# LV. FACTORS INFLUENCING ALKALOIDAL CONTENT AND YIELD OF LATEX IN THE OPIUM POPPY (*PAPAVER SOMNIFERUM*).

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## I. Introduction.

The present paper is a summary of a full account of the work of the writer and others which is being published elsewhere[1]. A very large amount of data has been accumulated which it is hoped may be of interest to physiologists but under present conditions it is impossible to reproduce all the figures here. Typical figures only will therefore be given in the present paper.

The work represents an investigation arising out of war conditions. In pre-war days the bulk of the opium required for alkaloidal manufacture and for medicinal purposes was provided by Asia Minor, the Balkans and Persia. The morphine content of Indian opium was too low to enable it to compete in that market. With the entry of Turkey into the war supplies of medical opium were threatened and it became imperative to make use of Indian opium. The writer was placed on special duty in October 1916 to investigate the reason for the low morphine content of Indian opium. The work met with success and there is no reason why India should not put opium on to the market of at least as high morphine content as the product of Asia Minor and the Balkans.

Fortunately for the work the writer has had the advantage of collaboration with Mr H. M. Leake, M.A., Economic Botanist to the Government of the United Provinces. Mr Leake had for several years previously been endeavouring to isolate races of poppy which would be resistant to the Blight disease (*Peronospora arborescens*) which causes very serious havoc annually in India. He was therefore able to supply seed of pure races of poppy for experimental work at once. Much of the work which has been carried out on drug plants appears to suffer from the fact that pure races of plants were not used. In addition to the use of pure races the work has been carried out on a large scale and at several centres, *e.g.* in the plains and in the Himalayas. Some five or six acres of the same pure race have been under experiment annually.

[1] *Memoirs of the Dept. of Agric. in India.*

## II. Description of the Method of Extraction of Latex.

It will make the description of the work easier if the method of extraction of opium as practised by the Indian cultivators is first described. The operation is commenced some 10 to 20 days after the fall of the flower petals. The capsules are considered to be ready for lancing as soon as they feel firm to the touch and are yet green. The instruments used are:

(1) A knife which consists of two to four parallel sharp pointed blades bound together with cotton, the binding being so done that the points of each blade are about one-twentieth of an inch apart and all in the same plane.

(2) A small iron scoop.

(3) Unglazed earthen pots in which to store the opium.

The cultivator then usually divides his fields into three portions *A*, *B*, *C*. The lancing is invariably commenced just after midday and is carried out by men, women and children. They begin at the edge of the field and work backwards as otherwise the exuding latex would be brushed off on their clothes. The hand is quickly passed over the capsules. If a capsule is considered to be ready it is grasped in one hand and the knife is drawn vertically upwards over its surface from just above the stalk to just below the stigmatic rays at the top of the capsule. Sometimes the cut is made in the reverse direction, *i.e.* downwards. The second finger of the hand holding the knife is placed near the points of the knife on the surface of the capsules to steady the motion of the knife. Great care is taken not to cut too deeply into the capsule. If the cut be made too deep, then the wall of the capsule may be cut completely through with the consequent secretion of the latex on the inner surface. Good operators can incise 150 to 200 capsules in an hour.

Immediately on incision the latex commences to flow. It varies in colour from milk white and smoky white through pale pink to a very bright pink. It rapidly begins to darken however. Next morning at 6 a.m. or later if there is a heavy dew, the opium is collected. It is scraped off the cut surface with the blunt edged iron scoop. From the scoop it is transferred to the earthen pots. Usually the cultivator lances only one-third of his field (*e.g.* portion *A*) on the first day. After collecting the opium next morning from that portion he then lances portion *B*, that is on the second day. On the third day portion *C* is lanced. On the fourth day he again returns to lance portion *A*. On this occasion many more heads are now ready for a first lancing. In addition a second lancing is given to those capsules which were lanced on the first day. On the fifth day portion *B* receives its second lancing and on the sixth day *C* receives its second lancing. On the seventh day *A* is lanced for the third time and on this occasion more heads are usually ready for a first lancing. Those already lanced once now receive a second lancing and those already lanced twice receive a third lancing. Portions *B* and *C* come in for similar treatment in regular rotation. The lancings are continued as long as the capsules continue to give appreciable yield of latex. Usually each field is lanced

four or five times and occasionally as many as eight times so that some heads would then have received eight lancings. In some poor seasons and on some poor crops the heads cease to yield opium after the second lancing. The whole opium produce of each field is then mixed together and, after three to six weeks' storage, is brought to an official of the Government Opium Department by whom each cultivator's opium is weighed and examined for consistency or dry matter by touch and for certain adulterations (*e.g.* starch by the iodine test). The opium officers reach such a high degree of skill that they can usually tell the moisture content of the opium by touch to within 2 or 3 %. The cultivator at once receives the value of his opium except for a small balance which is paid some two months later when the district officer's examination has been checked by analysis at the Government Opium Factory, to which place all the opium is sent.

### III. The Alkaloidal Content and Yield of Latex from each of a series of Successive Lancings of the same Capsules.

We have established the fact that on the first occasion on which a capsule is incised by the cultivator the opium is far richer in morphine than is the produce of the second lancing and this again than that of the third lancing and so on. Cases have been met with in which the opium obtained from the fifth lancing gave no morphine at all by the method of estimation laid down in the *British Pharmacopoeia*. The following analysis is typical of many hundreds of estimations we have carried out of the morphine content of the opium from a series of successive lancings of the same capsules.

| | No. of lancings | | | | | |
|---|---|---|---|---|---|---|
| | first | second | third | fourth | fifth | sixth |
| Morphine percentage on dry matter of opium | 13·9 | 9·6 | 5·8 | 3·6 | 2·2 | 1·6 |

We have met with no case in which the opium of a particular lancing was of higher morphine content than the opium of a previous lancing of the same capsules.

As regards the yield of latex at each successive lancing it is difficult to state typical figures. Thus in the hills one usually finds that the capsules will bear a larger number of successive lancings than they will in the plains so that as a rule in the hills the first three or four lancings may each produce about the same amount of opium. In the plains, however, one usually finds the first lancing of a particular capsule produces the highest yield of latex and the yield falls off with each subsequent lancing, provided there are no sudden changes in the weather conditions. The following figures are perhaps typical.

*Table showing yield of latex (dried at* 100° C.) *at each successive lancing.*

| | Yield of latex in g. dry matter per 1000 capsules No. of lancings | | | | | |
|---|---|---|---|---|---|---|
| Locality | first | second | third | fourth | fifth | sixth |
| Elevation 3500 feet (Himalayas) ... | 20·6 | 33·8 | 29·5 | 18·2 | 5·6 | 0·5 |
| Cawnpore ... ... | 35·5 | 12·4 | 5·4 | 2·1 | 0·4 | 0·1 |

Varying weather conditions, as will be shown later, exert very great influence on the yield on any particular day. Thus cloudy weather or East winds usually considerably reduce the yield. The author has found similar phenomena in his work on sugar yielding palms [1913].

The rapid fall in morphine content of the latex from each successive lancing, raises the question as to whether any substance is formed in place of the morphine in the later lancings. Indian opiums are particularly rich in codeine and it seemed probable as this alkaloid is so closely related to morphine in its constitution, that one would find an increase in the codeine content of each successive lancing. This however is not the case. Narcotine is the only other alkaloid present in sufficient quantity to be of interest in this connection but here again there is, as a rule, no rise in narcotine content in the later lancings. The following figures show the percentages of morphine, narcotine and codeine in the dried latex of the first four successive lancings of the same capsules.

| Number of lancing | Percentage of alkaloids in dry matter of latex | | | |
|---|---|---|---|---|
| | Morphine | Narcotine | Codeine | Total of morphine, narcotine and codeine |
| First ... | 11·18 | 6·85 | 2·25 | 20·28 |
| Second ... | 9·28 | 6·34 | 2·71 | 18·33 |
| Third ... | 6·38 | 4·18 | 2·94 | 13·50 |
| Fourth ... | 3·41 | 3·36 | 3·64 | 10·41 |

As previously stated one always finds the same rapid progressive decrease in morphine content in each successive lancing. As regards narcotine and codeine one does not observe the same regularity in behaviour. Codeine certainly usually shows a slight increase just as in the table. On the other hand narcotine sometimes increases in the later lancings. We are investigating this matter in greater detail. It may be stated here however that the total nitrogen content of the dry matter of the latex from each successive lancing remains practically constant, so that some nitrogenous substance takes the place of morphine in the subsequent lancings. At present our work indicates that the substance is not an alkaloid.

It seemed of great interest to discover if decreasing or increasing the interval of time between the successive lancings would affect the rate at which the morphine content of the later lancings falls off. The following intervals of time between the successive lancings were experimented with:

(1) 4 hours, (2) 1 day, (3) 2 days,
(4) 3 days, (5) 4 days, (6) 5 days.

Our results showed that when the interval between successive lancings was one day or more the morphine content of the opium of each successive lancing fell away in exactly the same manner whether that interval was one, two, three, four or five days. With an interval of only four hours between the successive lancings indications were obtained that the falling off in morphine

content of each successive lancing was not so marked. The bearing of this phenomenon will be discussed in the last section of this paper. Strong indications were obtained that after receiving the first lancing, the capsules require a certain minimum interval of time before the second lancing in order that they may give a free yield of latex at the second lancing.

## IV. The Variation in Rate of Flow and Morphine Content of the Latex at Different Periods of Time after Incision.

Numerous experiments were performed in which latex was collected in fractions at various intervals of time after incision of the capsules. Thus immediately on making the incision latex flows out. This was collected within one minute of making the incision. From the same incised surface latex continues to flow and collections were made at certain set times during the ensuing 16 hours after which time we have only on rare occasions observed any flow of latex from an incised surface. In the latex of each successive fraction there is again a steady fall in the morphine content. Moreover when some two days later a second incision is made on the capsule and the latex again collected in fractions the fall in morphine content again takes place in each successive fraction, the first fraction usually showing about the same morphine content as the final fraction of the previous lancing. Latex collected in fractions from a third successive incision behaves in an exactly similar manner. The table gives a typical result.

| | | Percentage of morphine in latex dried at 100° C. Time of collection | | | | |
|---|---|---|---|---|---|---|
| No. of lancing | Date of lancing | 9 a.m. (immediately on incision) | 12 a.m. | 3 p.m. | 6 p.m. | 6 a.m. (next morning) |
| 1 | 13–4–19 | 12·3 | 11·0 | 10·9 | 10·1 | — |
| 2 | 16–4–19 | 9·7 | 7·8 | 7·7 | 5·9 | — |
| 3 | 19–4–19 | 7·3 | 5·7 | — | — | 5·5 |

The writer considers that these figures indicate that morphine is stored principally in the capsule. When the capsule is lanced the latex first flowing out would therefore be of maximum morphine concentration. As the flow continues the latex would flow in from parts of the plant below the capsule, where the morphine is presumably present in lower concentration. The results also tend to disprove the theory that morphine does not occur as such in the plant [Winterstein and Trier, 1910, True and Stockberger, 1916]. Figures showing the rate of flow of latex at various intervals of time after incision are given in the main publication. It is sufficient to state here that 22·6 to 60·6 of the total yield of latex may be exuded within a minute after incision.

## V. The Effect of Different Systems of Lancing on the Yield and Composition of the Latex.

The methods of lancing in use in the various opium producing centres differ greatly. In Asia Minor and the Balkans one spiral cut is made round the capsule and no subsequent lancing is carried out on the same capsule. In Egypt transverse cuts are made. We have already stated that in India one vertical cut with a multiple bladed knife is made at each lancing. It seemed desirable in view of the high morphine content of the latex yielded at the first lancing, to obtain as high a yield as possible at the first lancing. With this end in view experiments were carried out in which two, three, four and five and even six vertical cuts with a four bladed knife were made at the first lancing. A very definite result was obtained from these experiments in that if one obtains a large yield at a first lancing by increasing the number of incisions, then the percentage of morphine in the latex diminishes. Following up this matter, further experiments were made in which only one incision, a very tiny one was made into the capsule so that only a small yield of opium was obtained. The opium obtained in this manner was of distinctly higher morphine content than that obtained by making incisions of the usual length.

Further when only a tiny incision is made at each lancing then the rate of fall in morphine content of the opium of each successive lancing is much less rapid.

Some interesting data have been accumulated both in field and pot experiments on the connection between the yield of latex and the number of incisions made in the capsule. The following table gives the result of a field experiment in which some 5000 capsules were taken for experiment. Some of them were lanced with a single vertical incision, some with three incisions and the remainder received a minimum of five incisions per capsule. In each case a four bladed knife was used. The first two groups received subsequent lancings also but only the results of the first lancing are given in the table.

*Effect of increasing the number of incisions at each lancing on the yield of latex per capsule.*

| | *A* 1 vertical incision | *B* 3 vertical incisions | *C* 5 or more vertical incisions |
|---|---|---|---|
| Gms. latex (dried at 100° C.) per 1000 capsules | 30·3 | 40·9 | 20·9 |

Thus by increasing the number of incisions per lancing from one to three a slight increase in yield of latex is obtained. When however the number of incisions per capsule is increased to five or more then the yield of latex per capsule is considerably diminished. These results were fully borne out in an experiment on poppy plants grown in pots. In this case experiments were made on capsules receiving one, two, three and six incisions at each lancing. The table shows the yield of latex dried at 100° C., per capsule for the first lancing only.

*Yield of opium per capsule as affected by using one, two, three and six incisions at each lancing. (Pot experiment.)*

| | Number of incisions at each lancing | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 6 |
| Gms. dry opium per capsule, 1st lancing only | 0·0294 | 0·0352 | 0·0374 | 0·0277 |

Here again it is seen that increasing the number of incisions does not result in a corresponding increase in the yield of latex, and beyond a certain point may cause a decrease.

The ordinary lancing knife as used in India contains four blades the points of which are set about $\frac{1}{20}$th inch apart. We have carried out experiments to determine the comparative effect of using knives with two, four and six blades. Increasing the number of blades does not increase proportionally the yield of latex. The six bladed knife in our experiments gave about a 20 % increase in yield of latex over the yield obtained with the two and four bladed knives which gave practically identical yields.

## VI. The Yield and Alkaloidal Content of the Latex from Different Capsules of the same Plant.

One may find as many as 40 capsules in various stages of development on a plant of the opium poppy. Usually, however, a plant has one or two or perhaps three capsules. The oldest is at the end of the main stem and will be referred to as the terminal capsule. The subsequent capsules are borne each at the end of a branch arising from an axil of a leaf on the main stem and the younger the capsule the farther down the main stem does its branch originate. These will be referred to as lateral capsules. We have found as a result of a large number of experiments carried out on 50 pure races of poppy plants that the oldest or terminal capsule produces opium of much higher morphine content than do the younger capsules and it appears that the morphine content of the latex diminishes progressively the later the order of origin of the capsule. It must be clearly understood that this phenomenon has no connection with the unripeness of the later capsules. Our lancings were not carried out until the capsules were at the correct latex yielding stage. Usually also, though not invariably, the terminal capsules yield more latex than the lateral capsules.

The table gives results typical of our experiments. In the experiment to which this table refers 1620 plants each with several capsules in process of development had their terminal capsule lanced. The second, third and fourth capsule on each plant was lanced when ready. The opium obtained from the terminal and from first, second and third lateral capsules was examined separately.

| Description of capsule | No. of lancing | Date of lancing | Yield of dried opium per 1000 capsules, g. | Percentage of morphine on dried opium |
|---|---|---|---|---|
| Terminal ... | 1 | 2–3–18 | 79·7 | 14·0 |
| | 2 | 5–3–18 | 27·0 | 8·4 |
| | 3 | 9–3–18 | 8·2 | 5·3 |
| | 4 | 12–3–18 | 3·5 | 3·3 |
| 1st lateral ... | 1 | 5–3–18 | 34·6 | 12·2 |
| | 2 | 9–3–18 | 8·0 | 7·1 |
| | 3 | 12–3–18 | 2·2 | 4·9 |
| | 4 | 15–3–18 | 0·3 | — |
| 2nd lateral ... | 1 | 9–3–18 | 13·7 | 10·1 |
| | 2 | 12–3–18 | 6·5 | 6·1 |
| | 3 | 15–3–18 | 0·7 | — |
| 3rd lateral ... | 1 | 12–3–18 | 14·3 | 8·0 |

In an examination of the latex from the terminal and lateral capsules of 50 pure races of poppy not a single case was met with in which the morphine content of the latex from the lateral capsules was higher than that of the latex of the terminal capsules.

Experiments were carried out to discover if by leaving the terminal capsules unlanced one would obtain latex of higher morphine content from the lateral capsules. It was found that the morphine content of the latex of the lateral capsules was not increased by leaving the terminal capsules unlanced.

Indications were however obtained which showed that by removing all lateral buds a large yield of latex could be obtained from the terminal capsules.

## VII. The Relation between the Stage of Development of the Capsule and the Yield and Alkaloidal Content of its Latex.

It has already been shown in Section III that the morphine content diminishes progressively in the latex from each of a series of successive lancings from the same capsules. As will be shown here this phenomenon has nothing whatever to do with alterations in morphine content of the latex with increasing age of the capsule as at first sight might appear possible. We have carried out experiments on this point during three seasons with identical results in each season. Some 20,000 plants have been included in each year's experiments.

Capsules have been lanced at the following intervals of time after flowering: 5, 9, 13, 17, 21, 25 and 29 days, *i.e.* at all stages from soft green to the ripe stage. Controls have been carried out by lancing side by side with the experimental capsules on each day a number of capsules in the same field which were considered by the cultivators to be ready for lancing. The results show that if the capsules are lanced only four or five days after flowering then the morphine concentration of the latex is distinctly low. About nine days from flowering the latex reaches its maximum morphine content and this maximum is maintained at whatever age beyond nine days the capsules are first lanced.

The yield of latex is low when the capsule is very immature but rises to a maximum when the capsules are first lanced at 15 to 17 days from flowering. When the first lancing is carried out later than this the yield of latex again diminishes. The table summarises the results for 1918–19 which are typical of those of the other seasons.

*Table showing connection between stage of development of capsule and the yield and morphine content of the latex.*

| No. of days between flowering and first lancing | First lancing: Latex dried at 100° C. | | Second lancing (2 days later): Latex dried at 100° C. | |
|---|---|---|---|---|
| | Percentage of morphine | G. per 1000 capsules | Percentage of morphine | G. per 1000 capsules |
| 5 | 10·2 | 27·7 | 6·5 | 23·0 |
| 9 | 11·7 | 28·2 | 8·4 | 19·7 |
| 13 | 12·1 | 30·4 | 8·3 | 26·1 |
| 17 | 11·4 | 36·9 | 8·7 | 21·1 |
| 21 | 12·5 | 26·3 | 10·7 | 22·1 |
| 25 | 12·5 | 22·1 | 9·1 | 16·9 |
| 29 | 12·2 | 25·3 | 10·4 | 8·4 |

## VIII. The Effect of Climate and Weather Conditions.

The literature records the results of numerous experiments carried out with the idea of tracing the influence of climate and weather conditions on drug production in various medicinal plants. This work is summarised in our main publication. As regards our own particular problem, the discovery of the reason for the low morphine content of Indian opium, it may be stated at once that climate as such does not control to any important extent the power of the poppy plant to produce morphine. Opium of high morphine content has been produced in every continent, in climates as different as those of the tropics and Sweden.

Our work in this connection has been carried out during four seasons, on a very large scale. We have been fortunate in being able to carry out trials with the same pure race in various places in the plains of northern India and in the same season at altitudes of 4000, 5000 and 6500 feet in the Himalayas. At the latter altitude our poppy was at times under snow. Our remarks apply especially to morphine. As regards other alkaloids our investigations are not so far advanced. Codeine for instance is always high in Indian opium. We have examined the produce of numerous pure races of Indian opium selected by Mr Leake and we have never found less than about 1·8 % codeine. Some samples yielded over 4½ % and the average for Indian opium we would put at about 3 %. Turkish opium only contains about 0·25 %.

Narcotine is usually present in opium in quantities amounting to 5 to 8 % but French opium has at times been reported to contain none of this alkaloid [van Ittalie and Kerbosch, 1911]. The matter is referred to at a greater length in the complete account of our work.

*Effect of Climate.*

Before dealing with our experimental results it will be advisable to point out that previous investigators on opium have not recognised the large difference which exists in the morphine content of the opium of each successive lancing, nor have they realised the variation in morphine content of the opium produced by the various capsules of the same plant. In our work on climatic conditions we have only used the terminal capsules of each plant as a source of opium and we have kept separate the product of each successive lancing. At each centre we have usually had ½ an acre of poppy at least, while at our main station at Cawnpore five to six acres of the same pure race have been under experiment. On each area very large numbers of samples were collected amounting to some hundreds at Cawnpore. Much attention has been paid in our work to the determination of the range of variation which occurs over a field which is all under similar treatment. Over a given field provided only terminal capsules are used the various samples of opium of the first lancings usually show quite a small range of variation say from 11 to 13 % morphine for a race averaging 12 % of morphine.

The following table summarises the figures obtained at the various stations in 1916–17, 1917–18, 1918–19 and 1919–20.

*Table showing effect of climate or locality on the morphine content of the opium. Produce of first incision of terminal capsules only.*

| No. of samples examined | Locality | Percentage of morphine calculated on opium dried at 100° C. | |
|---|---|---|---|
| | | Range of variation | Average |
| | 1916–17 | | |
| 10 | Sitoli (Alt. 5000 feet) ... ... | 10·9–13·9 | 12·7 |
| 1 | Douglas Dale (Alt. 4000 feet) ... | — | 10·6 |
| 1 | Chaubattia (Alt. 6500 feet) ... | — | 10·3 |
| 1 | Cawnpore ... ... ... ... | — | 11·1 |
| | 1917–18 | | |
| 91 | Cawnpore ... ... ... ... | 11·8–15·8 | 14·0 |
| 1 | Fatehgarh (Ankin) ... ... | — | 15·1 |
| 2 | „ (Mianganj) ... ... | 12·6–13·1 | 12·8* |
| 5 | Rae Bareli ... ... ... ... | 11·2–13·4 | 12·2* |
| 11 | Douglas Dale (Alt. 4000 feet) ... | 11·3–16·7 | 13·3 |
| 1 | Sitoli (Alt. 5000 feet) ... ... | — | 10·0 |
| 2 | Chaubattia (Alt. 6500 feet) ... | 16·1–16·3 | 16·2 |
| | 1918–19 | | |
| 119 | Cawnpore ... ... ... ... | 10·5–15·8 | 12·6 |
| 12 | Rae Bareli ... ... ... ... | 9·2–12·7 | 11·1 |
| 8 | Douglas Dale (Alt. 4000 feet) ... | 9·3–12·4 | 11·1 |
| | 1919–20 | | |
| 71 | Cawnpore ... ... ... ... | 14·0–17·7 | 15·4 |
| 8 | Rae Bareli ... ... ... ... | 11·5–15·0 | 13·1 |
| 1 | Fatehgarh ... ... ... ... | — | 16·6 |
| 2 | Etawah ... ... ... ... | 16·1–17·6 | 16·8 |
| 3 | Douglas Dale (Alt. 4000 feet) ... | 13·2–15·3 | 14·0 |

* These samples were mixtures of first and second lancings and would therefore be lower in morphine content than first lancings only.

It should be stated that the pure race grown in 1916–17 was a different one from that used in the subsequent three seasons. In 1916–17 the poppy producing the one sample at 6500 feet altitude was at one stage under snow.

It would not appear that climate is an all important factor in determining the power of the plant to produce morphine. At the same time during the past three seasons in which we have been very carefully studying the behaviour of a pure race of poppy grown at various stations in the plains, indications have been obtained that locality does exert a certain amount of influence on the morphine producing power of a plant. Thus at Cawnpore during the season 1919–20 our main pure race gave an average of 15·4 % of morphine in the opium of the first lancing. At Etawah and Fatehgarh it has given perhaps a slightly better result, whereas in Rae Bareli it has only produced about 13 % of morphine. In the previous season we noticed that the Rae Bareli crop behaved in a similar manner. The climate of all these places shows no marked difference and they are all within 100 miles of Cawnpore. It is hoped to follow up this matter further.

### *The Influence of Season.*

There is evidence that the morphine content of the opium produced by the same pure race may vary from season to season. We have grown a collection of pure races for three successive seasons and have found a distinct variation in the morphine content in successive seasons. Since only one analysis was carried out in each of these cases in each season it is hardly fair to generalise from these results. We have, however, grown a large area of one pure race at Cawnpore on the same farm during four successive seasons, and numerous analyses were made in each season. The table summarises the results of the four years' experiments.

*The seasonal variation in morphine content of opium produced by the same pure race of poppy. Produce of first lancings only.*

| Season | Area under experiment acres | No. of samples analysed | Percentage of morphine in dry matter of opium | |
|---|---|---|---|---|
| | | | Range of variation | Average |
| 1916–17 | 0·5 | 16 | 11·7–14·7 | 12·9 |
| 1917–18 | 5·5 | 91 | 11·8–15·8 | 14·0 |
| 1918–19 | 6·0 | 119 | 10·5–15·8 | 12·6 |
| 1919–20 | 1·5 | 71 | 14·0–17·7 | 15·4 |

We have accumulated similar data for the same pure race grown during three seasons at Rae Bareli in the plains and at Douglas Dale in the Himalayas.

### *Weather Conditions.*

Experiments were carried out in 1917–18 and in 1918–19 to trace any connection there might be between temperature and other weather conditions and yield or morphine content of the opium produced. A continuous record

of the temperature was kept by means of a thermograph. Observations were also made of barometric and humidity conditions but produced no results worthy of consideration here. Rainfall, direction of winds and amount of cloud were also recorded. A field of 0·5 acre in extent sown with seed of a pure race was used. The plan of experiment was to select 1000 capsules daily, which were evenly distributed throughout the field, and which the cultivators considered were ready for lancing, and to lance them. In the 1917–18 experiment 1000 capsules were so selected daily throughout the period 28th February to 26th March. The yield of opium and its morphine content were determined in each day's produce. At intervals of three days the capsules received a second and third lancing; after which the plants bearing them were pulled up to prevent confusion. The plants selected each day were labelled with cloth labels of a distinctive colour in order to simplify the finding of them on the field. Only terminal capsules were included in the experiment. The results for the first lancings only of the 1917–18 experiments are set out in the table.

*Table showing yield of opium per* 1000 *capsules and its morphine content recorded daily throughout duration of opium harvest. First lancings only.*

| Date of lancing | Gms. dry opium per 1000 capsules | Percentage of morphine on dry matter of latex |
|---|---|---|
| 28–2–18 | 52·1 | 14·9 |
| 1–3–18 | 62·1 | 14·5 |
| 2–3–18 | 60·3 | 14·9 |
| 3–3–18 | 53·1 | 14·9 |
| 4–3–18 | 60·1 | 14·6 |
| 5–3–18 | 61·1 | 14·5 |
| 6–3–18 | 64·5 | 13·9 |
| 7–3–18 | 53·7 | 14·2 |
| 8 3–18 | 51·3 | 13·6 |
| 9–3–18 | 50·4 | 14·0 |
| 10–3–18 | 43·2 | 14·2 |
| 11–3–18 | 45·4 | 14·1 |
| 12–3–18 | 41·5 | 14·8 |
| 13–3–18 | 33·0 | 15·3 |
| 14–3–18 | 33·0 | 15·1 |
| 15–3–18 | 27·5 | 14·8 |
| 16–3–18 | 24·4 | 14·6 |
| 17–3–18 | 19·4 | 15·0 |
| 18–3–18 | 18·1 | 15·0 |
| 19–3–18 | 12·3 | 15·7 |
| 20–3–18 | 5·4 | 13·8 |
| 21–3–18 | 7·7 | — |

As regards morphine content it will thus be seen that this has kept remarkably constant throughout the period of opium harvest. On comparing the daily yield of latex with the temperature records it appeared that the yield of latex diminished with a rise in temperature and increased with a fall in temperature. After the 9th February, however, the latex yield fell away rapidly probably owing to the drying up of the field and the drying of the capsules and this rapid and continuous fall masked any temperature effect.

It is very interesting to note that in spite of a very rapid fall in the latex yield with advance in season, yet the morphine content of the dried latex is not modified to a detectable extent.

The 1918–19 experiments completely bore out those of 1917–18.

## IX. The Influence of Manures.

The literature provided numerous references to the effect of manuring on drug production in plants but in the writer's opinion most of the work is very unsatisfactory. Pure cultures of plants appear to have been rarely, if ever, used and it would seem that lack of experience in conducting field experiments has handicapped certain of the investigators. These matters are referred to in our main publication. We have experimented on the same pure race in the seasons 1916–17, 1917–18, 1918–19 and 1919–20.

In 1916–17, on a field of one acre, we tested the effect of $K_2SO_4$, superphosphate and nitrate of soda, alone and in all possible combinations. Each manure was applied at the rate of 400 lbs. per acre. These large amounts were put on in order to magnify any possible manurial effect. Each plot was duplicated and the usual precautions taken in field trials were used. In 1917–18, exactly the same experiments were carried out, but castor cake and cattle dung plots were also added to the series. The first two years' experiments having indicated that nitrogen was the dominant manure for poppy, in the next two seasons the trials with superphosphate and $K_2SO_4$ were discontinued and the effect of increasing amounts of $NaNO_3$ was determined. Plots receiving castor cake and poppy seed cake were also added to the series in 1919–20. The germination in 1918–19 was so uneven that the experiment was unsatisfactory in that year.

The results obtained in the seasons 1916–17 and 1917–18 were practically identical. Superphosphate and potash alone, or in combination, had no appreciable effect on the yield of latex or on the morphine content. Nitrate of soda and castor cake, however, largely increased the yield of latex but the effect on the percentage of morphine in the dried latex was almost inappreciable.

The results for 1917–18 are summarised in the table.

*Table showing effect of manures on yield of latex and its morphine content.*

| Treatment | Percentage of morphine in dry matter of latex from first lancing | Total yield of latex from all lancings as dry matter. Unmanured plot = 100 |
|---|---|---|
| No manure ... ... ... | 14·1 | 100 |
| $K_2SO_4$ ... ... ... ... | 14·9 | 103 |
| Superphosphate ... ... | 15·8 | 120 |
| $NaNO_3$ ... ... ... | 15·0 | 188 |
| $K_2SO_4$ + Superphosphate ... | 13·8 | 99 |
| $K_2SO_4$ + $NaNO_3$ ... ... | 13·8 | 192 |
| Super. + $NaNO_3$ ... ... | 14·9 | 217 |
| Super. + $K_2SO_4$ + $NaNO_3$ ... | 14·7 | 253 |
| Cattle dung ... ... ... | 14·7 | 121 |
| Castor cake ... ... ... | 14·5 | 149 |

In considering the figures one must remember that control plots showed that these variations in morphine content on the different manurial plots are less than the variations to be expected over a field uniformly treated. At the same time the influence of nitrogenous manures on the yield of latex is undoubted.

Careful records have been kept of the weights of the plants, capsules, and of the seed out-turn on each of the plots. It has been shown that these weights are proportionate to the yield of total latex on each plot. The effect of the manure is apparently therefore to produce a bigger capsule which in virtue of its size produces a larger yield of latex. For a particular race of poppy it would seem that the morphine concentration of the latex of the capsule is practically constant.

Our 1919–20 experiments were designed to test the effect of increasing amounts of nitrate of soda on the yield and alkaloid content of the latex. Plots receiving castor cake and poppy seed cake, were also added to the series.

*Table showing effect of increasing amounts of nitrogen on yield of latex and its morphine content.*

| Treatment (per acre) | Percentage of morphine in dry matter of latex | Yield of latex at first lancing as dry matter. Unmanured plot = 100 | Yield of latex as dry matter, total of all lancings |
|---|---|---|---|
| Unmanured ... ... ... ... | 14·4 | 100 | 100 |
| $NaNO_3$ 80 lbs. ... ... ... | 15·3 | 106 | 127 |
| „ 160 lbs. ... ... ... | 15·5 | 124 | 142 |
| „ 320 lbs. ... ... ... | 15·4 | 113 | 103 |
| „ 480 lbs. ..... ... ... | 16·2 | 144 | 130 |
| „ 640 lbs. ... ... ... | 16·5 | 158 | 142 |
| Poppy cake 1600 lbs. containing N = $NaNO_3$ 530 lbs. ... ... | 16·1 | 153 | 166 |
| Castor cake 1600 lbs. containing N = $NaNO_3$ 580 lbs. ... ... | 17·0 | 146 | 193 |

The morphine content of the latex shows a small but regular increase with increasing amounts of nitrogen in the form of nitrate of soda. Castor cake produced latex of the highest morphine concentration, though it did not supply as much nitrogen as the maximum amount of $NaNO_3$ applied. There is however only a difference of 2·6 % in morphine content between the latex produced on the unmanured plot and that produced on the castor cake plot. This difference is no greater than that which it is possible to find in the opium harvested from different portions of the same field uniformly treated. We are convinced that the influence is just significant however, since there were four samples of opium collected corresponding to each treatment and the analyses of these four samples agreed closely in practically every case. The figures given in the above are the averages of these analyses.

As regards yield of latex at the first lancing there has been a uniform increase in latex yield with increasing amounts of $NaNO_3$ with the exception

of the plots receiving 320 lbs. $NaNO_3$ per acre. As regards the total out-turn of opium from all the lancings the plot receiving only 160 lbs. $NaNO_3$ per acre has given as big a yield as any of the nitrate plots. The cake plots have given the biggest total yields since they kept up the yields of latex at the subsequent lancings. The important point however is that though the yield of latex has been much increased yet there is little modification in the morphine content of the latex.

## X. The Influence of Starvation.

The plants on the unmanured plots in all our manurial experiments were certainly very poor specimens and ill nourished. It seemed of interest deliberately to produce the poorest plants possible and to examine the latex produced by them. For this purpose some hard uncultivated land at Cawnpore was ploughed up and poppy grown on it in the season 1918–19. A most miserable crop resulted and we were able to obtain complete plants only some 5 to 8″ high. A portion of this field was dressed with nitrate of soda and this certainly improved to a slight extent the plants growing there but only slightly. The capsules lanced were mostly from $\frac{1}{3}$ to $\frac{2}{3}$″ long; though they were slightly larger on the plants receiving nitrate of soda.

The opium from the first lancing only from the unmanured portion yielded 8·5 % morphine. The portion receiving nitrate of soda gave opium with 10·3 % morphine. With the same pure race of poppy grown in the same season on our experimental area the extreme range of variation in the morphine content of opium from first lancings only was from 11·1 to 15·0 %. In the season 1919–20 these results were checked at Cawnpore. Around the edges of fields, owing to the land receiving poor cultivation, there are always a certain number of stunted plants which usually only produce one capsule. The smallest of these plants were selected and their terminal capsules lanced for opium. These plants were not as small as those grown specially in the previous season.

Three separate samples of opium collected yielded 12·4, 12·7 and 10·1 % morphine reckoned in the dry opium. The normal plants in the same field yielded opium containing 15 to 16 % of morphine. Similar results were obtained in experiments in the Himalayas in 1919–20. The stunted plants from the edges of the fields produced opium with 10·3 % and 7·3 % morphine respectively in the two samples examined. The normal plants in the same field produced opium with 13·2–15·3 % morphine.

Undoubtedly therefore stunted plants yield opium very low in morphine content but the stunted plants examined by us were quite abnormal and would never be used by the cultivators for lancing. These results differ from those of Wayne Arny [1917] who working on belladonna found that small plants produced the highest percentage of atropine.

## XI. The Influence of Heredity.

An account of this will appear elsewhere written in conjunction with Mr Leake. The latex produced by more than 500 pure races isolated by him and of crosses made by him has been examined for morphine content and in many cases for codeine content. The pure races represent practically all types of poppy grown in India including white as well as coloured flowered races. Opium from the first lancings of terminal capsules only varied in its morphine content from 6·6 to 20·1 %, reckoned on the dry material. Most of them gave over 11·5 % morphine. The variation in codeine content is from 1·8 to 4·8 %. Indications have been obtained that a race of poppy producing latex of high morphine concentration maintains that power in subsequent seasons.

## XII. The Bearing of the Results on the Function of Alkaloids in Plants.

For the complete literature on the subject the reader must be referred to our main publications.

Our work has established the following facts:

1. When a capsule is lanced for the first time the concentration of morphine in the latex is at a maximum in the first latex to flow out. As the flow continues the morphine concentration diminishes.

2. At each successive lancing the morphine content of the opium obtained decreases rapidly and if sufficient successive incisions are made latex can eventually be obtained which contains no morphine as measured by the method of the British Pharmacopoeia. The morphine is not replaced in the latex by codeine or narcotine. The interval of time between each successive lancing whether one or five days has no influence on the rate of fall in morphine content of the opium from each successive lancing. If the interval of time between the first, second and third lancings is only a few hours, the fall in morphine content at each successive lancing is not so rapid.

3. If only small incisions are made at each successive lancing, and therefore only a small amount of latex removed each time, the fall in morphine concentration of the latex at each successive lancing is much less rapid than when larger incisions are made and more latex removed.

Similarly when for any reason, *e.g.* bad weather conditions or the making of smaller incisions than usual, the yield of the first lancing is low, then the falling off in morphine content is not so great at the second and third lancings as it would have been with a larger yield at the first lancing.

4. In very young capsules, say six days old, the latex is less concentrated in morphine. After the capsules have reached the stage at which they feel firm, say 16 days old in the writer's experiments, the morphine content of the dry opium is the same however long the first lancing is delayed after that stage and however much the yield of opium may vary from day to day.

5. Manuring with nitrogenous manures largely increases the yield of latex but the percentage of morphine in the latex is not largely modified. The yield of dry opium for a particular race of poppy is roughly proportional to the weight of the capsule and incidentally to that of the plant itself.

6. Morphine exists as such in the latex inside the plant.

7. Even though the capsules are lanced to exhaustion of morphine the seed shows no sign of deterioration, *e.g.* in germinating power. It is the universal practice in India to lance as long as the capsules yield latex and the writer knows of no evidence that the seed suffers in any way. The seed when ripe contains no alkaloids whereas the ripe capsules contain considerable quantities even after a year's storage.

8. Climate and weather conditions do not affect the morphine content of the latex to any appreciable degree. They may, however, have important effects on the yield of latex.

Starting with the above established facts it therefore appears that morphine is stored in the capsule more than in other parts of the plant.

This agrees with Clautriau's remarks [1889], who found that the alkaloids were most concentrated in the epidermis of the capsule and diminished in amount towards the lower part of the plant. That morphine is most concentrated in the latex of the capsule seems the only explanation of facts 1 and 2 above. This would explain why the latex first flowing from the cut surface is richest in morphine. As the flow continues, the latex in parts of the plant below the capsule, where it is poorer in morphine, has to be drawn on. This theory fits in well with fact 3 also, because if one only takes out very small amounts of latex at each lancing it will naturally take longer before the less concentrated latex below the capsule is drawn on. This may be accounted for by supposing that a few hours is not sufficient to exhaust the latex already in the capsule when only one incision is made. Actual observations show that the flow continues for at least 16 hours from one cut. If then the second cut is applied within a few hours of the first the latex exuding therefrom will be partly latex which was already in the capsule and therefore richer in morphine and which with one day's interval between the lancings would have all been exuded at the first lanced surface.

The plant loses its power to produce morphine about the stage that its capsules become firm to the touch (vide 4). This explains No. 2, *i.e.* that the morphine content of each successive lancing falls off. The flow of latex appears by No. 6 to be proportionate to the size and the weight of the capsule and incidentally to that of the plants. The effect of nitrogenous manures is to increase the size and weight of the capsules. Yet the morphine concentration in the latex remains nearly the same. It would therefore appear that the amount of morphine produced depends on the amount of plant tissue produced. Different races of poppy however produce latex of different morphine concentration. It may be that plants yielding larger amount of latex may produce

a lower morphine content in that latex and *vice versa.* Data are being accumulated to test this point.

It may be that the amount of morphine produced is proportional to growth whatever the race of poppy. The amount of latex vessels may however be a factor varying with race, and hence a capsule with few latex vessels, *i.e.* one which will give a low yield of latex, may produce opium especially rich in morphine.

It would therefore appear that, during the period of active growth of the plant, morphine is being produced at the same rate as the plant tissue. This fact is against the view of Bayliss [1915], that alkaloids are more or less an accidental product of chemical change. The plant no longer produces morphine when the seed begins to ripen, *i.e.* when its period of active growth has ceased. On the other hand the morphine does not seem to diminish (see No. 4).

The plant deposits morphine chiefly in the capsule, and one of the functions of the lactiferous system would seem to be the removal of alkaloids to the capsule. This theory is supported by the mode of growth of the lactiferous tubes in the case of *Euphorbiae* [Pfeffer, 1900], which send ramifications into each new portion of the tissue in which they occur. No. 7 indicates that the morphine is not used to nourish the seeds. Therefore it would seem that morphine is a useless end product of metabolism. The animal organism takes in complex food materials, and excretes its end products of metabolism which are mainly of a simple structure. The plant on the other hand feeds on simple substances and therefore it is not surprising if some of its end products are complex substances, which it finds difficult to excrete. That the amount of the morphine produced bears a definite relation to the amount of plant growth, would seem to support the theory that it is an excretory product, for the amount of excretory product would naturally depend on the amount of plant growth.

The large number of different alkaloids produced by plants is no objection to the theory that alkaloids are excretory products. Rather it is in favour of it, for certain alkaloids are characteristic of certain families and the metabolism of different families is certain to show differences.

A plant producing much oil in its seed might be expected to form different excretory products from plants producing much starch or protein as reserve material.

## Conclusion.

Morphine in the opium poppy is a useless end product of metabolism. The plant having no mechanism for excreting an end product of such complicated structure stores it in places where it can do no harm to its own metabolism, *i.e.* chiefly in the capsule. The lactiferous system would seem to represent a means of removing waste products of metabolism.

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