Daily Penicillin Serum Concentrations Following Injection of 2.4 Mega-units of “All-purpose” Penicillin

A. E. TINKLER & R. SHANNON

In order to investigate the reliability with which a given dose of benzathine penicillin will result in predictable ranges of penicillinaemia on any particular day during the week after injection, ambulant adult males were injected with 2 400 000 IU of “all-purpose” penicillin (600 000 IU potassium penicillin G, 600 000 IU procaine penicillin G and 1 200 000 IU of benzathine penicillin). Penicillin serum assays were performed, 24 each day, from the third to the seventh day after injection (120 assays in all).

Statistical evaluation of the results showed that the means of the groups of 24 assays fell within narrow ranges, indicating that the long-acting component (benzathine penicillin) gives reliable daily ranges in a high proportion of cases. The results of a previous trial, using half the present dosage (1.2 mega-units of “all-purpose” penicillin) were compared statistically with those of the present trial. A very satisfactory degree of correlation between dosage and resulting daily serum concentration was observed—in general double the dose yielded double the daily concentration. The many factors which affect absorption rate are discussed and it is suggested that preparations which depend on an oily gel to delay absorption add an avoidable factor to the list of variables which may play an important part in producing the significant differences in serum levels commonly reported after the use of PAM preparations. The narrow ranges of penicillinaemia observed after 1.2 mega-units and 2.4 mega-units of benzathine penicillin and the degree of correlation observed in general between dose and resulting serum levels suggest that a large-scale controlled series of parallel trials should be undertaken to compare the relative long-acting qualities of PAM and benzathine penicillin.

This paper reports the results of daily assays on the penicillin serum concentrations found in ambulant adult male patients during the week following the intramuscular injection of 2.4 mega-units of “all-purpose” penicillin. Each injection consisted of 0.6 mega-unit (600 000 IU) of potassium penicillin, 0.6 mega-unit of procaine penicillin and 1.2 mega-units of benzathine penicillin in aqueous suspension.

A previous investigation into the daily penicillin serum concentration found in ambulant male patients following the intramuscular injection of 1.2 mega-units (half the dosage investigated in the present trial) of a similar complex had indicated a high degree of reliability and predictability in the daily concentrations of the long-acting component of the complex, benzathine penicillin (Tinkler et al., 1965).

The present trial was conducted in order to obtain further evidence as to the reliability of the benzathine salt in producing reasonably predictable daily serum concentrations.

The results obtained in both trials might be criticized on the grounds that an “all-purpose” penicillin was used, although the trials are concerned only with the serum concentrations of the benzathine salt during the week following injection. However, the potassium penicillin does not influence the serum concentration observed in either trial, owing to its rapid elimination. Similarly, most of the procaine penicillin is eliminated within 24 hours (Welch et al., 1953), so that this penicillin is of progressively decreasing importance in the maintenance of serum concentrations after the first post-injection day. The results of the assays on the first and second post-injection days are, however, omitted in order to eliminate any influence on the reported serum concentrations due to the presence of the procaine penicillin salt.

1 Director, Department of Venereal Diseases, United Bristol Hospitals, Bristol, England.
2 Chief Technician, Public Health Laboratory, Bristol, England.
MATERIALS AND METHODS

Patients

The subjects were ambulant adult males suffering from acute gonococcal urethritis. An "all-purpose" penicillin was therefore chosen in order to give a high initial serum concentration and thus ensure a rate of cure comparable to that in other treatment schedules. All patients received a single injection in the upper and outer quadrant of the buttock, and all continued their normal occupations during the trial.

Since this investigation is not primarily concerned with the clinical condition of the patient, or with the results of treatment, these points are not discussed but will be the subject of a separate communication.

Twenty-four assays were performed each day from the third to the seventh post-injection day.

For other details concerning the materials and methods used, the reader is referred to pages 210 and 211 of the paper by Tinkler et al. (1965); the information given there under the headings Assay medium . . . Computation of potencies applies with no change whatever to the present study.

RESULTS

The investigation involved 120 assays, 24 each day from the third to the seventh post-injection day.

Table 1 shows the mean daily serum concentrations observed in this trial after intramuscular injection of 2.4 mega-units of "all-purpose" penicillin and, by way of comparison, the mean serum concentrations observed in the previous trial after intramuscular injection of 1.2 mega-units of "all-purpose" penicillin. The decay curves for these two dosages are compared in Fig. 1.

Since the observation of means, though providing a guide for the majority of patients, cannot be accepted as a standard by which to measure therapeutic effectiveness for all patients, a detailed statistical analysis of the observed results was undertaken in order to delineate:

(a) the daily ranges of penicillinaemia within which the mean serum concentrations of groups of 24 patients can be expected to fall following intramuscular injection of 2.4 mega-units of "all-purpose" penicillin, as shown in Fig. 2; and

(b) the estimated daily ranges of penicillinaemia within which the serum concentration of any individual patient of a large group can be expected to fall following intramuscular injection of 2.4 mega-units of "all-purpose" penicillin, as shown in Fig. 3.

Fig. 2 shows the narrow ranges within which the daily means for groups of 24 patients will fall in 95 out of every 100 cases following the dosage used.

Fig. 3 shows the extremely wide daily ranges which any individual of a large group may exhibit. These data make it clear that the failure of any individual patient of a large group to respond to standard treatment or prophylaxis against a sensitive organism may be due to factors quite unrelated to the quality and specificity of the product used or the resistance of the organism.

Comparison of serum levels following intramuscular injection of 2.4 and 1.2 mega-units of "all-purpose" penicillin

After the injection of 1.2 mega-units of "all-purpose" penicillin, the mean serum concentrations

<table>
<thead>
<tr>
<th>Dosage of all-purpose penicillin (mega-units)</th>
<th>Mean serum concentration (IU penicillin/ml serum)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 3</td>
</tr>
<tr>
<td>2.4</td>
<td>0.1</td>
</tr>
<tr>
<td>1.2</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*a Each IU of "all-purpose" penicillin contains 0.5 IU of benzathine penicillin, which is responsible for the long-term action.

FIG. 1
MEAN DAILY SERUM CONCENTRATIONS OF "ALL-PURPOSE" PENICILLIN FOR GROUPS OF 24 MEN

![Graph showing mean daily serum concentrations of all-purpose penicillin for groups of 24 men.](image-url)
of groups of 24 patients were found to fall within narrow daily ranges during the post-injection week (Tinkler et al., 1965). This was also found to be the case in the present trial using twice the dosage (Fig. 2). These findings suggest that the long-acting component of the complex, benzathine penicillin, will produce reliable daily serum concentrations in a high proportion of cases after a given dose. Within the limits of these trials, the results also suggest that by doubling the dose the resulting daily levels will be doubled.

If in fact there is a reasonably close correlation between dose and resulting daily serum concentrations, one would not expect a significant difference between the daily variances observed in the present trial and those calculated on the basis of doubling the results of the previous trial, unless some unexpected factor is introduced by doubling the dosage. A statistical statement of this expectation is as follows:

If the daily variance ($V$) of individual concentrations ($\bar{x}$) about their daily means ($\bar{x}$) is known to be $V(\bar{x})$, then the expected variance of twice the individual concentrations ($2\bar{x}$) about their daily means ($2\bar{x}$) is $2V(\bar{x})$, as long as no unexpected factor is introduced by doubling the dosage.

The two sets of results were compared by considering the ratio of:

(a) the degree of scatter from the daily means observed directly in the present trial and

(b) the degree of scatter from the daily means calculated as above from the results of the first trial.

The values of the variance ratio ($F$) for the various days of the test are shown in Table 2.

The more closely the daily scatters of the present trial approximate to those calculated as above from

<table>
<thead>
<tr>
<th>Day</th>
<th>Variance ratio ($F$)</th>
<th>Significance at 5% level</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>1.05</td>
<td>Not significant</td>
</tr>
<tr>
<td>4</td>
<td>1.1</td>
<td>Not significant</td>
</tr>
<tr>
<td>5</td>
<td>1.8</td>
<td>Not significant</td>
</tr>
<tr>
<td>6</td>
<td>3.2</td>
<td>Significant ($P &lt; 1%$)</td>
</tr>
<tr>
<td>7</td>
<td>1.0</td>
<td>Not significant</td>
</tr>
</tbody>
</table>

$F = \frac{\text{estimate of variance with 2.4 mega-units \"all-purpose\" penicillin}}{\text{estimate of variance with 1.2 mega-units \"all-purpose\" penicillin}}$
the results of the previous trial the nearer will the variance ratio approach unity ($F = 1$).

Table 2 shows good agreement between the two variances except on day 6, when the variance ratio was 3.2; but this single statistically significant difference does not invalidate the conclusion that, in general, the expected degrees of scatter were achieved and hence no unexpected factor operated on the distribution of serum levels when the dose was doubled.

In order to compare directly the mean daily levels observed in this trial with twice those of the previous trial, Student's $t$-tests were performed on the mean differences of each day's results:

$$\Delta \bar{x} = (x_2 - 2 \bar{x}_1)$$

where $\bar{x}_2 =$ mean levels for 2.4 mega-units of "all-purpose" penicillin and $\bar{x}_1 =$ mean levels for 1.2 mega-units of "all-purpose" penicillin.

Since the preliminary daily variance tests on the estimates of $V(\bar{x}_2)$ and $2^2 V(\bar{x}_1)$ were not found to differ significantly, except on day 6 ($F = 3.2$), a pooled estimate of $V(\Delta \bar{x})$ was used for the $t$ tests:

$$t = \frac{\Delta \bar{x}}{SE(\Delta \bar{x})}; \ SE(\Delta \bar{x}) = \sqrt{V(\Delta \bar{x})};$$

with $N_1 + N_2 - 2$, $(24 + 24 - 2) = 46$, degrees of freedom.

The pooled estimate of $V(\bar{x})$ could not be used for the 6th day; a modified $t$ test with 36 degrees of freedom was therefore used for the 6th day. The results of these calculations are shown in Table 3.

Table 3 shows that on three of the five days there was no significant difference in the mean daily levels, and that on day 3 the difference was barely significant. Only on day 4 was a really significant difference obtained, and this was due partly to the unexpectedly low variance observed on this day in the present trial. These differences do not provide convincing evidence against the proposition that in general double the daily serum concentrations were achieved when the dose was doubled.

**DISCUSSION**

The rate of absorption of any product administered by injection may be affected by many variable factors, resulting in very wide deviations from mean serum levels in a given individual of a large group (Fig. 3).

These factors fall into three categories, namely:

1. Patient variables.
2. Technical variables, e.g., type and size of needle and syringe, sterilization method, injection technique, volume/dose ratio, method of storage, etc.
3. Batch variables, due to physico-chemical differences between different batches of the same product or between similar products of different origins.

Patient variables cannot be avoided, but if the maximum degree of uniformity of results is to be obtained by different workers using the same dose of comparable products then variables due to factors in categories (2) and (3) above must be reduced to a minimum.

Technical variables can be reduced by standardization of techniques but batch variables which cannot be foreseen by the clinician may contribute to an individual patient’s failure to respond to treatment. In mass treatment campaigns, the nucleus of failures due to such causes may be sufficiently large for a disease to rebuild to endemic proportions.

In an editorial, Welch (1953) stated that benzathine penicillin seems to obviate all the disadvantages hitherto encountered with long-acting penicillin. Benzathine penicillin certainly has one obvious

---

**TABLE 3**

<table>
<thead>
<tr>
<th>Day</th>
<th>Difference in mean levels (IU/ml)</th>
<th>SE($\Delta x$)</th>
<th>Degrees of freedom</th>
<th>$t$</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.02</td>
<td>0.00925</td>
<td>46</td>
<td>2.16</td>
<td>0.025 &lt; P &lt; 0.05</td>
</tr>
<tr>
<td>4</td>
<td>0.036</td>
<td>0.00602</td>
<td>46</td>
<td>6.0</td>
<td>P &lt; 0.001</td>
</tr>
<tr>
<td>5</td>
<td>0.01</td>
<td>0.00676</td>
<td>46</td>
<td>1.5</td>
<td>0.1 &lt; P &lt; 0.2</td>
</tr>
<tr>
<td>6</td>
<td>0.01</td>
<td>— $^a$</td>
<td>36</td>
<td>1.65</td>
<td>0.1 &lt; P &lt; 0.2</td>
</tr>
<tr>
<td>7</td>
<td>0.006</td>
<td>0.00428</td>
<td>46</td>
<td>1.45</td>
<td>0.1 &lt; P &lt; 0.2</td>
</tr>
</tbody>
</table>

$a$ Pooled estimate not made for reasons given in text.
advantage over preparations which depend for their long-acting properties on the oily gel in which they are suspended, in that its long-acting properties are derived from the chemical linkage of the penicillin to a relatively insoluble salt \((N,N'\text{-dibenzylethylenedi-amine})\). They thus depend on molecular structure and particle size, both of which can readily be controlled in the manufacturing process and are relatively easy to check. PAM preparations, on the other hand, in which procaine penicillin is suspended in an oily vehicle, retard absorption by the addition of another variable to the list.

It is generally acknowledged that the oily vehicle may be an important factor in producing the significant variations in blood-level durations which are found to occur between different PAM preparations. For example, it was found \(^1\) that 21.4\% of 663 different batches of PAM failed to reach minimal requirements and that different batches of PAM varied significantly in their blood-level duration. This duration depended in large measure on the physico-chemical qualities of the oily vehicle and the degree of perfection of the gel.\(^1\)

The WHO Expert Committees on Venereal Infections and Treponematoses and on Biological Standardization have long been concerned about this problem and have initiated a series of investigations into the causes of the variable blood-level durations observed after the administration of different PAM preparations. These investigations have mainly been carried out in the National Institute of Medical Research, London, and are summarized by Bond et al. (1965). They have resulted in the establishment of provisional international minimal requirements for PAM preparations (WHO Expert Committee on Venereal Infections and Treponematoses, 1953), the revised penicillin serum assay method (Lightbown & Sulitzeanu, 1957), a preliminary (unpublished) blood-level duration test in the rabbit and finally the International Reference Preparation of Procaine Benzylpenicillin in Oil with Aluminium Monostearate (WHO Expert Committee on Biological Standardization, 1963).

All PAM preparations on the international market should meet these minimal standards, but significant differences in blood durations can still be found among preparations of different origin which comply with these standards.\(^1\) Even with the same batch, significant differences have been found to occur between different clinics. Batch PAM 1/9469 for example, which would have been considered suitable for use as an international reference preparation if sufficient quantities had been available, gave significantly different mean blood levels at three different London clinics at 24 hours, 48 hours and 7 days, but not at 72 hours (Bond et al., 1965).

An ideal long-acting penicillin preparation, regardless of origin or batch, should, on any particular day over a known period of time, produce serum levels which lie between narrow and predictable extremes, according to dosage, in a high proportion of cases. It is not suggested that the limited scope of this trial provides sufficient evidence for any such "ideal" claim for benzathine penicillin. Indeed, unavoidable patient variables together with technical variables, inevitable in the field, may make such ideal preparations impossible to achieve. Nevertheless, this ideal should provide the standard against which different long-acting preparations are measured. Those penicillins which depend for their long-acting property on the oily gel in which they are suspended fall very far short of this ideal, judging by reported experiences.

In contrast, the narrow daily ranges of mean levels in groups of 24 men obtained after injection of 1.2 mega-units of benzathine penicillin (Tinkler et al., 1965), and also in this trial after injection of 2.4 mega-units (Fig. 2) show that in both trials the benzathine penicillin produced reliable and predictable daily ranges in a high proportion of cases.

It must be emphasized, however, that the results of these two trials provide no valid grounds for a firm conclusion as to the relative merits of PAM and benzathine penicillin with regard to their long-acting qualities.

The WHO Expert Committee on Venereal Infections and Treponematoses in a series of reports, summarized in their fourth report, published in 1953, advocated PAM as the penicillin preparation of choice in the treatment of treponematoses both in mass campaigns and for individual clinic cases. Their observations apply equally to treatment or prophylactic measures against other sensitive organisms where a prolonged but low serum level is required.

As these reports were published before the introduction of benzathine penicillin, it is suggested that the results of this trial do at least indicate the necessity for a controlled series of parallel trials in man and rabbits to investigate the relative blood-level duration qualities of these two types of long-acting penicillin preparation.

\(^1\) Unpublished communications to WHO.
ACKNOWLEDGEMENTS

The authors wish to acknowledge the co-operation and advice of Dr A. J. Hedges, Department of Bacteriology, University of Bristol, with regard to the statistical analysis; of Dr G. Ewart Cree and Dr E. H. Jeanes, Consultant Venereologists, Bristol. They are also indebted to Mr W. J. Howell and Mr A. G. Martin, Male Nurses, Bristol Venereal Diseases Service; to Miss A. M. Thorn for preparation of the manuscript and to Mr H. J. Washer for the preparation of the original graphs.

RéSUMÉ

Poursuivant l’étude des pénicillinémies succédant à l’injection d’une préparation renfermant de la penicilline benzathine, les auteurs ont procédé à de nouveaux essais en utilisant des doses de pénicilline deux fois plus élevées.

Un certain nombre de sujets atteints d’urétrite blennorrhagique aiguë ont reçu en injection intramusculaire unique 600 000 UI de pénicilline G potassium, 600 000 UI de pénicilline G procainée et 1 200 000 UI de pénicilline benzathine en suspension aqueuse. Vingt-quatre dosages de la pénicillinémie ont été effectués quotidiennement du 3e au 7e jour suivant l’injection, soit 120 au total.

Les résultats ont confirmé ceux des essais précédents. Les valeurs moyennes quotidiennes de la pénicillinémie ont été très voisines chez tous les patients. Il semble que l’administration de la composante pénicilline benzathine permette d’obtenir des concentrations efficaces dans un grand nombre de cas. L’injection de doses deux fois plus élevées entraîne une augmentation correspondante de la pénicillinémie, fait corroboré par l’analyse statistique des résultats des deux séries d’essais.

Les auteurs examinent les différents facteurs qui peuvent exercer une influence sur la pénicillinémie: réactions individuelles, différences de technique, diversité des caractéristiques physico-chimiques des préparations utilisées. Ils soulignent à ce dernier égard les qualités de la pénicilline benzathine qui, au cours de leurs essais, a témoigné d’une action remarquablement constante. Il leur paraît opportun de procéder à une étude comparative des avantages respectifs de la pénicilline benzathine et de la benzylpénicilline-retard dans l’huile additionnée de monostéarate de potassium (PAM) qui, jusqu’à présent, a été considérée comme la préparation de choix pour le traitement individuel ou de masse des tréponématoïses.

REFERENCES


