simple device described by Rahn and Otis[10] and continuously analyzed as previously reported.[11] Readings were made at one minute intervals and the respiratory quotient calculated from the alveolar air equation (Fenn, Rahn, and Otis).[12]

Ventilation volume. The air was expired through a gas meter. Each passing of 457 cc of gas produced an electrical contact which closed the circuit of a magnetic writing pen recording on a constant-speed paper tape.

Frequency of respiration was recorded on the same tape by another pen. The slight negative mask pressure during each inspiration activated a sensitive aneroid which in turn closed the electrical circuit.

Three dogs each weighing approximately 23 kilos were fitted with individually constructed skin-tight masks. After an initial training period alveolar air could be sampled in the unanesthetized animal. Pentothal sodium was then injected into the saphenous vein and all the respiratory phenomena were recorded without interruption. After losing consciousness the dogs were placed in the lateral recumbent position with the head in hyperextension for the remainder of the experiment. Specific attention was placed on the maintenance of an adequate, unobstructed airway by position of the head.

Alveolar gas analyses were also performed in the operating pavilion on 9 patients undergoing elective operative procedures under pentothal anesthesia administered by an intermittent injection technic.

Results. 1. Single injection of 0.5 g of pentothal sodium in dogs.

Three dogs were each given a single injection of 0.5 g of pentothal over a 2-5 minute period with mask in place while continuous analyses were being carried out. Observations were continued until the animal had

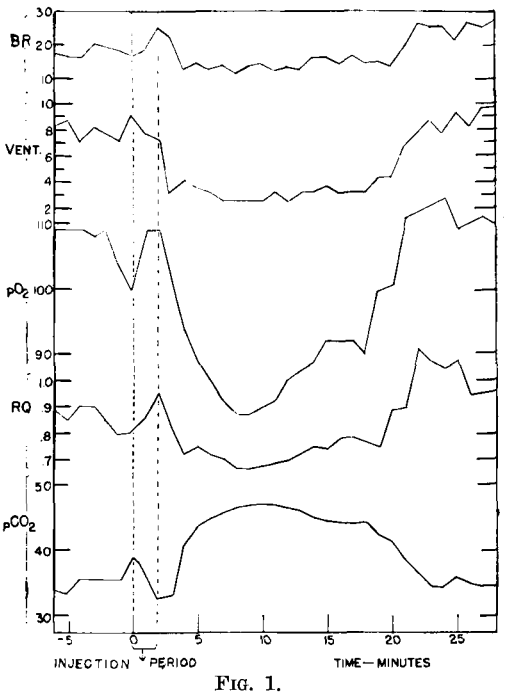

FIG. 1.

Respiratory responses of dog S to 0.5 g pentothal sodium. Injection occurred between 0 and 2 minutes. BR, breaths per minute; Vent, liter per minute expired at B.T.P.S. (body temperature, pressure and saturated): pO_2 and pCO_2 mm Hg alveolar pressure of the gases; R.Q., alveolar respiratory quotient.

[9] Suskind, M., unpublished.

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recovered to the point of restlessness, or for a period of 30 to 55 minutes. Maximum respiratory depression was reached in approximately 10 minutes and a gradual return to normal respiratory values followed. Signs of awakening and return of reflexes occurred as the respiratory picture of the animals approached control levels.

A typical record is shown in Fig. 1. It can be seen that the period of maximum depression is attained about 10 minutes after injection. This depression period is characterized by the lowest ventilation volume with its concomitant low pO_2, R.Q. and high pCO_2. The last period of recovery is associated with relative hyperventilation and an R.Q. above normal.

2. Intermittent injection of fractional doses of pentothal sodium.

Two dogs were given intermittent injections of approximately 0.25 g of pentothal sodium until a total of 1 g was administered. These experiments were carried on in precisely the same manner except that the recordings extended over a period of 140 minutes. The same general pattern of Fig. 1 and 2 repeated itself following each intermittent injection.

3. Intermittent injection of pentothal sodium in patients undergoing elective operative procedures.

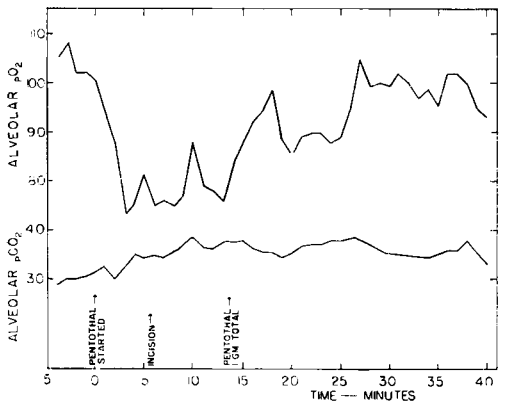

FIG. 2.

Alveolar pO_2 and pCO_2 recorded continuously in a patient before, during and after receiving pentothal sodium intermittently over a 14-minute period. Total dose 1 g. The fluctuations, particularly in the pO_2 level, arise from reflex hyperventilation.

Nine patients undergoing operative procedures were observed. Attempts to obtain control levels of alveolar air in the immediate preoperative period were not totally successful because of the apprehensive state of mind of the patient and the disturbances caused by preoperative medication. During light pentothal anesthesia operative stimulation always produced marked reflex hyperventilation which is reflected in the recorded values (Fig. 2). However, these responses are superimposed upon the respiratory pattern described above as characteristic of pentothal anesthesia in dogs.

Discussion. The question arises of a suitable quantitative criterion for the measurement of respiratory depression. If the total ventilation is used as an index one may make two errors. The first error is produced by changes in the oxygen consumption from the resting, conscious state to the anesthetized state. Thus a reduction of 30% of ventilation is without effect upon the alveolar gas concentration if likewise the oxygen consumption drops by the same amount. The second error is encountered when the breathing rate alters the dead space ventilation. For example a total ventilation of 6 liters per minute may break down into 4 liters alveolar ventilation and 2 liters dead space ventilation (10 respirations of a 200 cc dead space). The same total ventilation can be achieved by 20 respirations of the same dead space and a 2 liter alveolar ventilation. If the former produces a normal alveolar gas composition the latter situation would produce hypoxia and severe acidosis.

Thus unless these entities are known or can be measured the total ventilation may be a poor quantitative index of respiratory depression or stimulation. This is particularly so if one is interested in the effects of the altered ventilation upon the blood and tissue gas tensions.

The next best alternative is to measure *absolute alveolar ventilation* which eliminates the dead space ventilation but is dependent upon the oxygen consumption and for this reason is still unsatisfactory.[12] If respiratory depression or stimulation must be expressed

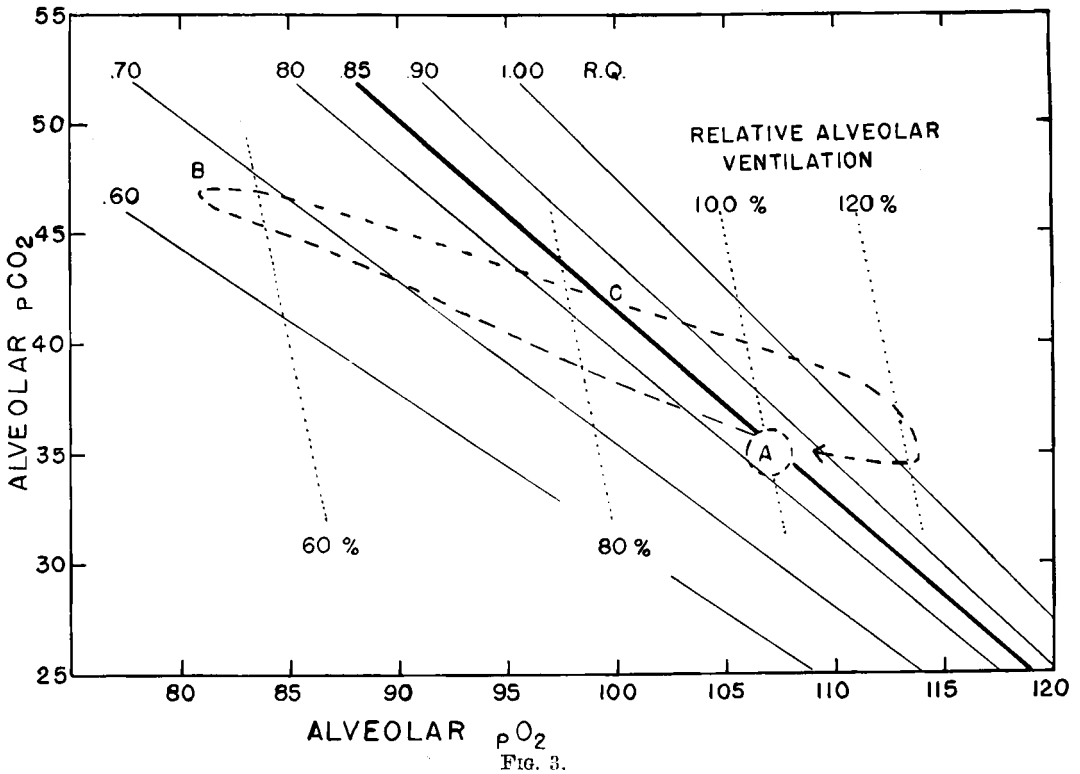

FIG. 3.

Typical alveolar pathway of dog S to single injection of pentothal sodium indicating the simultaneous alveolar pO_2, pCO_2, R.Q., and relative alveolar ventilation. Point A is the average, unanesthetized level prior to injection. Region B indicates the period of deepest depression, region B-C the period of compensation, and C-A the period of recovery characterized by high R.Q. For details see discussion.

in terms of ventilation volume it probably can be most satisfactorily done by calculating the *relative alveolar ventilation* from the alveolar gas concentration. This new derivation is discussed below.

On the other hand the difficulties in the foregoing discussion may be entirely avoided if respiratory depression or stimulation is measured in terms of the deviation it induces from the normal alveolar CO_2 and O_2. Thus the net result of the effective ventilation is expressed without involving the measurement of a ventilation volume. In Fig. 1 the various recorded values are plotted against time. In Fig. 3 the alveolar O_2 and CO_2 are plotted against each other and the whole period of anesthesia is represented by a point moving in a clockwise direction. Each point on such a graph simultaneously determines the alveolar respiratory quotient as well as the relative ventilation index. The iso-R.Q. lines are straight and originate at the inspired O_2 tension while the iso-ventilation lines have a negative slope of .209 when air is inspired.[12] The advantage of this type of graph is that it allows the simultaneous visualization of the various components of respiration.

Point A in Fig. 3 is the average alveolar air composition of the unanesthetized dog just prior to the injection of pentothal. The R.Q. is .85 and the alveolar ventilation is assumed to be normal or 100%. Following the injection, respiration is depressed. This is seen by a rapid fall in the alveolar pO_2 and R.Q. and a slower rise of the pCO_2 until point B is reached. The curve from A to B is very constant in dog and man and has been designated as the "respiratory acidosis pathway" by Rahn and Otis.[10] It is reproducible from time to time and has been observed whenever

the alveolar ventilation is reduced by dead space breathing, anesthesia or voluntary apnea.[10,13]

Point B indicates the deepest depression reached about 10 minutes after the injection and represents the greatest retention of metabolic CO_2 as indicated by the low R.Q. value. Since Suskind[9] has recently shown in this laboratory that there is no significant difference in the anesthetized dog between the arterial and alveolar pCO_2 one may estimate the changes in arterial pH accompanying the pCO_2 rise. Assuming a normal base bicarbonate level of the plasma the Henderson-Hasselbalch equation indicates a pH drop of .12 units between point A and B. Unpublished studies with standard doses of nembutal show much greater depression yielding CO_2 and O_2 values of 50 and 60 mmHg respectively.

This initial respiratory depression is followed by a period of compensation. The CO_2 stores of blood and tissues are slowly adjusted to the new alveolar CO_2 tension and as the rate of accumulation of CO_2 decreases, the R.Q. returns to its original value (Point C). However, the new steady state is not maintained. Throughout the period of compensation, the anesthetic is wearing off, and the sensitivity of the respiratory system to CO_2 is increasing. Thus we see during the final stages of recovery (region C-A) a relative hyperventilation which rids the blood and tissues of the recently acquired CO_2 stores and is manifested by an R.Q. above .85. The 3 respiratory phases described here, depression A-B, compensation B-C, and recovery C-A, all grade into one another and last approximately 10 minutes each. With complete recovery the CO_2 tension in blood, tissues and alveoli are readjusted to the original value of point A and the steady state with a normal R.Q. is observed.

At this time the dog becomes restless, and when an additional injection is given the whole cycle repeats itself. This was accomplished 3 times in succession in one dog and twice in another, the total period of anesthesia lasting over a period of 2 hours. This alveolar pathway cycle is typical not only for anesthesia but also in conscious man when the alveolar ventilation is reduced by various artificial means.

Should it be desirable to express the drug action in terms of a ventilation unit this can be done by assuming that the ventilation is normal (100%) only when it maintains an alveolar concentration of point A, the control value during the conscious state. This unit is called the *relative alveolar ventilation*, which has the advantage that it makes ventilation independent of changes in metabolic rate and thus offers a better physiological comparison for fluctuating metabolic rates as encountered in anesthetic studies.

The *relative alveolar ventilation* of point A is equal to $\frac{k.\ R.Q.\ 100}{alv.\ pCO_2}$ where k is a constant yielding a ventilation of 100% when the values of point A are substituted. (This equation is derived from the alveolar ventilation equation, Fenn[12]). The constant for our example is 41.2. When lines of equal "relative alveolar ventilation" are plotted they form straight lines as indicated in Fig. 3.

It can be seen that the deepest respiratory depression is obtained when the alveolar ventilation is 58% of the ventilation required to maintain the alveolar air composition at point A. Recovery, on the other hand, is associated with a relative hyperventilation reaching a maximal value just over 120%. Fig. 1, however, indicates a much larger relative change in the *total ventilation*, (from 100% to 38%) and is therefore misleading unless the marked drop in oxygen consumption and breathing rate is properly evaluated.

It is obvious from the data that respiratory stimulation or depression is a continuously changing state. This makes a quantitative comparison difficult unless some particular phase of the anesthesia cycle is employed as reference. Since *total ventilation* as an index may be quite misleading it is here suggested that the deviation from the normal alveolar pCO_2, R.Q. and/or *relative alveolar ventilation* be used as a quantitative and physiologically comparable approach to studies of res-

[13] Otis, A. B., Rahn, H., and Fenn, W. O., *Am. J. Physiol.*, 1948, **152**, 674.

piratory depression or stimulation until continuous arterial blood gas analyses become more practical.

Summary. 1. The alveolar O_2, CO_2 tension and R.Q., minute volumes and respiratory rates have been recorded continuously in animals and man under pentothal sodium anesthesia administered by single and intermittent injection.

2. When the alveolar gas concentrations are plotted with the aid of a CO_2-O_2 diagram a typical anesthesia pattern can be shown indicating the regions of depression, compensation and recovery.

3. With intermittent injections of pentothal sodium this typical pattern is repeated.

4. The theoretical significance of this respiratory pattern and its utilization for analysis of quantitative changes in respiration in the study of anesthetic agents is discussed.

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A Technic for the Recovery of Viruses from Crude Feces.

HERBERT R. MORGAN.*

From the Department of Epidemiology, School of Public Health, University of Michigan, Ann Arbor, Mich.

During the course of studies on the isolation of psittacosis and other viruses from feces, an attempt was made to develop a method, which would allow for direct inoculation of a crude fecal suspension into eggs, for the recovery of a variety of viruses by the use of anti-bacterial agents. Several different test agents were used to determine the applicability of the technic to known viruses in the hope that it might prove useful in the isolation of as yet unknown viruses from the gastrointestinal tract of man and animals. Since penicillin and the sulfonamides are known to inhibit certain viruses of the psittacosis-lymphogranuloma venereum group, they were eliminated from consideration.[1,2] Streptomycin has been shown to have no effect on them[1] nor on several other virus agents[3-6] so that it was useful for study. However, streptomycin alone is not efficacious against all gram positive organisms[7,8] so that it was necessary to find another agent to aid in the suppresion of the gram positive organisms in the feces.

The anti-bacterial agents tested were streptomycin, tyrothrycin in alcoholic solution, tyrothrycin in propylene glycol solution and crystal violet.

Since specimens are frequently submitted for virus isolation studies from patients receiving sulfonamide and/or penicillin therapy, it was of interest to determine whether psittacosis virus strains, known to be susceptible to their action, could be recovered from virus-fecal suspensions containing sulfadiazine or penicillin by the use of antagonistic or inactivating compounds such as p-aminobenzoic acid or cysteine hydrochloride.

The yolk sac route was chosen for the

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