STUDIES IN THE DETERIORATION OF AQUEOUS SOLUTIONS AND DISPERSIONS OF PROCAINE BENZYLPENICILLIN

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Among the more widely known properties of benzylpenicillin is its characteristic instability in the presence of water. This unfortunate feature gave rise to many problems in the course of its isolation and development, which were accentuated by the later production of a highly purified crystalline material. In addition to instability in solution, pharmacological examination revealed that benzylpenicillin was rapidly absorbed and excreted, thus necessitating administration by frequent injection or continuous drip. Many methods were attempted which were designed to prolong the therapeutic effect within the body, for example by dispersing the benzylpenicillin in an oleaginous, slowly diffusing vehicle, or by delaying renal excretion. Whilst some success was being achieved in the prolongation of benzylpenicillin blood levels, these techniques were quickly superseded when, in 1948, Sullivan *et al.*[1] first described an insoluble salt of benzylpenicillin formed by simple admixture in solution of procaine hydrochloride and sodium benzylpenicillin which, when injected, produced a prolonged blood level. By injecting a sparingly soluble salt in the form of an aqueous or oily suspension of fine crystals the need for frequent injection is reduced, and in most cases a single injection of 300,000 units of the procaine salt will provide a demonstrable serum level for 24 hours[2,3,4].

The procaine salt of benzylpenicillin is stable in aqueous media and it has been found possible to prepare an aqueous suspension of this material which, whilst in a form suitable for immediate injection, can be stored as a suspension for periods of 12 months or longer. Although it is thus known that procaine benzylpenicillin is relatively stable in aqueous suspension, it is also known that this stability is dependent on certain factors, including temperature of storage, *p*H of the suspension, presence of buffers and other agents, etc., and observations relating to studies on these aspects are described.

Part I

Deterioration in Dispersions of Procaine Benzylpenicillin

Procaine benzylpenicillin is frequently administered in the form of a ready prepared aqueous dispersion containing 300,000 I.U./ml., and it is the main purpose of this investigation to assess the stability of this form of the material. Benzylpenicillin, the product of a biosynthesis may show small batch to batch variations, in spite of careful efforts during the course of its manufacture to obtain consistently uniform material. The B.P. and T.S.A. Regulations lay down certain minimal standards of purity, e.g., moisture, toxicity, potency, etc., to which all batches must conform if they are to be suitable for clinical use. Beyond the scope of

these standards however, are small variations, including the presence of traces of iron or other metals, pigments, solvents, etc., any of which may influence the stability of the final product.

It is common practice in the manufacture of many pharmaceutical products, to select at random a number of samples from each batch, other than those which may be required for statutory or other tests, to be subjected to a range of storage conditions, so that observations may be made on their keeping properties. From a series of sterile filled, silicone-treated vials of suspension set aside at normal room temperature, i.e., 20° ± 5° C., samples were withdrawn and subjected to the tests described. Each sample tested was representative of a single batch of procaine benzylpenicillin and therefore factors other than time and temperature alone may account for the course of deterioration. The samples had been stored for periods varying from 1 to 46 weeks.

Formulation of Test Suspensions

Test Vehicle:

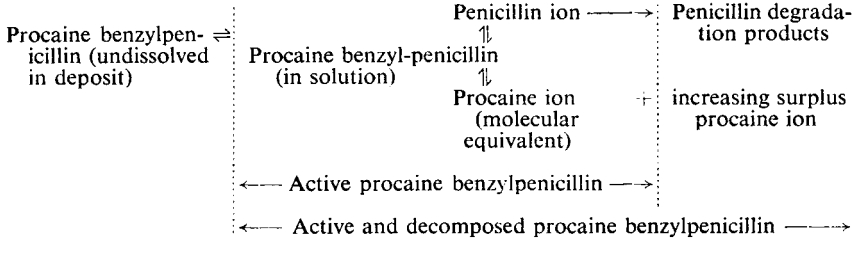
Sodium citrate, tribasic, anhydrous	1·44 g.
Sodium carboxymethylcellulose	0·481 g.
Polyoxyalkylene sorbitan mono-oleate	0·086 g.
Phenylmercuric nitrate	0·137 mg.
Water to 100 ml.	

Test Suspension:

Procaine benzylpenicillin	300,000 I.U.
Test vehicle to 1 ml.	

Theoretical Considerations

Procaine benzylpenicillin has been found to be unstable in aqueous solution (see Part II), the instability being partially mitigated by the inclusion of a buffering agent, e.g., tribasic sodium citrate. The pattern of degradation in a suspension is thought to be—



Thus on admixture of procaine benzylpenicillin with water, a small amount dissolves (solubility about 1 in 200), the remainder settling out as a deposit. The procaine benzylpenicillin molecule in solution may be expected to dissociate, giving rise to a procaine fraction which will remain in solution within the limit of its solubility, or may react with benzylpenicillin decomposition products; the benzylpenicillin moiety, being unstable, undergoes degradation. Therefore, the procaine fraction in

solution at any one time is made up of (*a*) procaine from decomposed procaine benzylpenicillin, as procaine ion or salt, and (*b*) procaine fraction of dissociated procaine benzylpenicillin in solution as such.

Practical Considerations

Since one may deduce the concentration of active procaine benzylpenicillin in solution at a given time from a microbiological assay, and that of total procaine benzylpenicillin (active and decomposed) from a spectrophotometric assay, a simple calculation will give the amount of procaine benzylpenicillin which has decomposed at that time. The samples of dispersions were separately centrifuged, the clear supernatant liquid removed and examined as follows:

(*a*) *Active and decomposed procaine benzylpenicillin concentration, from total procaine base.* Using the Unicam SP.500 Spectrophotometer, a purified sample of procaine benzylpenicillin had been found to show maximum absorption at 289·5 mμ, and to obey Beer's law in concentrations up to 80 μg./ml., although subsequent readings were not made on solutions in excess of 60 μg./ml. The specific extinction coefficient $E_{1\text{ cm.}}^{1\text{ per cent.}}$ 289·5 mμ was found to be 316. Supernatant liquids removed from test samples were diluted to less than the equivalent of 60 μg./ml. procaine as procaine benzylpenicillin and were assayed by measuring the optical density $E_{289\cdot5}$ and calculating:

$$\mu\text{g./ml.} = \frac{E_{289\cdot5}\text{ observed.}}{0\cdot0316}$$

Thus, assuming a potency of 1000 I.U./mg.

$$\mu\text{g./ml.} = \text{I.U./ml.}$$

The figures obtained for procaine ion were thereby translated in terms of the stoichiometric equivalent procaine benzylpenicillin (100 mg. of procaine benzylpenicillin contains 40·2 mg. of procaine ion), and are quoted as such in Table I, Column I.

(*b*) *Active procaine benzylpenicillin concentration.* This was obtained by microbiological assay of the supernatant liquid, using a cavity plate diffusion technique, with *Staphylococcus aureus* as the test organism. Results obtained are quoted as I.U./ml., in Table I, Column II.

(*c*) *Apparent loss of procaine benzylpenicillin.* Decomposed and active procaine benzylpenicillin concentration = I. Active procaine benzylpenicillin concentration = II. I — II = apparent loss of procaine benzylpenicillin, in I.U./ml. of supernatant liquid. Since the supernatant liquid represents about 65 per cent. of the total volume of the suspension, the actual loss in I.U./ml. of suspension is approximately two-thirds of the apparent loss I.U./ml. of supernatant liquid.

Samples were frequently taken which whilst incorporating different batches of procaine benzylpenicillin, were, nevertheless prepared during the same week and stored under identical conditions. Such results have been averaged for the particular week, the average, together with an indication of the number of samples involved being shown in Table I.

Assessment of Deterioration

When the results shown in Table I were plotted as graphs — (a) total procaine ion as procaine benzylpenicillin *v.* time, (b) active procaine benzylpenicillin *v.* time and (c) apparent loss of procaine benzylpenicillin *v.* time a linear relation was found to obtain in all cases, thus illustrating the regularity of the procaine penicillin deterioration.

TABLE I

PROCAINE BENZYLPENICILLIN—DETERIORATION IN TEST SUSPENSIONS

Period of storage (weeks)	Number of samples averaged	Total procaine ion as procaine benzyl-penicillin* Spectrophotometric assay (I.U./ml.) I	Procaine benzylpenicillin concentration Bioassay (I.U./ml.) II	Apparent loss procaine benzyl-penicillin (I.U./ml. of super-natant liquid) III
46	3	15,100	—	—
45	2	14,500	—	—
44	1	15,500	—	—
43	3	15,300	—	—
42	2	17,000	—	—
41	3	15,800	—	—
40	2	17,000	—	—
39	4	15,600	—	—
37	1	16,000	3,000	13,000
35	2	14,450	2,600	11,850
34	3	15,200	2,300	12,900
33	4	15,500	2,500	13,000
32	1	12,700	2,700	10,000
31	5	12,400	2,400	10,000
30	4	12,000	2,500	9,500
28	2	13,000	3,200	9,800
24	2	11,700	4,000	7,700
19	1	11,600	3,800	7,800
18	3	10,600	3,300	7,300
17	1	10,800	2,950	7,850
16	6	11,200	3,450	7,750
15	3	10,300	3,650	6,650
14	2	6,500	3,000	3,500
13	4	10,300	3,650	6,650
11	4	9,100	3,400	5,700
10	3	8,300	3,300	5,000
8	3	8,300	3,600	4,700
7	4	7,750	3,600	4,150
6	4	7,400	3,500	3,900
5	2	7,550	3,500	4,050
4	3	7,700	4,400	3,300
3	4	7,500	1,600	5,900
2	3	7,000	2,400	4,600
1	4	6,750	3,500	3,250

* Procaine benzylpenicillin activity = 1000 I.U./mg.

CONCLUSIONS

1. *Potency Stability*

When stored at normal room temperature (20° ± 5° C.) in silicone-coated, rubber capped vials, the rate of deterioration of procaine benzyl-penicillin in dispersions is regular. After 35 to 40 weeks storage the rate of decomposition was seen to decrease, probably because of the gradually increasing procaine ion concentration which, by exerting a common ion effect, depresses the solubility of the procaine benzyl-penicillin. The procaine ion concentration of the supernatant liquid was found to increase at a steady weekly rate equivalent to 0·2 to 0·3 mg. of procaine benzylpenicillin/ml. for the first 35 to 40 weeks, thence falling to 0·1 to 0·15 mg./ml. per week. That is to say, the supernatant demonstrates a weekly loss of procaine benzylpenicillin of about 200

to 300 I.U./ml. per week. Since the supernatant represents some 65 per cent. of the total volume of suspension, the actual loss in suspension would appear to be at the rate of 120 to 180 I.U./ml. per week, or 6 to 9000 I.U./ml. per annum, i.e., <3 per cent. of the activity.

2. p*H Changes*

Readings were made on 10 samples of suspension stored for 1 to 3 weeks, in addition to a further 10 samples stored for 11 to 14 weeks, both at room temperature. The readings were compared with those obtained at the time of preparation, and changes were seen to have occurred. Most samples showed a drop in *p*H (see Table II), the greatest drop occurring in the older preparations. This would suggest that the penicillin fraction decomposition gives rise to breakdown products with acidic properties, which is consistent with the accepted sequence of penicillin degradation.

TABLE II

PROCAINE BENZYLPENICILLIN—CHANGES IN *p*H OF TEST SUSPENSIONS

After 1 to 3 weeks	After 11 to 14 weeks
−0·11	−0·86
−0·06	−0·77
−0·23	−0·67
−0·12	−0·82
−0·42	−0·74
+0·07	−0·67
+0·05	−0·59
−0·10	−0·43
−0·07	−0·67
−0·00	−0·56
Average = −0·10	Average = −0·68

PART II

DETERIORATION OF SATURATED SOLUTIONS OF PROCAINE BENZYLPENICILLIN IN PRESENCE OF SPECIFIC STABILISING AGENTS

Since the supernatant liquid used for the determinations described in Part I represented a saturated solution of procaine benzylpenicillin in the presence of excess of procaine benzylpenicillin, it was considered of interest to study the stability of saturated solutions of procaine benzylpenicillin in the absence of excess of procaine benzylpenicillin, but instead with and without specific stabilising agents. The samples were subjected to various storage temperatures (i.e., 8° C. and 24° C.). The following agents were chosen.

(*a*) *Sodium citrate.* The value of this salt is associated with its buffering action for which purpose it is widely used in pharmaceutical products, especially in solutions of antibiotics.

(*b*) *Procaine hydrochloride.* The addition of a salt with a common ion decreases the concentration of the other ions of a sparingly soluble salt. Procaine hydrochloride, therefore, was selected to provide a source of procaine ion, and was confirmed experimentally by the author to depress the solubility of procaine benzylpenicillin appreciably when present in the solution in a concentration of 2 per cent. w/v. The lower concentration of procaine benzylpenicillin soluble in the presence of procaine hydrochloride proportionately reduces the quantity of the former available for decomposition.

(*c*) *Ethylenediamine tetra-acetic acid.* This material is a sequestering agent capable of removing from solution traces of metals such as iron,

considered to be a potential activator in the chemical decomposition of the dissolved procaine benzylpenicillin. It has been found by Swallow[5] to exhibit stabilising activity in solutions of sodium benzylpenicillin. The material was dissolved in a solution of sodium citrate during preparation, to form the sodium salt.

(*d*) *Hexamine.* Hobbs *et al.*[6] observed the specific stabilising action of hexamine in aqueous solutions of the soluble salts of benzylpenicillin. It was decided to investigate whether similar properties would be exhibited in favour of the procaine salt.

Method of Preparation of Samples

Saturated solutions of procaine benzylpenicillin were prepared in water as a control, or in aqueous solutions of the agents, as described in Table III. The solutions were prepared and filled 10 ml. each into silicone-treated vials of 26 ml. capacity, finally closed with white rubber caps,

TABLE III

SATURATED SOLUTIONS PROCAINE BENZYLPENICILLIN—FORMULATIONS (PER CENT. W/V)

	1	2	3	4	5	6	7	8	9	10	11	12
Procaine hydrochloride B.P.	—	1·0	2·0	3·0	—	—	1·0	2·0	3·0	2·0	—	—
Sodium citrate anhydrous	—	—	—	—	2·1	0·02	2·1	2·1	2·1	2·1	—	2·1
Ethylenediamine tetra-acetic acid ..	—	—	—	—	—	—	—	—	—	0·1	—	—
Hexamine B.P.C.	—	—	—	—	—	—	—	—	—	—	0·5	0·5

Procaine benzylpenicillin was added in excess to saturate the solution, stirred vigorously for 3 hours and finally filtered free from excess of procaine benzylpenicillin before filling for storage.

clamped firmly in position by means of aluminium seals of a type commonly in use for this purpose. Sufficient vials were prepared to permit one of each formulation to be withdrawn twice weekly from each storage temperature over a period of 13 weeks. The average for each weekly set of two results was used in the preparation of the Tables, which illustrate the course of deterioration (Tables IV and V). Assays were carried out microbiologically using a cavity plate diffusion technique, with *Staphylococcus aureus* as the test organism.

TABLE IV

STABILITY OF SATURATED SOLUTIONS OF PROCAINE BENZYLPENICILLIN

Storage temperature 8° C. I.U./ml.

Formulation number	1	2	3	4	5	6	7	8	9	10	11	12
Initial ..	5100	1450	1100	900	4800	4800	1000	820	650	700	6850	6500
After 1 week	5200	1500	1040	940	6200	4900	1100	1100	730	850	5650	5550
" 2 weeks	3900	1240	1070	840	4750	4800	1020	900	680	790	4400	4250
" 3 "	2450	1330	880	760	4550	4500	690	780	860	850	3850	4100
" 4 "	3000	1250	950	740	4850	4750	960	870	710	840	4200	4450
" 5 "	2450	1350	990	850	3450	3350	1060	680	620	735	4000	4200
" 6 "	2150	1320	1010	930	4750	4600	940	810	600	680	4100	4300
" 7 "	2000	1210	920	860	4700	4450	820	760	610	660	3700	3100
" 8 "	450	1260	920	720	4350	4550	960	750	480	530	3900	4300
" 9 "	720	1210	900	830	4900	4650	970	760	550	600	3900	4500
" 10 "	240	1200	890	850	4850	4650	1120	770	790	595	3700	4350
" 11 "	<100	1025	830	840	4450	4150	1000	820	680	620	4050	4300
" 12 "		1040	870	750	4500	4450	1090	720	550	610	3650	3900
" 13 "		1160	900	780	4750	4700	800	630	480	540	3700	2200

* Procaine penicillin activity = 1000 I.U./mg. † Not available.

TABLE V

STABILITY OF SATURATED SOLUTIONS OF PROCAINE BENZYLPENICILLIN

Storage temperature 24° C. I.U./ml.

Formulation number	1	2	3	4	5	6	7	8	9	10	11	12
Initial ..	5200	1500	1000	950	4800	4900	1650	1050	900	1120	6850	6500
After 1 week	1700	1000	840	780	5100	4400	1500	1080	980	1200	5250	5200
" 2 weeks	500	750	*	640	4550	*	1740	960	890	1080	3450	4000
" 3 "	100	630	200	440	3950	500	1710	990	930	1200	3300	3700
" 4 "	<100	250	500	180	4500	*	1650	890	870	960	3050	3800
" 5 "		<100	400	180	3700	140	1300	680	740	820	2400	3700
" 6 "			350	<100	3650	<100	1400	640	710	800	2200	3650
" 7 "			<100		2750		1500	770	760	890	2000	2900
" 8 "					3750		1680	810	770	770	2350	3100
" 9 "					3750		1650	710	590	590	2150	3550
" 10 "					3850		1250	670	660	790	650	3400
" 11 "					2750		1070	580	640	590	*	3700
" 12 "					3300		1310	620	710	870	850	2900
" 13 "					*		1170	640	690	890	790	3000

* Sample not available.

Observations

A. 8° *C. Storage*

(i) *Control*: A slow and steady deterioration, with complete loss after 10 weeks.

(ii) *Added procaine hydrochloride*: The addition of 1 to 3 per cent. of procaine hydrochloride effects a marked depression in the solubility of procaine benzylpenicillin. The reduction of solubility brought about by 1 per cent. of procaine hydrochloride is but slightly enhanced when the proportion is increased to 3 per cent. When sodium citrate is also present, the solubility is further depressed. The total loss of activity in solutions containing added procaine hydrochloride only, was less than 5 per cent. of that of the control, whilst on the further addition of sodium citrate, this loss is reduced to less than 1 per cent. of that of the control.

(iii) *Added sodium citrate*: (*a*) 2·1 per cent. exerts a marked stabilising action, resulting in very slight loss of penicillin activity after 13 weeks. (*b*) 0·02 per cent. (≡ 4 per cent. of the procaine benzylpenicillin content) exerts a marked stabilising effect, with a slight loss apparent after 13 weeks.

(iv) *Citrate/procaine hydrochloride*: The loss of activity in a combination of procaine hydrochloride and citrate is lower than that of either of the agents used alone.

(v) *Combined procaine hydrochloride/citrate with sodium ethylenediamine tetra-acetate.* The additions of sodium ethylenediamine tetra-acetate was found to enhance the stabilising action of the two agents.

(vi) *Hexamine*: This substance apparently increases the solubility of procaine benzylpenicillin, the initial concentration being 6000 to 7000 I.U./ml. A rapid loss of procaine penicillin occurs within the first 3 weeks, with or without citrate, diminishing to a slow deterioration in the absence of citrate, or very slight further losses if citrate is present.

B. 24° *C. Storage*

(i) *Control*: Very rapid loss of procaine benzylpenicillin, decomposition being complete within 2 weeks.

(ii) *Added procaine hydrochloride*: Deterioration is quite rapid, the whole of the material in solution being decomposed within 5 weeks.

(iii) *Added sodium citrate*: (*a*) 2·1 per cent. exerts a significant stabilising effect, but the sample shows steady and continuous decomposition until at the end of the 13 weeks test period it was approaching its half life, i.e., loss of one half of its original potency. (*b*) 0·02 per cent. shows only very slight stabilising activity; steady deterioration brings about complete loss after 5 weeks.

(iv) *Citrate/procaine hydrochloride*: Only slight losses were recorded after the test period. No significant difference between 1 and 3 per cent. of procaine hydrochloride.

(*v*) *Citrate/procaine hydrochloride/sodium ethylenediamine tetra-acetate*: The presence of the sequestering agent did not materially enhance the effect of the other two agents.

(vi) *Hexamine*: Very rapid loss observed over the first 3 weeks, once again diminishing to a steady loss in the absence of citrate, and a slower loss when citrate was present.

Conclusions

1. Procaine hydrochloride has been found to depress the solubility of procaine benzylpenicillin. Sodium citrate exerts a stabilising effect on solutions of procaine benzylpenicillin. Using a combination of the two agents, a reduction in the loss of procaine benzylpenicillin in aqueous solution is obtained. The best results were obtained with the following combinations—

Procaine hydrochloride	1 to 3 per cent.
Anhydrous sodium citrate	2·1 per cent.

2. Excepting in solutions containing hexamine, it has been found that deterioration in saturated solutions of procaine benzylpenicillin is regular, and may be represented by a linear graph.

3. Hexamine appears to increase the solubility of procaine benzylpenicillin thus making a higher concentration available for decomposition. Its incorporation in a dispersion of procaine benzylpenicillin may be expected to bring about a deterioration in the keeping properties.

Summary

1. Methods of assessing the chemical stability of procaine benzylpenicillin both in aqueous solution and in aqueous dispersion are described. The effect of the addition to solutions of specific stabilising agents are examined.

2. The addition of procaine hydrochloride 1 to 3 per cent. to solutions of procaine benzylpenicillin will depress the solubility of the latter from 5000 I.U./ml. to *ca.* 1000 I.U./ml.

3. Under these conditions, and especially in the presence of a buffer, procaine benzylpenicillin solutions have been found to retain their potency for prolonged periods. For the same reasons, the addition of a suitable

procaine salt to dispersions of procaine benzylpenicillin may be expected to lengthen their storage life[7].

4. Aqueous dispersions of procaine benzylpenicillin 300,000 I.U./ml., when suspended in the test vehicle and stored at normal room temperature (20° ± 5° C.) for a period of 46 weeks, have been found to show a loss of activity of about 6000 I.U./ml.

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DISCUSSION

The paper was presented by the Author.

Professor E. H. VOGELENZANG (Netherlands) asked how the author distinguished in the spectrophotometric assay between the procaine derived from the procaine benzylpenicillin in solution, that derived from the added procaine hydrochloride and that derived from decomposed procaine benzylpenicillin.

MR. J. L. LIVINGSTONE (London) said that citrate was by far the most potent stabiliser, and with other additions, particularly excess of procaine, it was not possible to detect any advantage. A disadvantage of adding excess of procaine was that owing to oxidative colour changes the appearance of the suspension might be adversely affected towards the end of its life.

MR. G. SYKES (Nottingham) said that the paper contained useful information concerning the solubility of procaine penicillin in the presence of other agents. It would seem that the substance achieved a certain balance. On the one hand the change of *p*H recorded by the author towards the acid state accelerated decomposition. On the other hand, it was known from experience that the addition of procaine retarded decomposition. He wondered whether the author had any information on the effect of phenol as an antiseptic in the preparation in place of phenylmercuric nitrate. What was the effect of ethylenediamine tetra-acetic acid in the presence of procaine?

MR. F. TAYLOR (London) expressed surprise at the figures for the solubility of the saturated solution at 24° C. in water. He would have expected it to be higher, and certainly a 1000 units difference for a 16° C.

rise in temperature. The figures appeared to show the same solubility at 8° C. as at 24° C. There appeared to be no improvement in stabilisation with a procaine hydrochloride content of between 1 and 3 per cent. Might it not be that the stabilising concentration of the soluble procaine salt was even less than 1 per cent.? Had the author any information about the effect of the preparation in causing a flaking effect on the side of silicone-treated vials? He believed that the effect was due to the breakdown of procaine and not to procaine itself.

DR. K. BULLOCK (Manchester) said that procaine in aqueous solution would break down rapidly at *p*H 7 and over. The process would continue until the *p*H fell to about 4·5, and then there would not be much subsequent decomposition.

MR. J. W. LIGHTBOWN (Mill Hill) referred to Table I, and said it seemed rather peculiar that the procaine benzylpenicillin concentration did not change significantly between the first week and the last recorded results. That might indicate that procaine was being decomposed, in which case the conclusions drawn as to the amount of procaine benzylpenicillin decomposition might be faulty? If procaine were to be built up in the supernatant liquid, it would seem desirable, in choosing the bacteriostatic, to ensure that it was compatible with the procaine. Would procaine be incompatible with the *p*-hydroxybenzoic acid esters?

MR. W. F. HARTE (Nottingham) asked the author to comment further on the point that the value of sodium citrate was associated with its buffering action.

MR. R. LEVIN, in reply, said that the question asked by Professor Vogelenzang did not arise, because where procaine hydrochloride was used the assays were all done microbiologically. The procaine ion could give rise to colour changes following oxidation. Small variations in the iron content of the procaine hydrochloride used in the manufacture of procaine penicillin or hydrochloride would cause variation in the extent of the discoloration. He had no experience of phenol as a bacteriostatic in the product, but he believed that it was not very satisfactory. Procaine hydrochloride when present in a concentration less than 1 per cent. would be effective in stabilising procaine penicillin, and he agreed that in the later stages of the deterioration which he had reported procaine ion would be present in a concentration of something like 0·5 per cent. At that concentration he believed it had exerted a stabilising effect on the suspension without having added procaine hydrochloride. The original *p*H of the suspension was in the region of 7 to 7·3, and no suspension was below *p*H 6·5; this showed the buffering action of sodium citrate. If the figures for the microbiological assay of penicillin in Table I were subjected to statistical analysis, it would be found that the penicillin content actually went down.