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RESEARCH PAPERS

PENICILLIN FORMULATIONS: THE EFFICACY OF OILY INJECTIONS

By J. C. Floyd

From the Pharmaceutical Research and Service Laboratories, Imperial Chemical Industries, Ltd., Blackley, Manchester, 9

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The rapid excretion of penicillin when administered parenterally in aqueous solution has led to the development of several non-aqueous formulations designed to prolong the therapeutic blood level after injection. The object of "slow-release" is two-fold; firstly to eliminate the inconvenience and pain to the patient of frequent injections and secondly to avoid wastage of penicillin. Its adoption is based on the now generally accepted principle that a continuous therapeutic level is more effective than intermittent "peaks." The three main principles so far employed in such preparations are: (a) the suspending of penicillin in vegetable oil, usually in presence of a thickening or dispersing agent, (b) the utilisation in these suspensions of penicillin salts which are only sparingly soluble in water, and (c) the control of particle size of the suspended penicillin. The first slow-release formulation was devised by Romansky[1], who suspended a water-soluble salt of penicillin in arachis oil containing 4·8 per cent. of beeswax. Numerous clinical reports have shown that this preparation achieves therapeutic levels (>0·03 u./ml.) for periods up to 24 hours after injection. Later, the preparation of sparingly soluble salts of penicillin containing either heavy-metal or organic bases was followed by their trial in slow-release formulations. Of these, the most effective so far has been procaine penicillin. Clinical reports on preparations containing procaine penicillin suspended in untreated arachis oil were made by Herrell, Nichols and Heilmann[2], and by Hobby Brown and Patelski[3], both claiming therapeutic levels for periods greater than 24 hours. At about the same time, attention was drawn to the significance of the particle size of the suspended penicillin by Romansky and Dowling[4], who reported that in oil/wax suspensions of soluble salts the best results were obtained when the particle size of the latter exceeded 50μ. This view was supported by Sullivan[5], who used large particle size procaine penicillin in untreated arachis oil. Shortly after the introduction of procaine penicillin Buckwalter and Dickison[6] developed a new technique of suspending penicillin by using an aluminium stearate/arachis oil gel instead of oil/wax or untreated oil. Clinical reports on the new suspensions were made by Thomas and his co-

workers[7], who obtained therapeutic levels for as long as 96 hours but who simultaneously showed that small particle size penicillin was more effective than large in stearate/oil suspensions. Beyond relatively simple theories such as "water-proofing" of penicillin, no satisfactory explanation of the mechanism of slow-release has been advanced. A survey of earlier work also shows that not all the variants of the slow release formulations have a common basis for comparison. This is largely due to the fact that clinical and laboratory evaluation has been by a variety of techniques. The object of the work described here was to compare as accurately as possible the efficacy of a series of preparations embodying the most important of the possible variants, viz. (a) soluble and sparingly soluble penicillin (potassium and procaine salts), (b) gelling and suspending agents (beeswax and aluminium stearate), and (c) particle size of penicillin. To permit comparison of a large number of variants with sufficient accuracy it was decided to adopt a laboratory animal test for evaluation, and to use statistical methods for the efficient design of the experiments and analysis of the results.

EXPERIMENTAL

This consisted essentially of two groups of tests described here as Experiments 1 and 2. In both experiments the orders of preparation of samples, dosing and inoculation were randomised to ensure that any extraneous non-random variation would not vitiate the comparison between the main factors of the experiments. The following standard techniques were used:—

(1) *Biological Evaluation.* Three groups, each of 10 mice, were injected subcutaneously with the test preparation, each mouse receiving 30,000 units in 0·1 ml. The mice were then infected intraperitoneally with a 24-hour culture of *Streptococcus pyogenes* Krüger strain. All three groups were infected simultaneously, but the test preparation was injected in advance at different time intervals for the three groups. The usual plan was to inject the first group 5 days before infection, the second 3 days, and the third 2 days. The number of deaths occurring up to 72 hours after infection was recorded for each group. A similar test has been used by Miller[8]. In general the number of deaths increased with the time interval between dosage and infection and the criterion used for comparing the effectiveness of the different formulations studied here was the estimated time between dosage and subsequent infection required to produce a 50 per cent. mortality in the mice. The greater this time the more effective the preparation.

(2) *Determination of Penicillin Potency of Test Preparation.* The penicillin salts were assayed both microbiologically (*Staphylococcus aureus*) and iodimetrically[9]. Suspensions were assayed iodimetrically only and the results interpreted by means of a factor obtained in the assay of the penicillin salt.

(3) *Control and Measurement of Particle Size of Penicillin.* Three ranges of particle size were selected for testing and are described here

arbitrarily as "coarse," "medium," and "fine." Reduction to appropriate particle size was effected by dry grinding, followed, where necessary, by grinding in oil (with subsequent removal of oil before incorporation in the arachis oil base). Representative particle size analyses are expressed graphically in Figure 1, the measurements being made using a technique based on that of Fairs[10]. The graph expresses the relationship between the ranges and shows that the distribution in each range does not exhibit any unusual feature.

(4) *Preparation of Base.* (i) *Stearate/Arachis Oil*: Four methods were used, differing essentially in the temperatures at which the oil and stearate

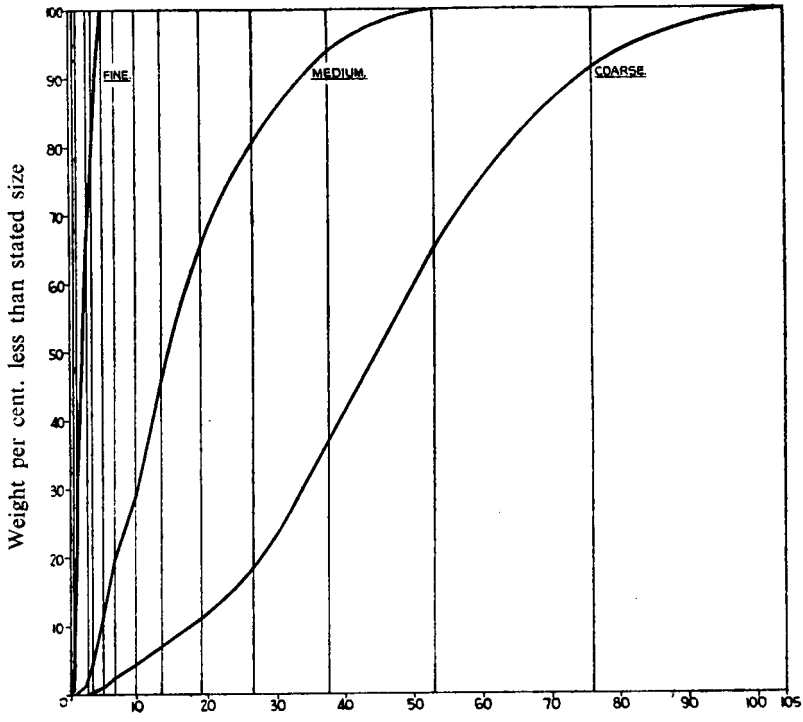

FIG. 1. Particle size distribution of typical preparations.

were gelled. (a) *High temperature*: Powdered aluminium stearate was dispersed in arachis oil, and the mixture heated slowly and with constant stirring to within the range 117° to 122°C. At this point the mixture became extremely viscous. The temperature was then raised to 150°C. and maintained at this level for 1 hour to effect sterilisation. The final product was rather less viscous than at 117°C. (b) *Medium temperature:* Aluminium stearate and arachis oil were separately sterilised by maintaining at 140°C. for 4 hours. The stearate was then dispersed in the oil, using aseptic technique, and the mixture raised slowly, and with stirring, to within the range 125° to 130°C. At this temperature the mixture

gelled and developed maximum viscosity, which was retained on cooling. (c) *Low temperature:* As for method (b), but the gelling temperature was raised to within the range 117° to 122°C. This gave a gel of slightly higher viscosity than (b). (d) *Unheated:* Aluminium stearate and arachis oil were separately sterilised by maintaining at 140°C. for 4 hours and then mixed by ball-milling for 24 hours. This yielded a fine dispersion of stearate in oil approximately equal in viscosity to the original oil.

(ii) *Beeswax/Arachis Oil:* The beeswax and arachis oil were mixed in the cold and the temperature raised slowly, with stirring, to 150°C., at which temperature it was maintained for 1 hour. The solution was allowed to cool and was agitated gently during cooling from 60°C. to room temperature to ensure dispersion of beeswax in a finely divided state.

(5) *Incorporation of Penicillin in Beeswax or Stearate Base:* The penicillin was mixed with the prepared sterile base in a mortar and the mixture tranferred to a standard vessel containing a fixed charge of steel balls. The vessel was sealed and rotated for 1 hour, the speed being adjusted so as to ensure efficient mixing with minimum alteration of particle size.

Experiment No. 1: Using the standard techniques already described, the effect of varying the following factors was investigated: (a) penicillin salt (procaine and potassium), (b) nature of suspending agent (beeswax and aluminium mono-, di-, and tristearates), (c) concentration of suspending agent (1 per cent. and 2 per cent. for both beeswax and stearate), (d) particle size of penicillin (medium and fine). The samples of aluminium stearate used are described here arbitrarily as mono-, di-, and tri-stearates since analysis showed that the base/acid ratio was approximately of this order. Typical analyses are given in the Appendix. All stearate/oil gels were prepared by the high temperature method, variations in the method of gelling being studied in Experiment 2. It was not considered desirable at this stage to examine the four factors in all combinations, and in the first part of the experiment (1a) one half of the combinations representing a half replicate of the full factorial design[11] was

TABLE I

LOGARITHMS OF ESTIMATED TIMES BETWEEN DOSAGE AND SUBSEQUENT INFECTION TO GIVE 50 PER CENT. MORTALITY

Penicillin Salt	Particle Size	Monostearate		Distearate		Tristearate		Beeswax	
			per cent.		per cent.		per cent.		per cent.
Procaine ...	Medium ...	0·36	(1)	0·85	(2)	0·55	(2)	−0·14	(1)
	Fine ...	0·68	(2)	0·44	(1)	0·99	(1)	0·57	(2)
Potassium ...	Medium ...	0·14	(2)	−0·48	(1)	All mice died	(1)	All mice died	(2)
	Fine ...	0·01	(1)	0·14	(2)	0·16	(2)	All mice died	(1)

The figures between the brackets refer to the concentration of stearate or beeswax.

carried out. The results are given in Table I expressed as logarithms of the time required between dosage and subsequent infection to produce 50 per cent. mortality. In deriving these results it was found that the mortality measured as a probit was linear with respect to the logarithm of the time between dosage and infection. The well-known method of probits[12] was then used to calculate the 50 per cent. point. The slope of the probit log-time line was calculated for each sample. Each slope had a fairly large standard error and the assumption could be made that all the slopes were equal. The combined slop was then calculated and used to obtain an estimate of the 50 per cent. point for all preparations.

The reliability of the results differed from sample to sample but the variation was not large and an average figure of 0·14 for the standard deviation could be used without risk of serious error. It is clear from this table that potassium is much inferior to procaine, and beeswax inferior to aluminium stearate but the 6 remaining samples of procaine penicillin in stearate gels do not give sufficient information on the comparison between the particle size of penicillin and the three types of stearate. A further 6 samples were therefore prepared (Experiment Ib), with the 2 different particle sizes of procaine penicillin, using the three stearates at concentrations of 1 per cent. and 2 per cent. but with these concentrations reversed. Table II shows the results of this experiment together with the relevant portion from Table I. The two halves of the experiment then consisted of samples prepared using all combinations of the variables, particle size, concentration, and type of stearate.

TABLE II

LOGARITHMS OF ESTIMATED TIMES BETWEEN DOSAGE AND SUBSEQUENT INFECTION TO GIVE 50 PER CENT. MORTALITY

	Particle Size	Monostearate		Distearate		Tristearate	
			per cent.		per cent.		per cent.
Experiment Ia	Medium ...	0·36	(1)	0·85	(2)	0·55	(2)
	Fine	0·68	(2)	0·44	(1)	0·99	(1)
Experiment Ib	Medium ...	0·32	(2)	0·32	(1)	0·34	(1)
	Fine	0·28	(1)	0·11	(2)	0·18	(2)

The figures between the brackets refer to the concentration of stearate.

It should be noted that the average result for Experiment Ib is appreciably lower than that for Experiment Ia. This is probably due to the different batch of mice or the different suspension of infecting organism, or both, the experiments being carried out at different times. The design is such, however, that all the important comparisons are made within experiments and the comparison between the experiments has been made to coincide with the higher and less important interactions between the factors. The analysis of variance is given in Table II.

The standard deviation of each result calculated from the detailed observation on the mice is approximately the same for both experiments, the average value being 0·130, which corresponds to a variance of 0·0169.

From Table III it is seen that the efficacy of the preparations is influenced by the amount of the stearate used. The magnitude of this

effect is shown by a comparison of the averages for all the 1 per cent. and all the 2 per cent. preparations given in Table II: mean for 2 per cent. stearate, 0·549; mean for 1 per cent. stearate, 0·355. The 2 per cent. preparations are clearly more effective.

Table III also shows that efficacy is influenced by the particular combination of type of stearate and particle size of the procaine penicillin

TABLE III

ANALYSIS OF VARIANCE

Source of Variation	Sum of Squares	D/F	Variance
Type of stearate	0·0258	2	0·0129
Amount of stearate	0·1121	1	0·1121
Particle size	0·0003	1	0·0003
Interactions :—			
Stearates with particle size	0·1347	2	0·0674
Stearates with amount	0·0230	2	0·0115
Between experiments	0·4454	1	0·4454
Remainder	0·0440	2	0·0220
Total	0·7853	11	—
Error	—	Large	0·0169

used. This is seen more clearly in Table IV, in which the results of Experiments 1a and 1b have been averaged.

Table IV shows that fine particles are better with mono- and tri-stearate, but worse with distearate, and that medium particles are best with distearate. This type of interaction was unexpected and requires verification. Should this interaction be fortuitous, it is unlikely to affect the further conclusion that fine particles are not significantly different from medium ones. Table IV also shows an apparent trend to higher results with increasing stearic acid content of the aluminium stearate.

TABLE IV

(*a*) INTERACTION OF TYPE OF STEARATE WITH PARTICLE SIZE

Particle Size	Type of Stearate			Means
	Mono	Di	Tri	
Medium	0·340	0·584	0·445	0·456
Fine	0·478	0·275	0·588	0·447
Mean	0·410	0·430	0·516	

Experiment No. 2: This was designed to assess the effect of varying (a) the gelling method (high, medium and low temperature and unheated), (b) the type of aluminium stearate (aluminium monostearate and an aluminium stearate of no specified composition, referred to as "Technical"), (c) concentration of stearate (2 per cent. and 3 per cent.), and (d) particle size of procaine penicillin (medium and coarse). The statistical design used to examine these factors was a half replicate of the factorial design. This involved 16 samples, and the results expressed as logarithms of the estimated time between dosage and subsequent infection to give 50 per cent. mortality in mice are given in Table V.

TABLE V

LOGARITHMS OF ESTIMATED TIMES BETWEEN DOSAGE AND SUBSEQUENT INFECTION TO GIVE 50 PER CENT. MORTALITY

Particle Size	Type of Stearate	High Temperature		Medium Temperature		Low Temperature		Unheated	
			per cent.		per cent.		per cent.		per cent.
Coarse ...	Mono- ...	0·40	(3)	0·25	(2)	0·39	(3)	−0·23	(2)
	Technical ...	0·49	(2)	0·38	(3)	0·40	(2)	−0·35	(3)
Medium ...	Mono- ...	0·50	(2)	0·43	(3)	0·60	(2)	0·06	(3)
	Technical ...	0·56	(3)	0·44	(2)	0·44	(3)	−0·03	(2)

The analysis of variance of these results is given in Table VI.

The only significant effects are particle size of penicillin and temperature of gelling. The residual variance, which is a mixture of interactions, is no greater than the error variance due to the inherent variability of the mice. Had the reverse been the case, it would have been necessary to carry out the second half replicate to complete the factorial design

TABLE VI

ANALYSIS OF VARIANCE

Source of Variation	Sum of Squares	D/F	Variance
Concentration of stearate	0·0038	1	0·0038
Particle size	0·0848	1	0·0848
Type of stearate	0·0002	1	0·0002
Temperature of gelling	1·1367	3	0·3789
Residual	0·0460	9	0·0051
Total	1·2715	15	—
Error	—	Large	0·0177

involving all combinations of the factors. The mean results are as follows:—

Particle Size: Coarse = 0·217; Medium = 0·362.

Temperature of gelling: High = 0·489; Medium = 0·378; Low = 0·485; Unheated = − 0·167.

Medium particle size is thus better than coarse, and unheated gels inferior to heated. There is no clear distinction between the high, medium and low temperatures of gelling. Similarly, there is no detectable difference between the two types of stearate and between 2 per cent. and 3 per cent. concentrations of stearate.

DISCUSSION

The reliability of the mouse protection test has already been discussed by Miller[8], whose conclusions are confirmed by comparison of his results and those reported here with the clinical data of Thomas[7]. Assuming that the test gives a good indication of the prolongation of blood levels achievable in man, it would thus appear that the most efficient of the oily injections so far examined consists of procaine penicillin (fine particle size) dispersed in oil gelled by aluminium stearate. Whether the three factors of penicillin salt, particle size, and gelling agent are closely inter-

related is not yet established, but the superiority of procaine penicillin over the potassium salt clearly extends to all formulations and all particle sizes. As far as penicillin salt is concerned, this might lead to a theory of delayed absorption due to reduced water-solubility. This explanation cannot be accepted, however, until a series of water insoluble salts have been studied. It has already been shown[7] that aluminium penicillin is less effective than the procaine salt and this difference cannot be explained on the grounds of solubility alone. The influence of particle size presents an interesting problem. The first reports[4,5] favoured large particles but the position was reversed when stearate gels were introduced, suggesting that the action of the latter is connected with the surface area of penicillin. The fact that gels of different viscosity do not differ significantly in biological behaviour indicates that aluminium stearate does not merely act as a thickening agent, although it is equally clear that it loses its effect if gelling does not take place. The chemical composition of the stearate is apparently not a significant factor and therefore samples may be selected on purely pharmaceutical grounds without risk of sacrificing activity. The optimum proportion of stearate seems to be approximately 2 per cent. of the oil base. Preparations containing more than this have no increased activity and are more difficult to manipulate.

In the light of the foregoing, it is suggested that the mechanism of aluminium stearate probably involves the formation of a protective film around the penicillin particles. Such a film might be created by interaction at the penicillin surface to produce " procaine stearate," the molecules of which could be orientated with the carbon chain in the oil phase. This would retain a film of oil over the penicillin surface, the former being held more tenaciously than would be a film of untreated oil. This theory would explain the effect of penicillin particle size since the area of adsorbed film would increase with reduction of the particle size. The theory is also compatible with the other observed effects of concentration of stearate and disappearance of activity in non-gelled material. In the former case the optimum concentration required to establish the protective film is apparently of the order of 2 per cent., and nothing is gained by raising this figure. For the latter, it is suggested that the interaction with procaine penicillin can only take place when the stearate is in the gelled state.

SUMMARY

(a) A comparison has been made of the efficiency of various oily injections of penicillin using a mouse-protection test.

(b) It is concluded that the most satisfactory preparation consists of procaine penicillin of small particle size dispersed in a stearate/arachis oil gel.

(c) A theory is suggested for the mechanism by which aluminium stearate induces " slow-release."

I am indebted to Dr. O. L. Davies for the design of the tests and the analysis of results, to Dr. A. R. Martin for supervision of the biological work, and to Mr. A. G. Fishburn for help in the preparation of the paper.

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APPENDIX

ANALYSIS OF ALUMINIUM STEARATES

Test	Monostearate	Distearate	Tristearate	Technical
	per cent.	per cent.	per cent.	per cent.
Loss at 100°C. for 3 hours ...	1·5	0·66	0·96	0·9
Ash	16·20	8·75	6·20	0·05
Water-insoluble ash ...	15·90	8·2	5·3	9·69
Aluminium (as Al_2O_3) ...	16·2	8·8	6·4	10·10

DISCUSSION

The CHAIRMAN said that the paper was an interesting illustration of the use of the mouse protection test as an alternative to the rabbit method of studying the persistence of penicillin in the blood. It was also a very good example of the value of physical chemistry in pharmacy.

DR. K. BULLOCK (Manchester) observed that procaine penicillin had been chosen from a number of salts of penicillin and organic bases. Could the author give the references to the other organic bases or indicate the types of other organic bases tried?

PROFESSOR H. BRINDLE (Manchester) said he was interested in slow release vehicles for penicillin and in the use of the mouse protection test as a criterion. He had always used the rabbit and, in common with other workers, had found some difficulty in getting a sample of the blood which was sufficiently sterile to give satisfactory results.

In the mouse protection test the solution or suspension was injected subcutaneously into the mouse. Everything depended on the relationship between the oily depot and the surrounding tissue and this might give very different results from those which one would get by intramuscular injection in human beings. He had not consulted the references cited by the author but thought that Thomas and Miller had worked with aqueous solutions, which were very different from oily suspensions. Did the author think it quite fair to relate his results on mice to what might happen when suspensions were administered intramuscularly in human beings?

DR. R. E. STUCKEY (London) said that various papers had been published recently giving varying opinions on the efficacy of penicillin suspensions. Had the author any experience with the aqueous procaine penicillin suspension tested by his method?

DR. F. HARTLEY (London) mentioned that the author suggested that the viscosity of the solution made very little difference to the duration of action of the material. Did the extent of gelling vary with the age of the suspension? If so, the ease of manipulation would also vary.

MR. J. C. FLOYD, in reply, said that a number of salts were mentioned in the literature, and they had used a number of organic bases, including *p*-chlorophenyl biguanide. In reply to Professor Brindle, he thought that the question was really concerned with whether the test gave results comparable with those obtained in man. He thought this question was answered by Thomas's findings which showed excellent correlation between the results in mice and in human beings.

They had examined the aqueous procaine penicillin suspension by the method described, but the results were not very good; the effect was so rapid that the test became too insensitive.

He was not quite clear what Dr. Hartley meant by the extent of gelling. If one gelled under optimum conditions that gel on standing would eventually break. He thought that all aluminium stearate gels eventually broke on standing, and presumably to incorporate penicillin into a broken gel would be a much more simple matter than to incorporate it into an extremely viscous one. Whether there was any biological difference between a gel before and after breaking he could not say.