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ORAL ADMINISTRATION OF PENICILLIN

WITH A NOTE ON METHODS OF ASSAY

BY

L. HENRY, M.B., Ch.B.

Senior House Officer

G. WHITE, M.B., Ch.B.

Senior House Officer

AND

M. J. MEYNELL, M.D., M.R.C.P.

Clinical Pathologist

*From the Department of Clinical Pathology,
General Hospital, Birmingham*

It has been noted that all preparations of penicillin, when given by mouth, are unstable in the acid medium of the stomach and are poorly absorbed from the gastro-intestinal tract. Oral penicillin therapy has thus been of value only in children, in whom the gastric acidity is lower (Cathie and MacFarlane, 1953).

In 1940 it was established that by the addition of various chemicals to the medium in which the mould was grown a variety of penicillins could be obtained, differing widely in their physical and antibacterial properties. Behrens *et al.* (1948), by using N-(2-hydroxyethyl)-phenoxyacetamide as the precursor substance, isolated phenoxyethyl penicillin. However, the *in vitro* effect of this differed only slightly from that of penicillin G, and it was not until Brandl *et al.* (1953) demonstrated its acid stability that its use as an oral penicillin was realized. Several articles appeared on the Continent confirming its value (Giovannini, 1954; Sjoberg *et al.*, 1955; Spitz, 1955), and the preparation was marketed under the name of "distaquaine V." The recent work in America (Jones and Finland, 1955; Martin *et al.*, 1955; Wright *et al.*, 1955) has been the subject of two leading articles (*B.M.J.*, 1956; *Lancet*, 1956), and we have already reported a preliminary survey of the serum levels obtainable (Henry and Meynell, 1956). Most papers have demonstrated that adequate and reliable absorption can be obtained with distaquaine V, but comparison is usually made with oral crystalline penicillin G. The latter is known to be

inadequately absorbed, the amount recoverable in the urine being in the region of 12% (Wright *et al.*, 1955). It was thus felt that injected penicillin would give a better basis for comparison.

The object of this paper is to compare the serum levels and urinary excretion rates obtained with phenoxyethyl penicillin (distaquaine V) and benzathine penicillin ("penidural") given orally, with crystalline penicillin G, and procaine penicillin ("avloprocil") given by intramuscular injection. In addition, the accuracy of the two standard methods of assay has been compared—the serial broth dilution method using *Staphylococcus aureus* (Oxford strain) and the plate method using *Sarcina lutea*.

Method

Six subjects were given 200,000 units of each preparation in turn, and thus acted as their own controls. The dose was given before meals and blood withdrawn at $\frac{1}{2}$, 1, 2, 3, and 4 hours, except in the case of procaine penicillin, when the intervals were $\frac{1}{2}$, 1, 2, 6, and 24 hours.

The bladder was emptied before the administration of the penicillin, and thereafter a 24-hour specimen of urine was collected; the volume was measured and an aliquot retained for assay. Serum levels were estimated on the same day by both methods, but in the case of the urine assays the *Staph. aureus* method was found to be unsuitable and the *Sarcina lutea* method only was used.

Staph. Aureus Method.—Serial dilutions of the serum are made in 1% glucose broth, containing Andrade's indicator, and to each tube is added one drop of an overnight culture of the Oxford strain of *Staph. aureus* (sensitive to 0.02 unit of penicillin per ml.). Readings are taken after 18 hours' incubation at 37° C. and the end-point is read according to colour change in the medium and visible growth, both being compared with a control estimation, using normal serum containing a known amount of penicillin. The end-point is sometimes difficult to judge, and it may be necessary to subculture the tubes on to a solid medium.

Sarcina Lutea Method.—Two media are used—a "base" layer with a "flood" layer superimposed. The base layer is made up of 1.5 g. of "lemco," 3 g. of "bacto" yeast, 6 g. of bacto peptone, and 15 g. of agar, suspended in 1 litre of water.

The flood layer is the same as the base layer with the addition of 1 g. of dextrose. The organism used is *Sarcina lutea* CPI—1001 (N.C.T.C. 8340). This is grown on slopes of flood layer agar, and after 18 hours' incubation is washed off with normal saline and the opacity is adjusted to that of a No. 8 Burroughs Wellcome opacity tube. If a standard Petri dish is used, base medium is poured in 10-ml. amounts and allowed to set. The flood medium is brought to 50° C. and is inoculated with the sarcina suspension, 2 ml. per 50 ml. of flood layer medium. Immediately after inoculation 4 ml. amounts of the medium are poured on to each plate containing the base medium. The plates are allowed to set and four holes punched in each, a No. 5 cork-borer being used, although the method is actually independent of the size of the hole.

Assay Procedure

Three plates are used for each assay. A standard solution of penicillin containing 0.1 unit per ml. is prepared, and the serum to be assayed is diluted to approximately the same strength with pH 7.5 phosphate buffer. Both these solutions are then diluted further (usually twice) with buffer, and four drops of a given dilution are introduced into the four holes on each plate, using a pipette delivering 70 drops per ml.

The plates are incubated at 26° C. for 18 hours, when the diameters of the clear areas are measured with a millimetre steel rule, and the average of the readings on the three plates is used in the calculation.

Calculation

The concentration of penicillin which diffuses through the agar diminishes exponentially with increasing distance from the punched hole. Since all four readings are on the same plate and are taken at the same time, the size of punched hole can be eliminated.

This leads to a formula of the type:

$$T_2 = S_2 \lambda \frac{(R_2 - r_2)(R_1 - r_1)[(R_2 - R_1) + (r_2 - r_1)]}{(r_2 - r_1)(R_2 - R_1)[(R_2 - r_2) + (R_1 - r_1)]}$$

which, on taking logs, becomes

$$\log_{10} T_2 = \log_{10} S_2 + \left[\frac{(R_2 - r_2)(R_1 - r_1)[(R_2 - R_1) + (r_2 - r_1)]}{(r_2 - r_1)(R_2 - R_1)[(R_2 - r_2) + (R_1 - r_1)]} \right] \log_{10} \lambda$$

However, if $\lambda = 2$ and the diameters are similar, a simpler formula may be used, thus:

$$\log_{10} T_2 = \log_{10} S_2 + \left[\frac{(R_2 - r_2) + (R_1 - r_1)}{(R_2 - R_1) + (r_2 - r_1)} \right] \log_{10} \lambda$$

where T_2 is concentration of test solution; S_2 is concentration of standard solution; λ is the dilution ratio used; r_2 is radius of clear area using solution S_2 ; r_1 is radius of clear area using diluted solution S_2/λ ; R_2 is radius of clear area using solution T_2 ; R_1 is radius of clear area using diluted solution T_2/λ . The diameters can be used in place of the radii without any alteration in the formula.

Results

The serum levels and urinary excretion rates for each preparation are given in Tables I-IV. The figures obtained with the *Sarcina lutea* method are given first, the corresponding levels for the *Staph. aureus* method being given in parentheses.

Side-effects were not noted in any subject.

Discussion

From the tables it is clear that parenteral penicillin is well absorbed from the site of injection, whether in the crystalline form or as a depot preparation. In the former a

TABLE I.—Crystalline Penicillin G, 200,000 U. (I.M.)

Subject	Serum Penicillin Levels (U./ml.)*					24-hour Urine			
	½ Hr.	1 Hr.	2 Hr.	3 Hr.	4 Hr.	Vol. (ml.)	U./ml. Conc.	Total Amount	% Excreted
L.H. . .	6.2 (5.12)	4.19 (2.56)	0.632 (0.32)	0.18 (0.16)	0.021 (0.08)	1,930	95	183,350	91.1
G.W. . .	3.9 (5.12)	2.97 (2.56)	0.44 (0.32)	0.17 (0.16)	0.027 (0.08)	1,125	88	99,000	49.5
M.J.M. .	4.3 (5.12)	3.9 (2.56)	0.41 (0.32)	0.16 (0.16)	0.15 (0.16)	1,820	92	167,440	83.7
P.J.D. .	4.2 (5.12)	3.5 (2.56)	0.38 (0.32)	0.15 (0.16)	0.042 (0.08)	905	208	188,240	94.1
A.L.T. .	3.9 (5.12)	2.6 (2.56)	0.13 (0.32)	0.076 (0.16)	0.021 (0.04)	815	180	146,700	73.3
B.J.B. .	5.7 (5.12)	4.6 (2.56)	0.47 (0.32)	0.25 (0.16)	0.03 (0.08)	1,710	88	150,480	75.2
Average	4.7 (5.12)	3.63 (2.35)	0.41 (0.29)	0.16 (0.19)	0.042 (0.09)				77.8

* Results with *Sarcina lutea* method are given first. Those with *Staph. aureus* method are given in parentheses.

TABLE II.—Procaine Penicillin, 200,000 U. (I.M.)

Subject	Serum Penicillin Levels (U./ml.)					24-hour Urine			
	½ Hr.	1 Hr.	2 Hr.	6 Hr.	24 Hr.	Vol. (ml.)	U./ml. Conc.	Total Amount	% Excreted
L.H. . .	0.62 (0.64)	0.88 (0.64)	0.74 (0.64)	0.48 (0.64)	Nil	1,620	110	178,200	89.1
G.W. . .	0.79 (0.32)	0.83 (0.64)	0.98 (0.64)	0.37 (0.32)	"	1,814	82	66,748	33
M.J.M. .	0.5 (0.32)	0.75 (0.64)	0.86 (0.64)	0.43 (0.64)	"	1,600	95	152,000	76
P.J.D. .	0.78 (0.64)	1.02 (0.64)	0.93 (0.64)	0.23 (0.16)	"	730	248	181,040	90
A.L.T. .	0.24 (0.16)	0.30 (0.16)	1.52 (1.28)	0.30 (0.16)	"	740	210	155,400	77
B.J.B. .	0.56 (0.32)	0.72 (0.32)	0.48 (0.32)	0.31 (0.16)	"	820	180	155,800	77
Average	0.58 (0.40)	0.75 (0.51)	0.92 (0.69)	0.35 (0.38)	"				73.7

See footnote to Table I.

TABLE III.—Phenoxymethyl Penicillin, 200,000 Units Orally

Subject	Serum Penicillin Levels (U./ml.)					24-hour Urine			
	½ Hr.	1 Hr.	2 Hr.	3 Hr.	4 Hr.	Vol. (ml.)	Conc. U./ml.	Total Amount	% Excreted
L.H. . .	0.88 (1.28)	4.82 (5.12)	0.72 (0.64)	0.18 (0.16)	0.02 (0.04)	2,280	15	34,200	17
G.W. . .	1.26 (2.56)	2.43 (1.28)	0.37 (0.32)	0.14 (0.16)	0.026 (0.08)	1,500	49	73,500	36.5
M.J.M. .	1.8 (1.28)	0.14 (0.32)	0.137 (0.16)	0.19 (0.16)	0.147 (0.16)	1,800	40	72,000	36
P.J.D. .	Nil (0.04)	2.67 (2.56)	0.228 (0.32)	0.14 (0.16)	Nil (0.04)	710	53	37,630	18
A.L.T. .	Nil (0.04)	0.24 (0.16)	3.8 (1.28)	0.77 (0.64)	0.05 (0.16)	1,100	55	60,500	30
B.J.B. .	2.6 (2.56)	2.5 (1.28)	0.52 (0.32)	0.18 (0.08)	0.05 (0.04)	1,620	54	87,480	43
Average	1.09 (1.29)	2.13 (1.79)	0.63 (0.51)	0.27 (0.23)	0.05 (0.09)				30.1

See footnote to Table I.

TABLE IV.—Benzathine Penicillin, 200,000 Units Orally

Subject	Serum Penicillin Levels (U./ml.)					24-hour Urines			
	½ Hr.	1 Hr.	2 Hr.	3 Hr.	4 Hr.	Vol. (ml.)	Conc. U./ml.	Total Amount	% Excreted
L.H. . .	0.06 (0.08)	0.120 (0.16)	0.026 (0.08)	Nil	Nil	1,210	9.6	11,616	6
G.W. . .	0.029 (0.04)	Nil (0.04)	Nil	"	"	730	27	19,710	10
M.J.M. .	0.13 (0.08)	0.18 (0.16)	0.052 (0.04)	0.013 (0.08)	"	1,290	11.4	14,706	7
P.J.D. .	0.26 (0.16)	0.063 (0.04)	0.035 (0.04)	"	"	720	17.1	12,312	6
A.L.T. .	0.045 (0.08)	0.13 (0.04)	0.012 (0.04)	"	"	720	16.2	11,664	6
B.J.B. .	0.21 (0.16)	0.14 (0.08)	0.03 (0.04)	0.01 (0.04)	"	820	12.6	10,332	5
Average	0.12 (0.10)	0.11 (0.09)	0.026 (0.003)	0.004 (0.003)	"				6.7

See footnote to Table I.

high level is reached after half an hour, falling below the therapeutic concentration of 0.1 unit per ml. after three hours; in the latter the levels are lower, but the effect lasts for over six hours. In both, approximately 75% of the injected penicillin (in terms of biological activity) can be recovered in the urine.

In contrast, oral penicillin is affected by a number of variable factors. These include the gastric acidity, the rate of stomach emptying, and the absorptivity of the intestinal mucosa. With phenoxymethyl penicillin this was reflected by the demonstration of only 30% of the ingested penicillin in the urine. The serum levels were correspondingly lower, but were still adequate, and remained above the therapeutic level (0.1 unit per ml.) for over three hours, although the rate of absorption varied in individual subjects.

With benzathine penicillin the results were much less satisfactory. Only 5-10% of the ingested dose was recoverable in the urine, and even when a therapeutic level was reached this was in no case sustained for over one hour. The levels obtained were in no way comparable with those of injected penicillin or phenoxymethyl penicillin.

Note on Methods of Assay.—The method using *Sarcina lutea* is reliable and has an accuracy within 5%, but it is laborious to carry out and requires an incubator set to 26° C. The *Staph. aureus* method is much simpler and requires no special equipment, but, being a serial dilution method, has an inherent accuracy of only 50% in that the actual concentration of penicillin lies somewhere between the end-point tube and the tube containing the next highest dilution. In no case should the level obtained with the *sarcina* method be lower than that with the *Staph. aureus* method. However, on comparing the results of both methods, in 44 out of 120 readings the *Staph. aureus* method failed to give levels even within this wide range of accuracy, and can thus be discounted as a method of assay when the absolute concentration of penicillin is to be determined. However, its simplicity renders it the method of choice when the sensitivity of an infecting organism is known, and assays are carried out

to ensure that a certain minimum concentration of penicillin is being obtained, the actual level being unimportant provided it is above the therapeutic one for the organism concerned.

Owing to the possible contamination of the urine, the *Staph. aureus* method is unsuitable for urine penicillin assays and the *Sarcina lutea* method must be used.

Summary and Conclusions

Serum penicillin levels and urine excretion rates were measured in six subjects, using two intramuscular and two oral preparations of penicillin. The two standard methods of assay are described in some detail, and the results obtained with both methods are compared.

The results obtained clearly indicate that oral penicillin is no substitute for parenteral therapy when consistently high levels are required. Where oral therapy is indicated phenoxymethyl penicillin gives adequate and reliable blood levels. Benzathine penicillin appears to give disappointing results in adults, and it is probably of more value in children.

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RAPID INTRAVENOUS TRANSFUSIONS IN CHILDREN

BY

LESLIE G. ANDREWS, M.B., D.C.H.

Paediatric Registrar, Institute of Child Health, Hammersmith Hospital and Postgraduate Medical School; Formerly Resident Medical Officer, Paediatric Department, St. George's Hospital, London

AND

DAVID J. COLEMAN, M.B., F.F.A.R.C.S., D.A.

First Assistant, Department of Anaesthesia, St. George's Hospital, London

Bayliss and Starling (1894) showed that rapid injections of crystalloid solutions and blood caused a rise in venous pressure in dogs. Previous cord transections at the level of T 1 had increased the capacity of their vascular system by one-quarter. In 1918 Starling pointed out that transfusions in anaemic patients caused the heart to behave as an overloaded heart-lung preparation, giving further rise to an already elevated venous pressure. This resulted in a fall of cardiac output and pulmonary oedema. Cabot (1926), Ellis and Faulkner (1939), and Sharpey-Schafer (1944) found the cardiac silhouette enlarged on radiological examination, electrocardiographic changes, and increased cardiac weight at necropsy in

anaemic patients. Sharpey-Schafer and Wallace (1942b) found increased cardiac diastolic size and electrocardiographic changes after transfusions in normal patients. In fatal cases necropsy has shown pulmonary oedema and cardiac dilatation (Plummer, 1936; Fishberg, 1937; Drummond, 1943). According to Riddell (1939) the commonest cause of fatality following blood transfusion is circulatory failure secondary to overloading.

This paper is a report on three anaemic children to whom 16 very rapid transfusions—35–50 ml. a minute—were given under general anaesthesia. There were no ill effects.

Case Reports

Case 1.—A boy aged 6, weighing 2 st. 1 lb. (13.2 kg.) and 3 ft. 8 in. (112 cm.) in height, with congenital hypoplastic anaemia, had a systolic murmur most clearly audible in the pulmonary area, and a liver enlarged to three fingerbreadths below the right costal margin. By May, 1955, he had received a total of 51 transfusions. Initially these consisted of 50 ml. of whole blood, but later he was being transfused with the packed cells from 1 pint (570 ml.) of blood. These earlier infusions were administered under basal narcosis—rectal thiopentone, 1 g. per 50 lb. (22.5 kg.) body weight—given in 20–30 minutes. Splenectomy in March, 1955, brought no improvement. Since June, 1955, he has received nine rapid transfusions under general anaesthesia, each consisting of the fresh packed cells from 2 pints (1,140 ml.) of blood. Blood-volume estimations were performed on three occasions.

Case 2.—The patient was a boy aged 7 weighing 3 st. 11 lb. (25 kg.) and 3 ft. 6 in. (107 cm.) in height, with congenital hypoplastic anaemia, probably of the Fanconi (1927) type—that is, with small head and pigmentation, though this latter may have been due to early haemochromatosis. The heart and respiratory systems appeared normal, but the liver was enlarged to two fingerbreadths below the right costal margin. By June, 1955, he had received a total of 93 intravenous blood infusions consisting of 50 ml. of whole blood, and later the packed cells from 1 pint (570 ml.) of blood. The operation was conducted under basal narcosis—rectal thiopentone, 1 g. per 50 lb. (22.5 kg.) body weight—and completed in 20–30 minutes. Splenectomy in May, 1953, produced no improvement. Since June, 1954, six very rapid transfusions have been given under general anaesthesia, each consisting of the fresh packed cells from 2 pints (1,140 ml.) of blood. On three occasions blood-volume estimations were performed and venous pressure changes were recorded manometrically once.

Case 3.—A girl aged 4, weighing 2 st. (12.7 kg.) and 3 ft. 3 in. (99 cm.) in height, had a neuroblastoma of the left adrenal gland. The cardiac and respiratory systems were normal. She had received two previous slow transfusions each consisting of 1 pint (570 ml.) of whole blood. We transfused her rapidly once under general anaesthesia with 1 pint (570 ml.) of whole blood.

Method

Each child was given a fat-free diet for 18 hours before transfusion. Pentobarbitone sodium, 0.6 gr. per stone (6 mg. per kg.), was given one hour before operation, and atropine, 1/100 gr. (0.65 mg.) hypodermically, half an hour pre-operatively. A quiet induction of anaesthesia was achieved with nitrous oxide, oxygen (30%), and trichlorethylene, and maintenance was with nitrous oxide, oxygen (30%), and ether. A Dinsdale (1954) respirator was used in order to assure minimal resistance to respiration. A high flow of gases was maintained in order to prevent accumulation of carbon dioxide.

The apparatus for transfusion was of a modified scalp vein type with Luer-Lock fittings (see Fig.) (Black *et al.*, 1951; Mollison, 1951). The packed cells were usually