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ON

# INDIGO-BLUE

FROM

# POLYGONUM TINCTORIUM

AND

OTHER PLANTS.

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Some papers read before the Society many years ago, and subsequently published in its 'Memoirs'\*, contain an account of my experiments with the leaves of the Isatis tinctoria, or common woad, the well-known plant employed in Europe for dyeing blue before the introduction of indigo from the East. I showed that the leaves of this plant do not, as some have supposed, contain either indigo-blue or its hydride ready formed, but yield by careful treatment a peculiar glucoside-indican-which, when acted on by acids and other reagents, splits up into indigo-blue and indigoglucine, the latter being a body resembling glucose. experiments also show that this substance, indican, is a highly unstable body, undergoing when its watery solution is heated for some time, or, more rapidly, by the action of caustic alkalies, an entire change, on the completion of which it no longer yields indigo-blue by decomposition with acids, but in place of the latter gives indigo-red, indifulvine, leucine, and other products. Though I succeeded in ascertaining the composition of indican and the relation in which it stands to indigo-blue, the difficulty of obtaining large quantities of it in consequence of its excessive liability to change, prevented my proceeding further with the investigation. It seemed to me, however, that it might be of some interest to ascertain whether other indigo-

<sup>\* &#</sup>x27;Memoirs,' ser. 2, vol. xii. p. 177, and vol. xiv. p. 181.

yielding plants contain ready-formed indigo-blue (as has been maintained with so much persistence), or whether the colouring-matter exists in the vegetable cells in the form of indican or some other glucoside; and I have accordingly examined such of the plants known to give indigo as I have been able to procure.

Before stating the results to which I wish to direct attention on this occasion, I may mention an observation belonging, strictly speaking, to the part of the subject previously treated of, which, however, I will now describe in a few words, as I may not have another opportunity of doing so. In my last memoir I stated that, among the products of decomposition of indican from Isatis tinctoria, leucine is usually found, sometimes, indeed, in considerable quantities. In some more recent experiments made with woad leaves I obtained, besides leucine, a substance having all the properties of tyrosine. This substance was only slightly soluble in cold water, but soluble in boiling water, from which it separated on cooling in long needles, forming, when dry, a snow-white felted mass. Its watery solution gave the well-known reaction with mercuric nitrate. Its solution in concentrated sulphuric acid, after neutralization with barium carbonate, gave a purple colour with ferric chloride. According to Prof. Gamgee, who had the kindness to examine the substance for me, it showed under the microscope the forms characteristic of ordinary tyrosine. There could be no doubt, therefore, of its identity with the latter. As the tyrosine in this case was not obtained from pure indican, but from the crude alcoholic extract of the leaves, it is impossible to say whether it preexisted in the plant or whether it was a product of decomposition of the indican of the extract. The latter supposition is the most probable one; for tyrosine, being almost insoluble in alcohol, could hardly be contained

in any appreciable quantity in the alcoholic extract of the leaves.

Some connexion between tyrosine and indigo-blue has frequently been suspected by chemists. Indeed a glance at the formulæ of the two bodies will show that some connexion is possible, since, by replacing H by CH<sub>3</sub> in indigo-blue and adding 2H<sub>2</sub>O, we arrive at the formula of tyrosine, thus:—

$$C_8H_5NO + CH_2 + 2H_2O = C_9H_{11}NO_3$$
. Indigo-blue. Tyrosine.

In order to explain the formation of tyrosine from indican, we may suppose the latter to split up into tyrosine, indiglucine, acetic acid, and carbonic dioxide, thus:—

$$\begin{split} \text{C}_{26}\text{H}_{33}\text{NO}_{18} + 3\text{H}_{2}\text{O} = & \text{C}_{9}\text{H}_{11}\text{NO}_{3} + 2(\text{C}_{6}\text{H}_{10}\text{O}_{6})\\ \text{Indican.} & \text{Tyrosine.} & \text{Indiglucine.} \\ & + 2(\text{C}_{2}\text{H}_{4}\text{O}_{2}) + \text{CO}_{2}. \end{split}$$

Supposing the tyrosine in this case to have been really formed from indican, the question suggests itself whether the leucine and tyrosine so frequently found in the animal organism as products of disease, may not be derived from some substance similar to indican rather than directly and immediately from albuminoids.

### Polygonum tinctorium.

This plant has long been known and employed as a source of indigo by the Chinese. According to Stanislas Julien\*, who has given translations from Chinese works of various processes for extracting indigo from the leaves, the plant is called in China Lân, the most productive variety being termed Tcha-Lân, i.e. the Lân resembling the tea shrub. It was introduced into Europe in the eighteenth

<sup>\*</sup> Comptes Rendus, t. vii. p. 703.

century, and at one time, particularly about the years 1838 to 1840, formed the subject of numerous investigations by eminent French botanists and chemists, such as Turpin, Joly, Baudrimont, Pelletier, Robiquet, and others, some hopes being entertained that the plant might be cultivated profitably in France. The numerous trials made with this view having led to no result, the matter fell again into oblivion, and this interesting plant remained what it was before, a mere curiosity.

I obtained the seeds of the plant from Messrs. Vilmorin, Andrieux & Co., the eminent horticulturists of Paris, and therefore felt sure of their genuineness. They were sown in a hotbed, and germinated rapidly. As soon as the young plants were a few inches high they were transplanted into the open ground, where they grew vigorously, producing an abundance of leaves and attaining during the summer season a height of nearly three feet. Towards the end of summer spikes of pretty pink flowers, resembling those of other species of *Polygonum*, made their appearance. The seed, however, did not ripen in the open air, the plant being cut down by the early frosts before this could take place; but, by growing a few plants under glass, I obtained a quantity of well-matured seeds, which yielded another crop in the following season.

For a botanical description of *Polygonum tinctorium* I must refer to Turpin\* and Joly†, the latter of whom has given a full account of its structure and affinities. Of the various organs, the leaves, being the seat of the blue colouring-matter, are alone of any interest to the chemist. These leaves, which are large, oval in shape, and glossy, show no indication of the presence within their tissue of any pigment beside the chlorophyll to which they owe

<sup>\*</sup> Comtes Rendus, t. vii. p. 806.

<sup>† &#</sup>x27;Sur le Polygonum tinctorium:' Montpellier, 1839.

their lively green colour, except, occasionally, in certain places where they have suffered injury from the bites of insects or from other causes, and where blue spots make their appearance—a phenomenon which I shall endeavour to explain presently. It is nevertheless easy to show that they contain a considerable quantity of what the French call a "matière colorable," i. e. a substance which, though colourless in itself, yields colouring-matter by appropriate treatment. A few leaves having been cut into pieces and rubbed up with a little water in a mortar to a thin paste, the mass is poured on a bit of calico, and yields, by squeezing and kneading, a green muddy liquid which, on the addition of a little sugar-of-lead solution, gives a green flocculent precipitate containing the chlorophyll, albumen, and other matters previously held in suspension. The liquid filtered from this precipitate is clear and yellow, and, on being mixed with sulphuric or hydrochloric acid and left to stand for several hours, yields a deposit consisting of tolerably pure indigo-blue. The amount of colouring-matter obtained in this way from Polygonum tinctorium is far greater than that which the same quantity of woad-leaves grown in the same locality would produce, proving that the yield is influenced not only by soil and climate, but also by the peculiar nature of the plant.

The isolation of the "matière colorable" of Polygonum tinctorium is still not a very easy task. The same precautions must be observed as in the case of Isatis tinctoria, particularly as regards the evaporation of the solvents employed, which must always be effected without applying artificial heat. Unless some means are at disposal for evaporating rapidly at the ordinary temperature, by means of a current of air or otherwise, success is very uncertain. The method formerly employed in preparing indican from Isatis tinctoria was first tried. The leaves of the plant were

dried in a stove moderately heated, and, while still warm, ground to powder. The powder, which had the colour of fresh hay, was passed through a hair sieve to separate the leaf-stalks and other fibrous portions, and then extracted in a percolator with spirit of wine. The green alcoholic extract was evaporated in a shallow tin dish, the evaporation being assisted by passing a current of air over the surface of the liquid in the apparatus formerly described. Chlorophyll and fatty matter were deposited during evaporation, leaving a brown watery liquid, which was poured off, agitated with freshly precipitated copper oxide, and filtered. The copper in the filtrate was precipitated with sulphuretted hydrogen, and the filtered liquid was evaporated as before. The residue was treated with absolute ether, which dissolved a portion, and left, on evaporation, a yellow syrup. This syrup is the indigo-producing body as pure as it is possible to obtain it. I prefer to this the following process, as being more expeditious and surer. The alcoholic extract of the dried leaves having been evaporated, the watery liquid which is left is mixed with acetateof-lead solution, which gives a dirty-yellow precipitate, consisting of chlorophyll and other impurities in combination with lead. To the clear yellow filtrate basic lead acetate is added; this gives a primrose-yellow precipitate, which is filtered off and, after being washed with water and then with alcohol, is suspended in absolute alcohol, through which a current of carbonic anhydride is passed. After the gas has passed through for some time the liquid acquires a yellow colour, and, after being filtered from the insoluble portion, consisting principally of lead carbonate, is evaporated at the ordinary temperature. Water added to the residue leaves a portion undissolved, which is filtered off. Sulphuretted hydrogen is passed through the filtrate to precipitate the lead contained in it; and having been again

filtered, is it evaporated, when it leaves a sirupy residue which may be treated with ether as before.

The indigo-producing body thus obtained is, if it be permitted to draw a certain conclusion from mere qualitative reactions, identical with the indican of Isatis tinctoria. Its appearance is that of a yellow transparent sirup, showing no tendency to assume a crystalline form. It is soluble in water, alcohol, and ether. The watery solution has a more or less acid reaction. It becomes of a deep vellow colour on the addition of caustic alkali, and gives with basic lead acetate a light-yellow precipitate. When the watery solution is mixed with a little sulphuric or hydrochloric acid and left to stand for some time, the surface of the liquid becomes covered with a film of indigo-blue, a deposit of the same substance being usually formed at the bottom. The filtered liquid shows, when tested with a salt of copper and an excess of caustic alkali, the well-known reaction of glucose. If, however, the watery solution is left to stand at the ordinary temperature for a considerable time, or if it is simply boiled for some time, or if it is mixed with caustic alkali and left for a short time, it no longer yields indigo-blue on the addition of acid. This is probably due, as I have shown to be the case with indican, to a molecular change, resulting, when completed, in the formation of a body which, when decomposed with acids, yields indirubine and brown resinous substances in place of indigo-blue. When a large quantity of watery solution is mixed with acid and left to stand, a portion of the substances undergoes, it seems, the same change; for the deposit formed when operating with one litre or more of the solution contains not only indigo-blue, but also indirubine, indifulvine, and other products. The deposit, which in this case is almost black, after being filtered off, washed, and dried, is treated first with caustic alkali and then with cold

alcohol, in order to remove the indifulvine and other resinous substances. On treating the residue with boiling alcohol, the indirubine dissolves, and is obtained, after several crystallizations, in the beautiful dark-red needles characteristic of the substance. The portion insoluble in boiling alcohol is indigo-blue, requiring for its purification merely to be dissolved in some suitable menstruum, such as boiling aniline. I am inclined to think that the same molecular change takes place in the cells of the plant during the later stages of its development; for I obtained from some leaves gathered late in the season when the flowers had begun to appear, a quantity of indican having the usual appearance, but giving, by decomposition with acid, far less indigo-blue and more indirubine and other products than the indican from younger leaves. This result was confirmed by experiments, to be described presently, made with the leaves themselves.

The preceding experiments lead to the conclusion that the leaves of Polygonum tinctorium contain a substance not to be distinguished from the indican of Isatis tinctoria, which, by decomposition with acids, yields indigo-blue and glucose, accompanied by some by-products, and that there is no proof of the existence of ready-formed colouringmatter in the plant while the latter is living and in a healthy state. The preexistence of indigo-blue, or of its hydride, indigo-white, in these plants was taken for granted forty or fifty years ago, when the class of bodies which we now call glucosides and the peculiar kind of decomposition which they undergo were unknown. Even now a superficial examination of some phenomena would almost certainly lead to the conclusion that the indigo-blue is formed by the action of air, i. e. in consequence of the oxidation of some easily oxidizable substance in the plant. Bearing in mind, however, with what extreme facility indican is decomposed, its watery solution on standing some time, even at the ordinary temperature, depositing indigo-blue, I think it will not be difficult to explain all the phenomena hitherto observed by myself and others.

On taking a plant of Polygonum tinctorium and making incisions with a penknife in the leaves between the main vessels, or crushing the soft parts of the leaves here and there with an agate pestle, then, after a short time, plunging the whole plant into boiling alcohol to remove the chlorophyll, it will be found that those parts of the leaf which have not been injured become white or retain only a faint yellow tinge, while those parts that have been cut, crushed, or otherwise injured, show a blue colour, the coloration extending for some distance inwards from the place where the lesion occurred, the most intense colour being at the edge of the cut or bruise. So, too, in the living plant, when some injury accidentally occurs to a leaf, the part injured will appear blue. Nothing can be more natural than to suppose that in these cases the blue colouring-matter is formed by the action of the air, i. e. by the oxidation of some substance which escapes from the cells in consequence of organic lesion, just as the surface of a freshly cut apple or pear becomes brown on exposure.

If a plant of *Polygonum tinctorium* be immersed in water, and the water be frozen by surrounding the vessel containing it with a freezing-mixture, it will be found, after complete thawing, that the leaves or parts of leaves which have been thoroughly frozen appear of a dark colour and are quite flaccid; and if the plant be then immersed in boiling alcohol so as to dissolve the chlorophyll and other matters, those very parts show afterwards an intense blue colour, while those portions which had remained unfrozen appear almost colourless. This experiment, which had already been made by Joly, was considered by him to prove

the preexistence of indigo-blue in the plant—though why, if this were the case, the colouring-matter should not make its appearance in the unfrozen portions of the leaf, I am at a loss to understand.

The fact that a fresh leaf of Polygonum tinctorium, if immersed in alcohol or ether, appears blue after the chlorophyll has been removed, has also been considered to prove the preexistence of indigo-blue in the cells. This phenomenon is always observed when the leaves are immersed in cold alcohol, and more distinctly when ordinaary spirits of wine are taken than with absolute alcohol. A very simple experiment suffices, however, to prove that in this, as in all the other cases, appearances are deceptive. If freshly gathered leaves of Polygonum tinctorium are plunged at once not into cold, but into boiling alcohol, the whole of the colour is soon removed, the leaves retaining only a faint yellow tinge. On now simply evaporating the green alcoholic liquid, not a trace of indigo-blue will be found in the residue. It is therefore absolutely certain that the leaves contain no ready-formed colouring-matter; for so stable a body as indigo-blue could not possibly be decomposed or be made to disappear by the action of boiling alcohol only. It must necessarily appear either in the alcoholic extract of the leaves or in the residual portion left by the alcohol.

A very simple explanation offers itself, I think, for all the phenomena hitherto observed. Indican, the mother substance of indigo-blue, is a body the molecules of which are in a state of unstable equilibrium. As long as it is contained within the cells of the plant the vitality of the cells keeps it in its original unchanged condition. As soon, however as this vitality is destroyed (whether by organic lesion, by extreme cold, or any other means), the indican begins to undergo decomposition, the molecules

rearrange themselves in the order to which their chemical affinities predispose them, and the compound splits up into indigo-blue and indiglucine, this taking place so rapidly that in certain cases it would appear as if the colouringmatter preexisted in the plant. If (to take the simplest case) the leaves as soon as gathered are immersed in cold spirits of wine or in cold ether, the vitality of the cells is thereby destroyed; and the indican contained in them is then in part decomposed, yielding indigo-blue, which remains undissolved, imparting a more or less distinct blue tint to the leaves. When boiling spirit of wine is taken, the indican is extracted before it can undergo decomposition, and dissolves in the spirit. It may be detected in the residue obtained on spontaneous evaporation of the alcoholic extract by its property of yielding indigo-blue on decomposition with acids, as above described. It is possible that the leaves contain some ferment which hastens the decomposition of the indican as soon as vitality has ceased; but I have no positive evidence to offer in favour of this view.

I may, in conclusion, describe another experiment, which, though it teaches nothing new, confirms what I have just stated, and is interesting in its way. Having cut some sprigs of *Polygonum tinctorium*, about six inches long, I immersed the cut ends in dilute hydrochloric acid (consisting of one part of acid of specific gravity 1.15 and ten parts of water), and left them to stand for several days exposed to the sun and air. The acid was gradually absorbed, ascending through the stems, first into the lower leaves, then into the higher ones. The gradual absorption of the acid was distinctly seen by the discoloration of the leaves, which commenced at the basis of each leaf and extended towards the apex, the lively green colour being changed into a dirty yellow. After some time this

coloration was followed by a dark blue one, commencing at the base of each leaf and extending towards the apex, but never quite reaching the latter except in the lower leaves. When the change in colour had begun to show itself in the upper tenderer leaves, the whole showed symptoms of fading, all further power of absorbing the acid liquid seemed to be lost, and the sprigs were then at once immersed in hot spirit of wine. After remaining in the spirit until the chlorophyll was removed, the part of each leaf which had undergone a change appeared blue, whilst the part into which the acid had not penetrated appeared almost colourless. In this case it is probable that the indican was decomposed not so much in consequence of the loss of vitality in the cells, as by the direct action of the acid. In some of the leaves there was another distinct blue coloration towards the apex, in the part to which the acid had not penetrated, separated from the blue part at the base by a white zone. This second coloration may be attributed to the loss of vitality in that part of the leaf.

All these experiments must be made with plants whilst in a state of vigorous growth. If made when the season is advanced (that is, after the flowers have begun to appear), the leaves, though apparently unchanged, show only traces of blue colour after treatment and subsequent immersion in hot alcohol. This shows either that the indican has disappeared, being applied to other purposes in the economy of the plant, or that it has undergone the peculiar molecular change before referred to, into a substance which no longer yields indigo-blue by decomposition, but indirubine and other products. The latter is the more probable way of accounting for the difference; for the parts of the leaf which have been accidentally injured by the bites of insects or from other causes at the later stage of the plant's development become red, not blue, as at the earlier stages.

This red colour disappears on immersion of the leaves in hot alcohol, the indirubine, to which it is probably due, being more soluble in that menstruum than indigo-blue.

The leaves of *Polygonum tinctorium* in which the blue colour has been developed by any of the means described, exhibit even to the naked eye, and still more distinctly when examined under the microscope, certain appearances which are not without some interest.

- I. The colouring-matter seems to be confined to the parenchyma of the leaf. The stem and its fibrous ramifications in the leaf are free from it, so that in the coloured leaf the vessels may be distinctly traced as white veins on a blue ground. Even the cells of the parenchyma adjacent to the vessels are much less coloured than those a little further off, which produces the effect of a gradual shading of colour from the white of the vessels to the dark blue of the remoter cells. The cells of the leaf-cuticle are also free from colouring-matter.
- 2. The younger leaves at the summit of each branch generally show a more intense colour than the older ones near the base. Each leaf probably contains the same amount of colouring-matter; but in the lower leaves it is more widely distributed.
- 3. The intense and apparently uniform coloration of some of the leaves might lead to the conclusion that the cellular tissue is itself dyed blue—which would not seem improbable, considering the affinity which indigo-blue shows for cellulose, as seen in the blue dyeing of cotton fabrics. On examining the leaf-cells under the microscope, this is found, however, not to be the case. The colouring-matter is discovered within the cells of the parenchyma in the shape of separate dots and parcels of various sizes, and apparently in an amorphous state, the cell-wall being quite colourless. These dots and parcels being very numerous,

produce, when seen in the mass, a uniform blue coloration, more or less intense. The darker colour of some leaves is simply the effect of a greater crowding of the blue particles in each individual cell, the cells of the paler leaves containing fewer of these little masses, sometimes hardly any.

Mr. Charles Bailey, to whom I gave some specimens of leaves of *Polygonnm tinctorium* coloured blue, had the kindness, at my request, to submit them to microscopic examination, and gave the following as his opinion thereon:—

"The colouring-matter left in these specimens would seem to be what Nägeli terms 'crystalloids;' and, with one exception, these bodies are, as far as my examination has gone, confined to the interior of the cells of the parenchyma. I do not see the least trace of any of this colouring-matter occurring in the intercellular spaces. The only part of the tissue where I find it, other than the parenchyma, is in the cells of the stomata; but it occurs nowhere else in the cuticle."

#### Bletia Tankervilliæ.

The occurrence of a blue colouring-matter in this and other plants belonging to the Orchidaceæ, such as Calanthe veratrifolia, was first noticed by Clamor-Marquart\* and by the late Dr. Crace Calvert†. The attention of these observers was directed to these plants by seeing a blue coloration appearing in the white petals of the flowers on their beginning to fade; and they found the blue colour to be due to indigo. In accordance with the views then prevailing, they assumed the preexistence of the colouring-matter, either as indigo-blue or as its hydride, in the tissues of these plants. It is easy to see, however, on reading the accounts of their experiments, that the colouring-matter was really formed during the processes

<sup>\*</sup> Buchner, Repertorium f. die Pharmacie, B. lvii. S. i.

<sup>†</sup> Journal de Pharmacie, t. vi. p. 198.

employed for its extraction; and it seemed to me, therefore, highly probable that the plant would be found to contain some glucoside similar to indican.

Bletia Tankervilliæ is not difficult to procure, being frequently grown for the sake of its handsome brown and white flowers and its general beauty. The leaves of the plant having been cut in pieces and ground with water between two stones to a pulp, which is strained through calico, yield a green muddy liquid which, heated to near the boiling-point, gives a thick green coagulum. The liquid filtered from this coagulum is clear, of a deep yellow colour, with a slight acid reaction and an acrid taste. When this liquid is mixed with sulphuric or hydrochloric acid and left to stand, it deposits dark-coloured flocks consisting of indigo-blue mixed with a substance which imparts a purple colour to boiling alcohol, probably indirubine. The presence of glucose may be detected in the filtrate by the usual test, while the same test applied before the addition of acid shows no indication of its presence. It may hence be inferred that the liquid contains in solution a glucoside similar to, if not identical with, indican. It undergoes, like indican, a complete change when submitted to the action of alkalies; for if its watery solution be mixed with caustic alkali and boiled, then with an excess of sulphuric acid and again boiled, it deposits brown flakes, which are found to contain but little indigo-blue, while in the filtrate only a trace of glucose can be detected. The same change takes place gradually when the watery solution is left to stand for several days at the ordinary temperature.

The gradual formation of indigo-blue in the leaves of Bletia Tankervilliae may easily be traced in the same way as with those of Polygonum tinctorium, by placing the lower ends, immediately after cutting, in dilute hydrochloric acid and leaving them freely exposed for a few days. The acid,

as it ascends, causes a dark discoloration; and the part discoloured, after immersion in boiling alcohol to remove the chlorophyll, appears blue.

### Indigofera tinctoria.

It would be a matter of some interest to ascertain in what state the colouring-matter exists in this the most important of all the plants yielding indigo. From what I have said, however, it will be apparent that, in order to arrive at a certain conclusion, it would be necessary to work with fresh leaves; for if they contain a glucoside resembling indican, this would, in a very short time, undergo complete decomposition. I obtained some seeds of *Indigofera tinctoria* from Messrs. Vilmorin, Andrieux & Co., and treated them in accordance with the directions they kindly gave me. The seeds germinated and the young plants lived for some time in a hothouse; but, unfortunately, they attained no great size, and soon decayed and died, so that I was unable to obtain a quantity of leaves sufficient for examination.

Mr. P. Michéa, an intelligent indigo-planter, with whom I have been in correspondance, gives me, however, some interesting information relating to this part of the subject. Mr. Michéa writes to me as follows:—"It was my finding in the *Indigoferæ* of India (in the wild plant which grows at many places in the three Presidencies as well as in the cultivated species of Bengal, the North West, and Madras) a glucoside substance perfectly similar to the indican of the *Isatis tinctoria* in all its properties, which made me declare that the colouring-matter of the Indian *Indigoferæ* was due to indican."

The experiments just described lead to the onclusion that in all the indigo-yielding plants hitherto examined, the colouring-matter is derived from a glucoside which splits up with great ease into indigo-blue and glucose, and that this glucoside is probably, in all cases, the same, and identical with the indican of *Isatis tinctoria*.

Various other plants have been supposed to yield indigoblue. Of these I have examined the following:—

Galega officinalis.

Hedysarum Onobrychis (Sainfoin).

Polygonum Fagopyrum (Buckwheat).

Polygonum Persicaria.

Rhinanthus Crista-galli.

Sophora japonica.

Spilanthes oleracea.

The leaves of these plants, when treated in the manner above described, yielded no trace of any substance resembling indican, and showed no indications of containing any blue colouring-matter like indigo.