The Effect of PaCO₂ on the Metabolism of Ischemic Brain in Squirrel Monkeys

John D. Michenfelder, M.D.,* and Thorolf M. Sundt, Jr., M.D.†

Cerebral ATP, lactate, and pyruvate concentrations were measured bilaterally in 21 squirrel monkeys 2 hours after occlusion of the right middle cerebral artery (MCA). All animals were mechanically hyperventilated beginning 30 minutes after MCA occlusion. Effects of addition of various concentrations of CO₂ to inspired gases were studied in three groups (seven animals in each): 1) hypocapnic (PaCO₂ = 20 mm Hg); 2) normocapnic (PaCO₂ = 40 mm Hg); 3) hypercapnic (PaCO₂ = 60 mm Hg). Results in these animals were compared with those obtained in six monkeys breathing room air spontaneously during the 2-hour occlusion period (PaCO₂ ≈ 30 mm Hg). In the hypocapnic animals, cerebral ATP was significantly less and cerebral lactate significantly greater than corresponding values in the hypercapnic and spontaneously breathing monkeys. We conclude that hypocapnia induced by mechanical hyperventilation does not improve, but rather aggravates, the cerebral metabolic effects of ischemia induced by MCA occlusion. Three possible mechanisms are suggested to explain the deleterious effects of hypocapnia: the effect of pH on glycolytic activity, the direct effect of CO₂ on cerebral blood flow, and the systemic effects of hypocapnia and mechanical hyperventilation. (Key words: Cerebral ATP, lactate, pyruvate; Cerebral ischemia and CO₂ tensions.)

Recent studies of animals and man have led some to believe that the management of patients with evidence of acute cerebral ischemia (for example, stroke) should include the induction of hypocapnia by mechanical hyperventilation.1-4 The rationale for this is based upon two assumptions: first, that cerebral lactic acidosis, known to occur during ischemia, may be decreased by inducing respiratory alkalosis and, second, that in ischemic brain, CO₂ may have a paradoxical effect on cerebral blood flow (CBF), an effect sporadically documented to occur in both patients and laboratory animals. A major stimulus to the use of mechanical hyperventilation was provided in 1968 by Soloway and associates,5 who demonstrated that in dogs hypocapnia dramatically diminished the size of the cerebral infarction which occurs following occlusion of a middle cerebral artery (MCA). In a subsequent study in rhesus monkeys,6 however, they were unable to reproduce these results, presumably because in these experiments hypocapnia was induced 1 hour after MCA occlusion, rather than simultaneously with occlusion. The present study offers a different approach to evaluating the effects of CO₂ on ischemic brain by comparing, at different CO₂ tensions, the cerebral ATP and lactate concentrations 2 hours after MCA occlusion in the squirrel monkey. The animal preparation and biochemical determinations used in this study have been the basis for a number of investigations in this laboratory; we believe that they constitute a useful model for examining the effects of focal and incomplete cerebral ischemia.

Material and Methods

Each unmedicated squirrel monkey (Saimiri sciuereus; weight, 800 to 1,500 g) was anesthetized with intraperitoneally administered sodium pentobarbital (15 mg/kg), placed prone, covered with a heating blanket, and the head fixed in a headrest. The scalp and muscle flaps were reflected bilaterally with an electro-surgical unit. The right middle cerebral artery was occluded by a miniature Mayfield clip; this was done through an intraorbital extradural approach that avoids retraction or manipulation of the
brain, with the aid of an operating microscope. Bilateral frontoparietal craniectomies were next completed without violation of the dura, utilizing a micropneumatic air drill and operating microscope. After the animal had been repositioned, a tracheostomy was fashioned, and a catheter was inserted in a femoral artery for measurement of blood pressure and collection of blood samples.

Thirty minutes after occlusion of the MCA, the tracheostomy tube was attached to a ventilator (Harvard pump), muscle paralysis was produced by succinylcholine (5 mg, intramuscularly, at 20- to 30-minute intervals), and ventilation was controlled with 30 per cent oxygen and nitrogen. In every animal the minute volume was sufficient to produce a $P_aCO_2$ of approximately 20 mm Hg. In some studies, $CO_2$ was introduced simultaneously into the inspired gases in various concentrations to produce $P_aCO_2$'s approximating either 40 or 60 mm Hg. The animals were thus divided into three groups (seven monkeys in each): hypocapnic ($P_aCO_2$—20 mm Hg), normocapnic ($P_aCO_2$—40 mm Hg), and hypercapnic ($P_aCO_2$—60 mm Hg). Thereafter, $P_aCO_2$, $P_aO_2$, and pH (electrodes, 37°C) were monitored by intermittent blood sampling. Arterial blood pressure (strain gauge) and brain temperature (parietal epidural thermistor) were continuously monitored. Blood loss during and following surgery was usually less than 5 ml, but in some animals fresh heparinized squirrel monkey blood was transfused as needed to maintain mean arterial pressure (MAP) above 70 mm Hg.

Ninety minutes after initiation of controlled ventilation (2 hours after MCA occlusion) final blood samples were obtained for determination of blood gases, hematocrit, lactate, and pyruvate. The dura overlying both exposed hemispheres was then excised and bilateral cerebral biopsies were obtained simultaneously, using the technique described by Kramer and associates.8 (With this method, a 100- to 400-mg core of brain is obtained and deposited in liquid nitrogen within 1 second.) The biopsies from both hemispheres were taken from the central area of brain previously shown to develop ischemic changes (or infarction) following MCA occlusion.9 Immediately preceding removal of the biopsy specimens, tagged $^{51}Cr$ erythrocytes were injected intra-arterially, and following biopsy a blood sample was obtained for determination of whole-blood radioactivity. The animal was then killed.

In studies of three additional animals, the protocol was identical, except that succinylcholine was not administered and spontaneous ventilation (room air) was permitted throughout the entire 2-hour period preceding cerebral biopsy.

The method for preparing the frozen brain tissue for determination of ATP, lactate, and pyruvate concentrations has been described.10,11 ATP concentrations were determined by the fiery luminescence method,10,12,13 and lactate and pyruvate concentrations were determined by standard enzymatic methods.14 In addition, the radioactivity of the brain tissue was determined, and this, with determination of blood ATP, lactate, and pyruvate concentrations, permitted correction for the error introduced into determinations of cerebral values by the variation in the amounts of blood present in the biopsy specimens (usually less than 5 per cent). Possible error introduced by variation in degrees of edema in biopsy specimens cannot be excluded but, on the basis of previous measurements, such error is considered insignificant.15

Significance of differences between values in the various groups was tested by Student's $t$ test for unpaired data using Dunnett's table of $t$ values for multiple comparisons ($P < 0.05$ considered significant). Regression equations were calculated by the method of least squares.

Results

Blood-Gas Values

Controlled-ventilation Groups. Immediately preceding cerebral biopsy, the blood-gas values in the three main groups differed significantly in accordance with the protocol of the study (table 1). The magnitudes of alkalosis and acidosis in the hypocapnic and hypercapnic monkeys, respectively, were, as anticipated, related to the differences in $P_aCO_2$. Buffer base values in all three groups were in the low-normal to below-normal range. Mean $P_aO_2$ values were similar. The blood lactate level in the hypocapnic group was significantly greater than that in the other monkeys, in which ventilation was controlled.
**Table 1. Prebiopsy Blood Values, MAP, and Brain Temperature (Mean ± SE)**

<table>
<thead>
<tr>
<th></th>
<th>Controlled Ventilation</th>
<th>Spontaneous Ventilation (N = 6)</th>
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<tbody>
<tr>
<td></td>
<td>Hypocapnia (N = 7)</td>
<td>Normocapnia (N = 7)</td>
</tr>
<tr>
<td>PaCO$_2$, mm Hg</td>
<td>20 ± 1*</td>
<td>40 ± 2*</td>
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<tr>
<td>PAO$_2$, mm Hg</td>
<td>168 ± 15</td>
<td>166 ± 14</td>
</tr>
<tr>
<td>pH</td>
<td>7.52 ± 0.02</td>
<td>7.37 ± 0.02*</td>
</tr>
<tr>
<td>Buffer base, mEq/l</td>
<td>45 ± 1</td>
<td>47 ± 2</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>89 ± 4</td>
<td>96 ± 7</td>
</tr>
<tr>
<td>Temperature, °C (brain)</td>
<td>37.0 ± 0.1</td>
<td>37.0 ± 0.2</td>
</tr>
<tr>
<td>Hct, per cent</td>
<td>45 ± 2</td>
<td>49 ± 3</td>
</tr>
<tr>
<td>Lactate, μmole/ml</td>
<td>2.17 ± 0.20*</td>
<td>1.05 ± 0.18</td>
</tr>
<tr>
<td>Pyruvate, μmole/ml</td>
<td>0.16 ± 0.03</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>L/P ratio</td>
<td>15 ± 2</td>
<td>11 ± 1</td>
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* Significantly different from all other values of the same variable, $P < 0.05$.

**Spontaneous-ventilation Group.** Blood-gas values of the controlled-ventilation groups were compared with those in six monkeys breathing room air spontaneously (three monkeys from the present study; three monkeys from a previous study$^{11}$). In these six monkeys, “normocapnia”* was not observed; instead, PaCO$_2$ values of approximately 30 mm Hg were consistently achieved (fig. 1). Buffer base values in these animals were also significantly higher than those in the controlled-ventilation groups. The significantly lower PaCO$_2$ is accounted for by the differences in minute ventilation and inspired oxygen concentration. Other differences in mean values were not significant.

**Cerebral ATP and Lactate Concentrations**

In the control cerebral hemispheres (that is, MCA not occluded) cerebral ATP concentrations did not differ among the three controlled-ventilation groups or in the spontaneously breathing group (mean values, table 2). The mean values in all four groups were approximately 5 per cent lower than those previously reported$^{11, 12}$ but this difference is not statistically significant. Cerebral lactate concentrations (control hemisphere) in the controlled-ventilation groups tended to be greater than those in the monkeys breathing spontaneously and greater than those previously reported$^{11, 15}$.

In two of the controlled-ventilation groups (hypocapnic and hypercapnic) these differences were significant. Cerebral pyruvate concentrations (control hemisphere) did not differ significantly among the groups, and, therefore, differences in lactate/pyruvate (L/P) ratios paralleled lactate concentration differences but were not significant.

In the cerebral hemispheres with occluded MCA’s large differences among cerebral concentrations of ATP and lactate were observed in the four groups (table 3; figs. 1 and 2). Mean ATP concentrations were lower in all of the controlled-ventilation groups than in the group breathing spontaneously; in the hypoxic group this difference was significant. The hypocapnic ATP concentrations were also significantly lower than those observed in the hypercapnic monkeys. Differences in cerebral lactate concentrations were striking. In the hypocapnic and normocapnic monkeys, the mean cerebral lactate concentrations were similar and almost twice those in the hypercapnic and spontaneously breathing monkeys. Since the pyruvate concentrations did not differ significantly, the large differences in L/P ratios reflected the lactate differences only.

The suggested direct and inverse correlations, respectively, between MAP and mean ATP and lactate concentrations in the MCA-occluded hemisphere were examined by plotting the individual values (figs. 3 and 4). In the controlled-ventilation groups, there was an apparent relationship; there was no relation-

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* To our knowledge, normal PaCO$_2$ of the resting, awake squirrel monkey is unknown.
TABLE 2. Control Cerebral Hemisphere: Cerebral ATP, Lactate, and Pyruvate Values (Mean ± SE)

<table>
<thead>
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<tr>
<td></td>
<td>Hypocapnia (N = 7)</td>
<td>Normocapnia (N = 7)</td>
</tr>
<tr>
<td>ATP, μmole/g</td>
<td>1.87 ± 0.08</td>
<td>1.89 ± 0.08</td>
</tr>
<tr>
<td>Lactate, μmole/g</td>
<td>3.58 ± 0.39*</td>
<td>2.89 ± 0.57</td>
</tr>
<tr>
<td>Pyruvate, μmole/g</td>
<td>0.26 ± 0.05</td>
<td>0.18 ± 0.04</td>
</tr>
<tr>
<td>L/P ratio</td>
<td>21 ± 8</td>
<td>15 ± 4</td>
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<td></td>
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<td>14 ± 2</td>
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<td></td>
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<td>11 ± 4</td>
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*Significantly different from spontaneous ventilation values, P < 0.05.

TABLE 3. MCA-occluded Cerebral Hemisphere: Cerebral ATP, Lactate, and Pyruvate Values (Mean ± SE)

<table>
<thead>
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<tr>
<td></td>
<td>Hypocapnia (N = 7)</td>
<td>Normocapnia (N = 7)</td>
</tr>
<tr>
<td>ATP, μmole/g</td>
<td>0.41 ± 0.11*</td>
<td>0.68 ± 0.14</td>
</tr>
<tr>
<td>Lactate, μmole/g</td>
<td>19.50 ± 2.12*</td>
<td>19.24 ± 2.23*</td>
</tr>
<tr>
<td>Pyruvate, μmole/g</td>
<td>0.17 ± 0.03</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>L/P ratio</td>
<td>135 ± 29*</td>
<td>124 ± 23*</td>
</tr>
</tbody>
</table>

*Significantly different from hypocapnic and spontaneous ventilation values, P < 0.05.

**Fig. 1.** Effect of PaCO2 on cerebral ATP concentration 2 hours after MCA occlusion. Individual values observed in mechanically ventilated monkeys at different PaCO2 levels are compared with values observed in monkeys breathing spontaneously. Hypocapnia (PaCO2 = 16 to 22 mm Hg) resulted in significantly lower ATP concentrations, whereas normocapnia (PaCO2 = 36 to 49 mm Hg) and hypercapnia (PaCO2 = 56 to 67 mm Hg) did not significantly alter ATP concentrations.
ship in the spontaneously breathing group (not plotted).

Discussion

Effects of CO$_2$ on Cerebral Ischemia

A wealth of information about cerebral blood flow and metabolism during both normal and abnormal circumstances has accumulated in recent years. Many investigations have been concerned with the pathophysiology of focal cerebral ischemia leading to the focal infarction seen in stroke patients. In several areas, information gained from such experimental studies has been applied to clinical situations, with varying degrees of success. Of particular interest to both investigators and clinicians has been the mechanism whereby CO$_2$ alters cerebral vascular resistance, how this effect is altered in the presence of cerebral ischemia, and ultimately how cerebral metabolism (and hence, function) is thus affected.

Recognition that cerebral ischemia, no matter how induced, results in cellular lactic acidosis has caused some 1,2,4 to speculate that it is the progressive acidosis itself that ultimately interferes with the still-functioning cellular metabolic processes and so initiates a vicious circle of cellular self-destruction. This consideration and the demonstration that, in certain pathologic circumstances, CO$_2$ may have a paradoxical effect on blood flow to ischemic areas of brain 16,17 have led some 3,4 to recommend that patients with clinical evidence of cerebral ischemia be made hypocapnic by mechanically controlled hyperventilation.

Proponents of this approach argue that the hypocapnic state will, by producing respiratory alkalosis, relieve the cerebral acidosis and, at the same time, possibly improve cerebral blood flow to ischemic areas by a paradoxical effect. Supportive experimental evidence was provided by the study of Soloway and associates,5 who showed that, in dogs, hypocapnia induced at the time of occlusion of one MCA strikingly decreased the size of the cerebral infarction 2 hours later compared with that produced in normocapnic dogs. However, Soloway's group later reported 6 that, in rhesus monkeys, hypocapnia induced 1 hour after MCA occlusion had no ultimate effect on the size of the infarction. They concluded that their initial work should be interpreted carefully and suggested that a critical period existed very early in the development of a cerebral infarction when hypocapnia might be effective. In clinical practice, several workers 7,18 have reported their early findings in patients treated with hypocapnia, and although a meaningful statistical analysis of the collected data is not possible, initial impressions generally have been favorable. More recent clinical studies have been less optimistic.19,20

The present study was designed to examine the metabolic effects of cerebral ischemia at different CO$_2$ concentrations, using tissue ATP and lactate concentrations as indicators of cel-
lular energy reserve and acidosis, respectively. The experimental model for this study has long been studied in these laboratories, providing a solid base from which, it is hoped, meaningful conclusions may be drawn. Previous studies with this preparation have demonstrated that permanent MCA occlusion in the squirrel monkey (as opposed to many other species) produces a relatively consistent area of infarction, similar in distribution to that which occurs in man. If, however, the MCA flow is re-established within 2 hours following occlusion, almost all animals survive without infarction or compromised cerebral function, and only after 4 hours of occlusion does death or infarction predictably occur. Studies of CBF during the initial 2-hour period of occlusion have demonstrated that flow in the eventual area of infarction is reduced to only about 40 per cent of normal and is relatively stable. Metabolic studies have shown that cerebral ATP concentrations decrease (and those of lactate increase) slowly during the 4-hour period of occlusion and, at 2 hours, ATP concentration is approximately 50 per cent of normal (following decapitation, canine cerebral ATP concentration reaches 25 per cent of normal in 4 minutes). Furthermore, the decrease in ATP concentration is paralleled by a progressive decrease in EEG activity; when the concentration of ATP is 50 per cent of normal or less, there may be almost electrical silence. Finally, it has been shown that the progressive decrease in ATP concentration (and increase in lactate concentration) can be reversed by re-establishing MCA flow within 4 hours of the time of occlusion. With this background of data, it is reasonable to assume that any therapeutic measure that is effective in the management of focal cerebral ischemia should alter these metabolic changes favorably, at least during the initial 2 hours of occlusion—a period during which the ischemic, metabolic, and functional changes are totally reversible by the re-establishment of flow.

From the results of this study we conclude that, compared with the situation in squirrel monkeys breathing spontaneously, hypocapnia has no beneficial effect on cerebral metabolic processes compromised by a 2-hour period of ischemia from MCA occlusion. Indeed, we must conclude the opposite: that hypocapnia in these circumstances magnifies the deleterious metabolic effects of ischemia by further significantly decreasing energy reserves and increasing lactic acidosis.

Possible Factors Affecting Cerebral Metabolic Changes

In addition to this conclusion, the data justify speculation about various other aspects of the effect of CO₂ on cerebral metabolism during ischemia. The assumption that change in blood [H⁺] might similarly change cerebral [H⁺] (or at least the degree of cerebral lactic acidosis) appears to be unwarranted. Again, the opposite seems to be the case: at a pH of 7.52 (hypocapnia), lactate concentration in the brain was more than twice that at a pH of 7.26 (hypercapnia). This apparent paradox may be related in part to the effect of pH on anaerobic glycolysis. In vitro studies have demonstrated that alkalosis stimulates glycolytic activity of the brain, whereas acidosis has an opposite effect. Thus, the large difference between lactate concentrations in hypocapnic and hypercapnic monkeys could be explained by such an effect of pH. However, this would leave unexplained the differences between ATP concentrations in these two groups, as well as the increased lactate concentrations observed in the normocapnic group (pH 7.37). And if alkalosis accounts for the cerebral lactic acidosis in the hypocapnic monkeys, then a similar degree of lactic acidosis should have been seen in the monkeys breathing spontaneously (pH 7.52). For these reasons, it seems unlikely that a pH effect is the primary explanation for the observed differences. Rather, it is reasonable to assume that a paradoxical flow response to CO₂ did not occur (as has been recently demonstrated by others) and that hypocapnia further restricted blood flow to the MCA-occluded hemisphere, while hypercapnia at least maintained the postocclusion flow.

These considerations cannot explain all the differences recorded. In the normocapnic monkeys, the degree of cerebral lactic acidosis was similar to that in the hypocapnic group; the ATP concentrations tended to be lower in the normocapnic group than in the groups of hypercapnic and spontaneously-breathing monkeys. This may be explained by the possible deleterious effects of mechanical hyperventila-
Fig. 3. Relationship of cerebral ATP concentrations to MAP 2 hours after MCA occlusion. In mechanically ventilated monkeys, a direct correlation is suggested.

Fig. 4. Relationship of cerebral lactate concentrations to MAP 2 hours after MCA occlusion. An inverse correlation exists.

tion on perfusion pressure and CBF. Since all of the controlled-ventilation groups were ventilated using similar minute volumes (sufficient to produce a $\text{P}_{\text{aCO}_2}$ of 20 mm Hg without the addition of $\text{CO}_2$ to the inspired gases), such an effect would be operative in all. Evidence supporting the factor of decreased systemic blood flow secondary to mechanical hyperventilation includes lower MAP's (not significant) in the controlled-ventilation groups compared with the group breathing spontaneously and significantly lower buffer base values in the controlled-ventilation groups. Evidence supporting decreased CBF secondary to mechanical hyperventilation as a factor includes the tendency for increased cerebral lactate in the control hemisphere of the controlled-ventilation groups compared with monkeys breathing spontaneously (significant in the hypocapnic and hypercapnic groups) and insignificantly higher L/P ratios in the controlled-ventilation groups.
A systemic effect of carbon dioxide itself is also suggested by the correlation of MAP with ATP and lactate concentrations in the MCA-occluded hemispheres of the mechanically ventilated monkeys. Although MAP in every animal was above 70 mm Hg, the finding that animals with MAP's between 70 and 100 mm Hg tended to have lower ATP and higher lactate concentrations than did animals with MAP's above 100 mm Hg indicated some relationship. A direct relationship between $P_a CO_2$ and cardiac output has been demonstrated. Presumably, in hypocapnic animals this effect contributed to a reduction in perfusion pressure; since autoregulation is impaired or absent in the MCA-occluded hemisphere, a further decrease in ATP concentration and increase in lactate concentration may have occurred. However, the absence of any correlation between MAP, ATP, and lactate in the spontaneously breathing monkeys suggests that this effect was of minimal importance in explaining our results.

The possible influence of pentobarbital on the metabolism of ischemic brain is unknown, but any such effect should have been the same in all animals. In normal brain, anesthetics have no influence on ATP and lactate levels, and in totally anoxic brain anesthetics do not alter rates of ATP depletion and lactate accumulation.

Combining all of these factors, it would appear that the cerebral metabolic changes in the hypocapnic group could be accounted for by a combination of these factors: a pH effect, a direct effect of CO$_2$ on CBF, an indirect systemic effect on CBF secondary to mechanical ventilation, and an indirect systemic effect on CBF secondary to CO$_2$ levels. In the normocapnic animals, the increased cerebral lactate concentration can be explained only by the systemic effects of mechanical ventilation. In the hypercapnic animals, the systemic effects were either countered by the direct effect of CO$_2$ on CBF or, because of a higher MAP, CBF was increased in ischemic areas due to loss of autoregulation. Also, the accumulation of lactate may have been attenuated by a pH effect on glycolysis.

The suggestion by Soloway and colleagues that early in the postocclusion phase there is a critical period during which the induction of hypocapnia is beneficial cannot be ruled out by this study, but this explanation seems unlikely. If such a critical period exists, it must be within the initial 30 minutes following MCA occlusion. We previously demonstrated that a rapid increase in cerebral lactate concentration does occur in the initial 30 minutes, compared with the rate of lactate accumulation thereafter. Otherwise, the postocclusion cerebral metabolic and functional changes progress gradually during a period of several hours and are potentially totally reversible with restoration of flow if the occlusion time has not exceeded 2 hours. That the studies of Soloway and his group were done in two different species might explain their different results, rather than would a critical time effect. The initial studies, demonstrating a protective effect of hypocapnia, were done in dogs. The notoriously complex and profuse collateral arterial circulation to the brain of the dog may well be altered favorably by hypocapnia in the event of cerebral ischemia. In the monkey, the cerebral circulation more closely resembles that of man, and hypocapnia may at no time have a beneficial effect. In any event, even if a brief critical period does exist, the opportunity to induce hypocapnia within 30 minutes of an ischemic event would rarely arise under clinical conditions.

References


