Summary
Cephalothin and 6-aminopenicillanic acid were antigenic in rabbits despite their inability to undergo a penicillenic acid-type rearrangement in vitro. Both hemagglutinating- and PCA-reacting antibodies formed in response to injection with these antigens cross-reacted with the benzyl-penicillin antigen.

TOXICITY OF THE PENICILLINS

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The absence of direct toxicity is one of the most remarkable properties of the penicillin molecule; that is to say, the penicillin molecule as we know it and as we should use it. If you try hard enough, you can make it toxic by altering it or abusing it, as I shall try to show later. With this reservation, one can say that penicillin G and the main therapeutic derivatives of 6-aminopenicillanic acid (6-APA) at present in use are virtually non-toxic. By this I mean that these drugs can be given in doses very much larger than those of any comparable biologically-active substance to some animals and to man without disturbing organic function or causing any signs or symptoms suggestive of damage to essential tissue (Table I). In mice, rats and dogs, penicillin G, the phenoxybenicillins, methicillin, the isoxazoles and ampicillin are all tolerated intravenously in doses of 2 g./kg. or more, and in twice that dosage subcutaneously or orally. With some derivatives (e.g. ampicillin) it is difficult to establish a toxic dose within the limits of solubility. Extremely high doses (5 g./kg.) may cause convulsions if given intravenously: this would be equivalent to about half a kilogram by injection or a kilogram by mouth to man! At about half this dose level, the isoxazoles cause transient hypotension but in other respects there is no interference with vital functions. The toxic level in man has never been established but it is known that methicillin and penicillin G can be given intravenously in doses of 20 g. per day for weeks on end, and that ampicillin and the isoxazoles can be given in doses of at least 4 g./day (80 mg./kg.) without any signs of immediate or delayed toxicity. This means that concentrations of penicillin G and methicillin of 50 mg./% can circulate harmlessly in the blood and tissues; in other words, that penicillin is no more toxic than glucose or urea, and much less toxic than many other physiological substances. When such large doses are being given, it is best to use the sodium salt as the potassium cation can be toxic: 15 mega-units of penicillin G supplies 25 mEq. of K+ which may cause cardiac dilation, especially if the heart is already damaged, as in bacterial endocarditis, which is the main disease requiring such high doses. Even the sodium cation may rise if there is any renal impairment and it should be remembered that normal doses of methicillin yield 5-10 mEq. of Na+. Some among you may recall that, in the early days, the toxicity of successively purified batches of crude peni-

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mouse</th>
<th>Rat</th>
<th>Guinea Pig</th>
<th>Rabbit*</th>
<th>Dog</th>
<th>Man</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin G</td>
<td>3,000</td>
<td>3,500</td>
<td>5</td>
<td>500</td>
<td>500</td>
<td>1,000</td>
</tr>
<tr>
<td>Methicillin</td>
<td>3,000</td>
<td>4,000</td>
<td>10</td>
<td>500</td>
<td>250</td>
<td>400</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>5,000</td>
<td>5,000</td>
<td>10</td>
<td>200</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>Cloxacillin</td>
<td>2,000</td>
<td>2,500</td>
<td>10</td>
<td>200</td>
<td>100</td>
<td>80</td>
</tr>
</tbody>
</table>

* More sensitive to oral dosage.

REFERENCES
Penicillin was almost exactly the same as that of sodium acetate (Florey, Chain, Heatley, Jennings, Saunders, Abraham and Florey 1949). The calcium salts are always more toxic than the sodium or potassium salts. In general terms, one might say that the toxicity of the simpler derivatives of 6-APA is the toxicity of the cation.

Apart from the cations, penicillins appear to be non-toxic when given systemically. What are the reasons for this? I would mention the following:

(a) **Chemical Structure**

The 6-APA molecule consists essentially of two amino-acids, L-cysteine and L-valine, twisted together biogenetically into an unusual dipeptide (Fig. 1). The derivatives are all formed by the addition of side chains to the only reactive group, the amino-group in the 6-position. The are all therefore simple acyl dipeptides, usually lacking in surface-activity or indeed in any great reactivity. They do not cause histamine-release like larger peptide molecules and they are too small to behave as antigens unless linked as haptns in multivalent penicilloyl conjugates.

(b) **Rapid Excretion**

All the penicillins are effectively cleared from the blood by renal and hepatic cells so quickly that, with the simpler penicillins, 70% of a given dose may be excreted in an hour. Metabolism is limited so that, no matter how great the load, very little energy is subtracted from the hepatic and renal cells during this process: even in high concentration, penicillins have little or no effect upon the respiration of these cells in vitro. With the exception of the isoxazoles, the penicillins are excreted by both the glomerulus and the tubules (Acred and Brown, 1963).

(c) **Metabolism**

Until recently, it was thought that most penicillins circulated in unchanged form and were excreted as such. It is now known that a number of metabolites are formed, especially from the phenoxy- and isoxazole-penicillins (Rolinson and Batchelor, 1962; Vanderhæghe, Parmenter and Evrard, 1963). One of these has been identified by Belgian workers as p-hydroxyphenylmethyl-penicillin, derived from penicillin V. The others await final identification. Some are biologically active: one of the isoxazole metabolites may be more active against certain bacteria than the parent compound. None is known to be toxic but it must be remembered that the side chains at present in favour are relatively simple substances. Renal clearance of the isoxazole metabolites proceeds at a slower rate. These metabolites are formed in the liver and it may be noted that some investigators (Medical Letter, 1962) have encountered a rise in serum transaminase during oxacillin therapy, which might indicate some disturbance of hepatic function. Ampicillin and methicillin are less likely to yield metabolites than the other penicillins. Apart from true metabolic conversion, most penicillins can degrade in solution to penicillenic acid, before or after injection. This substance can readily conjugate with proteins and may

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**Fig. 1.** Molecular structure of a penicillin.
act in this way as a sensitising antigen, in common with other penicilloyl conjugates.

(d) Selective Biological Activity

The lack of toxicity of the penicillins to mammalian cells and tissues contrasts strangely with their selective toxicity, in minute doses, to bacteria. From the work of Park and Strominger (1957) we know that the toxicity to bacteria is due to interference with mucopentide synthesis in the cell wall. The biochemical routes to this synthesis, via muramic and probably teichoic acid (Baddiley, 1964), are not needed in mammalian cells which are therefore indifferent to the presence of penicillins. As far as we know, all the therapeutic penicillins act similarly upon sensitive bacteria and are therefore likely to be equally inert in this respect toward mammalian cells. Studies with various cell lines, including human cells, in tissue culture show that respiration and growth proceed normally in concentrations of 100-500 μg./ml. of the main therapeutic penicillins. At higher concentrations, penicillin G, methicillin and ampicillin are still non-toxic but the isoxasoles can be lethal to certain cells at 250-1000 μg./ml.

Idiosyncracy

I have said that mammalian cells are normally indifferent to the penicillins but there may be some exceptions to this general rule. A few incidents have been recorded suggestive of some kind of idiosyncrasy to methicillin. The manifestations of this are variable (Table II). Hewitt, Finegold and Monzon (1962) observed fever, eosinophilia, oliguria and haematuria in three patients receiving moderately large doses between the 14th and 28th days of therapy. One of these patients was hypersensitive to penicillin G, the others were not. In any event, renal disturbance is an extreme rarity even during severe allergy. Grattan (1964) has recorded one similar case in a child with no known renal abnormality. Haematuria ceased when methicillin was stopped but reappeared twice when the drug was restarted. Vic-Dupont, Rapin and Hault (1963) have described, less convincingly, "Un syndrome pseudo-infectieux" without renal disturbance, occurring in a similar fashion. In two cases studied by Allen, Roberts, Evans and Kirby (1962) haematuria occurred during therapy but subsided while the drug was continued.

A fall in neutrophil granulocytes in the peripheral blood has also been noted in two of three patients receiving normal doses of methicillin, with spontaneous recovery when the drug was stopped. This occurred in patients who showed no signs of allergy but it should be remembered that agranulocytosis is known to be an occasional, though extremely rare, manifestation of hypersensitivity (Sigal, 1963). No other evidence of damage to the blood or blood-forming organs has been reported, so far as I know. A fall in granulocytes is of course part and parcel of the response of an infection to methicillin. I have only once seen this fall take the granulocytes below the lower limit of normal. This was in a child with a congenital anemia and haemoglobinopathy who subsequently tolerated methicillin well and excreted it in normal quantities.

These few renal and neutropenic episodes cannot possibly be direct toxicity. They must be due either to idiosyncrasy or hypersensitivity. The only derivative so far incriminated is methicillin and one would like to be sure that there is no possibility of an impurity or another toxic drug somewhere in the story.

The only laboratory animal in which penicillins are directly and uniformly toxic is the guinea pig. The lethal dose varies but may be as low as 10 mg. and is higher by the
oral than by parenteral routes. This peculiar toxicity seems to apply to the newer derivatives as well as to penicillin G. Death usually occurs in 3-5 days and the explanation may lie in a suppression of the guinea pig's normal gram-positive flora, with superinfection by gram-negative organisms to which this animal is highly susceptible. Signs of shock with generalised vaso-dilatation are present and adrenal haemorrhage may also be found. The Syrian hamster is said to show similar susceptibility, less acutely (Schneierson and Perlman, 1956). About 1948 there was a report from France that an injection of 1 mega unit of penicillin G diminished the sexual activity of bulls, (Hennaux, Dimitropulos and Cordiez (1948). Curiously enough, this alarming report does not seem to have been confirmed or denied, perhaps because these days sexual activity is an outmoded virtue in bulls. There is an impression however that you cannot keep a good bull down so easily, and that the report is exaggerated.

**Local Toxicity**

So far I have been dealing with systemic toxicity. Any penicillin can be locally irritant and, in that sense, toxic if too much is injected into sensitive sites like the subarachnoid space or anterior chamber of the eye. We find that, in the subarachnoid space or cerebral ventricles, 2-5 mg. of any penicillin causes no reactions but, if we judge by penicillin G, doses of 10 mg. or more may be irritant. Pure preparations are harmless to veins and to the lungs (as aerosols) though they may delay coagulation when applied topically to dental cavities.

**Indirect toxicity**

This can arise in two ways:

(1) **By Suppression of the Competitive Bacterial Flora**

During therapy, especially oral therapy, the gram-positive buccal flora is suppressed or eliminated. Sensitive organisms are also suppressed in the intestine, permitting overgrowth of resistant coliforms or secondary
forms-stomatitis, necessitate further to coliforms and malabsorption of penicillinase-forming staphylococci in the intestine is superinfection.

The absorption and excretion of 6-APA, presumably from such a cause, has been recorded (English, Huang and Sobin, 1960). We have looked for 6-APA in urine without finding any in patients receiving oral penicillins. We have also fed cultures of amidase-forming coliforms along with penicillins but again we were unable to detect free 6-APA in the urine even when fecal cultures proved survival of the amidase-forming coliforms.

The most serious consequence of suppression of the normal flora of the mouth and intestine is superinfection. This can be due to penicillinase-forming staphylococci as well as to coliforms and Candida spp. The major forms of this superinfection are too well-known to necessitate further description here but the minor forms—stomatitis, pruritus ani, diarrhea and malabsorption of the drug—often escape attention. Superinfection by penicillinase-forming staphylococci is not seen if the penicillinase-resistant isoxazoles are given, though coliform superinfection is at least as liable to occur but is fortunately much less dangerous except in debilitated patients and in infants.

(2) Hypersensitivity

This can be regarded as a form of indirect toxicity and is undoubtedly the most serious hazard of penicillin therapy (Table III). You have heard the experts talking earlier today so I need not dwell upon this subject except perhaps to remind you of Bertrand Russell's aphorism that even when the experts all agree they may still be wrong. In this field there is agreement now among the experts that penicillins combine with body proteins to form penicilloyl and penicillenate conjugates which are antigenic (Parker, 1963). Parker (P. 141) and Levine (P. 146) have described the practical advantage of using penicilloyl-polylysines of lower antigenicity for detecting cutaneous sensitivity. The precise role of these various conjugates in different types of human hypersensitivity is still debatable. My own experience leaves me in no doubt that some penicilloyl proteins are

<table>
<thead>
<tr>
<th>Tissue affected</th>
<th>Immediate (0-30 minutes)</th>
<th>Manifestations</th>
<th>Delayed (30 minutes-several days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>Pruritus, urticaria</td>
<td>Cheiropompholyx dermatitis</td>
<td></td>
</tr>
<tr>
<td>Mucosae</td>
<td>Conjunctivitis, pruritus</td>
<td>Glosisisis, stomatitis</td>
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<tr>
<td>Airways</td>
<td>Asthma, laryngeal oedema</td>
<td>Loeffler's syndrome</td>
<td></td>
</tr>
<tr>
<td>Lungs</td>
<td>Oedema</td>
<td>Purpura</td>
<td></td>
</tr>
<tr>
<td>Blood vessels</td>
<td>Anaphylaxis, erythema</td>
<td>Eosinophilia, thrombocytopenia</td>
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</tr>
<tr>
<td>Blood</td>
<td></td>
<td>Neutropenia, eosinophilia</td>
<td></td>
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<td>Marrow</td>
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<table>
<thead>
<tr>
<th>Ring</th>
<th>Side-chain</th>
<th>No. sensitive/No. tested</th>
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</thead>
<tbody>
<tr>
<td>6-APA</td>
<td>nil</td>
<td>7/10</td>
</tr>
<tr>
<td></td>
<td>benzyl-</td>
<td>10/10</td>
</tr>
<tr>
<td></td>
<td>p-amino-</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td>α-amino-</td>
<td>6/6</td>
</tr>
<tr>
<td></td>
<td>dimethoxy-</td>
<td>9/10</td>
</tr>
<tr>
<td></td>
<td>5-chlor-isoxazole-</td>
<td>2/2</td>
</tr>
<tr>
<td></td>
<td>α-amino-adipyl-</td>
<td>3/3</td>
</tr>
<tr>
<td>7-ACA</td>
<td>nil</td>
<td>0/1</td>
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<td>benzyl-</td>
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</tr>
<tr>
<td></td>
<td>α-amino-adipyl-</td>
<td>0/5</td>
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</table>

TABLE III

Allergic Reactions to Penicillins

TABLE IV

Cross-allergenicity to β-lactam Antibiotics. Intradermal Tests in Sensitised Individuals
capable in minute doses of provoking an anaphylactic as well as a local response. This has to be borne in mind when conducting cutaneous tests with these conjugates. Also, if the penicillenate story is correct, 6-APA which cannot undergo this degradation should not be cross-allergenic with other penicillins, which it is (Stewart, 1962; De Weck, 1962) by more than one criterion. For this and other reasons, it is likely that other penicilloyl proteins and other mechanisms are also involved, alternatively or additionally, in the induction of the hypersensitive state. We have to accept too the fact that all the therapeutic penicillins are cross-allergenic and that the hypersensitive state cannot be adequately suppressed by any known measures, including adrenaline, antihistamines or steroids. There is a ray of light, therapeutically as well as scientifically, in the fact that the cephalosporins with a different nucleus are not cross-allergenic (Table IV) and one wonders therefore if the difficulty might be overcome by changes in the sulphur-containing ring.

If a patient is allergic to one penicillin, he is unlikely to tolerate others; but this is not to say that they all have the same degree of allergenicity (Stewart, 1962). Depot preparations, whether used locally or intramuscularly, are more likely to cause sensitisation than soluble preparations. Benzathine penicillin is said to sensitisate 2% of subjects in one injection (Hoigne, 1962). Ampicillin, if judged from some recent reports, is prone to cause erythematous skin rashes but it is not proved that this is always due to hypersensitivity.

Conclusions

In the light of this evidence, one must qualify the idea that penicillins as a class are non-toxic. They may acquire direct toxicity from their cations and from substituents such as benzathine or procaine if very large doses are given. Idiosyncrasy seems to be a rarity, but indirect toxicity arising from hypersensitivity is far from rare and is so varied in origin and symptomatology that it constitutes a constant, unpredictable hazard. The side chains so far attached to 6-APA appear to carry little or no added danger, but it is too early to be certain of this, especially since naphthalenes and other groups with intrinsic toxicity of their own are being introduced, (Hopper, Yurchenev, Gillen and Warren, 1962). Adding naphthalene to penicillin seems to me like putting moth balls into champagne—distasteful as well as poisonous, and unnecessary anyway. Something should undoubtedly be done to abolish cross-allergenicity but the evidence available suggests that this calls for modification of the nucleus rather than the side chain.

REFERENCES