The problems of acidosis

GABRIEL G. NAHAS
From the Department of Anesthesiology, College of Physicians and Surgeons, Columbia University, New York, USA

After trauma the failure of various organ functions tends to disturb acid-base equilibrium and produce acidaemia. Circulatory failure produces stagnation of blood in tissues and pulmonary failure, impairment of CO₂ elimination and O₂ uptake. Liver failure impairs the metabolic conversion of acid metabolites such as lactate or ketones into oxidizable substances and kidney failure impairs the excretion of acid metabolites. It is evident that the degree of acidosis during shock will be a function of the severity of the impairment of organ function, mostly of the heart and lungs.

The physiological effects of acidaemia are well established. It depresses the functional activity of all vital organs. The cardiovascular system is primarily affected (Clowes, Sabga, Konitaxis, Tomin, Hughes, and Simeone, 1961). Both cardiac conduction and the contractile force of the myocardium are depressed and there is vaso-dilatation in the peripheral circulation and cerebral vessels. By contrast, vasoconstriction develops in the lung vasculature. In the liver enzymatic activity is generally depressed. Acidaemia has a thromboplastic effect and increases coagulation, contributing to intravascular clotting, as described by Hardaway (1966).

It is also known that acidosis decreases catecholamine activity (Burget and Visscher, 1927). Adrenaline and noradrenaline, at acid pH, influence heart rate and blood pressure less than at normal pH. It should, however, be remembered that acidosis is also a profound stimulus to catecholamine production (Nahas, Zagury, Milhau, Manger, and Pappas, 1967). These two antagonistic effects of acidosis result in a new, unstable condition which will vary from one individual to the next and will depend upon the degree of sympatho-adrenal stimulation and the degree of inhibition of catecholamine activity produced by a given level of acidosis. In the present paper I will describe primarily the effects of acidosis on catecholamine activity and some steps of intermediary metabolism.

I Effects of Acidaemia on Adrenal Function

Fenn and Asano reported in 1956 that hypercapnia was accompanied by an increase in sympatho-adrenal activity in the cat. Subsequently Tenney (1956) showed that CO₂ serves as a potent stimulus to increase the titre of circulating sympatho-adrenal catecholamines in the cat. In 1960 we attempted to quantitate these changes. Hypercapnic acidosis was produced in dogs by the process of 'apnoic oxygenation' and arterial blood pH was decreased to 7.0 and PaCO₂ increased to about 100 mm Hg (Nahas, Ligou, and Mehlman, 1960). Under these conditions, plasma concentrations of adrenaline and noradrenaline increased, while oxygen uptake did not, although an increase in catecholamine is usually accompanied by increased oxygen uptake (Steinberg, Nestel, Buskirk, and Thompson, 1964). Furthermore, mean blood pressure did not change much in these animals (Fig. 1). However, when the acidosis was corrected there was a sudden increase in oxygen uptake and blood pressure. These observations were interpreted as indicating that correction of acidosis restored a normal hydrogen-ion concentration and catecholamines exerted their optimal metabolic activity.

Similar observations were made on animals ventilated with 10% CO₂ and 30% O₂ so that arterial pH fell to 7.0. There was no increase in oxygen uptake and no increase in non-esterified fatty acids (FFA) or glycerol concentrations in plasma. When pH was restored to normal, there were marked increases in FFA and glycerol con-
Catecholamines and pressure
Arterial CO₂

Fig. 1 Effects of a rapid restoration of an acid blood pH to normal on \(QO_2\), blood pressure, and plasma catecholamine levels. Mean of three experiments. (From Nahas et al., 1960; US Army photograph ANW50.)

It averaged 70 ng/gland/min. Adrenal catecholamine output was increased by 100% following perfusion with hypercapnic mixtures at a pH of 6.96 to 7.10 (\(PCO_2\) 70-118 mm Hg) and 660% with a mixture at a pH of 6.79 to 6.92 (\(PCO_2\) 125-210 mm Hg). This increase was primarily due to a rise in adrenaline concentration. Similar results were obtained following perfusion with media made acid by the addition of lactic acid. These results indicate that increases in \([H^+]\) directly stimulate adrenal medullary secretion.

It was also observed (Mittelman, Dos, Barker, and Nahas, 1962) that hypercapnic acidosis in the dog significantly increased cortisol output. The physiological importance of catecholamine release during hypercapnia was demonstrated by observations (Nahas and Cavert, 1957) which showed that giving adrenaline or noradrenaline reversed or prevented the acute myocardial failure produced by respiratory acidosis which was first observed by Jerusalem and Starling (1910) in the heart-lung preparation (Fig. 5).

The increased catecholamine output of acidemia was related to an augmentation of catecholamine synthesis in rats (Nahas and Steinsland, 1968). It was first observed that exposure to hypercapnia was not accompanied by significant depletion of catecholamine stores: in a group of rats exposed to 20% \(CO_2\), 25% \(O_2\), balance \(N_2\), for periods of up to five hours, there were no significant changes in the noradrenaline content of the heart, salivary glands and brain and the catecholamine content of the adrenal gland compared with a control group breathing air. Another group of rats was given intraperitoneally 300 mg/kg of L-\(\alpha\)-methyl-p-tyrosine in three divided doses before a similar period of hypercapnia. They presented a 37-7% decrease in the noradrenaline content of the heart and a 34-3% decrease in the catecholamine content of the adrenals (Fig. 6). Rats treated with L-\(\alpha\)-methyl-p-tyrosine and breathing room air did not present significant changes in the catecholamine content of heart or adrenal gland. Forty hours after the administration of dopa-\(3^H\), exposure to hypercapnia significantly decreased specific activity of catecholamines in the adrenal gland. Conversion of tyrosine-\(3^H\) to labelled catecholamines was also increased in rats during hypercapnia. These results indicate that the rate of catecholamine synthesis is increased during hypercapnia and enables the animal to sustain the elevated catecholamine release required to maintain vital physiological functions.

Lotspeich (1967) has reported that metabolic acidosis produces in the rat an increase in glutaminase enzymes and in the hexose monophosphate shunt enzymes. Kidney hypertrophy is also present and can be explained by an incorporation of the glutamine carbon skeleton into renal tissue components. When protein synthesis was blocked by actinomycin D, the hypertrophy of metabolic acidosis did not occur. This led Lotspeich to postulate that the increased \([H^+]\) of NH₄Cl...
The problems of acidosis

![Graph showing changes in pH, GLYCEROL (μmol/l), F.F.A. (meq/l), GLUCOSE (mg%), and B.P. (mm Hg) over time.]

**Fig. 2** Changes in oxygen consumption \((VO_2)\) and plasma free fatty acids, glycerol, glucose, mean blood pressure, and pulse rate in six dogs during and after a 60-min period of hypercapnic acidosis induced by ventilation (10% CO₂, 25% O₂, balance N₂). (From Poyart and Nahas, 1966.)

![Diagram of the apparatus used for the perfusion of the isolated adrenal gland.](http://jcp.bmj.com/)

**Fig. 3** Diagram illustrating the apparatus used for the perfusion of the isolated adrenal gland. Perfusing medium is kept under pressure in two flasks by means of a gas mixture. Pressure is recorded by means of a Hg manometer. Perfusing medium is made to pass through a water bath which is maintained at the required temperature before reaching the gland. (From Nahas et al, 1967.)
Fig. 4  (Left) Electron micrograph of a section of the adrenal medulla. The adrenal was fixed in vivo with buffered glutaraldehyde. Cells are seen between a venule (V) and a sinusoid (S).  (Right) Electron micrograph prepared from an adrenal gland which was perfused in vitro with diluted blood for two hours. Portions of two chromaffin cells are seen around a sinusoid (S). (From Nahas et al, 1967.)

Fig. 5  Selected records taken from a Starling heart-lung preparation. Reversal of acidotic heart failure is obtained by administration of 4 μg/min of adrenaline. (From Nahas and Cavert, 1957.)
acidity might derepress a gene site in kidney cells responsible for protein and enzyme synthesis. In our experiments, the adrenals of hypercapnic rats were not weighed; however, it was previously reported (Schaefer, King, Mego, and Williams, 1955) that, after one hour of exposure to 30% CO₂, the weight of the adrenal gland in guinea pigs was significantly increased, indicating that hypercapnia might also produce a hypertrophy of the adrenals. However, further investigations are required to ascertain if the increased rate of synthesis of adrenal catecholamine is also accompanied by an increased synthesis of the enzymes which convert tyrosine into dopa and dopamine. Acidosis profoundly alters sympatht-adrenal regulation, producing an increase in catecholamine synthesis and release, and at the same time decreasing the metabolic activity of these amines.

II Effects of Acidaemia on the Metabolic Activity of the Catecholamines

During hypercapnia, the increase in enzyme activity which controls catecholamine synthesis contrasts with the inhibitory effect of [H⁺] on enzymes which regulate intermediary metabolism, and which are activated by these catecholamines. This inhibition was observed in vivo as well as in vitro.

EXPERIMENTS IN VIVO

For the experiments in vivo, pedigree beagles were used because they have constant and reliable substrate and acid-base levels (Poyart and Nahas, 1966). They were given a standard dose of noradrenaline or adrenaline (1.5 µg/kg/min for 30 min) while being mechanically ventilated with room air. (Arterial pHa was 7.42 and pCO₂ 30 mm Hg.) A week later, the animals received the same dose of catecholamine, but this time were ventilated with a mixture of 10% CO₂ and 25% O₂ in N₂ so that their average pHa was 7.0 and average PaCO₂ was 100 mm Hg. These results are summarized in Figure 7. Noradrenaline infusion, when ventilation was with room air, produced 25-30% increments in O₂ uptake which persisted for at least an hour after the end of the 30-minute infusion. By contrast, when the same animals were made hypercapnic, a week later, oxygen uptake was not significantly different from the control uptake. Changes in free fatty acid levels with noradrenaline infusion are also shown in Figure 7. There was a marked increase in free fatty acids when pH was maintained at normal. At pH 7.0 the lipolytic activity of noradrenaline was depressed by 70%. Similar alterations in free fatty acids, glucose, and lactic acid blood concentrations with adrenaline infusion are shown in Figure 8. At normal pH, there was a marked increase in free fatty acid and glycerol

Fig. 6 (Top) Adrenal catecholamine (CA) content (µg/kg BW) of rats breathing room air or exposed to acute hypercapnia. The rats received 100 mg/kg body weight of L-a-methyl-p-tyrosine at the 0, sixth, and 12th hours. (Bottom) Heart noradrenaline content (µg/g tissue) of rats breathing room air or exposed to acute hypercapnia. The rats received 100 mg/kg body weight of L-a-methyl-p-tyrosine at the 0, sixth, and 12th hours. (From Nahas and Steinsland, 1966.)

Fig. 7 Changes in oxygen consumption (VO₂) and plasma free fatty acids in six dogs with noradrenaline infusion at normal pH and during hypercapnic acidosis induced by ventilation with a gas mixture of 10% CO₂, 25% O₂, balance N₂. Bars indicate ± 1 SE of the mean. (From Poyart and Nahas, 1966.)
Fig. 8  Changes in oxygen consumption ($V_O^2$) and plasma FFA, glycerol, glucose, and lactic acid (LA) concentrations in dogs receiving adrenaline (E) (1.5 $\mu$g/kg/min) and breathing room air and a hypercapnic mixture (10% $CO_2$, 25% $O_2$, balance $N_2$). Bars represent ± 1 SE of the mean. (From Poyart and Nahas, 1966.)

Fig. 9  Comparative changes in oxygen consumption ($V_O^2$), FFA, and glycerol plasma concentrations produced by noradrenaline (NE) infusion in two groups of dogs: one with normal pH, the other with acid pH produced by $NH_4Cl$ administration. Vertical bars indicate ± 1 SE of the means. Stars indicate that the values are significantly different ($p < 0.05$) from initial control measurements. (From Nahas and Poyart, 1967.)
concentrations; during acidosis, these substances did not increase significantly. Under conditions of normal pH, glucose concentrations rose from 100 to 300 mg% and lactic acid levels also rose. During hypercapnic acidosis, there was still a significant increase in glucose, but no change in lactic acid. This would indicate that while glycolysis does proceed during acidosis, glucose utilization might be impaired.

In an additional group of dogs metabolic acidosis was induced by the intravenous administration of ammonium chloride (0·15M) for 48 to 60 hours until pHa was decreased to between 7·05 and 7·15 (Nahas and Poyart, 1967). During the experiment, ventilation was adjusted to maintain pH and PaCO₂ at this level and noradrenaline was then infused for 30 minutes. Before the noradrenaline infusion, blood concentrations of free fatty acids, glycerol, glucose, and lactic acid were the same as at normal pH while VO₂ was slightly higher. No significant increases in VO₂ and free fatty acids occurred following noradrenaline infusion, and the increase in glycerol, although significant, was small and comparable to the change in free fatty acids. As compared with controls, the increase of VO₂ induced by noradrenaline during acidosis was inhibited by 60% free fatty acids by 68% and glycerol by 80% (Fig. 9). There were no significant changes in blood glucose or lactic acid concentrations.

Similar experiments were performed to study the effect of respiratory and metabolic alkalosis on noradrenaline-induced calorigenesis and lipolysis (Nahas and Poyart, 1967). Three groups of dogs were either hyperventilated mechanically or alkalosis was induced by the infusion of THAM (0·3M) or sodium bicarbonate (0·3M). Neither metabolic nor respiratory alkalosis significantly altered control values. When noradrenaline was simultaneously infused with alkali, there was a slight but not significant enhancement of lipolysis. There were no significant differences in changes in blood glucose and lactic acid except for a marked hypoglycaemia while THAM was being infused. When noradrenaline was infused during hyperventilation, the increase in VO₂ was significantly higher than the increase produced by noradrenaline at normal pHa. At the end of noradrenaline infusion during hyperventilation, plasma free acid concentrations were also significantly higher than at normal pHa but there was only a slight change in glycerol (Fig. 10).

Alterations of the metabolic effects of noradrenaline are, therefore, related to changes in [H⁺] rather than to changes in PaCO₂ or [HCO₃⁻]. Acid pH, produced either by hypercapnia or by infusion of NH₄Cl, inhibits to the same extent noradrenaline-induced lipolysis and calorigenesis. On the other hand, a decrease in [H⁺], either by means of hyperventilation or by administration of base, results in higher VO₂ and slightly higher release of free fatty acids. Figure 11 shows the correlation between the increase in VO₂ and the plasma concentration of free fatty acids, the latter being determined by the [H⁺] of the blood at the end of the noradrenaline infusion.

**Experiments in vitro**

This inhibition of activated lipolysis by acid pH was also observed in vitro. Rat epididymal adipose tissue was incubated in Krebs-Ringer phosphate medium with 5% albumin without glucose and with glucagon, ACTH, noradrenaline or cyclic 3',5'-AMP dibutyrate; the pH of the medium was varied from 7·4 to 6.6. Glycerol release was measured and taken as the index of lipolytic activity. In a first series of experiments at pH 7·4, noradrenaline, ACTH, and glucagon-activated lipolysis was potentiated by increasing doses of theophylline. In a second series, with the pH of the medium at 6·6, the lipolytic effects of these three hormones were significantly inhibited. When theophylline (10⁻²M) was added in com-

---

**Fig. 10** Changes in oxygen consumption (VO₂) and FFA and glycerol plasma concentrations produced by noradrenaline (NE) in four dogs at normal pHa (hyperventilation). Each dog was studied twice at a week's interval. (From Nahas and Poyart, 1967.)
bination with optimal doses of the hormones, the rate of glycerol release was similar at normal and acid pH (Fig. 12). These results were interpreted as indicating that H+ might exert its inhibitory effect on a common mechanism which results in cyclic 3′,5′-AMP formation (Triner and Nahas, 1965). This hypothesis was confirmed: when cyclic 3′,5′-AMP dibutyrate (10⁻³M) was added to the medium, the same glycerol release was found at pH 7.4 and 6.6 (Poyart and Nahas, 1968). When combined with noradrenaline, cyclic 3′,5′-AMP dibutyrate also reversed the inhibitory effects of acidosis (Fig. 13). These results, when analysed according to the drug-receptor theory, would indicate that acidosis might inhibit, at least in part, the different lipolytic drugs used in this study by hindering the formation of the drug-receptor complexes which activate lipolysis.

### III Effects of Acidosis on Glucose Metabolism

The effects of variations in extracellular pH on glucose metabolism have been studied in different tissues. In rat liver slices, epididymal fat pad, and diaphragm muscle, a direct relationship was found between the rate of lactate production and the pH of the incubation medium (Gevers and Dowdle, 1963). During acidosis, glucose uptake in human red blood cells was inhibited and lactate production decreased. It was suggested that this inhibiting effect of acidosis was exerted between the hexose phosphate and the triose phosphate stages (Murphy, 1960). Conversely, during alkalosis, accelerated glycolysis and a higher glucose uptake and production of lactate were observed in erythrocytes (Triner, Kypson, Mráz, and Zicha, 1964). In the perfused rat heart Delcher and Shippl (1966) observed increased glucose uptake, lactate production, and glycogen breakdown. The activities of several enzymes are sensitive to changes of pH: Reynolds and Haugaard (1967) observed decreased phosphorylase activity in diaphragm muscle and Trivedi and Danforth (1966) demonstrated a significant fall in the activity of phosphofructokinase from frog skeletal muscle at low pH. The purpose of
the study performed on the rat diaphragm was to investigate further the effect of acid pH on the concentration of some intermediates in the glycolytic pathway in skeletal muscle. At pH 6.8, glucose uptake was significantly decreased (by 34%), while glycogen content was significantly increased over control (+22%) at pH 7.4 (Table I). Both lactate and pyruvate production were significantly decreased (-38% and -20% respectively). In the muscle there was a significant increase in glucose-6-phosphate (G-6-P, +22%) but not in glucose-1-phosphate (G-1-P, +12%) or fructose-6-phosphate (F-6-P, +11%) concentrations while fructose-1,6-diphosphate (F-1,6-P) concentration was significantly lower (-29%) (Table II). These changes indicate that acid pH inhibits in mammalian skeletal muscle phosphofructokinase activity, the rate-limiting step in the glycolytic pathway. There were no significant changes in O2 consumption or citrate concentration in the muscle with acid pH (Table III), indicating that the Krebs cycle and electron transfer through the respiratory chain are not inhibited by this degree of acidosis. Similarly, there was no change in O2 uptake in vivo during hypercapnic or metabolic acidosis.

<table>
<thead>
<tr>
<th>Medium</th>
<th>Krebs-Ringer Phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 7.4</td>
</tr>
<tr>
<td>Glycogen</td>
<td>7.15 ± 0.56</td>
</tr>
<tr>
<td>(μmol glucose/g wet tissue)</td>
<td>(11)</td>
</tr>
<tr>
<td>Glucose uptake</td>
<td>15.36 ± 1.39</td>
</tr>
<tr>
<td>(μmol glucose/g wet tissue)</td>
<td>(14)</td>
</tr>
<tr>
<td>Lactate</td>
<td>31.58 ± 1.26</td>
</tr>
<tr>
<td>(μmol lactate/g wet tissue)</td>
<td>(16)</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>1.41 ± 0.00</td>
</tr>
<tr>
<td>(μmol pyruvate/g wet tissue)</td>
<td>(13)</td>
</tr>
</tbody>
</table>

Table I The effect of acid pH on glycogen content in muscle and on lactate and pyruvate concentrations in medium

<table>
<thead>
<tr>
<th>Medium</th>
<th>Krebs-Ringer Phosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH 7.4</td>
</tr>
<tr>
<td>Glucose-1-phosphate</td>
<td>4.0 ± 0.7</td>
</tr>
<tr>
<td>(μmol/liter) (mg dry tissue)</td>
<td>(14)</td>
</tr>
<tr>
<td>Glucose-6-phosphate</td>
<td>16.1 ± 0.8</td>
</tr>
<tr>
<td>(μmol/liter) (mg dry tissue)</td>
<td>(17)</td>
</tr>
<tr>
<td>Fructose-6-phosphate</td>
<td>4.3 ± 0.3</td>
</tr>
<tr>
<td>(μmol/liter) (mg dry tissue)</td>
<td>(13)</td>
</tr>
<tr>
<td>Fructose-1, 6-diphosphate</td>
<td>7.5 ± 0.5</td>
</tr>
<tr>
<td>(μmol/liter) (mg dry tissue)</td>
<td>(13)</td>
</tr>
</tbody>
</table>

Table II The effect of acid pH on the concentration of hexose phosphates in muscle (μmol/100 g wet tissue)

Metabolic changes occurring in K+-free medium are similar to those at acid pH. When K+ is not present in the medium, there is a shift of K+ and other ions from the intra- to the extracellular compartment and it is known that similar shifts of K+ occur at low pH (Fenn and Cobb, 1934). The importance of ions, such as K+, in the activation of certain enzymes of the glycolytic pathway has often been demonstrated and recently reviewed by Bygrave (1967). An elevated K+ concentration in the incubation medium increases glucose metabolism in liver slices (Ashmore, Cahill, Hastings, and Zottu, 1957) and glycogen breakdown in heart muscle (Stadie, Haagard, and Perlmutter, 1947).

Changes in intermediates of the glycolytic pathway at low pH suggest that the activity of the enzymes, phosphorylase and phosphofructokinase, which require phosphate ions for their actions, are inhibited. These enzymes are also very sensitive to changes in concentration of cyclic 3',5'-AMP. The results of this study do not show whether or not metabolic changes due to acid pH and ouabain are caused by an inhibition of the adenyl cyclase system. However, since the effect of cyclic 3',5'-AMP on phosphorylase activity of skeletal muscle is not pH dependent (Reynolds and Haagard, 1967), at low pH there might be a decreased formation of cyclic 3',5'-AMP.

One can ask, what is the relevance of these studies to actual injury? A series of experiments was designed to examine this problem (Nahas, Triner, Small, Manger, and Habif, 1966). Oxygen uptake, free fatty acid, and glucose plasma concentrations were measured in mechanically ventilated animals following moderate haemorrhage. The haemorrhage was graded so that, although oxygen-carrying capacity was decreased, normal peripheral oxygenation was still maintained. The animals, after a control period, were bled 25 ml/kg of body weight, over 30 minutes. They remained hypovolaemic for one hour, were retransfused, and then observed for another hour. When they were mechanically ventilated so that pH remained within 0.1 unit of 7.4, we observed an increase in oxygen uptake of about 12%. The hypovolaemic period was also characterized by an initial increase in blood glucose which, however, tended to decrease as the hypovolaemia progressed. Free fatty acid levels were also elevated during the period of hypovolaemia.
Another group of animals was treated in the same way but, at the end of bleeding, were hyperventilated, so that the pH fell to an average of 7-2. In these dogs, there was no increase in oxygen uptake, but stimulation of the sympathetic nervous system seemed to be as vigorous as before or even more so. Blood pressure was increased above control, and there was a marked and sustained increase in glucose levels. Under these conditions, free fatty acid levels did not change (Fig. 14).

In conclusion, the ability of the body to mobilize and utilize its fuel stores and to increase metabolism above basal levels is significantly inhibited by acid pH. These fuel stores are those which are normally mobilized in all conditions of stress. If the trauma is not too severe, the body is able to compensate and the traumatized individual who is conscious tends to maintain his pH at normal by hyperventilation. However, in cases of severe trauma, these compensatory mechanisms are not always operative and alterations of the metabolic effects of the catecholamines as a function of [H+] might be one of the factors which will alter fuel mobilization and utilization. These alterations must be better known in order to devise more efficient forms of therapy. Two therapeutic implications may be derived from our observation: the use of pressor amines in trauma should be carefully controlled, since there is such a marked endogenous production of catecholamines and, furthermore, correction of acidaemia should precede administration of pressor amines so that they may exert their full activity.

This work was supported in part, by a National Institutes of Health grant GM-09069-06 and 07-

army contract DADA-17-67-C-7126, the Robert Sterling Clark Foundation Inc and the Dolores and Bob Hope gift for the study of shock.

References


The problems of acidosis.

G G Nahas

*J Clin Pathol* 1970 s3-4: 73-83
doi: 10.1136/jcp.s3-4.1.73

---

Updated information and services can be found at:
[http://jcp.bmj.com/content/s3-4/1/73.citation](http://jcp.bmj.com/content/s3-4/1/73.citation)

---

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

---

**Notes**

To request permissions go to:
[http://group.bmj.com/group/rights-licensing/permissions](http://group.bmj.com/group/rights-licensing/permissions)

To order reprints go to:
[http://journals.bmj.com/cgi/reprintform](http://journals.bmj.com/cgi/reprintform)

To subscribe to BMJ go to:
[http://group.bmj.com/subscribe/](http://group.bmj.com/subscribe/)