THE STABILITY OF INJECTION OF MORPHINE SULPHATE

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Received June 23, 1950

OPIUM preparations have been used medicinally for several hundred years but, until well into the nineteenth century, only crude preparations were available. Morphine, the most important opium alkaloid, was first isolated by Sertürner[1], a young German pharmacist, in 1805, but it was not until he published a further paper in 1817[2] that the importance of his work was appreciated.

The inclusion of a hypodermic injection of morphine acetate in the Additions, published in 1874, to the British Pharmacopœia 1865, constituted the first official recognition in this country of morphine for injection. The same salt was retained in the British Pharmacopœia 1885, but in the 1898 Pharmacopœia an injection of morphine tartrate was included and this appeared again, although its strength was reduced, in the 1914 Pharmacopœia. In all of these formulæ no directions were given for sterilisation of the injections, which were simply solutions of the appropriate salt in distilled water.

No formula for morphine injection appeared in the British Pharmacopœia 1932 but the British Pharmaceutical Codex 1934 included an injection containing 2·5 per cent. of morphine hydrochloride in water. This was sterilised by heating in an autoclave, by tyndallisation or by filtration. The B.P.C. 1934 formula was shown by Davis[3] to be unsatisfactory, particularly in allowing fungoid growth, and after testing several preservatives he suggested two formulæ. The first contained 2·5 per cent. of morphine hydrochloride with 0·05 per cent. of chlorocresol in distilled water, the injection to be prepared using aseptic precautions and sterilised by heating at 80°C. for one hour. The second contained 2·5 per cent. of morphine hydrochloride with 0·1 per cent. of chlorocresol and was sterilised by autoclaving. A modified formula based on this work was included in the second supplement, published in 1941, to the B.P.C. 1934.

Berry[4] reported that solutions of morphine salts may develop colour during storage or on heating. He concluded that although the hydrochloride, the sulphate and the tartrate of morphine were all used in medicine, it was the sulphate which gave the most stable solutions. He also found that the development of colour is dependent on the *p*H of the injection, the colour developing rapidly in alkaline solution and being retarded by acid. According to Berry a *p*H no higher than 3 is required if discoloration is to be prevented; he was of the opinion, however, that this *p*H was rather too low for an injection. The effect of *p*H on pain, at the site of injection, was investigated by Lupton[5] and was the subject of discussion at the 1945 meeting of The British Pharmaceutical Conference.

An alternative suggestion by Berry in the same paper was the inclusion of 0·05 per cent. of potassium or sodium metabisulphite which is very effective in preventing darkening in colour of the injection. In a footnote, however, he reported that later work had shown that certain qualities of rubber affect metabisulphite and reduce its protective action.

A solution of morphine sulphate, based upon Berry's formula, was introduced into Charing Cross Hospital in 1943 and found to be very satisfactory but, on storage for a few weeks, a brown colour was found to develop in solutions stored in clinbritic bottles. On examination of the solutions it was found that in many cases the bottoms of the rubber caps were bleached and it appeared that the caps were probably absorbing sulphur dioxide or sodium metabisulphite from the solutions. When the rubber caps were soaked in a 0·2 per cent. solution of sodium metabisulphite before use no further cases of darkening were noted. Similar results with injection of adrenaline have been reported by West and Whittet[6].

Morphine sulphate found a place in the Fourth Addendum, published in 1941, to the B.P. 1932 and an injection of morphine sulphate has been included in the B.P. 1948. It therefore seemed of interest to investigate the effect of sodium metabisulphite on the darkening of solutions of morphine sulphate and the effect of caps, treated and untreated with sodium metabisulphite, on such solutions when stored in vaccine bottles. There appears to be little data available on the correlation of colour change to loss of activity and it was thought desirable to ascertain whether such relationship does in fact exist.

Preparation of Morphine Injection. Samples of a solution of morphine sulphate 2·5 per cent. together with 0·2 per cent. of chlorocresol in water for injection were prepared, placed in clinbritic bottles, sealed and sterilised by heating at 98° to 100°C. for 30 minutes. These correspond to the B.P. 1948 injection of morphine and were used as controls. Even when freshly prepared they had a distinct brown colour.

Further samples were prepared exactly as above except that 0·1 per cent. of sodium metabisulphite was added. Half of these samples were stored in clinbritic vaccine bottles sealed with rubber caps which had been soaked for 48 hours in a 0·2 per cent. solution of sodium metabisulphite. The remainder were stored in bottles sealed with rubber caps which had not been so treated. All these solutions were water-white after sterilisation. These samples were stored under various conditions and were examined and tested from time to time.

Spectrographic Studies. Morphine, $C_{17}H_{19}O_3N$, readily undergoes oxidation and one of the main products is pseudomorphine, $C_{34}H_{36}O_6N_2$, isolated from opium by Pelletier[7]. This oxidation product is also known as oxydimorphine, dehydromorphine and oxymorphine and, as its formula indicates, it is formed by a reaction involving 2 molecules of morphine. The suggestion that some conversion of morphine into the comparatively inactive pseudomorphine may occur during sterilisation of solutions by heat[8] and the possibility of similar transformation occur-

ring in the body[9] has created a special interest in methods of detecting and estimating either alkaloid in the presence of the other. Dietzel and Huss[8] found that measurement of the ultra-violet absorption afforded a useful means of differentiating the two alkaloids and applied this technique to the study of solutions of morphine hydrochloride, under varying conditions of oxidation.

In preliminary experiments we were able to confirm the findings of Dietzel and Huss regarding the absorption spectra of the alkaloids. For this work we used a sample of morphine sulphate B.P. $(C_{17}H_{19}O_3N)_2,H_2SO_4,5H_2O$, and a sample of pseudomorphine prepared from morphine by the process of Dietzel and Huss[10] using potassium ferricyanide as oxidising agent. Figure I shows the ultra-violet absorption curves, measured with a Hilger medium quartz spectrograph, of the alkaloids using 0·1N sulphuric acid as solvent. It will be seen that morphine exhibits a well-defined curve with a maximum at $\lambda = 283$ mμ and a minimum at $\lambda = 262{\cdot}5$ mμ. In the case of pseudomorphine, however, only general absorption occurs and the minimum at $\lambda = 262{\cdot}5$ mμ has completely disappeared. Figure 2 shows the ultra-violet absorption curve of morphine sulphate injection, prepared as described above, containing chlorocresol and sodium metabisulphite. Although the chlorocresol and sodium metabisulphite cause some slight increase in intensity of absorption, amounting to about 10 per cent., the shape of the morphine curve is well maintained. It was therefore concluded that measurement of the absorption at $\lambda = 262{\cdot}5$ mμ and $\lambda = 283$ mμ would afford a satisfactory means of following the formation of pseudomorphine in our injections.

Samples of morphine injection, prepared and filled into clinbritic bottles as already described, were stored for 9 months after which their ultra-violet absorption spectra were measured. Examination of several samples immediately after preparation, when they were practically colourless with the exception of those containing no sodium metabisulphite, confirmed that their absorption spectra were substantially as recorded in Figures 1 and 2. After 9 months' storage at room temperature the samples containing sodium metabisulphite had developed a slight colour, there being very little difference between those with caps which had been treated with sodium metabisulphite and those with untreated caps. Samples stored in an incubator at 80°F. had darkened in colour to a greater degree. Under all storage conditions those samples containing no sodium metabisulphite exhibited the greatest degree of discoloration.

For the purpose of measuring their ultra-violet absorption all samples were diluted to contain 0·025 per cent. of morphine sulphate and determinations were made using 1 cm. cells. Table I summarises the results.

These spectrographic figures gave little, if any, indication of the formation of pseudomorphine in the injections although, under the experimental conditions, it was expected that the presence of 5 per cent. or more of pseudomorphine would have been detectable.

TABLE I

ULTRA-VIOLET ABSORPTION OF SAMPLES, AFTER 9 MONTHS' STORAGE, DILUTED TO CONTAIN THE EQUIVALENT OF 0·025 PER CENT. OF MORPHINE SULPHATE

Sample	Log $\frac{I_0}{I}$ for 1 cm. cell at λ=283 mμ	Log $\frac{I_0}{I}$ for 1 cm. cell at λ=262·5 mμ
2·5 per cent. morphine sulphate (2.6.48)	0·95	0·25
2·5 per cent. morphine sulphate + 0·2 per cent. chlorocresol (2.6.48)	1·00	0·30
2·5 per cent. morphine sulphate + 0·2 per cent. chlorocresol + 0·1 per cent. sodium metabisulphite (2.6.48)	1·00	0·35
2·5 per cent. morphine sulphate + 0·2 per cent. chlorocresol steamed 30 minutes (9.7.48)	0·98	0·32
2·5 per cent. morphine sulphate + 0·2 per cent. chlorocresol + 0·1 per cent. sodium metabisulphite ; cap soaked ; steamed 30 minutes (9.7.48)	0·98	0·34
2·5 per cent. morphine sulphate + 0·2 per cent. chlorocresol + 0·1 per cent. sodium metabisulphite ; cap unsoaked ; steamed 30 minutes (9.7.48)	0·95	0·30
2·5 per cent. morphine sulphate + 0·2 per cent. chlorocresol ; steamed 30 minutes (8.4.49)	1·00	0·40
2·5 per cent. morphine sulphate + 0·2 per cent. chlorocresol + 0·1 per cent. sodium metabisulphite ; cap unsoaked ; steamed 30 minutes (8.4.49)	1·00	0·35
2·5 per cent. morphine sulphate + 0·2 per cent. chlorocresol + 0·1 per cent. sodium metabisulphite ; steamed 30 minutes ; cap soaked ; stored in inverted position (8.4.49)	0·95	0·40
2·5 per cent. morphine sulphate + 0·2 chlorocresol, steamed 30 minutes inverted and incubated at 80°F. (8.4.49)	1·00	0·30

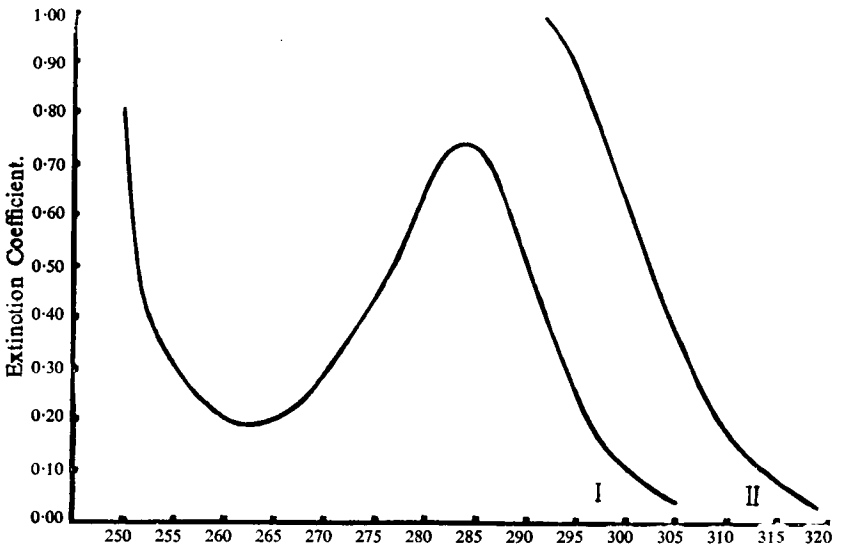

FIG. 1. Ultra-violet absorption curves of morphine and pseudomorphine: I, morphine sulphate 2·289 mg./10 ml. of 0·1N sulphuric acid, II, pseudomorphine 2·372 mg./10 ml. of 0·1N sulphuric acid.

Colorimetric Estimation of Pseudomorphine. Thörn and Ågren[11] recently reported that both morphine and pseudomorphine give colours with aromatic aldehydes in the presence of sulphuric acid. Using a 1 per

cent. solution of vanillin in sulphuric acid (95 per cent.), they found that solutions of pseudomorphine gave a characteristic green colour having maximum absorption at $\lambda = 600$ mμ while morphine afforded a reaction mixture exhibiting little absorption in this region. The reaction was claimed to be suitable for the estimation of pseudomorphine in

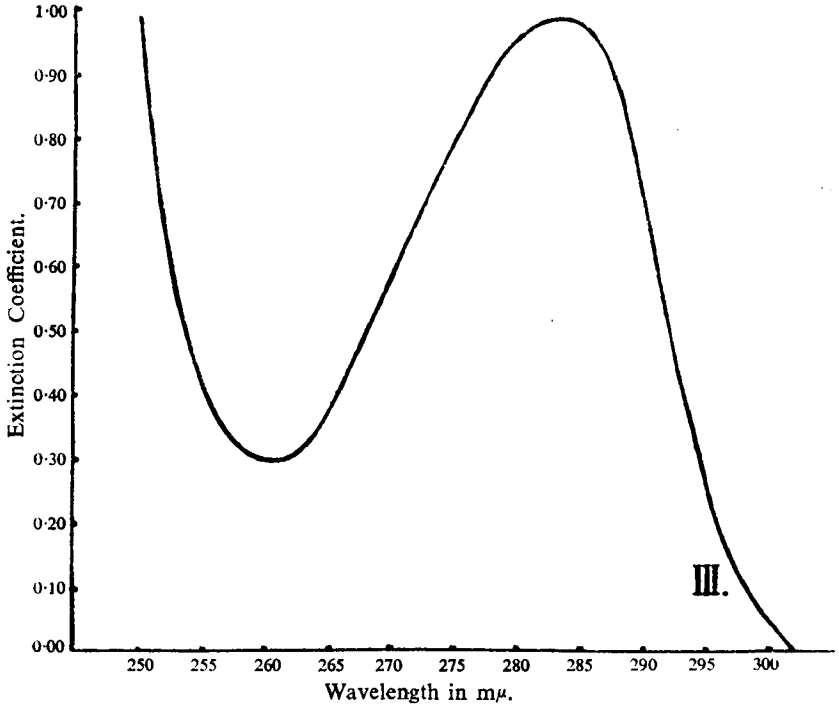

FIG. 2. Ultra-violet absorption curve of 2·5 per cent. morphine sulphate solution containing 0·1 per cent. of sodium metabisulphite and 0·2 per cent. chlorocresol; steamed for 30 minutes (diluted 1 in 100).

morphine injections. For this purpose 0·5 ml. of injection to be tested is placed in a dry test tube and treated with 10 ml. of vanillin reagent, which is slowly added from a pipette with continuous shaking and cooling. The mixture is heated in a boiling water-bath for 20 minutes, cooled in running water and the absorption at $\lambda = 600$ mμ measured. Thörn and Ågren[11] have published absorption curves for the reaction mixtures obtained both with morphine and pseudomorphine.

On trying the vanillin reagent we at once confirmed its sensitivity for the detection of pseudomorphine. A series of standard solutions was prepared by adding known amounts of pseudomorphine to samples of our morphine injection, containing 2·5 per cent. of morphine sulphate, 0·2 per cent. of chlorocresol and 0·1 per cent. of sodium metabisulphite. The vanillin reaction was then carried out on each sample. The technique which we adopted was very similar to that of Thörn and Ågren but we cooled the test tube in ice during the addition of the reagent and the subsequent mixing operation. Readings on each reaction mixture

were then taken with a Spekker absorptiometer using No. 607 Ilford filters and employing a water cell as the blank. Figure 3 shows the calibration curve constructed from the figures obtained. Different batches of vanillin reagent often gave different absorptiometer readings and we found it desirable to use freshly prepared reagent and to construct a calibration curve for each batch.

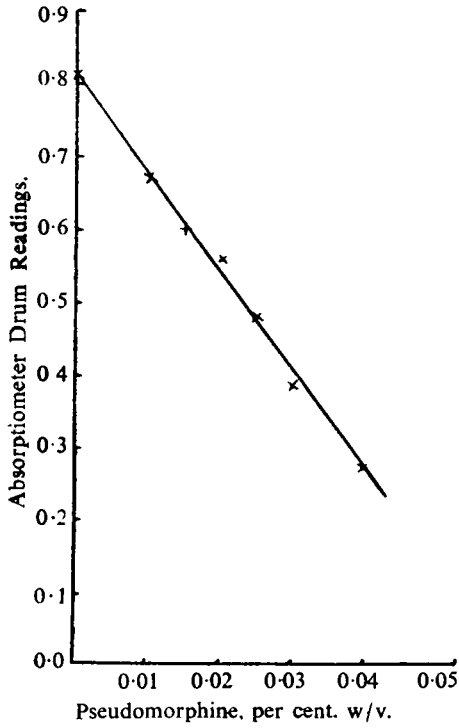

FIG. 3. Calibration curve for pseudomorphine in morphine injection; with Ilford 607 and heat resisting H503 filters.

Samples of morphine injection, prepared as for the spectrographic investigation, were divided into two groups which were stored at room temperature and in an incubator at 55°C. respectively. When freshly prepared the maximum amount of pseudomorphine estimated by the vanillin reagent in any sample tested did not exceed 0·002 per cent. At the end of three months samples when tested gave the results summarised in Table II.

It was thought of interest to carry out colorimetric determinations on some of the samples which had been

TABLE II

PSEUDOMORPHINE CONTENT, ESTIMATED BY VANILLIN REAGENT, IN MORPHINE INJECTION AFTER 3 MONTHS STORAGE

Sample	Pseudomorphine content, calculated from Absorptiometer readings	
	Stored at room temperature	Stored at 55°C.
	per cent.	per cent.
2·5 per cent. morphine sulphate + 0·2 per cent. chlorocresol steamed for 30 minutes (12.12.49)	0·0029	0·0083
2·5 per cent. morphine sulphate + 0·2 per cent. chlorocresol + 0·1 per cent. sodium metabisulphite, steamed 30 minutes caps unsoaked (12.12.49)	0·0016	0·0030
2·5 per cent. morphine sulphate + 0·2 per cent. chlorocresol + 0·1 per cent. sodium metabisulphite, steamed 30 minutes caps soaked (12.12.49)	0·0015	0·0022
2·5 per cent. morphine sulphate + 0·2 per cent. chlorocresol + 0·1 per cent. sodium metabisulphite, steamed 30 minutes caps unsoaked, inverted (12.12.49)	0·0014	—

opened after 9 months and had been subsequently stored at room temperature for a further 3 months. The results are shown in Table III.

TABLE III

PSEUDOMORPHINE CONTENT, ESTIMATED BY VANILLIN REAGENT, OF MORPHINE INJECTIONS, OPENED AFTER 9 MONTHS, RESEALED AND STORED FOR A FURTHER 3 MONTHS AT ROOM TEMPERATURE

Bottle	Contents	Pseudomorphine content, calculated from absorptiometer readings
		per cent.
H	M.S.C. Caps soaked	0·012
E	M.S.C. Caps soaked	0·013
B	M.S.C. Inverted caps soaked	0·018
C	M.S.C. Caps unsoaked	0·021
U	T.M.S.C. Inverted caps unsoaked	0·024
R	M.S.C. Inverted caps unsoaked	0·027
A	M.S.C. Inverted caps unsoaked	0·030
Y	M.S.C. Inverted caps unsoaked	0·033
V	M.S.C. Caps unsoaked	0·033
Q	T.M.S.C. Inverted caps soaked	0·035
K	T.M.S.C. Caps soaked	0·035
W	T.M.S.C. Caps unsoaked	0·037
X	M.C.	0·035
T	M.C.	0·036
D	M.C. Inverted	0·043
S	T.M.C.	0·055
Z	T.M.C. Inverted	0·056

M=2·5 per cent. morphine sulphate solution. S=0·1 per cent. sodium metabisulphite solution
C=0·2 per cent. chlorocresol solution. T=Stored at 80°F. for first 9 months.

Pharmacological Tests. In view of the small amount of pseudomorphine found in the injection, even when exhibiting discoloration, it was felt desirable to test two of the most discoloured samples, which had been stored at 80°F. for 9 months, for analgesic activity. Mr. A. F. Green, of the Wellcome Research Laboratories, Beckenham, carried out these experiments and we are indebted to him for the results which are summarised in Table IV.

TABLE IV

ANALGESIC TESTS ON MORPHINE INJECTIONS

The morphine was injected subcutaneously in rats using a quantal heat response

Solution	Dose of morphine sulphate mg/K.		
	2·0	*1·5*	*1·125*
Standard 2·5 per cent. morphine sulphate	28/30	21/30	17/30
2·5 per cent. morphine sulphate + 0·2 per cent. chlorocresol steamed 30 minutes. Incubated at 80°F.	26/30	20/30	13/30
2·5 per cent. morphine sulphate + 0·2 per cent. chlorocresol + 0·1 per cent. sodium metabisulphite, steamed 30 minutes caps soaked. Incubated at 80°F.	27/30	19/30	17/30

There did not appear to be any significant difference between the activities of the injections and of the laboratory standard morphine sulphate

solution. Any observed differences were obviously within the experimental error of the tests.

Effect of Rubber Caps on Metabisulphite Solutions

Since the results of storage tests in this instance were indecisive with respect to absorption of metabisulphite the following tests were performed:—One litre of 0·1 per cent. sodium metabisulphite solution was prepared and divided into two parts. One half was placed in a corked flask and one dozen unused clinbritic rubber caps was added. The other half was placed in a similar flask and kept as a control. Another batch of caps was placed in distilled water as a control on the appearance of the caps. The flasks were stored at room temperature.

5 ml. quantities of the solution from the caps and the control solution were titrated regularly against 0·01N iodine solution. The results are shown in the Table V and are plotted in the graph (Fig. 4). They show

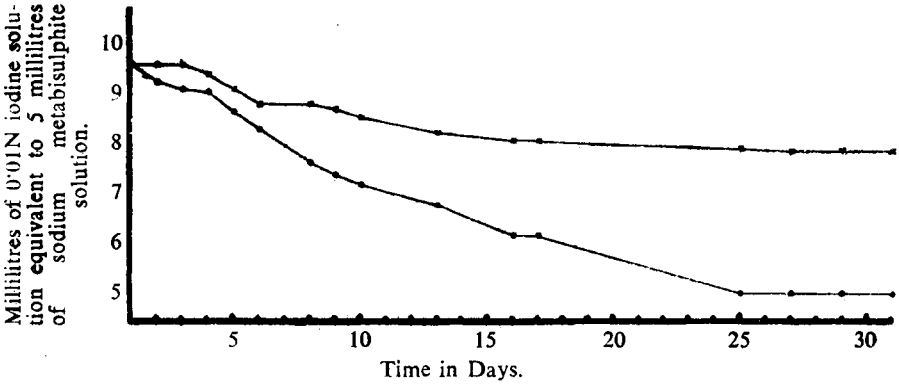

Fig. 4. The effect of storing clinbritic rubber caps in sodium metabisulphite solution (0·1 per cent.). Upper graph, solution only; lower graph, solution containing caps.

that there is considerable absorption of metabisulphite for between 18 to 26 days after which the loss in strength of the solution containing the caps was practically the same as that of the control (unfortunately it was not possible to take daily readings between the 18th and 26th days) probably indicating that the caps had become saturated with metabisulphite solution.

The caps stored in sodium metabisulphite were bleached almost white and were very much lighter than the control caps stored in water, although these were lighter than unused caps. The solution containing the caps had a slight odour of rubber with practically none of sulphur dioxide, whereas the control sodium metabisulphite solution had a very marked odour of sulphur dioxide.

From the readings obtained with the control solution it appears that

there is a slight deterioration of sodium metabisulphite in simple solution.

The above results suggest that it might be advisable either to increase the strength of sodium metabisulphite used for the preliminary soaking of the caps or to increase the duration of the soaking.

TABLE V

TITRATION OF SODIUM METABISULPHITE SOLUTIONS (0·1 PER CENT.) AGAINST 0·01 N IODINE. 5 ML. OF SODIUM METABISULPHITE SOLUTION USED.

Day	Test solution	Control solution
1	10·10	10·10
2	9·75	10·10
3	9·60	10·10
4	9·55	9·90
5	9·15	9·60
6	8·55	9·30
8	8·15	9·30
9	7·90	9·20
10	7·70	9·05
13	7·30	8·75
16	6·70	8·60
17	6·70	8·60
25	5·55	8·45
27	5·55	8·40
29	5·55	8·40
31	5·55	8·40

DISCUSSION

The work described in the present communication has confirmed that the B.P. injection of morphine sulphate progressively darkens in colour on storage. This colour change is accelerated at tropical temperatures. Addition of 0·1 per cent. of sodium metabisulphite retards, but does not entirely prevent, the development of colour.

As a result of our spectrographic and colorimetric studies it appears that the development of colour in the injection is not due to the formation of pseudomorphine, which occurs as colourless crystals yielding colourless solutions in dilute mineral acids. Further evidence in support of this conclusion emerged when an old sample of morphine hydrochloride injection, adjusted to *p*H 3·2 in order to retard colour formation, was examined. This injection contained 1·6 per cent. of morphine hydrochloride and 0·1 per cent. of chlorocresol and had been sterilised by heating in an autoclave. A colorimetric assay indicated that it contained 0·1 per cent. of pseudomorphine although it had become only slightly discoloured during 7 years' storage at room temperature.

The small amount, not exceeding 0·05 per cent., of pseudomorphine which we found in our injections is in harmony with the results obtained by Dietzel and Huss[8] with morphine hydrochloride solutions. These workers, using a spectrographic technique, failed to detect pseudomorphine in injections, adjusted to *p*H 3·24, after 120 minutes' heating in a boiling water-bath. Under the same conditions very little change occurred when nitrogen was passed through the solution. When nitrogen was replaced by oxygen, pseudomorphine was progressively formed. A similar change was brought about by heating in an autoclave at 150°C.

The vanillin colour reaction, introduced by Thörn and Ågren[11], has been found much more sensitive for the detection of pseudomorphine than the spectrographic method. The latter, however, affords a useful indication of the morphine content of an injection by virtue of the prominent absorption band with maximum at $\lambda = 283$ mμ. With injections which have become discoloured the vanillin reagent affords brownish green colours instead of the pure green yielded by pseudomorphine. For this reason our results have been recorded as "pseudomorphine content, calculated from absorptiometer readings," as it is appreciated that some absorption in the region of $\lambda = 600$ mμ might be due to products other than pseudomorphine. Nevertheless, for purposes of following the changes in morphine injection on storage the solution of vanillin in sulphuric acid (95 per cent.) is a valuable reagent.

One of the chief purposes of this investigation was to examine the effect on the stability of morphine injection containing sodium metabisulphite, of treating the rubber caps of the containers with sodium metabisulphite. In our experiments using clinbritic vaccine bottles very little, if any, difference between treated and untreated caps was observed. This was in marked contrast to the experience at Charing Cross Hospital during the war, and we have concluded that the composition of the rubber has much to do with the behaviour of the caps.

It appears that with the rubber caps now supplied with clinbritic bottles there is little danger of the protective action of sodium metabisulphite being seriously reduced but, in view of the earlier experience of Berry[4] and of West and Whittet[6], the preliminary soaking in sodium metabisulphite solution as directed by the Pharmacopœia would seem a wise precaution, unless the caps are known to be satisfactory.

We are well aware that the quality of commercial morphine salts varies, and even if two batches of salt are obtained from the same manufacturer they may yield solutions which discolour at different rates under identical conditions. It is against this background that our results must be assessed but we are of the opinion that the addition of sodium metabisulphite to morphine injection provides the best means so far available of preventing discoloration. This discoloration appears pharmacologically to be of little importance but pharmaceutically its prevention is most desirable.

Summary

1. Samples of morphine injection, containing 2·5 per cent. of morphine sulphate and 0·2 per cent. of chlorocresol have been prepared, filled into clinbritic vaccine bottles and sterilised by steaming for 30 minutes. Some samples contained, in addition, 0·1 per cent. of sodium metabisulphite.

2. The clinbritic bottles were divided into two groups, closed by rubber caps which had and had not been soaked in 0·2 per cent. sodium metabisulphite solution respectively.

3. Samples of injection were stored at room temperature at 80°F. and at 55°C. and examined at intervals.

4. Spectrographic measurements and colorimetric estimations, using

the vanillin reagent of Thörn and Ågren, have been made in order to follow the changes in morphine injection on storage. The colorimetric method has proved useful for the detection of psuedomorphine.

5. The presence of sodium metabisulphite retards but does not entirely prevent the discoloration of morphine injection. This discoloration does not appear to be due to the formation of pseudomorphine.

6. A pharmacological test revealed no decrease in the analgesic activity of discoloured samples of morphine injection.

7. In the experiments recorded little, if any, difference in behaviour was observed between caps treated and untreated with sodium metabisulphite.

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DISCUSSION

An abstract of the paper was read by Mr. Whittet.

THE CHAIRMAN said that he had found discoloration in sealed glass ampoules, particularly with morphine sulphate, which was accompanied by a fall in *p*H, presumably due to the decomposition of the base and the setting free of a certain amount of sulphuric acid. Had the authors come across that particular type of discoloration?

DR. G. E. FOSTER (Dartford) pointed out that in the paper the vanillin colour reaction with pseudomorphine had an absorption curve with a maximum of 600 mμ. This statement might be misleading, as it depended on what was used as the blank, whether one obtained the maximum at the right wavelength or not. It was possible, if one used the vanillin reagent, but not if water was used as the blank. Recently, he had measured the absorption spectrum of a discoloured sample of morphine injection without sodium metabisulphite in the visible region of the spectrum, using water as the blank. When the result was compared with that obtained with a similar injection containing sodium metabisulphite, there was a difference in the two curves which actually corresponded to a maximum at about 375 to 400 mμ. By setting the spectrophotometer at that wavelength and using it as a colorimeter he had compared the actual discoloration in samples of injection of morphine under various conditions. The density readings were as follows:—without metabisulphite, 1·2; with metabisulphite, 0·36. Soaking the caps previously in metabisulphite solution and storing the bottles in an inverted position had little effect

on the result. Possibly, further examination of the absorption at 375 mμ would give more information about the discoloration.

PROFESSOR H. BERRY (London) said that the problem also involved the quality of rubber. Attempts had been made to achieve a standard mix. However, even if the mix was standardised it was still possible to obtain different results with the rubbers made from it. The lubricant used in moulding the rubber was a source of trouble. For instance, if a mixture of sodium laurylsulphate and lauryl alcohol was used it was very difficult to remove it from the rubber. Other sources of trouble were the antioxidants and accelerators used in rubber manufacture. The age of the sample of morphine salt was a further factor: an old sample would discolour much more readily than a fresh one.

DR. F. HARTLEY (London) said that he understood that the discoloration was held to be pharmacologically unimportant by the authors. He did not think that the authors' tests were adequate and there was the question of whether the discoloration had any influence on the toxicity of the morphine sulphate or whether it increased the obnoxious side effects shown by morphine. He doubted whether the discoloration was only of pharmaceutical importance.

MR. T. D. WHITTET referred to the Chairman's remarks about the effect of *p*H on the stability of morphine salts. Previous reports had demonstrated that morphine salts were more stable in acid solutions. The advantage of metabisulphite was that it exerted a protective action without producing such a low *p*H. The subject of rubber was certainly important. The quality had recently been improved and the rubber caps were now made from pale crepe rubber instead of a mixture of half pale crepe and half smoked sheet. This might account for the difference between the earlier results and those obtained recently. Another point which might account for the different results was that in the work on adrenaline with Dr. West, they had used a stronger solution of sodium metabisulphite and had soaked the caps for a longer time. Mr. Coulthard had mentioned that he had not been able to confirm that rubber caps did in fact remove metabisulphite from adrenaline solutions, but, on the other hand, Mr. Soulsby had encountered examples of discoloration in the injection when stored in rubber-capped bottles. He agreed that further pharmacological and toxicity tests on the morphine solutions would be advisable.