LUNG FUNCTION IN PROVOKED ASTHMA:
RESPONSES TO INHALED UREA, METHACHOLINE
AND ISOPRENALINE

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SUMMARY

1. Lung function was studied in fifty-six symptom-free asthmatic subjects after
the inhalation of urea, an agent not previously used for asthma provocation. The
effects of urea were compared with those produced by the inhalation of methacholine
and of isoprenaline.

2. After urea ventilatory capacity was impaired in 68% of subjects and improved in
16%. This response was a relatively constant phenomenon in the same subject. Urea
appears to possess the unusual property of being able to produce both broncho-
constriction and bronchodilatation. The response could not be related to any of the
clinical or other physiological variables examined.

3. Mild hypoxaemia, ventilation–blood-flow inequality and impairment of gas
transfer also occurred after urea administration.

4. Methacholine produced considerable impairment of ventilatory capacity with
consistent hyperinflation but with variable changes in ventilatory pattern and
distribution of ventilation.

5. Isoprenaline rapidly and completely reversed the changes which occurred after
urea and methacholine administration.

Key words: asthma provocation, urea, methacholine, isoprenaline.

The general pattern of disturbances of pulmonary function that occur in asthma have become
well recognized (Woolcock & Read, 1966; Tai & Read, 1967; McFadden & Lyons, 1968a, b,
However, by the time a patient with acute asthma can be investigated, various complicating or
compensatory changes may have occurred. Moreover, depending on the severity and duration
of the episode, the patient may be exhausted, difficult to study or partly treated. Thus, the
initial functional abnormalities in spontaneous asthma have not generally been amenable to
detailed study. Because of this and because the functional abnormalities in spontaneous and

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provoked asthma are indistinguishable, provocation of susceptible subjects provides a convenient means for investigating the pattern of physiological changes that are likely to occur in early and uncomplicated asthma.

Asthma provocation has been used as an investigational tool for many years. Following the administration of a variety of bronchoactive agents, changes have been studied in ventilation (Colldahl, Holmgren, Pegelow & Svanborg, 1964; Stanescu, Teculescu, Pacararu & Popa, 1968), mechanical function (McIlroy & Marshall, 1956; Constantine, Dautrebande, Kaltrieder, Lovejoy, Morrow & Perkins, 1959; Dautrebande, Lovejoy & Constantine, 1960; Lovejoy, Constantine, Flatley, Kaltrieder & Dautrebande, 1961; Dubois & Dautrebande, 1958; Bouhuys, Hunt, Kim & Zapletal, 1969; Cade, Woolcock, Rebuck & Pain, 1971), ventilation–blood-flow relationships (Raine & Bishop, 1964; Norris & Bishop, 1966) and gas transfer (Bouhuys, Georg, Jonsson, Lundin & Lindell, 1960; Stanescu & Teculescu, 1969).

The purpose of the present study was to make a detailed examination of the effects on pulmonary function of inhaled urea, a substance which has not previously been used as an agent for deliberate asthma provocation. Its application in this regard seemed to be worth exploring, following the observation by Pain & Denborough (1967) that inhaled urea impaired the ventilatory capacity of some asthmatic subjects but not of others. The effects of urea were compared with those produced by inhaled methacholine, an established agent for asthma provocation (Curry, 1947; Herxheimer, 1951; Parker, Bilbo & Reed, 1965; Itkin, 1967; Cade & Pain, 1971). Ventilatory capacity, lung volumes, distribution of ventilation, ventilatory pattern, arterial-blood-gas tensions, ventilation–blood-flow relationships and gas transfer were measured before and after the inhalation of urea, methacholine and isoprenaline in fifty-six symptom-free asthmatic subjects. The acute changes in these indices were examined and their physiological implications considered.

MATERIALS AND METHODS

Patients

Fifty-six asthmatic subjects were studied. The criteria for selection were clinical: episodes of dyspnoea with wheeze, symptomatic response to bronchodilators, intervals with complete remission of abnormal clinical features, absence of chronic disease (cough, sputum, dyspnoea) and absence of complicating factors (localized disease, cor pulmonale). All subjects were symptom-free at the time of study, but major reversibility of airways obstruction had been demonstrated by spirometric measurements on previous occasions. Once selected for study, no patient was excluded on the basis of subsequent physiological measurements. The patients had been referred from the out-patient clinics of a general teaching hospital to the respiratory laboratory for routine functional assessment.

There were thirty-two males and twenty-four females and their ages ranged from 16 to 78 years (mean 42 years). Asthma had developed in childhood in twenty-one subjects and in adult life in thirty-five, an adult onset arbitrarily being defined as occurring after the age of 20 years. Twenty-eight subjects were receiving corticosteroid therapy and twenty-eight were not. None had been receiving treatment with disodium cromoglycate before the study. No bronchodilator agent was permitted during the 6 h before the study. The best forced-expiratory volume in 1 s (FEV₁) recorded in the previous 12 months ranged from 34 to 140% of predicted normal (mean 82%). Within each of the clinical subdivisions, there was no bias in the distribution of
the other clinical variables. Informed consent was given by all patients to the experimental procedures.

**Bronchoactive agents**

Urea aerosol was inhaled as a 4 m solution from a Wright (1958) nebulizer for 10 min (Pain & Denborough, 1967). Methacholine was delivered as a 2.5% solution from a Bennett 'Vaponephrin' nebulizer, as previously described by Cade & Pain (1971). Single slow maximum inspirations of the aerosol were taken at approximately 30 s intervals until definite symptoms of chest tightness and dyspnoea occurred or until five inhalations had been given, which ever was sooner. Isoprenaline sulphate was given as a 1% solution for 90 s from a Wright (1958) nebulizer.

**Methods**

Standard spirometric measurements were made of vital capacity (VC), forced-expiratory volume in 1 s (FEV₁) and FEV₁/VC ratio. Peak-expiratory flow (PEF) was recorded with an air-flow meter (Wright & McKerrow, 1959). The functional-residual capacity (FRC) was measured by the closed-circuit helium-dilution method of Gilson & Hugh-Jones (1949) and residual volume (RV), total lung capacity (TLC) and RV/TLC ratios were then derived. Tidal volume (Vₜ), respiratory rate (f) and hence minute ventilation (Vₑ) were also taken from this recording. Lung-clearance index (LCI) was calculated as the volume of ventilation to 90% mixing divided by the FRC. Compartmental analysis was made to permit calculation of the ventilatory defect (VD) of Read (1958). For the purpose of comparison between patients of different sex, age and height, all spirometric and lung-volume measurements (apart from ratios which are shown as percentages) have been expressed as percentages of predicted values at BTPS, obtained from the tables of Goldman & Becklake (1959).

Arterial oxygen and carbon dioxide tensions (Pa,O₂ and Pa,CO₂) were measured using a Beckman triple-electrode system. Expired gas was analysed for carbon dioxide using a calibrated katharometer (Pulmo Analyzer, Godart). Inspired and expired gas were analysed for carbon monoxide using an infra-red analyser (Infra Red Development Company). The physiological dead space to tidal volume ratio (VD/Vₜ) was corrected for the dead space of the valve-box. The diffusing capacity of the lung for carbon monoxide (DL,CO) was performed by Filley's steady-state method, as described by Holland & Blackett (1958). The fractional carbon monoxide uptake (FCO) was also calculated (Bates, 1952). Corrections were made for ambient temperature and barometric pressure and for the difference between inspired and expired volume.

**Procedures**

Spirometric and lung-volume measurements were made before and 2 min after the period of inhalation of urea. After 30 min, when symptoms and spirometric values had returned to baseline values, methacholine was given and the spirometric and lung-volume measurements were repeated. Isoprenaline was then given and all measurements were repeated after 5 min. For the studies of gas exchange, 0.1% carbon monoxide was inspired from a Tissot spirometer via a one-way valve-box (dead space 140 ml). Collections of arterial blood and expired air were made over 2 min and in duplicate immediately before and after the period of inhalation of urea. This sequence thus occupied 18 min: the first 4 min for a gas-exchange study, the next 10 min for urea inhalation, and the next 4 min for a gas-exchange study.
RESULTS

Urea was given to all fifty-six subjects and produced mild but variable impairment of ventilatory capacity with small and insignificant changes in lung volumes, ventilatory pattern and distribution of ventilation (Table 1). Methacholine was given to twenty-one subjects and produced considerable impairment of ventilatory capacity with marked hyperinflation but variable

| Table 1. Changes in ventilatory capacity, lung volumes, ventilatory pattern and distribution of ventilation after urea and methacholine administration. Values are mean changes (with SD in parentheses) and are given as percentages, except for FEV₁/VC, RV/TLC, LCI and VD which are absolute changes. Abbreviations are defined in the Materials and Methods section. Significance of changes is derived from the t-test and is indicated by ***P<0.001 and **P<0.01. |
|---|---|---|
| Index | + Urea | + Methacholine |
| VC | -13*** (17) | -36*** (20) |
| FEV₁ | -12*** (20) | -44*** (19) |
| FEV₁/VC | -0.2 (7-0) | -8*** (9) |
| RV | +9 (44) | +63** (73) |
| TLC | -2 (17) | +3 (12) |
| RV/TLC | +3.5 (9.7) | +21*** (14) |
| Vr | -2 (22) | +1 (27) |
| f | +5 (16) | +12.5 (29) |
| LCI | +0.5 (2.8) | +0.4 (3.5) |
| VD | +6 (27) | +10 (21) |

| Table 2. Correlations between changes and initial values of ventilatory capacity, lung volumes, ventilatory pattern and distribution of ventilation after urea and methacholine administration. Values are correlation coefficients. For abbreviations see the Materials and Methods section. Significance is indicated by **P<0.01, *P<0.05 and N.S., P<0.05. |
|---|---|---|
| Index | + Urea | + Methacholine |
| VC | N.S. | N.S. |
| FEV₁ | N.S. | N.S. |
| FEV₁/VC | N.S. | -0.50* |
| RV | -0.42* | -0.56** |
| TLC | N.S. | N.S. |
| RV/TLC | N.S. | -0.48* |
| Vr | -0.56** | N.S. |
| f | -0.45** | N.S. |
| LCI | -0.43* | -0.66** |
| VD | -0.51** | -0.74** |
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changes in ventilatory pattern and distribution of ventilation (Table 1). The significant changes after methacholine were all significantly greater than the corresponding changes after urea.

After urea changes in ventilatory capacity were correlated with each other and with changes in the degree of hyperinflation (for FEV₁; VC, \( r = 0.87 \) and for FEV₁; RV/TLC, \( r = -0.48 \)). After methacholine similar correlations were found with the addition of a significant inverse relation between the changes in tidal volume and respiratory rate (for FEV₁; VC, \( r = 0.86 \), for FEV₁; RV/TLC, \( r = 0.82 \) and for \( V_T; f, r = -0.48 \)).

After urea administration the changes in RV, \( V_T \), \( f \) and distribution of ventilation were inversely correlated with the initial values (Table 2). After methacholine administration the changes in the FEV₁/VC ratio RV, RV/TLC ratio and distribution of ventilation were inversely correlated with the values (Table 2).

### Table 3. Changes in ventilatory capacity, lung volumes, ventilatory patterns and distribution of ventilation after isoprenaline administration. Values are mean changes (with SD in parentheses) and are given as percentages, except for FEV₁/VC, RV/TLC, \( V_T \), \( f \), LCI and VD which are absolute changes. For abbreviations see the Materials and Methods section. Significance of the difference between these changes after isoprenaline administration and the preceding changes after urea or methacholine administration is derived from the t-test and is indicated by **\( P < 0.01 \) and *\( P < 0.05 \).

<table>
<thead>
<tr>
<th>Index</th>
<th>+ Isoprenaline after urea</th>
<th>+ Isoprenaline after methacholine</th>
</tr>
</thead>
<tbody>
<tr>
<td>VC</td>
<td>+19** (12)</td>
<td>+38 (18)</td>
</tr>
<tr>
<td>FEV₁</td>
<td>+21** (12)</td>
<td>+32 (13)</td>
</tr>
<tr>
<td>FEV₁/VC</td>
<td>+6* (7)</td>
<td>+4 (9)</td>
</tr>
<tr>
<td>TLC</td>
<td>+5 (18)</td>
<td>-3 (19)</td>
</tr>
<tr>
<td>RV/TLC</td>
<td>+14 (10)</td>
<td>-24 (11)</td>
</tr>
<tr>
<td>( V_T )</td>
<td>+122 (172)</td>
<td>+209* (248)</td>
</tr>
<tr>
<td>( f )</td>
<td>-2 (5)</td>
<td>-1 (4)</td>
</tr>
<tr>
<td>LCI</td>
<td>+0.5 (2)</td>
<td>-0.7 (3)</td>
</tr>
<tr>
<td>VD</td>
<td>-12 (29)</td>
<td>-19 (23)</td>
</tr>
</tbody>
</table>

The changes after isoprenaline are shown in Table 3. In the group given urea alone, the increase in ventilatory capacity after isoprenaline administration was significantly greater than the preceding decrease after urea administration. Insignificant changes were recorded in the other indices, both after urea and after isoprenaline administration. In the group given both urea and methacholine, the changes after isoprenaline were all similar in degree (but opposite in direction) to the preceding changes after methacholine, with one exception: tidal volume, which remained virtually unchanged after methacholine and increased significantly after isoprenaline.

Gas exchange was studied before and after administration of urea in eighteen subjects and the changes are shown in Table 4. There was a mild but significant decrease in \( Pa_{O_2} \) with no
significant change in $P_{a, CO_2}$ or ventilation. There was a significant increase in $V_{D}/V_{T}$ ratio and a decrease in FCO and PEF.

With one exception there was no statistically significant relation between any of the clinical variables (sex, age, age of onset of asthma or treatment with corticosteroids) and any of the physiological variables (changes in ventilatory capacity, lung volumes, ventilatory pattern or distribution of ventilation after urea, methacholine or isoprenaline administration). The exception was that increases in TLC after methacholine were lowest in older subjects (from analysis of variance, $F = 4.09$, $P < 0.05$).

**Table 4.** Changes in arterial blood gas tensions, ventilation, ventilation–blood-flow inequality, gas-transfer and peak-expiratory flow after urea administration. Values are mean changes (with SD in parentheses). For abbreviations see the Materials and Methods section. $V_A$ is the calculated alveolar ventilation. Significance of the changes is derived from the $t$ test and is indicated by ***$P < 0.001$ and **$P < 0.01$.

<table>
<thead>
<tr>
<th>Index</th>
<th>Units</th>
<th>Change after urea</th>
</tr>
</thead>
<tbody>
<tr>
<td>$P_{a, O_2}$</td>
<td>(mmHg)</td>
<td>$-9^{***}$</td>
</tr>
<tr>
<td>$P_{a, CO_2}$</td>
<td>(mmHg)</td>
<td>$+1$</td>
</tr>
<tr>
<td>$V_T$</td>
<td>(ml)</td>
<td>$+37$</td>
</tr>
<tr>
<td>$f$</td>
<td>(/min)</td>
<td>$-0.2$</td>
</tr>
<tr>
<td>$V_E$</td>
<td>(litre/min)</td>
<td>$-0.45$</td>
</tr>
<tr>
<td>$V_A$</td>
<td>(litre/min)</td>
<td>$-0.32$</td>
</tr>
<tr>
<td>$V_{D}/V_{T}$</td>
<td>(%)</td>
<td>$+3.2^{**}$</td>
</tr>
<tr>
<td>FCO</td>
<td>(%)</td>
<td>$-3^{**}$</td>
</tr>
<tr>
<td>DLCO</td>
<td>(ml/min per mmHg)</td>
<td>$-6$</td>
</tr>
<tr>
<td>PEF</td>
<td>(litre/min)</td>
<td>$-73^{***}$</td>
</tr>
</tbody>
</table>

Details of the findings above are contained in a thesis for the degree of Doctor of Philosophy in the University of Melbourne (Cade, 1970) and are available from the authors upon request.

**DISCUSSION**

Acute provocation, as outlined above, provides the only practical means at present available for examining the initial functional changes that occur in asthma. This is especially so since there is no satisfactory animal model of bronchial asthma and since efforts to render a normal subject asthmatic usually fail. However, there are other advantages of using provocation as a model of acute asthma for laboratory study. Its production is simple, rapid, easily controllable and readily reversible. It appears to be without undesirable side-effects and to be symptomatically and physiologically indistinguishable from spontaneous asthma. It permits the planned study of an acute asthmatic episode from its genesis to its lysis on the one occasion and in an otherwise healthy patient. The disadvantages of asthma provocation are less obvious, but include (1) the potential hazard of producing excessive airway obstruction, (2) the possibility
that in a subject free from recent asthma to 'remind' the bronchial tree of its hyper-reactivity could increase the risk of the subsequent recurrence of spontaneous asthma, and (3) the objection that provoked asthma could in some way be physiologically different from spontaneous asthma. Provided a reasonably careful 'titration' of the dose is made, the first objection is not borne out in practice, nor can the second and third objections be supported by any currently available evidence.

Urea

The time-course of response to urea was explored during detailed study of the small group of subjects whose mechanical function has previously been reported (Cade et al., 1971). With the subject seated in a body plethysmograph, airway resistance was measured continuously during and after 10 min of urea inhalation. Airway resistance began to increase after 3 min of inhalation, reached a plateau by 10 min when the inhalation was discontinued and began to subside gradually by 13 min. In those subjects who noted symptoms of mild dyspnoea and wheeze, the time-course of subjective awareness corresponded closely to that of the objective measurement. In subjects who showed little or no change by 10 min, we have been unable to elicit any response by extending the period of urea inhalation up to 30 min. A standard procedure was therefore used in all subjects and all measurements were made within 5 min immediately following the cessation of urea inhalation, since this period was one of a relative 'plateau' of both subjective and objective abnormalities. During lung-volume measurements 90% helium mixing was complete on average in less than 2 min (SD approx. 1 min), so that it seems reasonable to suggest that the measurements were made during the 'plateau'. Similarly, the studies of gas exchange were completed within the first 4 min after the cessation of urea inhalation. The close similarity of duplicate measurements further supports the belief that the study was made in an already changed and not in a still-changing situation.

The effects of inhaled urea were first investigated in asthmatic subjects by Pain & Denborough (1967) to assess the potential therapeutic value of its powerful in vitro mucolytic effect (Waldron-Edward & Skoryna, 1966). Their finding that urea produced ventilatory impairment in some asthmatic subjects has been confirmed and extended by the present study. Although 68% of subjects showed a significant decrease in FEV$_1$ (ranging from 5 to 66%), 16% showed no significant change, i.e. the FEV$_1$ changed by less than 5%. In the remaining 16% of subjects, the FEV$_1$ showed a significant increase (ranging from 5 to 33%). The urea response was not related to the type of asthma, to treatment with corticosteroids, or to the initial functional status of the subject.

The paradoxical effect of urea is difficult to explain. It cannot be attributed to psychological expectations on the part of the subject, since in the previous study (Pain & Denborough, 1967) control inhalation of saline produced no significant changes in FEV$_1$. It seems likely that urea is capable of exerting a dual action on the bronchial tree, but it is not possible from the present findings to determine whether both effects can occur together in the same subject (but usually to a different degree) or whether only one effect is produced at any one time. The latter possibility may be more likely since serial observations of the urea response at monthly intervals for 2-5 months in fifteen subjects showed a highly significant consistency of response within individuals (J. F. Cade & M. C. F. Pain, unpublished work).

The bronchoactive effects of urea were confined to asthmatics and could not be demonstrated in normal subjects. The bronchoconstrictive effect is most likely caused by a non-specific
irritant action similar to that of dusts, since urea has no known relevant pharmacological action. The bronchodilator effect did not appear to be related to any subjective mucolytic effect, although this is difficult to assess accurately. Since subjects who improved after urea could not be predicted on the basis of clinical or initial physiological criteria and since the degree of improvement was generally small, inhaled urea does not appear to possess any therapeutic usefulness in asthma.

Associated with the mild increase in airway obstruction after urea was a small but significant decrease in $PaO_2$ of 9 mmHg. There was no change in ventilation or in $PaCO_2$, but significant worsening of ventilation–blood-flow relationships was suggested by the increased $Vd/VT$ ratio. A small but significant decrease in gas transfer also occurred, although the values still remained well within the normal range.

The particular advantages of using urea to study gas exchange after acute provocation are that its onset of action is gradual, its full effects reach a plateau of mild degree and suitable duration and, in particular, it has no known primary vasoactive effects, since it is already present as a physiological substance in the bloodstream.

**Methacholine**

Methacholine has been shown to be a potent bronchoconstrictor in the present and in previous studies (Parker *et al.*, 1965; Itkin, 1967). The VC and the FEV$_1$ were both invariably decreased, a mean decrease of 36% in the former and 44% in the latter occurring within 2 min of inhalation. A significant although much smaller mean decrease in FEV$_1$/VC ratio occurred, but in 13% of subjects this ratio actually increased.

Since methacholine may reasonably be considered to produce pure acute airway obstruction, uncomplicated by sputum plugging, mucosal oedema or atelectasis as may occur in spontaneous asthma, one may conclude from these observations that the FEV$_1$ is the most sensitive of the simple spirometric indices in demonstrating acutely changing airway calibre, the FEV$_1$/VC ratio is the least sensitive and VC is intermediate. These conclusions emphasize the semantic problems involved in spirometric definitions of airway obstruction. If, for example, one follows a recent definition which considered obstruction to have occurred when there was a decrease in the FEV$_1$/VC ratio and restriction to have occurred when there was a decrease in VC (Palmer & Diament, 1968), one is forced to the uncomfortable conclusion that in 13% of subjects in the present study, methacholine produced 'restriction' and 'bronchodilatation'.

Methacholine-induced obstruction was accompanied in every case by considerable hyperinflation, the mean increase in RV being over 60% and the mean increase in RV/TLC ratio being over 20%. The strong correlation between these changes in RV and RV/TLC ratio and the changes in VC and FEV$_1$ arise in a different situation from that in which Woolcock & Read (1966) sometimes noted a disparity between measurements of lung volumes and of FEV$_1$, since they were following the slow resolution of asthmatic exacerbations rather than as here the acute production of airway obstruction. It is probable that associated changes in compliance can complicate these relations, especially in less-acute situations.

Although there was no mean increase in TLC, values tended to increase more with greater obstruction and significantly more in younger subjects. Changes were not related to baseline values. Since the helium-dilution method tends to underestimate lung volumes in the presence of airway obstruction (Woolcock, Rebuck, Cade & Read, 1971), individual increases in true TLC were probably greater than indicated here. Thus, TLC appears to be an acutely change-
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Further maldistribution of ventilation generally followed the inhalation of methacholine. There was a considerable variation in the degree of change and a significant negative correlation between the changes and the initial values for lung-clearance index and ventilatory defect. In the presence of some initial airway obstruction, with ventilation already maldistributed, any further increase in obstruction may cause less change and may even appear to improve distribution, because partially obstructed and poorly ventilated spaces could become completely non-communicating. This paradoxical improvement in distribution of ventilation despite increasing airway obstruction appeared to occur in about one-third of patients. In these patients one would expect to find an increased discrepancy between FRC measurements by dilution (or washout) methods and by body plethysmography.

Increased airway resistance is usually expected to result in larger and slower breaths to minimize the resistive component of ventilatory work. On the other hand, decreased compliance is usually expected to result in smaller and faster breaths to minimize the elastic component of ventilatory work. In our patients, we found that increased airway obstruction was regularly accompanied by increased hyperinflation and thus possibly by decreased compliance. The lack of any significant changes in ventilatory pattern after inhaled methacholine suggests that increased airway resistance and decreased compliance could have effectively cancelled the effects of each other on the ventilatory pattern.

**Isoprenaline**

Rapid and striking bronchodilatation with isoprenaline aerosol occurred after provocation with either urea or methacholine. Except for tidal volume, the degree of improvement in all indices was similar in magnitude and opposite in direction to the changes previously produced by urea or methacholine. Tidal volume increased considerably after isoprenaline administration, although it had remained virtually unchanged after urea and methacholine administration. The increase in tidal volume was more marked when isoprenaline was given after methacholine than when it was given after urea and may therefore have been due to a lag in the process which readjusts ventilatory work in the face of a sudden decrease in load, rather than to a direct central respiratory stimulant effect.

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