LOSS OF MORPHINE IN INDIAN OPIUM ON STORAGE.

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Conflicting views are held with regard to the loss of morphine in opium on storage. Debourdeax[6] (1912) found that the morphine content of opium decreased in powdered opium and that this loss was greater in some instances than in others. Annett and Singh[2] (1918) reported that no morphine was lost when opium was kept at 97-98°C. during the first five days but Miss Iles (*ibid.*, *idem*) stated that prolonged heating leads to a progressive loss of morphine. Macmillan and Tingle[12] (1920) reported that Persian opium lost 30% of its morphine in 96 hours but no further loss was recorded when the heating was continued up to 288 hours whilst Indian opium lost only 3% in 96 hours. Abraham and Rae[1] (1926) suggested that, in the presence of an ample supply of air, an oxidising action takes place which reduces the morphine content. If the amount of air is materially reduced and fresh air is not admitted, the reducing agents present in morphine more than counterbalance this reaction and either stop the oxidation entirely or even cause a regeneration of morphine but, according to these authors, the loss of morphine is not greater than 0·09% per month in any case. Annett and Singh[3] (1922) observed a large loss of morphine in Indian opium which had been stored for two to four years, whilst Dott[8] (1926) found that, whether a sample of opium was dried at 60° or at 100°C., it showed practically no loss after about ten months. (The loss from opium dried at 60° was 0·04%, while the morphine content of that dried at 100° fell from 11·04% to 10·58% and subsequently rose to 10·88%.).

Annett[4] (1923) detected no fall in the percentage of morphine in samples which had been stored for only one year, the loss only occurring in those kept for two years or more.

Jermstad[10] (1928) investigated two samples which had been tested ten years previously, the fall being from 11·26% to 10·94% (0·32%) in one case and from 16·20% to 15·82% (0·38%) in the other.

In view of these conflicting views, the Government of India ordered that this problem should be re-examined.

Two varieties of fresh opium (Malwa and Benares) were stored at room temperatures (10°C. to 35°C.) under the following conditions forming three different sets :—

Set I—*Moist Opium* : as received at the Opium Factory, Ghazipur and stored in corked bottles (consistence 80·0%).

Set II—*Powdered Opium dried below* 60°*C.* The opium was dried by spreading it in thin layers on glazed plates and exposing to the heat of the tropical sun. In three or four days' time the opium was fairly dry and then it was powdered. The drying was completed by keeping the powder in an electric oven at 50°–60°C. for about two hours. This powder was stored in corked and paraffined bottles.

Set III—*Powdered Opium dried at* 95°–100°*C.* Moist opium was first dried and powdered with the aid of spatulas on glazed plates heated on a steam table and then kept in a steam oven at 95°–100°C. for 20 minutes. This was also stored in corked and paraffined bottles.

These three sets of opium were analyzed in quick succession during the first two months and then left undisturbed for about a period of two years when the morphine content was again determined.

The B.P. 1932 method was followed in assaying with only one modification, namely, that the opium mixture, after being shaken with lime and water, was filtered with slight suction through a sintered glass filter (Schott and Gen. No. 17G3). This prevents any concentration of the filtrate which occurs if the liquor is filtered through an ordinary filter taking a longer period.

Several experiments were conducted with each set of which a typical one is recorded below. Duplicate estimates were made in every case :—

TABLE I.

Date of Assay.	Percentage of morphine in Malwa opium calculated on dry matter.	Date of Assay.	Percentage of morphine in Benares opium calculated on dry matter.
	Moist opium : Consistence 80%.		
8-5-33	14·10	12-5-33	9·40 9·25
7-6-33	13·90	16-6-33	9·40
10-7-35	14·05 14·10	10-7-35	9·04 9·25
	Powdered opium dried below 60°C.		
8-5-33	14·10	12-5-33	9·40
29-5-33	13·60	16-6-33	9·00
8-6-33	13·20	10-7-35	7·74 7·60
10-7-35	11·90 12·00		
	Powdered opium dried at 98°C.		
8-5-33	14·10	12-5-33	9·40 9·25
17-5-33	13·80	6-6-33	9·00
5-6-33	13·90	11-7-35	8·80
11-7-35	13·80		

The results in Table I show that—

(1) There is practically no loss of morphine from moist opium on storage;
(2) After two years, powdered opium dried below 60°C. lost about 2·0% of morphine or 0·085% per month (*cf.* Abraham and Rae who found a rate of 0·09% per month); and
(3) Powdered opium, stored after heating at 95°–100°C., lost only 0·4% after two years.

A sample of Malwa opium dried at 60°C. and stored at room temperature in a bottle with a cotton plug lost 10% of its morphine content in three months as the following table shows :—

TABLE II.

Date of Assay.	Morphine content.
1-1-33	12·10%
13-2-33	11·80%
21-2-33	11·69%
23-3-33	11·10%
1-4-33	10·85%

These figures show that free access of air is responsible for the rapid loss of morphine and that the process is one of oxidation.

The same sample was dried at 60° and also at 98–100° and stored under identical conditions at various temperatures. The following table shows the loss in two cases :—

TABLE III.

Malwa Opium, 1931-32.

Sealed tin of moist opium received in June, 1932; opened on 19th February, 1933.

Storage temperature.	Date of Assay.	Morphine content of opium stored after drying at 60°C.	Morphine content of opium stored after drying at 98–100°.
At start	1-3-33	12·3 12·2	12·3 12·4
55-60°	15-3-33	11·8	12·4
	29-3-33	11·7	12·2
30–35°	16-3-33	11·4	12·1
	30-3-33	11·5	12·2
Room temperature	17-3-33	11·7	12·3
	31-3-33	11·6	12·3
At 0°	18-3-33	11·5	12·2
	1-4-33	11·6	12·4

From this it will be evident that drying the opium at 100° prevents the oxidation to a great extent, the loss being negligible, whilst the sample dried at 60°C. lost about 8% of its morphine content. The temperature at which it was stored had not any great influence, but this point requires further elaboration.

It has been suggested that this loss of morphine is due to enzymic activity. Of a number of methods devised for the isolation of an enzyme from opium, the following gave the most satisfactory result :—

Method for the Isolation of Enzymes from Opium.

On the day of its arrival, 500 gms. of fresh Malwa opium were extracted at the Opium Factory with 1·5 litres of distilled water by shaking in a 3 litre flask for 3-4 hours. The mixture was set aside overnight. The shaking was continued next morning for 2-3 hours more and the resulting liquid filtered under slight suction. The extract was carefully neutralized with calcium carbonate and refiltered. The filtrate was concentrated in vacuo at 35-40° to 1/10th of its original volume. To this highly concentrated solution, 4-5 volumes of neutral alcohol were added and a flocculent precipitate was thrown down. The precipitate was collected, dissolved in water and reprecipitated with alcohol. Impurities were removed as far as possible by repeating this procedure 3 or 4 times, the final product (about 0·5 g.) being dried in a vacuum desiccator.

The fine white powder, so obtained, was readily soluble in cold water. The solution was dialyzed from a colloidon thimble for ten days in running water, concentrated and then reprecipitated with alcohol.

The final preparation gave the following tests :—

1. To one ml. of the enzyme solution (1%) the addition of 2-3 ml. of freshly prepared tincture of guaiacum (0·3 g. in 10 ml. alcohol) produced a blue colour immediately in the presence of hydrogen peroxide (2 ml. of a 3% solution). The colour did not appear in the absence of H_2O_2. This indicates the presence of peroxidase only.

2. A similar test with pyrogallol gave a deep brown colour immediately in presence of hydrogen peroxide but no reaction was obtained in its absence.

3. Resorcin also gave a similar test in the presence of hydrogen peroxide but the colour developed only after a few minutes.

Attempts to isolate enzymes from Benares opium have not been successful so far. The dirty gummy substance obtained did not respond to any of the tests for peroxidase and oxidase.

Further studies on the enzyme preparation will be made as soon as possible.

It has been suggested that the first oxidation product of morphine is pseudomorphine. Balls[5] (1927) has shown that silicotungstic acid at a suitable pH precipitates pseudomorphine but no reliance can be placed on this method as, with small quantities, the error is about 30%. As no suitable known procedure exists to test whether pseudomorphine is developed as a result of enzymic activity in opium, the following method for the quantitative separation of pseudomorphine and morphine was devised.

Pseudomorphine was specially prepared for these experiments by oxidizing morphine with potassium cuprocyanide at pH=6·5–6·8 (Balls)[5] (1927)

and then purified through its hydrochloride. Pseudomorphine gives a fairly insoluble picrate. It seemed probable that a separation from morphine could be effected through this salt.

As a result of the determination of the solubility of pseudomorphine picrate in a number of solvents, it was found that it was least soluble in acetone, but, even in this solvent, it dissolved to the extent of 0·84% at 35°C.

Ultimately it was found that the sulphate of pseudomorphine is almost completely insoluble in ethyl alcohol while the sulphates of morphine and codeine are soluble.

Method.—An artificial mixture of pseudomorphine (0·1 to 0·2 g.) and morphine (1 to 3 g.) was made from weighed quantities of each and transferred to a conical flask. To this mixture, 20 to 30 ml. of ethyl alcohol (98%) was added and the calculated amount (on the basis that the whole of the sample was 100% morphine) of normal sulphuric acid was run in from a burette. The flask was then warmed under reflux on the steam bath for 20–30 minutes and cooled. The precipitate was collected in a weighed Jena sintered glass crucible, No. 1G4. The flask and the precipitate in the crucible were washed three times with alcohol (using not more than 15 ml. in all) and the crucible dried to constant weight at 100–105°C. The increase in weight representing pseudomorphine sulphate. Pseudomorphine sulphate × 0·83 = pseudomorphine.

The table below gives the results in a few typical cases of artificial mixtures prepared as described above :—

TABLE IV.

Experiment No.	Pseudomorphine taken.	Morphine taken.	Codeine taken.	Pseudomorphine Found.	Pseudomorphine Error.
	g.	g.	g.	g.	%
1	0·0897	1·5310	*Nil*	0·0924	plus 3·01
2	0·0945	2·2539	,,	0·0942	minus 0·31
3	0·1858	1·0905	,,	0·1874	plus 0·86
4	0·1870	2·7000	,,	0·1918	,, 2·04
5	0·1361	*Nil*	,,	0·1362	,, 0·07
6	0·1150	0·2596	0·2124	0·1155	,, 0·43

From the above results it appears that the error by this method is within 2-3% on the weight of pseudomorphine taken. In all experiments, except No. 2, the error is positive. According to Kollo [11] (1919) and Dietzel [7] (1928) this may be due to a slight decomposition of morphine sulphate on heating.

When this method of separation was employed to ascertain the amount of pseudomorphine in the morphine precipitated during assay, it was found that the precipitated morphine is practically free from pseudomorphine. This was expected, because the solubility of pseudomorphine in lime water is negligible and therefore very little is extracted from the opium by lime water. This provides direct evidence that the precipitate of morphine obtained by the B.P. method of 1932 is also free from pseudomorphine for

practical purposes and the results given in Table I are not vitiated by the contamination of the morphine precipitated by pseudomorphine.

Foulton [9] (1933) has recently shown that many oxidation processes of morphine yield no more than traces of pseudomorphine and that it is almost certain that morphine, when oxidized, does not necessarily pass through the pseudomorphine stage. Even if it is assumed that loss of morphine from opium is an oxidation process and pseudomorphine is one of the products formed, it is almost certain that the pseudomorphine does not appear in the final morphine crystals obtained by the B.P. method in quantity sufficient to affect the results obtained.

A thick fungoid growth appears on moist samples of opium. We are much indebted to Dr. Chaudhuri, Head of the Panjab University Botany Department, for identifying the fungus as *Scopulariopsis brevicavtis*, var. *glabra* Thom.

A culture medium containing—

0·356 g. of ammonium nitrate,
0·080 g. of potassium dihydrogen phosphate,
0·080 g. of magnesium sulphate,
0·0046 g. of ferrous sulphate,
0·0046 g. of zinc sulphate,

n a litre was prepared. Enough morphine hydrochloride was added to give a 0·5% (morphine) solution. After sterilization, five flasks containing the same volume of the solution (100 ml.) were inoculated and kept at 29-30°C. A blank, diluted to 250 ml., showed a rotation $[\alpha]_D = -97{\cdot}5°$ in a dcm. tube. Samples withdrawn after two and two and a half months respectively showed the following results on assay :—

TABLE V.

	Initial morphine content.	After two months.	After 2·5 months.
	0·50 gm.	0·364 gm.	0·346 gm.
Rotation $[\alpha]_D$ =	—97·5°	—95·0°	—92·5°

The morphine content was determined by Eaton's method and the purity of the morphine precipitated was determined by titration. The specific rotation did not show much variation, probably because the product of metabolism is also optically active.

A sample of moist opium on which a profuse growth of the fungus had developed did not show any loss of morphine on assay.

This growth of *Scopulariopsis brevicavtis* showed considerable reluctance to grow in the synthetic medium but, after repeated culture, a strain was obtained which grew fairly copiously in the solution. It does not follow that on moist opium it uses morphine as its natural substrate, whereas it

is forced to use morphine for its growth in the culture solution. Further work is necessary before a definite conclusion can be drawn.

Sage [13] (1922) suggests that the loss of morphine in dry opium is accompanied by the formation of ammonium salts but no data have been advanced by him to support the view.

A sample of dried (at 60°) opium was kept, after the determination of its 'ammonia' content, in an ammonia free atmosphere. The following table shows that no variation in the percentage of ammonia was noticed after $2\frac{1}{2}$ months although there was 8·5% loss in morphine content :—

TABLE VI.

Date of Estimation.	Percentage of ammonia found.	Morphine content.
26-11-32	0·45	11·7%
30-11-32	0·42	11·6%
7-1-33	0·39	11·1%
	0·37	11·1%
10-2-33	0·42	10·8%
	0·41	10·7%

Hence it appears that the loss of morphine is not accompanied by any increase in the 'ammonia' content of the opium and the results recorded in Table V tend to support this view.

SUMMARY AND CONCLUSIONS.

(1) Moist opium does not lose morphine on storage.

(2) Opium dried at 60°C. stored in contact with air suffers a rapid loss of morphine. This is not completely prevented by storage in corked and paraffined bottles.

(3) Opium dried at 98–100° and stored out of contact with air does not lose morphine to any appreciable extent.

(4) There is no evidence of the formation of ammonium salts as a result of the oxidation.

(5) An enzyme (peroxidase) has been isolated from Malwa opium which may be the factor responsible for the decomposition of morphine.

(6) A fungoid growth noticeable on moist opium has been identified as that of *Scopulariopsis brevicavtis*, var. *glabra* Thom.

(7) When this fungus is made to grow in a dilute solution of morphine hydrochloride in a suitable nutrient medium, a slight fall in the concentration of morphine is observed but the specific rotation of the solution does not change appreciably.

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