

## Activity units for allergen extracts

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*(Received 6 February 1979; accepted for publication 20 February 1979)*

### Summary

The two present systems of units for the strength of allergen extracts, Noon and p.n.u. are both unsatisfactory. A new activity unit, based on skin prick in humans, is described, and its relationship to *in vitro* tests is explored. Potency limits for provocation materials used for skin prick tests are suggested.

### Introduction

Two systems of units for expressing the concentration of allergen extracts are currently in use. The first, devised by Noon (1911) is 'that quantity of pollen toxin which can be extracted from the thousandth part of a milligram of Phleum pollen'. An extract made from 1 mg pollen in 1 litre extracting fluid contains 1 Noon unit/ml. Although this unit was restricted by definition to Phleum pollen it has been applied to many other allergens. It is doubtful if this was ever intended initially, as a procedure is given in the paper for comparison of other extracts by intra-ocular assay in humans. Whatever was the original intention is probably lost in history, but the problems with this unit remain. These are:

(i) Different antigens have very different solubilities, for example 1 mg ovalbumin dissolves completely in 1 litre of aqueous fluid, whilst 1 mg cat fur gives only a few  $\mu\text{g}$  of soluble substance. This means that the size of the Noon unit varies for each allergen.

(ii) Solution of components from relatively insoluble material is time and temperature dependent. Thus whilst all the soluble substances from pollen may be extracted in 12 hr at 4°C, horse hair might still be yielding allergens in solution after 3 days.

(iii) Different extracting fluids leach out different amounts of soluble substances. The precise conditions of extraction must therefore be stated, and the use of other conditions is not necessarily comparable.

(iv) There is a tacit assumption that the 'toxin' is a single entity. This may well have been acceptable in 1911 when the unit was devised but there is abundant evidence now

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that allergen extracts are complex mixtures of substances of different properties and stabilities. Variation of the concentration of these in the extract disorganises the constitution of the unit from batch to batch and it is not possible to regard it as a repeatable entity.

The other commonly used system is that of protein nitrogen units (p.n.u) devised by Stull, Cooke & Tennant (1933). This is defined as  $10^{-5}$  mg nitrogen precipitated with phosphotungstic acid.

If allergen extracts contained only one, pure, biologically stable allergenic substance that precipitated with phosphotungstic acid this method of measuring strength might be satisfactory. However many workers have shown that the extracts that are usually made contain allergenically inactive as well as active components. The method on which the p.n.u. is based does not differentiate either the proportions of different active substances or the total amounts of active and inactive components and cannot therefore be anything other than a guide to total macromolecular nitrogen.

Neither of these systems of units is related to the biological activity of the material. The present progress in standardization of allergen extracts requires some method of stating strength and preferably this should be related to biological activity (Report of 1st Meeting of International Joint Steering Committee for Allergen Standardization). The present report proposes the use of a unit based on biological activity in man, but which can also be used in *in vitro* assays, and so relate activity in man to results of radioimmunoassays and isoelectric focussing.

#### Definition of the proposed biological activity unit for allergen extracts

An extract, having been shown by previous tests to be free from irritants or non-specific toxicity, contains 10 units of activity/ml if when prick tested in thirty individuals who clinically are sensitive to the extract a geometric mean weal size of 75 mm<sup>2</sup> is obtained.

For materials that have a high specific activity, i.e. a high biological activity shown by a small amount of material, the gravimetric content of the unit will be small, and where the specific activity of the material is lower, the dry weight of the unit will be greater in proportion.

#### Method

Each participant is asked to test one allergen extract. Four dilution levels of the allergen extract, the most concentrated of which has been shown to be free from irritants or non-specific toxicity, are prepared in sterile Coca's solution containing 50% glycerol and 0.4% phenol and subjected to a sterile filtration (Coca, 1922).

Eight solutions are supplied for prick tests in human skin, coded A-G, which between them contain four tenfold dilution levels, each dilution level occurring twice.

The prick tests should be made in a randomized block design on the forearm only by pricking through a drop of the extract into the superficial layers of the epidermis with a gentle lifting motion, using a separate No. 20-25 disposable needle for each solution. The drop of allergen extract should then be removed from the test sites by careful application of absorbent paper tissue, so as to avoid spread to the other sites.

The weal size observed 20 min after the prick has been made should be recorded by marking the perimeter of the weals with a felt tip or ballpoint pen, then transferring the mark to a piece of card by means of 'Sellotape'. The weal size areas are then calculated.

The solutions should be tested in thirty patients using the randomised block design which are chosen so that each solution is tested in each patient, but in a different order.

### Results

The technique has been applied to the 1st British Standard cocksfoot (*Dactylis glomerata*) pollen extract 75/506, and to the proposed standard extracts of *Aspergillus fumigatus* and *Dermatophagoides pteronyssinus*.

Thirty patients clinically sensitive to cocksfoot pollen were prick tested, on the arm, with tenfold dilutions of the pollen extract in duplicate and the weal sizes observed 20 min after prick testing were recorded with sellotape, photocopied, cut out and weighed. The weights were then converted to sq. mm from the knowledge of the unit weight of paper. The geometric mean of the weal sizes at corresponding dilutions was calculated, and was found to plot as a straight line on log-log graph paper (Fig. 1). It follows that

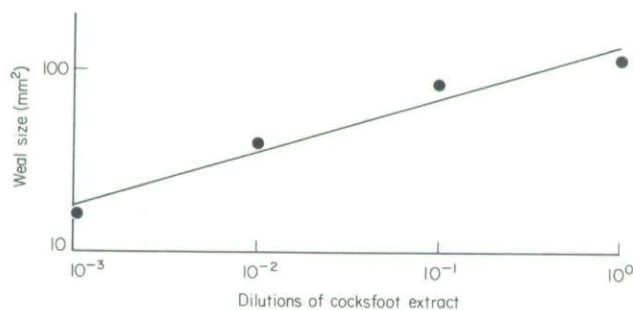


Fig. 1. Relationship between the concentration of allergen solution used in the prick test to the geometric and weal size.

since the weal size versus dose is a straight line on log-log paper, the relationship between weal size and dose is

$$\log A = \log k + m \log x$$

where  $A$  = area of weal size

$k$  = constant

$x$  = dose of allergen

$m$  = slope of the line

and therefore  $A = kx^m$ .

In practice the slope of the line is shallow, and this is the reason for selecting serial tenfold dilutions. From Fig. 1 it can be seen that at a dilution of  $10^{-1}$  the extract gives a mean weal size of  $68 \text{ mm}^2$ , thus showing that the undiluted extract contains 71 activity units/ml.

### Relationship of the results from *in vitro* tests

It is also possible to express the results of the *in vitro* tests of isoelectric focussing and inhibition RAST in the same units as skin tests.

The gel and densitometric trace produced from it by the method of Topping, Brighton, Stokell & Patterson (1978) and shown in Fig. 2 was made from  $100 \mu\text{l}$  of a solution of the 1st British Standard cocksfoot (*Dactylis glomerata*) pollen extract of 71

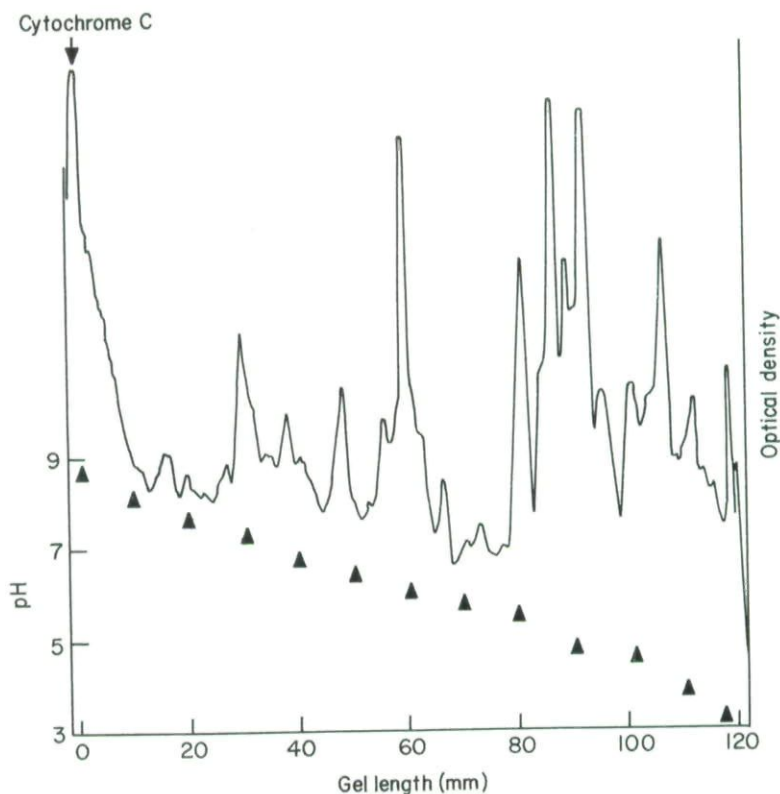


Fig. 2. Densitometric trace of the staining pattern observed after an isoelectric separation of the Cocksfoot pollen extract in polyacrylamide gel pH 3-10 gradient. ( $\blacktriangle$ ) pH.

units/ml. It follows therefore that 7.1 units of the extract contained the components seen in the gel.

The RAST inhibition curve, produced by a method developed from that of Yman, Ponterius & Brandt (1975) (Fig. 3), was also made with an inhibiting solution of the pollen standard of 71 units/ml. The end point for 50% neutralization in this assay is seen to be 1  $\mu$ l of the solution and this contains 0.07 unit.

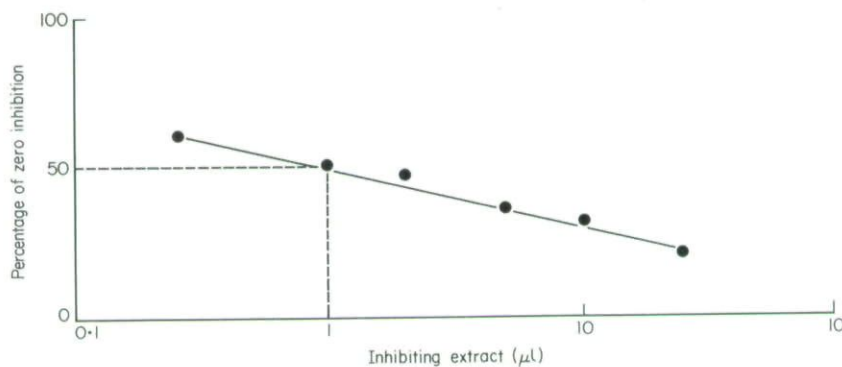


Fig. 3. RAST inhibition of Cocksfoot allergen discs by the British Standard cocksfoot extract.

Thus all three of the tests could be compared by means of the proposed biological activity unit.

### Discussion

It should be stressed that whilst inhibition RAST is a sensitive method for demonstrating 'total' strength or potency of an extract, it is also necessary to take into consideration the constitution of the components that are responsible for this potency. For this, isoelectric focussing in polyacrylamide gel is a method which, when calibrated in the proposed new units, produces a pattern of lines from which identification of biologically active components may be made. (Topping, Brostoff, Brighton, Danks & Minnis, 1978).

It is also possible using the proposed system to suggest limits for the potency of commercially available provocation solutions for skin prick tests.

The dose response curve in Fig. 1 is that for the population tested, and shows that a relatively large increase in dose is needed to effect a significant change in the weal size, and therefore that the limits of activity or potency for provocation materials used for skin test can be wide.

It is suggested that geometric mean weal sizes for the population of between 47 mm<sup>2</sup> (approx 8 mm diameter) and 120 mm<sup>2</sup> (approx 12 mm diameter) are suitable units for the activity of provocation materials used for skin prick tests. On the proposed scale of units these limits correspond to 2 and 50 units respectively. (Fig. 4.)

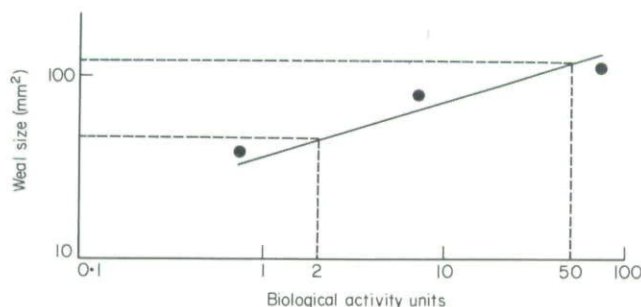


Fig. 4. The relationship between the concentration of allergen in activity units and geometric mean weal size, showing the suggested limits for the potency of provocation materials.

The new biological activity units can also conveniently be linked to the HEP (histamine equivalent) units used at present in some Scandinavian countries (Aas, 1978). At the time of the calibration in the thirty test patients, an additional solution of 1 mg/ml histamine is included in the tests, and the weal sizes recorded separately from those for the dilutions. It is then a simple matter to make an arithmetic equivalent between the two units by the method of Schultze-Werninghaus & Gonsior (1976).

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