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THE EFFECTS OF MORPHINE ON pH HOMOEOSTASIS IN THE UNANAESTHETIZED RABBIT

BY

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A routine method has been sought for examining the respiratory depressant effects of narcotic analgesics in the unanaesthetized rabbit. Since respiratory minute volume and the tension of carbon dioxide in the blood (P_{CO_2}) are closely related (Lambertsen, 1960), estimations have been made of P_{CO_2}, pH and standard bicarbonate by the Astrup method.

METHODS

Apparatus

The apparatus has been fully described by Astrup, Jørgensen, Andersen & Engel (1960). It consists of a microelectrode unit and microtonometer (maintained at 39.4° C and a pH meter accurate to ±0.002 pH units.

General methods

The rabbits were of mixed strain and of either sex; their weights varied between 2.5 and 3.5 kg. Five rabbits were used for each experiment, unless otherwise stated.

Blood was sampled anaerobically from the marginal vein of the ear after it had been warmed for about 1 min at 45–50° C by a 15 watt light bulb. Samples were taken into at least three capillary tubes, each of volume 60–80 µl. and containing 48 µg solid sodium heparin.

Blood pH, P_{CO_2}, actual and standard bicarbonate (Jørgensen & Astrup, 1957) were estimated by the method of Astrup *et al.* (1960) after temperature corrections had been applied, because rabbit body temperature is slightly greater than that of man.

Sixteen rabbits were used to establish control values.

Examination of effects of inhaled CO_2 and of administration of drugs

In all experiments control values were established for the animal under investigation during the 1 hr period immediately before the experiment. Two identical (±0.004 pH units) or three consistent readings (within 0.010 pH units) over at least 20 min were regarded as control values.

Inhalation of CO_2

After control values had been established, each rabbit was placed in a 7.5 l. Perspex equilibration chamber constructed to allow permanent local warming of the ear. Mixtures of CO_2 in O_2 were passed through the chamber at about 20 l./min. During the experimental period of 5 hr the outflowing gas was repeatedly analysed by Haldane's method in order to determine the ambient P_{CO_2}. Immediately after gas analysis, a small flap was opened in the roof of the chamber directly above the ear and blood sampled; this was usually completed within 10 sec of opening the chamber.

Administration of drugs

After control readings for 1 hr, morphine was injected at a rate of about 0.1 ml./sec. Blood was sampled 7, 15, 30, 45, 60, 90 and 120 min after injection, and then at hourly intervals until control values were regained. Doses of morphine used were 0.3, 0.6, 1.2, 2.4 and 4.8 mg/kg. Eight experiments were performed with 4.8 mg/kg and four with 0.3 mg/kg. The effects of nalorphine (1 and 5 mg/kg) and dextromoramide (0.05 and 0.10 mg/kg) were investigated in a similar manner.

Pretreatment with morphine

Groups of rabbits were given six consecutive daily doses of 4.8 mg/kg morphine. The effects of morphine (4.8 mg/kg) were investigated in these rabbits, the times of sampling being identical with those used in untreated rabbits.

Five rabbits pretreated with morphine were exposed to different ambient CO_2 concentrations with or without a further injection of morphine.

Drugs

These were: morphine sulphate injection B.P. (B.D.H.); nalorphine hydrobromide injection B.P. (Lethidrone, Burroughs Wellcome and Co.); dextromoramide bitartrate (Palfium injection, M.C.P. Pure Drugs Ltd.). All doses are expressed in terms of the salts. The route of injection was always intravenous.

RESULTS

Control experiments investigating acid-base changes during the day

Each experimental day was divided into five consecutive 90 min periods starting at 09.30 hr. Blood was sampled and pH, P_{CO_2} and standard bicarbonate determined for each rabbit five times, so that 80 sets of readings were collected at intervals distributed evenly throughout the day.

There was a fall in pH with time; however the points could not be fitted to a straight line. There was a consistent fall in standard bicarbonate at a rate of 0.53 m-equiv/l./hr (Fig. 1). The fall was significant for any 5 hr period ($P<0.05$).

When values of P_{CO_2} were plotted against time there was a slight fall at a rate of 0.27 mm Hg/hr but the change was not as uniform as that of standard bicarbonate and was not significant statistically.

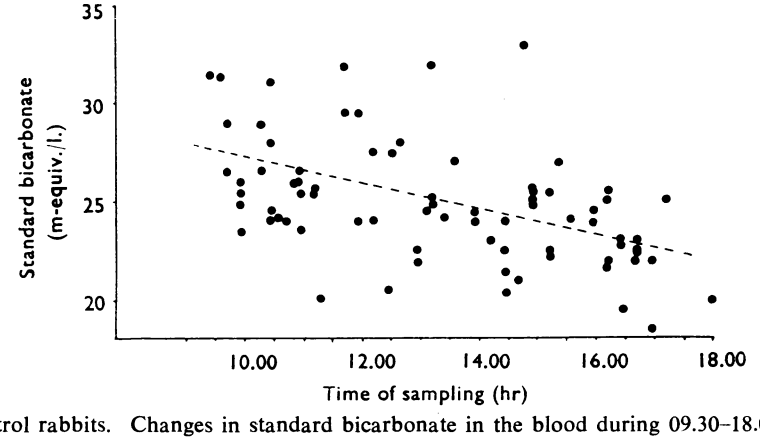

Fig. 1. Control rabbits. Changes in standard bicarbonate in the blood during 09.30–18.00 hr. Each point represents one reading; the broken line is the best-fitting straight line.

TABLE 1

THE MEAN CHANGES IN BLOOD P_{CO_2}, STANDARD BICARBONATE AND pH (WITH S.E. OF MEAN), DURING INHALATION OF 4 AND 8% CO_2 IN GROUPS OF FIVE RABBITS

The ambient P_{CO_2} was 36·7±2·0 in (a) and 58·1±1·3 in (b)

Duration of exposure (min)	P_{CO_2} (Δ mm Hg)	Standard bicarbonate (Δ m-equiv/l.)	pH (Δ)
(a) 4% CO_2			
60	+11·6±2·0	−0·3±0·6	−0·067±0·007
120	+11·1±1·1	−1·2±0·5	−0·082±0·007
180	+11·7±1·1	−1·6±0·3	−0·085±0·009
240	+11·9±1·7	−1·8±0·4	−0·092±0·013
300	+11·9±1·9	−2·1±0·5	−0·094±0·013
(b) 8% CO_2			
60	+25·3±3·2	−0·9±0·7	−0·116±0·035
120	+30·9±2·6	−1·3±0·6	−0·165±0·028
180	+33·4±2·0	+0·7±1·0	−0·141±0·024
240	+33·5±0·7	+2·9±1·7	−0·123±0·017
300	+33·5±3·7	+0·2±1·3	−0·126±0·022

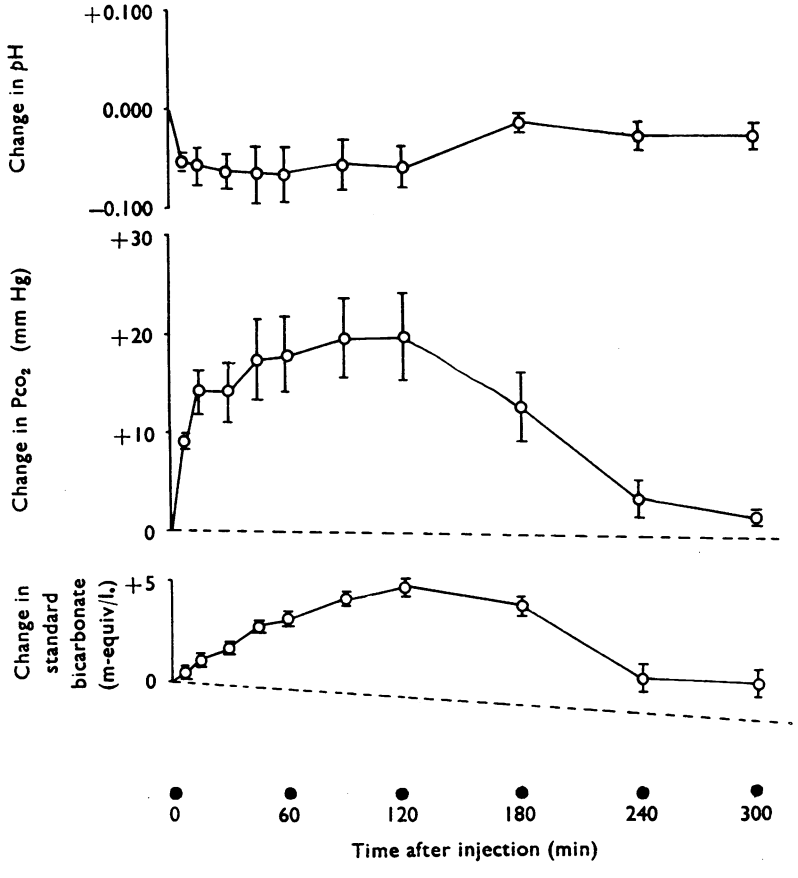

Fig. 2. The effects of morphine (2.4 mg/kg) on blood pH, P_{CO_2} and standard bicarbonate in 5 rabbits. Vertical bars, S.E. of means; broken lines, changes in P_{CO_2} and standard bicarbonate in control rabbits without morphine.

Response to inhaled CO_2

The changes in P_{CO_2} and standard bicarbonate on exposure to approximately 4 and 8% CO_2 are shown in Table 1. Equilibration of P_{CO_2} was nearly complete after 1 hr. At equilibrium the extent of the rise in P_{CO_2} was related to the concentration of CO_2 breathed but there were only negligible fluctuations in standard bicarbonate.

These results define the nature of acute respiratory acidosis in the rabbit and demonstrate that a change in the metabolic component plays no significant part. Also, since there was no consistent rise in standard bicarbonate over a period of 5 hr renal retention of bicarbonate was negligible. It is against this background that one must view the respiratory acidosis induced by morphine.

Effects of morphine

All doses of morphine produced qualitatively similar effects; those obtained with 2.4 mg/kg morphine are shown in Fig. 2. After injection, blood P_{CO_2} rose rapidly, accompanied by a slower increase in standard bicarbonate; because of this the decrease in pH was less than would have been expected for the observed rise in P_{CO_2}. This was different when P_{CO_2} was raised to a similar extent by CO_2 inhalation when no increase in standard bicarbonate occurred.

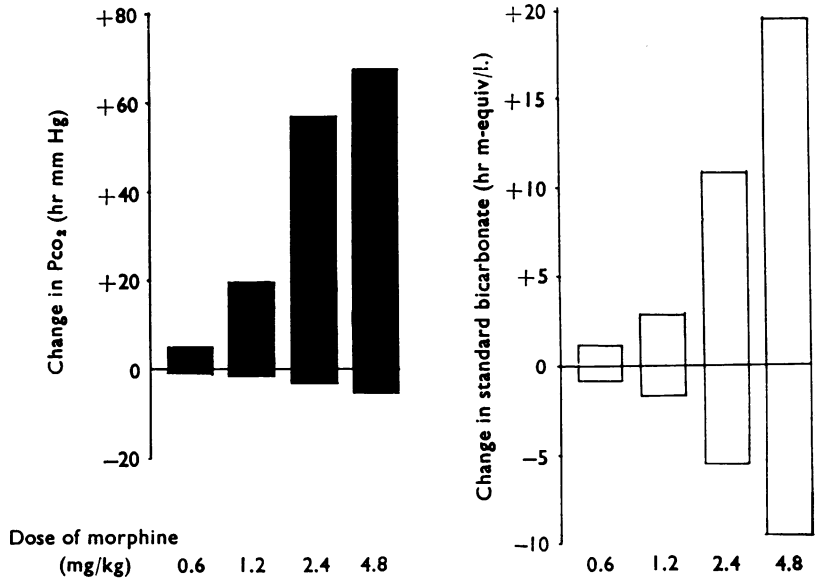

Fig. 3. Changes in P_{CO_2} and standard bicarbonate, integrated for the duration of morphine action; this was done by calculating the area defined by the curves describing these two parameters and the abscissa passing through the control values obtained prior to injection of morphine. Effects of 0.6, 1.2 and 2.4 mg/kg morphine given to groups of 5 untreated rabbits and 4.8 mg/kg morphine given to a group of 8 untreated rabbits. Shaded column, P_{CO_2}; clear columns, standard bicarbonate. The negative portions of the histograms indicate the values which would have been obtained in untreated rabbits not given morphine.

In order to express a dose-response relationship, the response to morphine was plotted as the area enclosed by each curve and the base line, and not as the maximum change in the measured parameters. The dose-response relationships for P_{CO_2} and standard bicarbonate are shown in Fig. 3. The negative portions of the histograms indicate those changes which would have occurred in the absence of morphine.

Of four animals treated with 0.3 mg/kg morphine, two showed no effects and the others small, insignificant changes. This dose was regarded as threshold, corresponding well to that observed by Wright & Barbour (1935) to produce a minimal change in respiratory minute volume in rabbits.

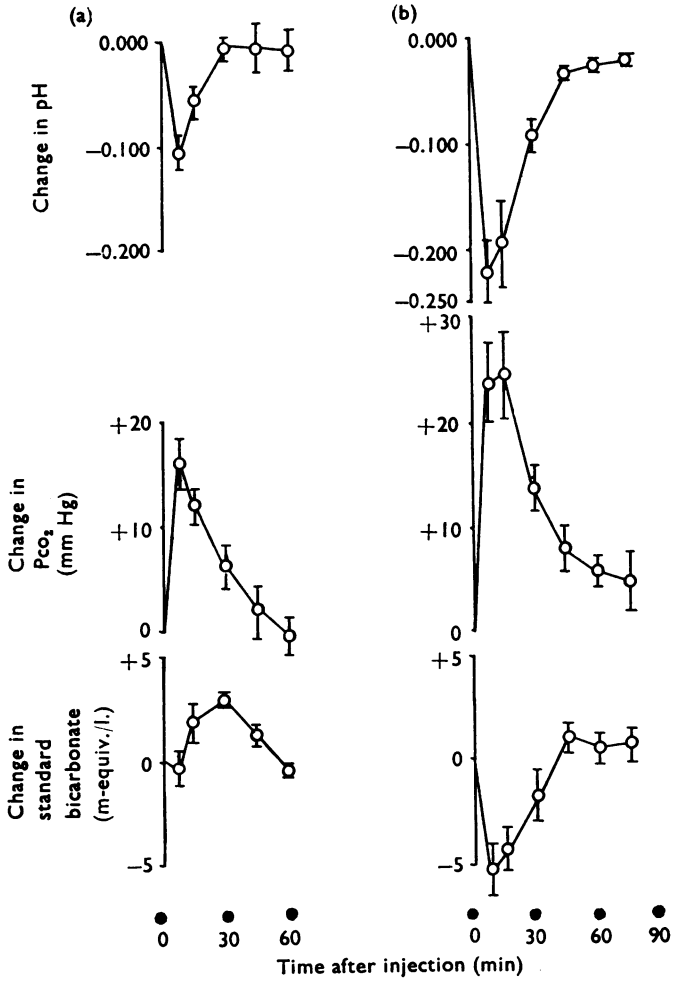

Fig. 4. The effects of 0.05 (a) and 0.10 (b) mg/kg dextromoramide on blood pH, P_{CO_2} and standard bicarbonate in two groups of 5 rabbits. Vertical bars, S.E. of means.

At all the other doses investigated there was an approximately constant ratio between the respiratory and non-respiratory responses. In other words, the extent of metabolic change expressed in this way was always a constant proportion of the respiratory response.

Effects of some other related drugs

Nalorphine in doses of 1.0 and 5.0 mg/kg had no significant effect on either P_{CO_2} or standard bicarbonate.

The responses to 0.05 and 0.10 mg/kg dextromoramide are shown in Fig. 4. The onset of the rise in P_{CO_2} was far more rapid than that produced by any dose of morphine. Control values were regained after not more than 90 min at both doses. After the smaller dose there was a slight fall in the standard bicarbonate, followed by a rise; at the higher dose the initial fall was much more marked. The fall in pH after 0.10 mg/kg was far greater than could be explained by the rise in P_{CO_2} alone (Table 2).

TABLE 2

MAXIMUM CHANGES IN P_{CO_2} AND THE CORRESPONDING CHANGES IN pH, WITH S.E. OF MEAN, AFTER INTRAVENOUS INJECTION OF MORPHINE AND DEXTROMORAMIDE

All values were obtained on 5 rabbits, except those for 4·8 mg/kg morphine, which was given to 8 rabbits

Drug	Dose (mg/kg)	P_{CO_2} (Δ mm Hg)	pH (Δ)	Interval between injection and maximum change (min)
Morphine	2·4	+19·5±4·6	−0·062±0·024	120
	4·8	+17·5±1·2	−0·034±0·010	120
Dextromoramide	0·05	+16·2±2·4	−0·105±0·016	7
	0·10	+24·5±4·6	−0·227±0·029	15

Effects of morphine in the rabbit pretreated with morphine

The effects of 4.8 mg/kg morphine in a group of untreated rabbits and in a group pretreated with morphine are shown in Fig. 5. In the pretreated group the change in P_{CO_2} was marginally smaller, indicating that little tolerance had developed to the depressant action of morphine on respiration. The change in standard bicarbonate, however, developed much more rapidly in the pretreated group, which showed scarcely any variation in pH despite the marked increase in P_{CO_2}.

The relationship between the respiratory and non-respiratory responses is shown in Fig. 6.

The ratio between P_{CO_2} and standard bicarbonate elevations may be compared with those shown in Fig. 3 at the same dose in the untreated rabbit.

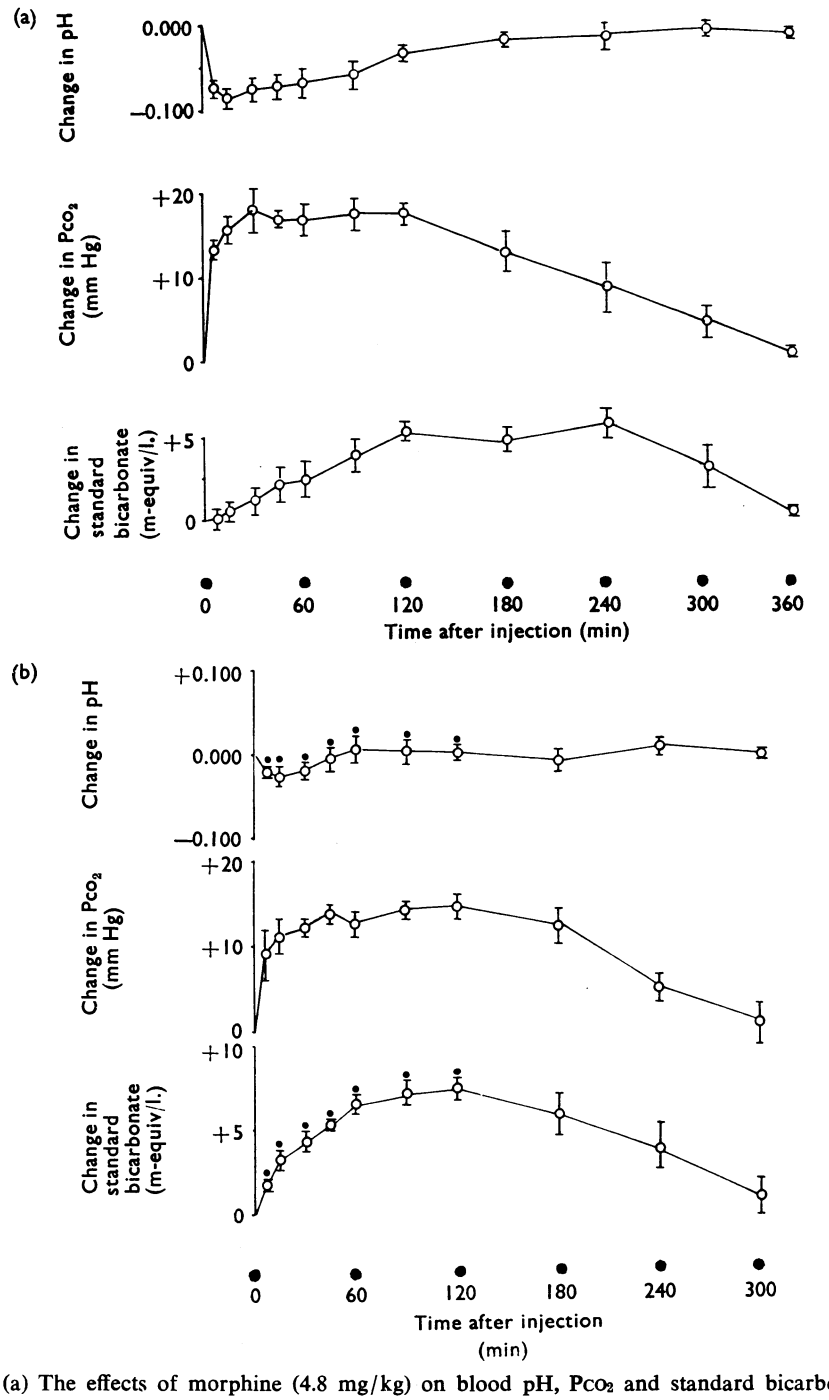

Fig. 5. (a) The effects of morphine (4.8 mg/kg) on blood pH, P_{CO_2} and standard bicarbonate in 8 untreated rabbits. (b) The effects of morphine (4.8 mg/kg) on blood pH, P_{CO_2} and standard bicarbonate in 5 rabbits pretreated with morphine. Vertical bars, S.E. of means; a closed circle in (b) indicates that the mean is significantly different ($P<0.05$) from the corresponding mean in (a).

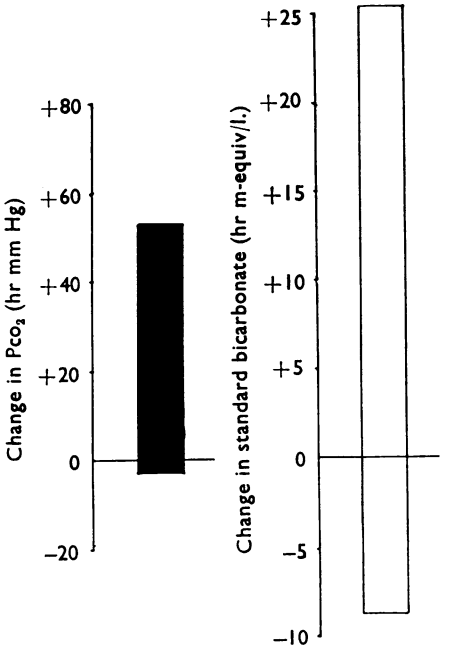

Fig. 6. Changes in P_{CO_2} and standard bicarbonate, integrated for the duration of morphine action; this was done by calculating the area defined by the curves describing these two parameters and the abscissa passing through the control values obtained prior to injection of morphine. Effects of morphine (4.8 mg/kg) given to 5 rabbits pretreated with morphine. Shaded column, P_{CO_2}; clear column, standard bicarbonate. The negative portions of the histograms indicate the values which would have been obtained in untreated rabbits not given morphine.

Effects of simultaneous injection of morphine and inhalation of CO_2

Table 3 shows the changes in P_{CO_2} and standard bicarbonate in two rabbits pretreated with morphine and exposed to 8% CO_2. One rabbit received an injection of morphine (4.8 mg/kg) at the start of CO_2 inhalation. In this animal P_{CO_2} settled, after inhalation for 3 hr, at the level found in untreated control rabbits inhaling the same concentration of CO_2 (Table 1). Standard bicarbonate rose to a higher level than that produced by an

TABLE 3

CHANGES IN P_{CO_2}, STANDARD BICARBONATE, AND pH IN 2 RABBITS PRETREATED WITH MORPHINE INDUCED BY INHALATION OF APPROXIMATELY 8% CO_2 WITH (a) AND WITHOUT (b) AN INJECTION OF MORPHINE (4·8 mg/kg) AT COMMENCEMENT OF CO_2 INHALATION

Duration of exposure to 8% CO_2 (min)	(a) P_{CO_2} (Δ mm Hg)	(a) Standard bicarbonate (Δm-equiv/l.)	(a) pH (Δ)	(b) P_{CO_2} (Δ mm Hg)	(b) Standard bicarbonate (Δm-equiv/l.)	(b) pH (Δ)
30	+50·6	+ 0·9	−0·229	—	—	—
60	+52·6	+ 4·3	−0·172	+34·9	−0·4	−0·213
120	+43·6	+ 9·9	−0·099	+32·4	+0·1	−0·193
180	+35·1	+12·6	−0·056	+33·9	−0·2	−0·200
240	+31·6	+15·9	−0·018	—	—	—
300	+33·1	+14·9	−0·015	+30·9	+1·4	−0·164
360	+33·6	+15·3	−0·016	—	—	—

injection of morphine, without CO_2 inhalation, in rabits pretreated with morphine. This rise in standard bicarbonate was sufficiently large to prevent a fall in blood pH, although P_{CO_2} was raised by 35 mm Hg. This persisted for 6 hr while the effect of morphine on standard bicarbonate without CO_2 inhalation wore off after 4 hr (Fig. 5).

The second rabbit, also pretreated with morphine, inhaled 8% CO_2 without a further injection of morphine ; in this case standard bicarbonate did not differ significantly from that of untreated control rabbits (Table 1).

Three other rabbits were exposed to different CO_2 concentrations and showed qualitatively similar responses.

DISCUSSION

The method described here demonstrates the effects of morphine on pH, P_{CO_2} and standard bicarbonate in the unanaesthetized rabbit and indirectly examines the effects of this drug on respiration. An important advantage of this approach is that disturbance of the rabbit is minimal and the experimental technique causes little respiratory change from the resting state.

It is generally accepted that both pH and P_{CO_2} are important parameters in the chemoregulation of respiration ; thus another advantage of the technique is that it describes the respiratory depression in more informative parameters than does measurement of change in blood P_{CO_2} alone or change in respiratory minute volume.

The use of the method is well illustrated by a comparison of the actions of morphine and dextromoramide. The latter is known to have a short duration of action. Figure 4 demonstrates the rapidity of onset of respiratory depression and respiratory acidosis ; there is also a metabolic acidosis not seen with morphine. Though the mechanism of this has not been investigated, bicarbonate redistribution (Brackett, Cohen & Schwartz, 1965 ; Brown & Clancy, 1965), and increased lactic acid production may be contributing factors. Hunter (personal communication) has demonstrated that a 50% depression in respiratory minute volume in the rabbit is produced by 3.6 mg/kg morphine, and 0.04 mg/kg dextromoramide. For similar doses dextromoramide produced a far more profound decrease in pH than did morphine (Table 2).

The magnitude of the rise in blood P_{CO_2} (11–34 mm Hg) observed when rabbits inhale 4 and 8% CO_2 approximately covers the range of rise produced by morphine and dextromoramide in the doses used ; however only when morphine is given is there a consistent rise in standard bicarbonate.

The question arises as to whether this appearance of additional bicarbonate is a true effect of morphine or an artefact. In this context changes in body temperature and in oxygen saturation of the blood would produce the greatest distortion of the Astrup nomogram. However, a fall in oxygen saturation could not explain the change observed because application of the necessary correction would lead to a more alkaline value.

Wright & Barbour (1935) and Stewart & Rogoff (1922) demonstrated that doses of morphine of the order used in the present study did not affect body temperature, although higher doses lowered it.

In the present experiments six daily doses of morphine produced a " pH tolerance." Schoen (1924) demonstrated a similar effect in human subjects after the fifth daily dose.

However, he regarded the original acidosis as metabolic in origin, and the diminishing effects on pH as being due to rapid development of tolerance to the depressant action of morphine on metabolic rate. Chin (1940) showed that morphine depresses the metabolic rate in rabbits as in man. This would be more likely to result in a fall rather than a rise in standard bicarbonate. In addition Chin (1940) demonstrated that this type of tolerance developed extremely slowly, therefore it is unlikely that the effect of morphine on metabolic rate is responsible for the rise in standard bicarbonate after morphine.

SUMMARY

1. The Astrup technique has been used to investigate the effects of morphine and dextromoramide on pH, P_{CO_2} and standard bicarbonate in the blood of the unanaesthetized rabbit.

2. The respiratory acidosis which results from morphine-induced respiratory depression is accompanied by a rise in standard bicarbonate while a similar rise in P_{CO_2} produced by inhaling 4 or 8% CO_2 in O_2 is not.

3. A " pH tolerance " to morphine-induced respiratory acidosis develops in the rabbit which depends more on an increase in standard bicarbonate than on a decrease in P_{CO_2} due to reduced respiratory depression.

REFERENCES

ASTRUP, P., JØRGENSEN, K., ANDERSEN, O. S. & ENGEL, K. (1960). The acid-base metabolism—a new approach. *Lancet*, **i**, 1035–1039.

BRACKETT, N. C., JR., COHEN, J. J. & SCHWARTZ, W. B. (1965). Carbon dioxide titration curve of normal man. Effect of increasing degrees of acute hypercapnia on acid-base equilibrium. *New Engl. J. Med.*, **272**, 6–12.

BROWN, E. B., JR. & CLANCY, R. L. (1965). *In vivo* and *in vitro* CO_2 blood buffer curves. *J. appl. Physiol.*, **20**, 885–889.

CHIN, K. (1940). The influence of morphine hydrochloride on the basal metabolism of rabbits. *Folia pharmac. jap.*, **28**, 212–242. (English Abstract 85–86.)

JØRGENSEN, K. & ASTRUP, P. (1957). Standard bicarbonate, its clinical significance, and a new method for its determination. *Scand. J. clin. Lab. Invest.*, **9**, 122–132.

LAMBERTSEN, C. J. (1960). Carbon dioxide and respiration in acid-base homeostasis. *Anesthesiology*, **21**, 642–651.

SCHOEN, R. (1924). Zur Kenntnis der Morphinwirkung beim Menschen. II. Mitteilung: Die Veranderungen des Grundumsatzes und der Einfluss mässiger Gewöhnung auf Grundumsatz, Blutreaktion und Atmung. *Arch. exp. Path. Pharmak.*, **102**, 205–217.

STEWART, G. N. & ROGOFF, J. M. (1922). Morphine hyperglycemia and the adrenals. *Am. J. Physiol.*, **62**, 93–112.

WRIGHT, C. I. & BARBOUR, F. A. (1935). The respiratory effects of morphine, codeine and related substances. III. The effect of morphine, dihydromorphine (dilaudid) and dihydrocodeinone (dicodid) on the respiratory activity of the rabbit. *J. Pharmac. exp. Ther.*, **53**, 34–45.