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# The effects of pentobarbitone sodium on the carbon dioxide response and production, and oxygen consumption of the rabbit

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The effect of intravenous doses of pentobarbitone sodium on the ventilatory response to $CO_2$, $CO_2$ production and $O_2$ consumption were investigated in the rabbit. Pentobarbitone sodium depressed the ventilatory response to $CO_2$, but the time course of this effect showed a biphasic character whereas depression of unchallenged minute volume did not. $O_2$ consumption and $CO_2$ production were reduced by pentobarbitone, but although the degree and duration of the depression increased with increasing dose, the values returned to control values before depression of respiratory minute volume and response to $CO_2$. The possibility that these results may explain the lack of progressive depression of respiratory minute volume with increasing dose of barbiturate, previously reported by the authors, is discussed.

It has been demonstrated, in the rabbit, that increasing doses of pentobarbitone, amylobarbitone and barbitone sodium do not cause progressive depression of respiratory minute volume (Hunter, Pleuvry & Rees, 1968). There is a plateau in the dose response curves so that, for example, between 7·5 and 30 mg/kg pentobarbitone there is no significant increase in the depression of minute volume.

The present study describes further investigation of this phenomenon, by means of a detailed examination of the effects of pentobarbitone sodium on the rabbits respiratory response to carbon dioxide ($CO_2$). Since such measurements can be modified by changes in $CO_2$ production and $O_2$ consumption, these two parameters were also measured.

## METHODS

Groups of five Flemish rabbits, 2 to 4 kg, were used. The three parameters measured in this study were examined independently.

*Responses to inhaled gases.* $CO_2$ gas mixtures were fed into the Gaddum respirometer circuit (Gaddum, 1941) avoiding positive pressure. The mixtures were 4·5, 7·2, and 14·3% $CO_2$ in oxygen. Respiratory minute volume was recorded until no further displacement of respiration was seen over a 30 s period (usually 2–3 min exposure).

After stable control values had been obtained, pentobarbitone sodium was administered into the lateral ear vein of the rabbit. Recordings of minute volume with the rabbit breathing air and then challenged with $CO_2$ were taken at 10 min intervals, the first being 5 min after injection, and continuing until responses had regained control values.

The effects of inhalation of 100% $O_2$ on the respiratory effects of 30 mg/kg of pentobarbitone sodium were also investigated.

*$CO_2$ production.* Minute volume was measured in the usual way using the Gaddum respirometer. After the rabbit had been breathing through the apparatus for about half a minute, the outlet valve was connected to an evacuated rubber bag and a 90 s sample of expired air collected. The gas filled bag was attached to the inlet of a $CO_2$ analyser (Hartman & Braun A.4 URAS 4) and the % $CO_2$ read from the scale. $CO_2$ production in ml/min $kg^{-1}$ was calculated from the minute volume, $CO_2$ percentage in the expired air and the weight of the rabbit.

*$O_2$ consumption.* A sample of room air was pumped into an $O_2$ analyser (Servomex Type O A 101 Mk.2.) to obtain the percentage of $O_2$ in the inspired air. The expired air, collected as above, was then passed through the apparatus and the two readings, when subtracted from each other, gave the $O_2$ consumption expressed as a percentage of the total gas expired. Thus $O_2$ consumption in ml/min $kg^{-1}$ can be calculated from the minute volume and weight of the rabbit.

To obtain stable control readings, it was necessary to accustom the animals to the procedure and to maintain a relatively constant sound level, e.g. a radio playing light music, during experiments.

After consistent control readings had been obtained for both $O_2$ consumption and $CO_2$ production, pentobarbitone was injected intravenously. Measurements were taken at 5 min intervals for the first 30 min and then at 10 min intervals until control values were regained. One group of five animals was injected with 1 ml of sterile saline and $CO_2$ production changes measured.

Percentage changes were generally used to express results in this study as these showed less variability than absolute values, however, some raw data have been included so that comparisons can be made with the findings of other workers.

## RESULTS

### *Effect of inhaled $CO_2$ in pentobarbitone-treated animals*

The effect of $CO_2$ inhalation in control rabbits has been fully described by Pleuvry & Rees (1969). Minute volume and tidal volume increase with increasing concentrations of $CO_2$, but respiratory rate is unchanged or falls slightly.

The time course of the effect of 30 mg/kg of pentobarbitone on resting minute volume and $CO_2$ challenged minute volume is shown in Fig. 1. The results are expressed as % change from pre-injection values. Although the increase in respiratory minute volume depends upon the concentration of $CO_2$ inspired, the % change from control $CO_2$ challenged minute volume in the presence of pentobarbitone is similar for all three concentrations of $CO_2$ and thus the values obtained for Fig. 1 are the bulked results for all three concentrations of $CO_2$.

The time course of the changes induced in the $CO_2$ challenged minute volume by pentobarbitone sodium is significantly different from the changes in unchallenged minute volume.

Fig. 2 shows the results expressed as % change in respiratory minute volume induced by $CO_2$ compared with the immediately preceding unchallenged minute volume. In the absence of drug this is a constant measure for a given concentration of $CO_2$, but in the presence of various doses of pentobarbitone the patterns shown in Fig. 2 are obtained. Although the results illustrated were obtained with 4·5% $CO_2$, similar patterns were obtained with 7·2 and 14·3% $CO_2$, except that the actual % changes were higher with greater concentrations of $CO_2$.

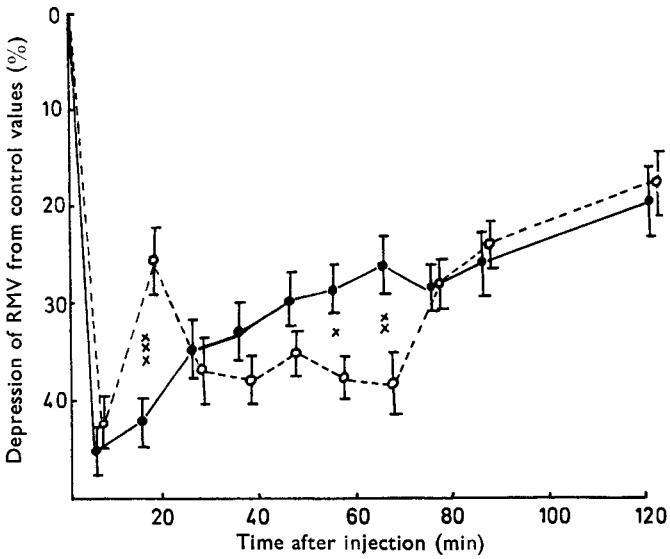


FIG. 1. The effect of 30 mg/kg pentobarbitone sodium on resting and $CO_2$ challenged minute volume in the rabbit. The closed circles represent the % change in unchallenged minute volume from pre-injection controls and the open circles are the % change of $CO_2$ challenged minute volume from pre-injection controls. Although the control values of $CO_2$ challenged minute volume increase with increasing concentration of $CO_2$, the % change from these values in the presence of pentobarbitone are similar and thus bulked results from all 3 concentrations of $CO_2$ are shown in the figure.

The means and standard errors of both groups were calculated on the basis of 15 experiments and significant differences between them are indicated by crosses; 1 cross $P$ 0·02, 2 crosses $P$ 0·01, 3 crosses $P$ 0·001.

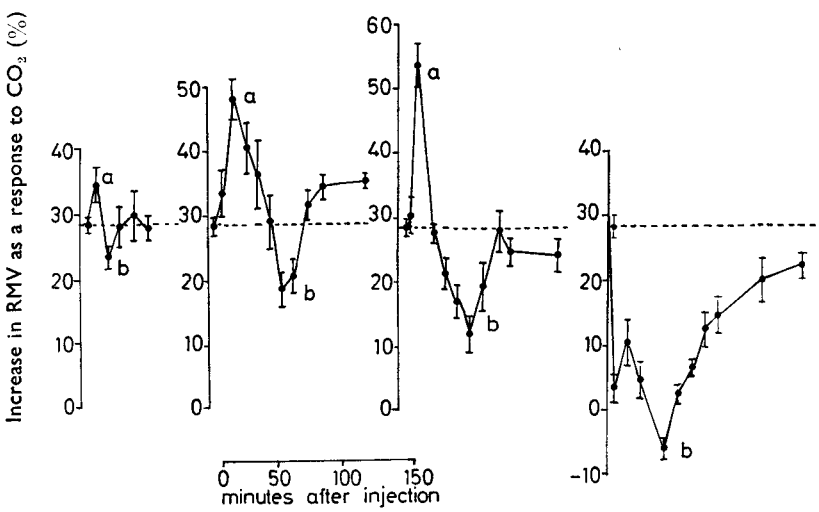


FIG. 2. The effect of various doses of pentobarbitone sodium on the minute volume response of the rabbit to 4·5% $CO_2$, measured as the percentage increase in minute volume caused by $CO_2$ inhalation over the immediately preceding unchallenged minute volume. All means and standard errors were calculated from five experiments and the first point on each graph indicates the mean preinjection response to $CO_2$.

The lettering a and b indicate the highest and lowest response to $CO_2$ measured after the various doses of pentobarbitone. The doses used were (from left to right) 7·5, 15, 30, 45 mg/kg.

Table 1. *The effect of pentobarbitone sodium on the $O_2$ consumption and $CO_2$ production of the rabbit.*

| Time after injection (min) | Percentage change from control values (mean ± standard errors) $CO_2$ production | $O_2$ consumption |
|---|---|---|
| 1. *Saline controls* | | |
| 5 | −0·2% (±1·5) | −0·9% (±2·5) |
| 10 | −3·8% (±2·1) | −0·2% (±2·8) |
| 15 | −2·2% (±2·6) | −1·1% (±3·5) |
| 20 | −4·8% (±2·6) | −2·7% (±2·9) |
| 25 | −2·3% (±2·3) | −1·0% (±2·3) |
| 35 | −1·8% (±2·6) | −1·2% (±4·2) |
| 45 | −3·0% (±2·1) | +3·1% (±4·1) |
| 55 | −3·2% (±2·9) | +2·4% (±8·6) |
| 65 | −0·6% (±0·4) | +8·8% (±5·6) |
| 2. 3·75 *mg/kg pentobarbitone sodium* | | |
| 5 | −11·8% (±2·2) | −13·7% (±1·5) |
| 10 | +2·9% (±2·2) | −0·7% (±3·1) |
| 15 | +1·7% (±1·7) | +2·7% (±6·4) |
| 20 | −1·4% (±3·5) | +6·2% (±2·5) |
| 25 | +1·0% (±3·5) | +1·2% (±3·2) |
| 3. 7·5 *mg/kg pentobarbitone sodium* | | |
| 5 | −14·9% (±3·3) | −18·7% (±2·4) |
| 10 | −12·6% (±3·6) | −13·3% (±2·5) |
| 15 | +1·6% (±3·0) | +3·8% (±4·5) |
| 20 | +4·1% (±3·0) | +1·9% (±2·1) |
| 25 | +2·4% (±4·8) | −2·9% (±2·2) |
| 35 | +12·1% (±7·0) | −3·9% (±3·8) |
| 45 | +3·3% (±3·2) | −3·0% (±2·8) |
| 55 | +4·7% (±5·2) | +0·9% (±2·0) |
| 4. 15 *mg/kg pentobarbitone sodium* | | |
| 5 | −29·3% (±2·5) | −21·7% (±2·5) |
| 10 | −23·2% (±4·9) | −20·2% (±3·2) |
| 15 | −28·5% (±5·2) | −13·5% (±3·7)* |
| 20 | −22·2% (±5·1) | −9·0% (±3·9)* |
| 25 | −11·1% (±6·6) | −4·1% (±5·5) |
| 35 | −13·1% (±6·9) | −6·1% (±5·5) |
| 45 | −6·5% (±2·6) | −3·4% (±6·4) |
| 55 | +2·2% (±2·6) | +3·5% (±9·0) |
| 65 | +0·9% (±5·6) | +7·8% (±5·0) |
| 75 | −1·4% (±6·5) | +8·7% (±5·7) |
| 85 | −0·8% (±5·2) | +8·0% (±6·4) |
| 120 | +0·5% (±3·2) | +11·9% (±7·2) |
| 5. 30 *mg/kg pentobarbitone sodium* | | |
| 5 | −34·8% (±3·2) | −30·5% (±0·3) |
| 10 | −32·5% (±3·8) | −22·2% (±1·7)* |
| 15 | −34·7% (±3·8) | −18·0% (±2·1)* |
| 20 | −28·9% (±4·2) | −15·2% (±5·3)* |
| 25 | −24·7% (±3·9) | −11·5% (±1·8)* |
| 35 | −24·9% (±1·7) | −8·6% (±1·5)* |
| 45 | −9·0% (±4·4) | −9·0% (±2·4) |
| 55 | −3·7% (±6·6) | −6·5% (±5·2) |
| 65 | −8·4% (±7·8) | −8·9% (±2·8) |
| 75 | −1·6% (±7·3) | −7·6% (±3·5) |
| 85 | −1·9% (±6·8) | −4·9% (±4·5) |
| 120 | −4·5% (±2·7) | −4·7% (±2·2) |

| Time after injection (min) | Percentage change from control values (mean ± standard errors) $CO_2$ production | $O_2$ consumption |
|---|---|---|
| 6. 45 *mg/kg pentobarbitone sodium* | | |
| 5 | −44·6% (±2·6) | −44·0% (±12·4) |
| 10 | −36·2% (±2·1) | −40·3% (±9·8) |
| 15 | −31·9% (±6·4) | −40·5% (±3·8) |
| 20 | −32·2% (±5·8) | −34·0% (±4·4) |
| 25 | −31·4% (±2·9) | −33·2% (±9·7) |
| 35 | −30·5% (±4·9) | −38·1% (±5·2) |
| 45 | −29·5% (±5·3) | −38·8% (±4·4) |
| 55 | −22·3% (±9·7) | −33·9% (±6·8) |
| 65 | −19·3% (±10·4) | −32·5% (±5·5) |
| 75 | −11·6% (±11·8) | −33·7% (±5·1) |
| 85 | +2·2% (±11·8) | −29·7% (±6·9) |
| 120 | −12·5% (+9·4) | −29·8% (±7·8) |

Note: Except for 45 mg/kg of pentobarbitone sodium, all means and standard errors were calculated on the basis of 5 experiments. With 45 mg/kg, the $CO_2$ production was calculated from 5 experiments, but the $O_2$ consumption was calculated from 3 experiments.
* Significant differences ($P < 0·05$) between the % change in $O_2$ consumption and $CO_2$ production.

The points a and b on Fig. 2 represent the highest and lowest % increase in minute volume obtained in the presence of pentobarbitone. Fig. 3 is obtained when the points a and b are plotted for each concentration of $CO_2$ in the inspired air. The % increase in minute volume for each concentration of $CO_2$ in the absence of drug is included for comparison. Fig. 3 illustrates that the highest % change increases and the lowest percentage change decreases in value with increasing dose of pentobarbitone.

Although the actual value of the highest % increase in minute volume increases with dose, the duration of its occurrence above control levels decreases until there is no initial increase in minute volume with the LD 50 dose of 45 mg/kg of pentobarbitone sodium.

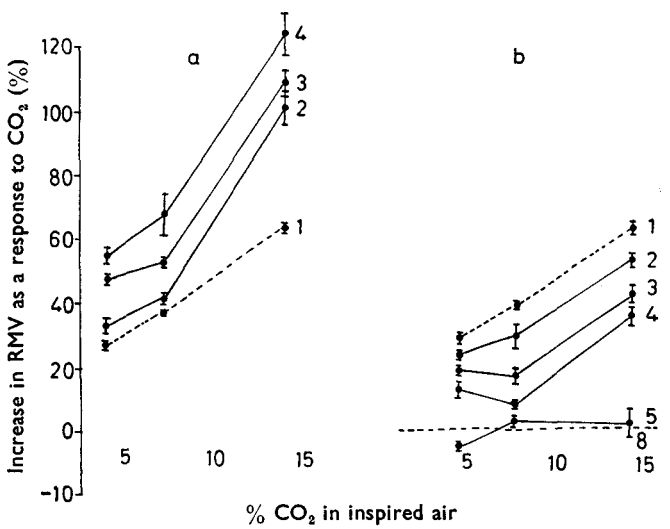


FIG. 3. The effect of increasing concentrations of $CO_2$ on the highest and lowest responses to $CO_2$ obtained after injection of pentobarbitone sodium. The highest responses to $CO_2$ obtained with 4·5% $CO_2$ are labelled a on Fig. 2. and the lowest are labelled b. 1. Dotted line. Control values in the absence of drug. 2, 7·5 mg/kg; 3, 15 mg/kg; 4, 30 mg/kg; 5, 45 mg/kg of pentobarbitone sodium.

Inhalation of 100% $O_2$ had no effect on control minute volume or on minute volume depressed by the administration of 30 mg/kg of pentobarbitone sodium.

*The effect of pentobarbitone on $CO_2$ production and $O_2$ consumption*

All doses of pentobarbitone sodium examined (3·75 to 45 mg/kg) depressed both $CO_2$ production and $O_2$ consumption of the whole rabbit. The maximum depression increases with increasing dose. Table 1 shows the results obtained.

Table 2. *Selected raw data from which Figs* 1, 2 *and* 3 *and Table* 1 *were constructed*

A. $CO_2$ challenge

| | | Respiratory minute volume ml/min ± s.d. | | |
|---|---|---|---|---|
| | Pentobarbitone | resting | $CO_2$ challenged | |
| 4·5% $CO_2$ | Control | 829±189 | 1051±218 | (23) |
| | 5 min after 7·5 mg/kg | 554±174 | 771±244 | (5) |
| | 25 min ,, | 734±250 | 920±275 | (5) |
| | 15 min after 15 mg/kg | 420±92 | 715±81 | (5) |
| | 55 mins ,, | 573±48 | 678±47 | (5) |
| | 15 min after 30 mg/kg | 489±59 | 752±97 | (5) |
| | 55 mins ,, | 622±96 | 694±74 | (5) |
| | 15 mins after 45 mg/kg | 373±11·5 | 411±11 | (3) |
| | 55 mins ,, | 447±11 | 457±5·7 | (3) |
| 7·2% $CO_2$ | Control | 769±148 | 1045±163 | (25) |
| | 5 min after 7·5 mg/kg | 589±39 | 796±103 | (5) |
| | 25 min ,, | 572±99 | 844±146 | (5) |
| | 15 min after 15 mg/kg | 609±104 | 945±202 | (5) |
| | 55 min ,, | 672±136 | 806±247 | (5) |
| | 15 min after 30 mg/kg | 469±65 | 779±164 | (5) |
| | 55 min ,, | 529±103 | 602±161 | (5) |
| | 15 min after 45 mg/kg | 398±131 | 462±147 | (5) |
| | 55 min ,, | 506±220 | 526±260 | (5) |
| 14·3% $CO_2$ | Control | 870±106 | 1380±127 | (23) |
| | 5 min after 7·5 mg/kg | 619±38 | 1242±46 | (5) |
| | 25 min ,, | 726±102 | 1121±146 | (5) |
| | 15 mins after 15 mg/kg | 636±105 | 1242±205 | (5) |
| | 55 min ,, | 725±139 | 1042±246 | (5) |
| | 15 min after 30 mg/kg | 563±65 | 1169±182 | (5) |
| | 55 min ,, | 651±103 | 870±74 | (5) |
| | 15 min after 45 mg/kg | 343±85 | 426±139 | (3) |
| | 55 min ,, | 392±56 | 400±78 | (3) |

B. $O_2$ consumption of the rabbit

| | ml/min/kg ± s.d. | |
|---|---|---|
| Control | 6·6 (±0·9) | (25) |
| Minimum value after | | |
| 3·75 mg/kg | 5·4 (±0·7) | (5) |
| 7·5 mg/kg | 5·4 (±0·5) | (5) |
| 15 mg/kg | 4·7 (±0·4) | (5) |
| 30 mg/kg | 4·3 (±0·3) | (5) |
| 45 mg/kg | 4·0 (±1·7) | (3) |

C. $CO_2$ production of the rabbit

| | ml/min/kg ± s.d. | |
|---|---|---|
| Control | 5·5 (±0·7) | (25) |
| Minimum value after | | |
| 3·75 mg/kg | 4·6 (±0·6) | (5) |
| 7·5 mg/kg | 4·5 (±1·0) | (5) |
| 15 mg/kg | 3·8 (±0·4) | (5) |
| 30 mg/kg | 3·5 (±1·1) | (5) |
| 45 mg/kg | 2·9 (±0·4) | (5) |

Note: the numbers in brackets at the end of each line indicate the number of animals in the group from which the means and standard deviations (s.d.) were calculated.

There is no significant difference between the maximum % depression of $CO_2$ production and $O_2$ consumption and the duration of the depression, which also increases with increasing dose of pentobarbitone, is approximately the same. However, there is a significant difference between depression of $O_2$ consumption and depression of $CO_2$ production, 15 to 20 min after 15 mg/kg pentobarbitone and 10 to 35 min after 30 mg/kg, $CO_2$ production is depressed more than $O_2$ consumption indicating a fall in the respiratory quotient. A similar observation has been reported by Ament, Suskin & Rahn (1949) for thiopentone.

Some of the raw data, from which the preceding figures and tables were constructed are shown in Table 2. The standard deviations of the means are included to show the wide scatter values between rabbits, particularly with minute volume measurements.

## DISCUSSION

The respiratory effects of pentobarbitone sodium described are in marked contrast to the respiratory effects of morphine. Depression of respiratory minute volume increases progressively with increasing dose of morphine (Hunter, Pleuvry & Rees, 1968); the time course of the action of morphine on $CO_2$ challenged minute volume shows no biphasic character (Pleuvry & Rees, 1969) and unpublished work of the authors' indicates that although morphine does initially lower $CO_2$ production, its effects are very transient and control values are regained 10 min after injection.

We have demonstrated that pentobarbitone can cause a reduction in minute volume by two mechanisms.

(*a*) Depression of metabolism. Less $CO_2$ is produced per minute and thus a lower alveolar ventilation is required to maintain the $P_{CO_2}$ of the blood at normal levels.

(*b*) Depression of the response of the respiratory reticular formation to $CO_2$ (Fig. 1). Changes in $CO_2$ response will mean that less increase in ventilation will result from rises in $P_{CO_2}$ of the blood. Since ventilation is normally driven by $P_{CO_2}$, ventilation will fall and $P_{CO_2}$ rise.

It is not possible to point to the exact mechanism by which a drug may depress $CO_2$ response as this may occur at a variety of sites described fully by Lambertsen(1964). Some of these sites are unlikely to apply to the barbiturates as it has been shown that the barbiturates only raise the threshold for stimulation of skeletal muscle in very high doses (Thesleff, 1956); there is no evidence that the barbiturates possess any local anaesthetic activity in central depressant doses and Dripps & Dumke (1943) have shown that they have little effect on peripheral chemoreceptors. In view of this last point it might be suggested that, as minute volume and respiratory rate are depressed by the barbiturates, the ensuing hypoxic stimulation of the aortic and carotid body chemoreceptors might tend to flatten the depression of minute volume/log dose graph and contribute to the plateau. However, it has been demonstrated that 100% $O_2$ does not affect the minute volume depressed by 30 mg/kg of pentobarbitone indicating that hypoxic drive is absent.

The principal alternative sites of action of pentobarbitone on $CO_2$ response are the chemosensitive areas of the respiratory reticular formation or generalized depression of the neurons within the respiratory reticular formation.

It has been shown that the barbiturates depress the reticular formation as a whole (French, Verzeono & Magoun, 1953) and both Harris & Borison (1954) and Robson, Houseley & Solis-Quiroga (1963) suggested that depression of conduction through the

respiratory reticular formation was important in the respiratory depressant effects of the barbiturates. Thus although depression of central chemoreceptors cannot be ruled out, it appears that barbiturates cause a more generalized depression.

Both depression of metabolism and depression of $CO_2$ response increase progressively with dose of pentobarbitone. However, depression of minute volume does not increase progressively with dose, there being a plateau in the log dose response curve where increasing the dose of pentobarbitone causes no further depression of minute volume.

It is possible that this anomaly, together with the curious pattern of changes in minute volume challenged with $CO_2$ after pentobarbitone, is related to the interactions of the two mechanisms by which pentobarbitone causes a fall in minute volume, i.e. depression of metabolism and general depression of the respiratory reticular formation.

Jennett (1968) demonstrated, in man, that, in the presence of metabolic depression, the alveolar ventilation "metabolic hyperbole" when breathing $CO_2$ moved to the left. Thus there might be a similar result in terms of alveolar ventilation for a given inspired $CO_2$ concentration when metabolism alone is depressed and when respiration is depressed. It was pointed out that it was impossible to distinguish the two unless alveolar or arterial $P_{CO_2}$ were measured during the $CO_2$ response readings. In the rabbit this is very difficult in practice.

However, the depression in $CO_2$ response obtained after $CO_2$ production and $O_2$ consumption have returned to normal is probably indicative of true respiratory depression.

Before this, the $CO_2$ response obtained will be reflecting the effects of both depression of metabolism and depression of the respiratory reticular formation. Table 1 shows that the maximum effect of pentobarbitone on $CO_2$ production and $O_2$ consumption occurs almost immediately after injection and then returns rapidly to normal values, the actual duration of the return to normal being dependent upon the dose of pentobarbitone. During this time there is a reduction in the sensitivity of the respiratory reticular formation to $CO_2$. Bearing in mind Jennett's findings described above, the possible effects of these two factors on $CO_2$ challenged minute volume will be considered.

Depression of metabolism and depression of the respiratory reticular formation will cause a fall in challenged minute volume, but as the metabolism returns to normal the challenged minute volume will tend to rise. However this will be partially overcome by the depression of $CO_2$ sensitivity, the greater the depression of $CO_2$ sensitivity the shorter the duration of the rise. A mechanism such as described above may account for the shape of the time course of depression of challenged minute volume shown in Fig. 1.

It was suggested earlier that the respiratory depressant activity of pentobarbitone sodium was more likely to be due to direct depression of the respiratory reticular formation as a whole rather than a specific effect on receptor systems. In view of this it seems reasonable to suppose that the actions of all factors centrally modifying respiration will be depressed including changes in metabolism. The graph constructed by Jennett (1968) showed that even in respiratory depression of undefined aetiology the alveolar ventilation "metabolic hyperboles obtained breathing air at different metabolic rates tend to come closer together as respiratory depression increases".

The maximum depression of resting minute volume obtained with pentobarbitone,

in the rabbit, occurs during the first 15 to 20 min after injection when depression of metabolism and presumably its effects on minue volume are at a maximum. As the dose of pentobarbitone increases, the depression of the respiratory reticular formation increases faster than the depression of metabolism. Thus the actual effect of metabolism depression on minute volume will tend to be reduced as the effects of depression of the respiratory reticular formation on minute volume increase. It is possible that this interaction could cause a flattening of the log dose/depression of minute volume curve obtained with pentobarbitone sodium in the rabbit.

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