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OXYGEN CONSUMPTION IN THE NEW-BORN RAT AND THE EFFECTS OF MATERNALLY ADMINISTERED ANALGESICS

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Despite the extensive use of pregnant animals in laboratory tests for drug teratogenicity little attention has been given by pharmacologists to the period shortly before or after birth. By contrast, recent clinical literature confirms the growing awareness among anaesthetists and obstetricians that most of the drugs they use, with the notable exception of the highly ionized skeletal-muscle blocking agents, are rapidly transferred from mother to foetus through the placenta. Adequate reviews have been presented by Snyder (1949); Marx (1961); Moya & Thorndike (1962); Nyhan & Lampert (1965) and Crawford (1966).

It is fairly routine clinical practice to give an analgesic drug to the mother at or shortly before the onset of the second stage of labour. All the existing agents may be identified in cord blood within a few minutes of administration, and tend to depress respiration in the new-born. Our interest in a new series of potent analgesics related to thebaine, some of which have been described before (Bentley & Hardy, 1963; Bentley, Boura, Fitzgerald, Hardy, McCoubrey, Aikman & Lister, 1965; Blane, Boura, Fitzgerald & Lister, 1967), led to the development of the test-system described here, in which the effects of maternally administered analgesics on the oxygen consumption of new-born rats can be studied.

METHODS

Mothers and young

Female S.P.F.-derived Sprague Dawley rats were caged with males and examined daily for the presence of spermatozoa in the vaginal smears. Positives were called 1-day pregnant and housed individually for 19 days in the animal unit. On the 20th day mothers were brought into the laboratory and given 2 mg progesterone subcutaneously to delay spontaneous delivery. The experiments described below were performed on day 21, which is the average full term.

Respiratory rate of the mothers was determined before any treatment and again 30 min later using a chest tambour method as first reported by Green (1953) and recently described in detail by Boura & Fitzgerald (1966). Any change at 30 min was expressed as a % of the control values.

Drugs were dissolved in physiological saline and administered subcutaneously to the mothers, which were killed, 30 min later, by dislocating the neck.

The uteri were exposed immediately through a mid-line abdominal incision. The umbilical cords were tied with thread and all the young put into a moist reservoir partially immersed in a constant temperature water-bath. Any dead were counted at the end of the respirometer experiment—that is, about 1 hr after delivery.

Since the position occupied by a foetus in the uterus might possibly influence development and vascularity, and hence the rate of passage of drugs across the placenta, the new-born used for the assessment of oxygen consumption were always taken from the ovarian end of the uterus, 3 from the right-hand and 2 from the left-hand cornua. They were placed separately and at random in the 5 chambers of the respirometer described below.

Apparatus

The construction of the apparatus for measuring oxygen consumption of individual new-born rats is shown diagramatically in Fig. 1.

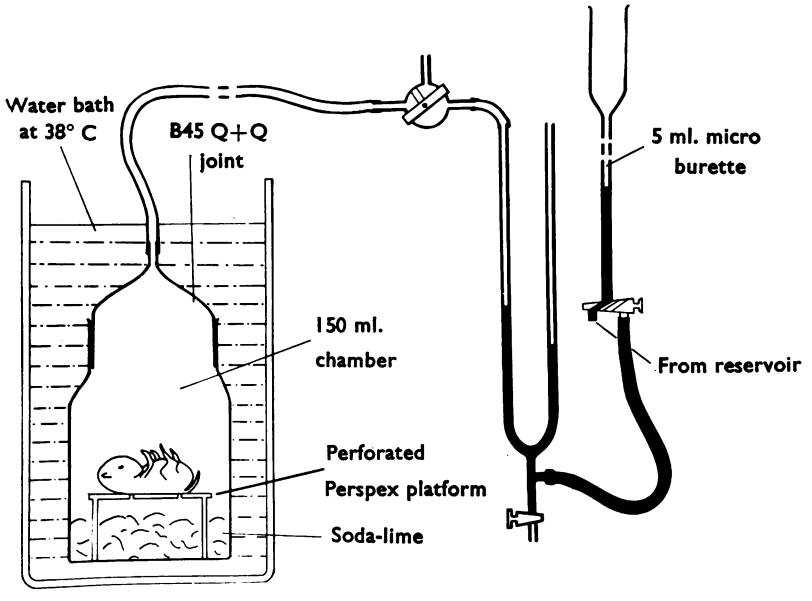

Fig. 1. Diagram (not to scale) of a unit of the respirometer used to measure oxygen consumption of young rats. A bank of five such units made up the complete apparatus. The manometer fluid was coloured water.

Each animal was placed on the perforated Perspex platform inside glass chambers of approximate volume 150 ml., access to the interior being by way of ground glass joints which were freshly greased. The vessels contained 10 g self-indicating soda-lime (Carbosorb, B.D.H.) and this was replaced at the first sign of colour change, or every 5 days, whichever was the sooner. It was established that although Carbosorb is rapidly effective, to the extent that there is no increase in the concentration of carbon dioxide within the chambers during the course of an experiment, it is not a powerful dehydrating agent. The complete apparatus consisted of 5 chambers totally immersed in a warm water-bath maintained at the maternal body temperature (38° C) and each connected *via* a three-way tap to a separate simple manometer-burette arrangement containing coloured water. The manometers were constructed from $\frac{1}{8}$-in. internal diameter glass tubing to have arms of approximately 14 in. height while the 5 ml. microburettes had two-way stopcocks permitting refilling from a common reservoir as required.

The three-way tap was adjusted initially to leave the system open to the atmosphere, and under these conditions equilibrium would normally be reached within 15 min provided that the apparatus was already warm. However, to allow for variation and errors, 30 min were allowed after the new-born rats had been positioned before the taps were turned to close the system and connect manometers to respiration chambers.

As oxygen was consumed, and expired carbon dioxide absorbed by the soda-lime, pressure in the system fell proportionately as indicated by a rise of fluid level in the arms of the manometers connected to the respiration chambers. Every 4 min fluid was run in from the burettes to balance the arms and restore pressure within the respirometers to atmospheric. This volume was recorded and is equal to twice the volume of oxygen consumed. Six such readings taken at consecutive 4-min intervals were used to calculate directly the oxygen consumption per 100 g/animal/min at s.t.p.

It was established statistically that there was positive correlation between weight at birth and oxygen consumption at a bath temperature of 38° C, that there was no significant variation in oxygen consumption over the 24-min test-period for a given animal, and that no difference existed between chambers. Not surprisingly, there was less variation between litter mates than in the untreated new-born population as a whole.

During the 24-min test-period the absolute oxygen concentration in the respiration chambers fell while that of nitrogen rose slightly. This artefact would be greatest with control animals since all drug studies were made with respiratory depressants. The following example illustrates the theoretical scale of these changes:

At a bath temperature of 38° C a group of 35 control new-born animals, of mean body weight 5.7 g, consumed oxygen at the rate of 1.8 ml./100 g/min (corrected to s.t.p.). The average net oxygen consumption in the 24-min test-period was therefore:

$$\frac{5.7\times1.8\times24}{100}=2.46 \text{ ml.}$$

The 150 ml. respirometer chamber contained 31.5 ml. oxygen at the start of the experiment where the atmospheric oxygen concentration was assumed to be 21%. The volume of oxygen present after 24 min would, in the example taken, be $31.5-2.46=29.04$ ml. Oxygen concentration had fallen from 21 to 19.7%.

The validity of this interpretation has been confirmed by direct measurements using an oxygen electrode.

Several studies have been made on the effect of lowered environmental oxygen concentration on oxygen consumption of young animals. Thus, Hill (1959) found that for kittens maintained at the neutral temperature, the oxygen concentration of inspired air could be reduced to 10% before oxygen consumption was affected. However, more relevant is the report of Taylor (1960) who worked with new-born rats and showed that the mean oxygen consumption was slightly less in 18% oxygen than in air. Only one group of 9 animals contribute to his figure and no comment was made on the statistical significance of this observation.

It is assumed here that any fall in oxygen consumption at the calculated minimum oxygen concentration of about 19.7% would not be sufficient to invalidate the comparison between drug-treated and control animals. In support of this contention it is worth emphasizing that the oxygen consumption of all animals was recorded at regular 4-min intervals and that no upward or downward trend developed during the 20-min test-period.

Drugs

All doses are expressed as the weight of the salt used and were administered to pregnant females on a body weight basis. Methadone, pethidine and etorphine [7α-(1-(R)-hydroxy-1-methylbutyl)-6,14-*endo*ethenotetrahydro-oripavine] were administered as the hydrochlorides, and morphine and atropine as the sulphates.

RESULTS

Caesarian-delivered controls at 38° C

Interspersed with the drug-treated rats described in the next section were a total of 23 control mothers which received only a saline injection. Observations of maternal

respiratory rate changes at the beginning and end of the pre-delivery 30-min period were made on 22 of these animals and the small fall which occurred was insignificant and presumably adaptive (Table 1).

From the 23 mothers 110 new-born were tested in the respirometer and gave an overall oxygen consumption corrected to s.t.p. of 1.80 ml./100 g/min (Table 1).

TABLE 1

COLLECTED CONTROL MEANS OF MATERNAL RESPIRATORY RATES AND OXYGEN CONSUMPTION OF NEW-BORN RATS

The mothers received saline only, and 30 min elapsed between the initial and final respiratory rate determinations. The offspring were delivered by Caesarian section and were maintained at 38° C

	Initial resp. rate	Final resp. rate	% change	P
22 mothers	39·05	36·77	5·77	0·4–0·5
	Wt.	O_2/100 g/min	S.E.	95% limits
110 new-born	5·81	1·80	0·02	1·76–1·83

The investigation covered a period of 4 months and to check for the existence of any trend attributable to seasonal variation the mean oxygen consumption from the 50 new-born tested in the first 2 months was compared by *t* test with that of the 60 new-born tested in the second 2-month period. The respective means were 1.81 and 1.79 ml./100 g/min, the difference between which is insignificant ($P = 0.6 - 0.7$). The combined control means have, therefore, been used as a standard for comparison with any drug treatment during the same time-period.

Comparative studies with narcotic analgesics

Detailed studies were made with four potent narcotic drugs to provide a background of information against which to compare newly evolving agents. Of these, morphine, methadone and now pethidine (Shnider & Moya, 1964) are well konwn to cross the placenta after maternal administration and to influence the foetus in both man and laboratory animals. Etorphine had not previously been studied in this context, although there is much in the acute pharmacology (Blane *et al.*, 1967) to suggest that it too, when given to mothers in the immediately prenatal period, would affect the foetus and the new-born.

The doses of drug used were related in the first instance to analgesic activity, as assessed in separate experiments using the tail-pressure method described by Green & Young (1951), but were later raised towards toxic levels. Each of the points from which the dose-response lines for oxygen consumption were constructed represent the mean value from, usually, 30 new-born from 6 mothers. The young were delivered by Caesarian section and incubated at 38° C. Satisfactory regression lines were obtained for morphine, methadone and etorphine, and did not deviate significantly from parallelism at the 95% level of confidence, except with very large doses of morphine (Fig. 2). By contrast, the lowest dose of pethidine to produce any appreciable reduction of oxygen consumption in the young rats was 100 mg/kg (7.2% reduction from control mean, $P = 0.01$) and this effect was not significantly intensified by raising the dose to levels which were above the maternal LD50 ; for clarity these points are not shown in Fig. 2.

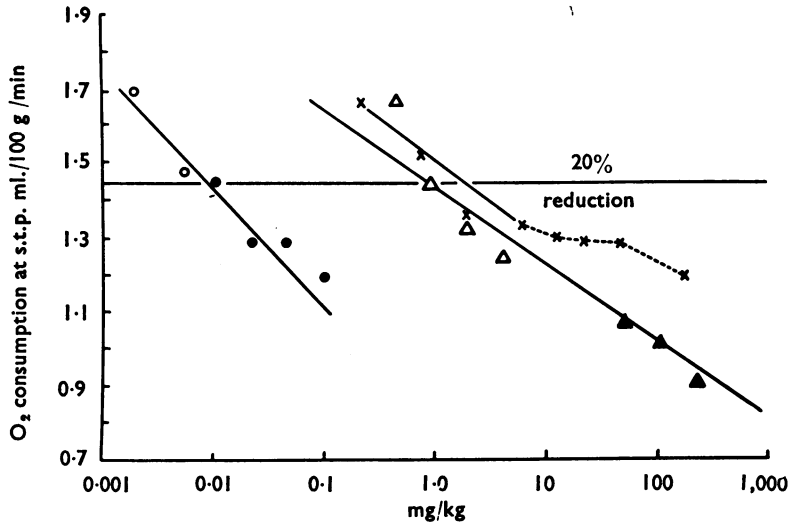

Fig. 2. Oxygen consumption of new-born rats from mothers treated with etorphine ○——●, methadone Δ——▲, or morphine ×——×. The dose-response line for morphine is broken (×- - - -×) where it deviates significantly from parallelism with the etorphine and methadone lines. At dose levels where any deaths occurred the symbols are blocked-in. All points differ significantly from the control mean of 1.80 ml./100 g/min (the largest value for P is less than 0.01) and 20% reduction from the mean is indicated by a horizontal line. The most usual group size was 30 new-born.

For morphine, methadone and etorphine a dose reducing the oxygen consumption of the new-born by 20% from the control mean value was used as an index of comparative activity. The values, with their 95% confidence limits, are given in Table 2 together with a figure for reduction of oxygen consumption at twice the maternal analgesic ED80.

TABLE 2

EFFECTS OF FOUR POTENT ANALGESICS ON PREGNANT RATS AND THEIR CAESARIAN-DELIVERED OFFSPRING

Non-pregnant rats were used in the evaluation of analgesic activity. All the values for percentile reduction of maternal respiratory frequency at analgesic ED80×2 differ from each other at significance levels between 1·0 and 0·1%. Etorphine is significantly less depressant on neonatal oxygen consumption at the maternal analgesic dose of ED80×2 than either morphine or methadone (P=<0·001).

	Maternal effects			Neonatal effects		
Drug/Mother s.c.	Analgesic ED80 (mg/kg) 95% confidence limits in parentheses	Dose reducing respiratory frequency by 30% (mg/kg)	Percentile reduction of respiratory frequency at analgesic ED80×2	Dose reducing O_2 consumption by 20% (mg/kg) 95% confidence limits in parentheses	Percentile reduction of O_2 consumption at maternal analgesic ED80×2	Minimum dose at which deaths occurred (mg/kg)
Morphine	3·36 (2·57–4·40)	10·4	11·5	1·65 (0·80–3·80)	27·8	>200
Methadone	1·54 (0·59–4·05)	2·9	32·0	1·15 (0·60–3·80)	25·6	50·0
Etorphine	$2{\cdot}52\times10^{-3}$ ($1{\cdot}85\times10^{-3}$–$3{\cdot}42\times10^{-3}$)	$3{\cdot}1\times10^{-3}$	52·0	$8{\cdot}65\times10^{-3}$ ($6{\cdot}0\times10^{-3}$–$16{\cdot}0\times10^{-3}$)	16·7	$12{\cdot}0\times10^{-3}$
Pethidine	19·24 (8·31–44·53)	46·0	27·5	>300	<7·2	>300

This is useful in any discussion of apparent " therapeutic ratio," where ED80 × 2 is chosen as a dose level which will consistently produce analgesia in all mothers. Thus, morphine and methadone produce severe (>20%) reduction of oxygen consumption at analgesic doses, whereas pethidine has no such effect and etorphine is intermediate.

Although no true measurements of maternal respiration were made, such as minute-volume, the information on rates is taken as being relevant and meaningful. The decrease in maternal respiratory rate at 30 min after drug administration is shown graphically in Fig. 3. It is apparent that the dose-response lines for morphine, methadone and pethidine begin to flatten out at about 40% reduction and that increasing the dose beyond this point results in little further depression of respiratory rate. With etorphine, on the other hand, the dose-response line in mothers rises steeply to a maximum above 70% depression, this intense effect being consistent with other observations in various species of non-pregnant animals (Blane *et al.*, 1967). The middle part of the response-lines for all four drugs are roughly parallel (*P* for deviation from parallelism is >0.5), and a convenient point at which to take values for comparative assessment is the 30% level of reduction in rate.

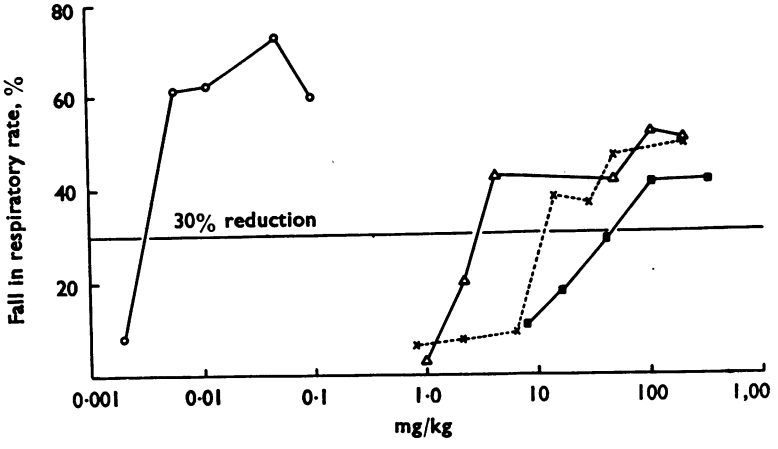

Fig. 3. Fall in respiratory rate of pregnant rats treated with etorphine ○——○, methadone Δ——Δ, morphine ×----× or pethidine ■——■. Results are expressed as a % of the pre-treatment value for each dose-group. The minimum group size was six mothers.

As with the new-born, a value is also given in Table 2 for reduction of maternal respiratory rate at the analgesic ED80 × 2 for each drug. Morphine is shown to advantage by this criterion (11.5% reduction) since analgesic doses are relatively non-depressant to the mother, whereas methadone and pethidine cause a very significant degree of respiratory depression (32.0 and 27.5% respectively). These results differ from those reported by Green (1959) who used young non-pregnant rats. He found the ratio of the analgesic to the respiratory rate-depressing potency to be the same for a number of drugs including morphine and methadone. The high value of 52% for etorphine reflects the relatively more powerful C.N.S. depressant action of this drug.

The young delivered from all control mothers and those receiving any dose of pethidine did not die within the test period, nor were there any deaths with even very large doses

of morphine despite severe reduction in oxygen consumption. With 50 mg/kg methadone and larger doses there were a few mortalities, but death of the new-born was a consistent and dose-dependent feature of etorphine treatment at and above 12 μg/kg—that is, beginning at about five times the analgesic ED80, although in no instance were the young dead when delivered.

Treatment of mothers with atropine 15 min before administration of very large doses of etorphine effectively prevented the young from dying without altering the pattern of either maternal respiratory depression or the diminished oxygen consumption of the new-born (Table 3), suggesting the possibility of some involvement of a cholinergic mechanism in the cause of death. That reduced oxygen consumption was not of itself entirely responsible for deaths was further confirmed by the survival of young with very severely lowered oxygen consumption from pentobarbitone given to the mothers.

TABLE 3

EFFECTS OF ATROPINE ON RESPONSES OF MOTHERS AND NEW-BORN TO 24 μG/KG ETORPHINE

Atropine given subcutaneously to groups of 6 mothers 15 min before administration of the fixed dose of etorphine. Neonatal oxygen consumption assessed in groups of 30 animals and expressed as a % of the normal control mean of 1·80 ml./100 g/min (see Table 1). The values for mortality are based on the total number of young born from each group of mothers, as indicated in parentheses. The effects of atropine in reducing mortality are highly significant ($P = <0{\cdot}001$).

Dose of atropine (mg/kg)	Reduction of maternal respiratory rate (%)	Reduction of neonatal oxygen consumption (%)	Neonatal mortality (%)
0	64·3	28·5	39·3 (91)
1·0	63·0	26·1	20·7 (58)
10·0	56·2	24·6	7·8 (77)

DISCUSSION

The mean value of 1.80 ml./100 g/min obtained for oxygen consumption of control new-born rats at the near-neutral temperature of 38° C agrees well with observations reported in the recent literature by other workers (Table 4). This, together with the small scatter and absence of seasonal change, provides grounds for confidence in the described technique, within its limitations, which might be included among tests to be used for the investigation of drugs administered at the time of childbirth.

TABLE 4

RECENT DETERMINATIONS OF OXYGEN CONSUMPTION IN THE NEW-BORN

Author	Ambient temp (°C)	Age of rat	O_2/100 g/min at s.t.p.
Kibler & Brody (1942)	30·0	24 hr	3·96
Fairfield (1948)	35·0	2–3 days	2·1–3·6
Gelineo & Gelineo (1951)	35·9	3–5 hr	1·90
Taylor (1960)	38·0	4 hr	1·97
Present study	38·0	½ hr	1·80

The oxygen consumption of new-born from mothers treated with morphine, methadone or etorphine was reduced in proportion to the maternal dose level of narcotic, over a limited range, but no explanation can be offered for the apparently significant change

in slope of the morphine response line at high doses (Fig. 2). The maternal respiratory rate was similarly decreased. However, qualitative as well as quantitative differences were discernible between the drugs. Thus, while morphine and methadone treatment led to severe reduction of oxygen consumption in the young at a dose level which was analgesic for all mothers, an equianalgesic dose of etorphine seems to have less effect on the new-born. This result presents something of an enigma in the light of the known potent respiratory depressant properties of etorphine in adult animals (Blane *et al.*, 1967) and its 3-acetyl derivative M183 in man (Campbell, Lister & McNicol, 1964). In the present study, doses of etorphine only slightly in excess of the analgesic ED80 (6 μg/kg) provoked a greater than 60% reduction in maternal respiratory rate, whereas the response to the other analgesics reached a maximum between 40 and 50% reduction (Fig. 3).

Before discussing further the results obtained with etorphine, it is relevant to outline the mechanisms by which drugs of this type might reduce oxygen consumption in the new-born rat. There are at least three possibilities:

(1) That the drug has so affected maternal respiration, or placental gas exchange, or both, that the capacity of the infant to maintain a normal metabolic rate after delivery has been seriously impaired.

(2) That the drug, acting directly upon the infant, has reduced its metabolic rate either by reducing muscular tone—for example, as in anaesthesia—or by some other cellular mechanism.

(3) That the drug may have acted directly on the infant to reduce O_2 transport either by an action on respiratory movements or on the circulation.

It has been shown by radioisotope techniques that there is rapid transport of etorphine from mother to foetus with selective uptake into brain tissue and these observations form the subject of a separate report (Blane & Dobbs, 1967). However, the effect of etorphine on the new-born rat itself has been found to be relatively harmless, for when the drug is injected intraperitoneally to new-born maintained at 38° C, the LD50 is very high (74 mg/kg).

At maternal dose levels of etorphine substantially above the analgesic ED80, some animals died within an hour after birth and, although some similar activity was seen with methadone, there were no deaths after large doses of morphine despite a severe reduction of neonatal oxygen consumption, nor did doses of maternally administered pentobarbitone, which were equally depressant on oxygen consumption of the new-born, cause any deaths.

The efficacy of pretreatment of mothers with atropine in reducing the numbers of young dying after etorphine treatment presents a second paradox, since neither the reduction of oxygen consumption in the new-born nor the severe decrease in maternal respiratory rate (see above) was alleviated. Thus, although maternal respiratory depression after etorphine undoubtedly weakened the foetuses by partial asphyxia, there was some other factor involved in the cause of post-natal deaths. This hypothesis is supported by the long-established fact that the foetus is peculiarly resistant to intrauterine anoxia (Harvey, 1651). The existing evidence does not permit an unequivocal answer, but it seems possible that the vagally induced maternal bradycardia, which is provoked by large doses of etorphine (Blane *et al.*, 1967) and is blocked by atropine, may tip the

delicate balance of foetal life near the time of birth so that, although the young do not die *in utero*, a proportion are so weakened that they are unable to survive the trauma of birth, and readjustment to the extrauterine environment, for as long as 1 hr. Such readjustment, including the initiation of independent respiration, will in any case be seriously hampered by the presence in the brain of the new-born of high concentrations of etorphine, as indicated by the radioisotope study.

The absence of reduction of oxygen consumption in the new-born in these experiments after even large maternal doses of pethidine strengthens the clinical impression that this is a relatively good drug in obstetrics (see Little & Tovell, 1949 ; Shnider & Moya, 1964). However, the safety factor is clearly overrated in the test situation described here since, in the new-born child, pethidine is well known to cause depression, even though this may be less severe than that seen after equianalgesic doses of other less commonly used obstetric analgesics such as morphine or diamorphine.

The results with pethidine emphasize the inadequate predictive value of the test with reference to safety in man. Thus, absence of a serious reduction in neonatal oxygen consumption in rats under the conditions described might be taken as a favourable indication for further investigations, but it would be incautious to draw any more sweeping conclusions. Consideration must be given, not only to the species difference, but also to the fact that observations of the type recorded here are made on mature new-born animals in good condition. In clinical practice account would need to be taken of the effects of drugs on, for example, prematurely delivered infants and infants already suffering from respiratory distress. Other important factors include the timing and number of drug injections relative to the time of delivery. On the other hand, where new drugs are found to produce severe adverse reactions in the test described here, there is a clear risk of potential danger to the young of other species.

SUMMARY

1. A simple manometric apparatus is described for the measurement of oxygen consumption in new-born rats.

2. Rats delivered by Caesarian section from 21-day pregnant mothers and incubated immediately at 38° C consumed oxygen at the mean rate of 1.80 ml./100 g/min, corrected to s.t.p.

3. Although morphine, methadone, etorphine and pethidine all reduced maternal respiratory rates this effect was most severe after etorphine. By contrast, normal analgesic doses of etorphine produced less depression of oxygen consumption in the new-born than did equianalgesic maternal doses of morphine or methadone.

4. Nevertheless, larger doses of etorphine caused some deaths in the new-born, an effect not observed with even severely depressed young delivered from mothers treated with large amounts of morphine. A few young also died where the mothers had received large doses of methadone.

5. The young delivered from mothers treated with atropine before administration of large doses of etorphine did not die, although their reduced oxygen consumption was unaffected. Largely on this evidence a hypothesis is presented to suggest that an important factor in the observed toxicity might be cholinergically mediated derangement of

the maternal cardiovascular system as an additional insult to the anoxic infant rat *in utero*.

6. Large maternal doses of pethidine did not reduce oxygen consumption in young rats. This supports the known clinical usefulness of the drug but implies a degree of safety for the infant which is not completely borne out in practical obstetrics. For this, and other reasons discussed, results obtained in the rat should be interpreted with caution in respect of their predictive value for the analogous human situation.

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