The Chemical Interpretation of some Mendelian Factors for Flower-Colour.

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The inheritance of flower-colour in *Antirrhinum majus* has been worked out by one of us* and also by Baur.† Investigation has shown that the flower-colour of the type in *Antirrhinum* is due to the presence of at least six factors and that these, in various combinations, produce a series of colour-varieties. Full accounts of the factors have been given in the papers cited, but for convenience of reference four are mentioned again here, *i.e.*:—

Y. A factor representing the power to form ivory pigment in the tube, accompanied by yellow pigment in the lips.
I. A factor representing the power to form ivory pigment in the lips.
R. A factor representing the power to form red pigment in the flower.
B. A factor representing the power to convert red into magenta pigment.

The factorial constitution of the type and varieties can be expressed as follows:—

YY(y)iirrB(b)B(b)	Yellow.
YY(y)II(i)rrB(b)B(b)	Ivory.
YY(y)iiRR(r)bb	Bronze.
YY(y)II(i)RR(r)bb	Red.
YY(y)iiRR(r)BB(b)	Crimson.
YY(y)II(i)RR(r)BB(b).....	Magenta.
yyI(i)I(i)R(r)R(r)B(b)B(b) ...	White.

In 1909 a series of researches was commenced with a view to the interpretation of the above factors in terms of chemical substances, or possibly

* Wheldale, M., "The Inheritance of Flower-colour in *Anturrhinum majus*," 'Roy. Soc. Proc.,' 1907, B, vol. 79, p. 288; "Further Observations on the Inheritance of Flower-colour in *Antirrhinum majus*," 'Rep. Evol. Com. Roy. Soc.,' V, 1909, p. 1.

† Baur, E., "Einige Ergebnisse der experimentellen Vererbungslehre," 'Beihefte zur Med. Klinik,' Berlin, 1908, Heft 10, p. 265; "Vererbungs- und Bastardierungsversuche mit *Antirrhinum*," 'Zs. indukt. Abstammungslehre,' Berlin, 1910, vol. 3, p. 34.

enzymes. Since some of the pigments involved (red, magenta) belong to the group of so-called anthocyanins, *i.e.* soluble red, purple and blue pigments of plants, general investigations were at first made by one of us* on anthocyanin pigments. As a result of qualitative reactions, in conjunction with evidence from cross-breeding, it was suggested that anthocyanins, as a group, are oxidised products of the natural yellow colouring matters, the flavones and xanthones. At the same time it was pointed out that a number of the latter substances had been isolated by Perkin and others† from various plants and several had been shown to be widely distributed. The existence of many flavones and flavone derivatives was mentioned and attention was drawn to the fact that, as a group, they have similar properties but differ among themselves in the number and position of their hydroxyl groups and in other points. It was further suggested that the oxidised products (anthocyanins) might, in a similar way, form a group of closely related substances, differing individually according to the flavone from which each had been derived.

In view of evidence collected from various sources, it was again suggested by one of us,‡ that since the flavones are known to be present in many cases as glucosides in the plant, the reactions involved in the formation of anthocyanin might be stated in very general terms as follows:—

$$\text{Glucoside} + \text{water} \rightleftarrows \text{flavone} + \text{sugar},$$

$$x\,(\text{flavone}) + \text{oxygen} \rightarrow \text{anthocyanin},$$

and also that, in addition to oxidation, there might be condensation of the flavone molecules. It was likewise stated that the first reaction might be controlled by a glucoside-splitting enzyme and the second, if due to oxidation, by an oxydase.

Subsequent work has strengthened the view that anthocyanins are, in all probability, derivatives of the flavones, though we ourselves have no further evidence as to the actual nature of the reactions involved in their formation.

Since we find little reliance can be placed on results given by crude water or alcoholic extracts from flowers, in all later investigations an attempt has been made to deal with the isolated and purified pigments. In a paper by one of us,§ the methods of preparation and purification of the crude pigment

* Wheldale, M., "The Colours and Pigments of Flowers with special Reference to Genetics," 'Roy. Soc. Proc.,' 1909, B, vol. 81, p. 44; "On the Nature of Anthocyanin," 'Phil. Soc. Proc.,' Cambridge, 1909, vol. 15, p. 137.

† Perkin, A. G., various papers in 'Chem. Soc. Trans.,' from 1895 to 1904.

‡ Wheldale, M., "On the Formation of Anthocyanin," 'Journ. Genetics,' 1911, vol. 1, p. 131.

§ Wheldale, M., "The Flower Pigments of *Antirrhinum majus*. I.—Method of Preparation," 'Biochem. Journ.,' 1913, vol. 7, p. 87.

have been described. In a more recent paper by both authors,* an account has been given of the identification of the ivory pigment of *Antirrhinum* with apigenin, a flavone of known constitution, isolated by Perkin† from apiin, a glucoside occurring in the parsley, *Apium petroselinum*. Apigenin is a very pale yellow crystalline substance, readily soluble in hot alcohol, slightly so in ether and almost insoluble in water. Melting point, 347° C. In the *Antirrhinum* plant, apigenin undoubtedly exists as a glucoside, in which state it is more soluble than after hydrolysis.

Attention has been given subsequently to the yellow pigment and the results are included in the present paper. The crude pigment prepared from yellow flowers was extracted with ether by methods described in previous papers. The ether extract contains apigenin from the tube and inner tissues of the corolla, and yellow pigment from the epidermis of the lips, including the patch on the palate. It was at first thought that the yellow pigments in the epidermis of the lips and in the patch on the palate might be identical. After removing the bulk of the apigenin from the ether extract by crystallisation from alcohol, the remaining yellow pigment, which is very soluble in alcohol, gave, on fractional crystallisation from dilute alcohol, products of which the melting points varied from about 250° to 338° C.

The wide range of the melting points, combined with certain qualitative reactions of these extracts, led to the conclusion that the palate contained the lip pigment mixed with other pigments, or even other pigments without the lip pigment. Since, however, the patch on the palate is common to all varieties (except white), the factorial difference between ivory and yellow is only concerned with the yellow lip pigment. Hence, in order to simplify the problem, the pigments of the palate have been disregarded for the time being, and investigations have been limited to crude material (unfortunately prepared only in small quantity) from the upper lips of the yellow variety. In this product, it seemed more likely that there would only be two pigments present to any extent.

Even the more simple mixture presented very great difficulties in the separation of yellow from ivory, both pigments having almost the same solubilities in all solvents used. Such separation as was possible by means of different solubilities gave products which indicated by their melting points, 300–328° C., the presence of luteolin, this substance being the only

* Wheldale, M., and Bassett, H. Ll., "The Flower Pigments of *Antirrhinum majus*. II.—The Pale Yellow or Ivory Pigment," 'Biochem. Journ.,' 1913, vol. 7, p. 441.

† Perkin, A. G., "Apiin and Apigenin," 'Chem. Soc. Journ., Trans.,' 1897, vol. 71, p. 805; 1900, vol. 77, p. 416.

known flavone melting above 300° C. and having at the same time the solubilities and properties of the yellow pigment.

Proceeding on the assumption that the yellow pigment might be luteolin, a fairly satisfactory separation was brought about by hydrobromic acid, which, according to Perkin,* forms, in glacial acetic acid, a compound with luteolin but not with apigenin. The luteolin hydrobromide remains in solution unless excess of hydrobromic acid is added, when it separates out in ochre-coloured crystals which are decomposed by water into luteolin and hydrobromic acid. The method of procedure in our case was as follows: The ether extract containing the mixed pigments was ground into a thin paste with glacial acetic acid, heated to boiling, and hydrobromic acid added, but not in excess. On cooling, the bulk of the apigenin separated out, while the yellow pigment remained in solution. The apigenin was filtered off, and on addition of much water to the filtrate the yellow pigment separated out and was also filtered off. A repetition of this process ensures greater purity of the yellow. After drying, the yellow was further purified by extraction with ether.

The pigment prepared in this way, except for its melting point, which varied from 310° to 328° C., resembled luteolin in properties. According to Perkin,† luteolin is a bright yellow crystalline substance, readily soluble in alcohol, fairly soluble in ether, and very slightly soluble in water, even when hot. With ferric chloride solution luteolin gives at first a green, later a red-brown, coloration. The melting point of luteolin was for many years given as "above 320° C." More recently Perkin has obtained luteolin by two different methods of purification, giving, in one case, a product melting at 327–329° C., in the other, at 323–326° C.

Luteolin occurs in *Genista tinctoria*‡ and in leaves of *Digitalis*§ and also, together with small quantities of apigenin, in *Reseda luteola*.‖

The structural formulæ of luteolin and apigenin are as follows:—

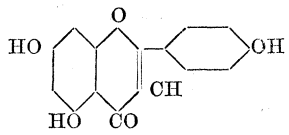
* Perkin, A. G., "Luteolin.—Part I," 'Chem. Soc. Journ., Trans.,' 1896, vol. 69, p. 206.

† Perkin, A. G., "Luteolin.—Part I," 'Chem. Soc. Journ., Trans.,' 1896, vol. 69, p. 206; "Luteolin.—Part II," 'Chem. Soc. Journ., Trans.,' 1896, vol. 69, p. 799; Perkin, A. G., and Horsfall, L. H., "Luteolin.—Part III," 'Chem. Soc. Journ., Trans.,' 1903, vol. 77, p. 1314.

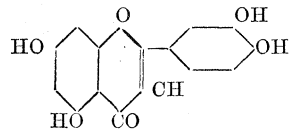
‡ Perkin, A. G., and Newbury, F. G. "The Colouring Matters contained in Dyer's Broom (*Genista tinctoria*) and Heather (*Calluna vulgaris*)," 'Chem. Soc. Journ., Trans.,' 1899, vol. 75, p. 830.

§ Fleischer, F., "Digitoflavon, ein neuer Körper aus der *Digitalis purpurea*," 'Ber. D. Chem. Ges.,' 1899, vol. 32, p. 1184; Killiani, H., u. Mayer, O., "Ueber die Identität von Digitoflavon und Luteolin," 'Ber. D. Chem. Ges.,' 1901, vol. 34, p. 3577.

‖ Perkin, *loc. cit.*



Apigenin.



Luteolin.

As pointed out by Smiles,* the more intensely coloured flavones contain two hydroxyl groups in the ortho position with respect to one another, whereas the arrangement in apigenin is not so productive of colour.

The close connection between the structure of the two substances, and the fact of their occurrence together in *Reseda luteola*, also favour the assumption that the yellow *Antirrhinum* pigment is luteolin. The presence of luteolin in the allied genus *Digitalis* is also of interest.

In order to corroborate this suggestion, attempts were made to form both the acetyl and benzoyl derivatives of the yellow pigment. The attempts failed, owing partly to the small amount of pigment available, and partly to the following difficulties. In the case of the acetyl derivative, the method of dissolving the pigment in caustic soda or pyridine and adding acetyl chloride apparently failed to acetylate the pigment completely. The method employed by Perkin and others of boiling the pigment with acetic anhydride and anhydrous sodium acetate was not found satisfactory when dealing with such small amounts of substance, since there were produced simultaneously brown decomposition products, from which it was impossible to isolate the derivative. In attempts to benzoylate the yellow pigment by the Schotten-Baumann method, the same difficulties arose, together with a further one, namely, the fact that the melting point, 201° C., of the benzoyl derivative of luteolin is only about 10° lower than that, 210–212° C., of the benzoyl derivative of apigenin; hence the possibility that the small amount of product formed might be impure apigenin derivative produced from apigenin retained in the luteolin used. It has been shown by Perkin† that in the Schotten-Baumann method, under certain conditions, a tribenzoyl, instead of a tetrabenzoyl, derivative may be formed.

Finally, attempts were made to form the benzol sulphonyl derivative described by Fleischer‡ as obtained from digitoflavone, the latter substance being extracted from *Digitalis* leaves and subsequently shown to be identical with luteolin. Fleischer's benzol sulphonyl derivative was obtained by

* Smiles, S., 'The Relations between Chemical Constitution and some Physical Properties,' London, 1910.

† Perkin, A. G., "Notes on Luteolin and Apigenin," 'Chem. Soc. Journ., Trans.,' 1902, vol. 81, p. 1174.

‡ Fleischer, F., *loc. cit.*

treating the digitoflavone, in caustic soda solution, with benzol sulphochloride and crystallising the product from a mixture of chloroform and ether. Melting point, 189° C.

By treating a specimen of yellow pigment, purified by means of the hydrobromide method and subsequent extraction with ether, in a similar way with benzol sulphochloride, an almost white product was obtained, which crystallised from a mixture of chloroform and ether and melted at 188–190° C.

By hydrolysing a small quantity of the benzol sulphonyl derivative with alcoholic soda for three hours, a sample of luteolin was obtained, melting at 324° C.

There is no doubt that the yellow *Antirrhinum* pigment is luteolin. The factorial difference between the yellow and ivory varieties can, therefore, be expressed as follows:—The ivory variety has the power to form apigenin throughout the tissues of the flower, whereas the yellow variety has the power to form luteolin, either in addition to, or more probably instead of, apigenin, in the upper epidermis of the lips. It appears most likely that the yellow variety has lost the power to form apigenin in the epidermis and produces luteolin instead, though there does not seem to be any particular reason why the power to form apigenin, instead of luteolin, should be a dominant character.* The different flavones synthesised in either case may be regarded rather as an expression of a fundamental difference in structure of the living molecule in the two varieties, affecting, perhaps, the production of different hydroxybenzoic acids, from which the flavones may be synthesised. Little can be gained at present by postulating the existence of a special organic catalyst or enzyme, representing the "I" factor, and concerned with the removal or addition of an hydroxyl group.

From the white variety no flavones could be extracted, and this is in accordance with Mendelian evidence. We must suppose, therefore, that either the substances from which the flavones are synthesised are absent, or the power of synthesis fails.

As regards the yellow patch on the palate, it appears likely that other flavones, having lower melting points and slightly deeper colour than luteolin, are present in this region.

It seems highly probable that the anthocyanin pigments are derived from the flavones by oxidation, or condensation, or both, though only accurate analyses of the pure pigments can ultimately decide this question. With regard to the suggestion made by one of us as to the mode of formation of

* There are probably very small quantities of other flavones in the lips of both yellow and ivory, but these do not affect the mass colour of the flowers.

anthocyanin from the flavone, *i.e.*, that the hydroxyls of the flavones may be protected by sugar, so to speak, and only after hydrolysis can changes take place at these points, there is no very definite evidence as to the number of sugar molecules attached to flavones in the plant. Careful isolation and analysis would be necessary to ascertain the actual condition in the living plant, owing to the great ease with which hydrolysis takes place after death.

Red and magenta anthocyanin have been obtained by us from *Antirrhinum* in a fairly pure state, and certain derivatives have been made. The fact that these, as well as the pigments, are practically amorphous indicates that they probably have very high molecular weights. The lack of melting points in the pigments supports this view.

In a recent paper Keeble, Armstrong, and Jones* bring forward an hypothesis to explain the loss of colour when coloured petals are treated with strong alcohol, and the subsequent restoration of colour when they are treated with water.

The phenomena recorded are as follows:—When coloured (anthocyanin) petals of Stocks (*Matthiola*) are placed in strong alcohol, some pigment passes into solution in the alcohol, which at first is coloured but fairly rapidly becomes colourless. The petals also become colourless though more slowly. When the colourless petals are taken out and placed in water the colour returns; in hot water the recovery is more rapid. When the extract is filtered from the petals and evaporated to dryness on a water-bath the colour returns to the residue. In addition we have noted that colour returns to the alcoholic filtrate on dilution with water, and this also happens even after evaporation to dryness and taking up again with alcohol.

The above phenomena are exhibited by most pigments of the anthocyanin class, and have been noted by various authors working on anthocyanin, among whom may be mentioned Hansen,† Molisch,‡ and Grafe.§

The hypothesis brought forward by Keeble, Armstrong, and Jones to explain these phenomena is the following:—The petals contain an oxydase and a reducing agent, which is probably not an enzyme. The oxydase is responsible for the production of anthocyanin from the chromogen, and the

* Keeble, F., Armstrong, E. F., and Jones, W. N., "The Formation of the Anthocyan Pigments of Plants. Part IV.—The Chromogens," 'Roy. Soc. Proc.,' 1913, B, vol. 86, p. 308.

† Hansen, A., 'Die Farbstoffe der Blüthen und Früchte,' Würzburg, 1884.

‡ Molisch, H. J., "Ueber amorphes und kristallisiertes Anthokyan," 'Bot. Zeit.,' Leipzig, 1905, vol. 63, p. 145.

§ Grafe, V., "Studien über das Anthokyan.—Mittheilung 3," 'Sitzb. Ak. Wiss. Wien,' 1911, vol. 120 (1), p. 765.

reducing agent reverses the reaction. With a decrease in amount of water in the cell the reducing agent becomes active and the oxydase inert, but with an increase in amount of water the oxydase becomes active and its effect is greater than that of the reducing agent. Hence, when petals are treated with strong alcohol the oxydases can no longer function, and the reducing agent is then able to reduce the anthocyanin to a colourless leuco-compound. On addition of water the oxydase again becomes active and re-oxidises the leuco-compound.

Such is the hypothesis, but we are not clear as to the explanation offered by the authors for the reappearance of colour in the alcoholic solution apart from the petals. Two alternatives offer themselves. First, that both oxydase and reducing agent are extracted by 95–99-per-cent. alcohol and are present in the alcoholic extract and that neither is affected by heating to 100° C.* (in spite of the fact that extraction by absolute alcohol and resistance to heat is not characteristic of oxydases), and that, although the authors quote experiments to prove that the oxydase can oxidise to some extent in 95-per-cent. alcohol, the reducing agent is more powerful in this medium. Or, that the reducing agent alone is extracted by alcohol and its influence is removed by evaporating the alcohol or by diluting, when re-oxidation occurs merely on exposure to air. If the latter be the case, the presence of the oxydase is superfluous to the recovery of colour in the petals themselves. We must also conclude that the reducing agent is very widely distributed, is unaffected by temperature of 100° C., and can only act in presence of alcohol.

To us the reduction and oxidation hypothesis appears directly opposed to essential experimental facts, although the original production of anthocyanins in the plant is, in all probability, either partly or wholly due to the action of an oxydase on a chromogen, most likely a flavone or xanthone.

In our experiments, various coloured petals of Stocks were used, and these were the flowers also used by Keeble, Armstrong, and Jones.

Experimentally we found that the same results are given both by the decolorised petals and by the alcoholic solution.

We find that if a little acid is added to absolute alcohol containing decolorised petals, the usual red colour reaction of acid with an anthocyanin is obtained both in the solution and in the petals. Moreover the same result is obtained equally well when dry hydrochloric acid gas or dry hydriodic acid gas is passed through the alcohol. Also, contrary to the observations of Keeble, Armstrong, and Jones, we find that prussic acid gas acts quite as

* In a later paper (Jones, W. N., "The Formation of Anthocyan Pigments. Part V.—The Chromogens of White Flowers," 'Roy. Soc. Proc.,' 1913, B, vol. 86, p. 318) the author definitely states that oxydase is destroyed by boiling 50-per-cent. alcohol.

well as any other acid, which would not be the case if an enzyme were responsible for the restoration of colour.

In any of these cases when the anthocyanin restored by acid is made alkaline, the greenish colour reaction of anthocyanin is obtained, showing that the restored colour is actually due to anthocyanin. The greenish reaction is also produced directly when a drop of a solution of caustic soda in absolute alcohol is added to the alcohol containing the decolorised petals.

If water is boiled to expel oxygen and carbon dioxide, and, while still hot, a stream of hydrogen is bubbled through it, this water, while the hydrogen is still passing, restores the colour to decolorised petals. In this case the medium is neutral.

It is not conceivable that oxidation can take place in all these experiments, particularly in that with dry hydriodic acid gas. Clearly also water is not necessary for the change, and another explanation for the restoration of colour must be sought.

Further, if reduction is the cause of decolorisation, the conditions in some of these experiments are exactly those most suited for the continued stable existence of the leuco-compounds, so that it would seem that this explanation must also be abandoned.

There may be a reducing agent present in the petals, but its reducing power cannot be responsible for the loss of colour in alcohol.

In support of their theory that reduction is the cause, Keeble, Armstrong, and Jones, in a later paper,* quote the fact that an extract from the petals is reduced to a colourless state by treatment with zinc dust and acid, and that the colour is restored by exposure to air. We would note in passing that this does not seem to be simply a reducing action, as we find that the restored colour is much fainter with acetic than with sulphuric acid. This observation has been made previously by Kastle,† who also does not consider it simply a reducing action. Untreated anthocyanin gives exactly the same colour with acetic as with sulphuric acid.

A point we wish to emphasize, however, is that we find the slightly acid solution to be easily decolorised by warming with a little hydrogen peroxide and colloidal platinum. The colourless oxidation product so formed is unstable, and the colour is restored if the solution is made more strongly acid.

* Keeble, F., Armstrong, E. F., and Jones, W. N., "The Formation of Anthocyan Pigments in Plants.—Part VI," 'Roy. Soc. Proc.,' 1913, B, vol. 87, p. 113.

† Kastle, J. H., "A Method for the Determination of the Affinities of Acids Colorimetrically by Means of certain Vegetable Colouring Matters," 'Amer. Chem. Journ.,' 1905, vol. 33, p. 46.

Since anthocyanin can thus be decolorised by oxidation as well as by reduction, in each case giving a product in which the colour is easily restored, there is as much reason, on the evidence of these experiments, to postulate one process as the other for the cause of decolorisation by treatment with alcohol. As a matter of fact, the conditions in both experiments are so different from those obtaining when petals are treated with alcohol, that probably neither experiment has any real bearing on the question at all.

That an alternative to the reduction and oxidation hypothesis can be offered, is shown by the parallel series of changes produced by using phenolphthalein solution, made red by ammonia, as a pigment. This red solution is decolorised by alcohol and the colour restored by diluting largely with water or by addition of a drop of alkali. On evaporating the decolorised alcoholic solution to dryness, a red residue is obtained. As it happens, phenolphthalein is colourless with acids, while anthocyanin gives colour reactions with both acid and alkali. Apart from this accidental difference, the two cases are strikingly similar.

Without wishing to insist on the parallel too rigidly, it would seem that the two series of phenomena might well have similar explanations. The present authors tentatively offer two alternative suggestions without attempting to decide between them.

It may be that strong alcohol dehydrates the anthocyanin, giving a colourless compound, and that colour is restored by subsequent addition of two radicals, either H and OH, or some other pair, such as H and I. Such an effect might perhaps be accounted for by the production in anthocyanin of a lactone grouping. A somewhat similar explanation has been advanced to account for the phenolphthalein changes.*

Or, the loss of colour when the petals are treated with alcohol may be due to combination of the anthocyanin with alcohol to an unstable colourless compound, which is easily decomposed by various reagents. A similar explanation has been advanced by Hantzsch to account for the differently coloured solutions given by certain substances in different solvents.

A few minor points in connection with the above work may be considered.

First, Keeble, Armstrong, and Jones state that the restoration of colour to petals is accelerated by a drop or two of hydrogen peroxide either in acid or alkaline medium, and, further, that the reappearance of colour is not due to the acidity or alkalinity of the medium, because the original colour, purple, red or pink in differently colored petals, is first restored, and the acid or alkaline anthocyanin colour only appears later.

* Meyer, R., u. Spengler, O., "Zur Constitution der Phtaleïnsalze," 'Ber. D. Chem. Ges.,' 1905, vol. 38, p. 1318.

Since we find that the return of colour in water is always accelerated by acid or alkali, we suggest that the acceleration by hydrogen peroxide is merely a function of the amount of acidity or alkalinity of the medium in which the hydrogen peroxide is dissolved. Moreover, although the exceedingly small amount of acid or alkali which at first diffuses into the petal from the very dilute solution may be sufficient to accelerate the actual return of colour, it is not sufficient to give the usual acid or alkaline coloration with the anthocyanin present. Further addition of the hydrogen peroxide solution would, and in fact does, bring about this result. In support of this, we observe that the extract, which at once comes into contact with the full amount of acid or alkali, immediately gives the acid or alkali colour, and not the original purple, pink, etc., of the petals.

To confirm this suggestion we carefully neutralised some laboratory hydrogen peroxide, which is, of course, always decidedly acid, and found that this neutral reagent actually retarded the recovery of colour as compared with control experiments on decolorised petals in cold, hot, or very faintly acidified water.

This result is not surprising in view of the decolorisation of petals by hydrogen peroxide and colloidal platinum, already described in this paper, and, we think, clearly demonstrates that the oxidising properties of hydrogen peroxide have nothing to do with the recovery of colour by the use of this reagent when it has not been neutralised.

Secondly, the same authors state that the purple coloration of a petal can be restored by re-oxidation in an acid medium. For this purpose purple petals of Stocks are incubated with 99-per-cent. alcohol with just enough citric acid to render the alcohol acid. The petals become almost decolorised, but retain a faint pink colour. When transferred to distilled water the pigment is reproduced in considerable quantity, at first red and then purple.

We should explain the phenomenon as follows: The purple pigment is rendered colourless by the alcohol, but, owing to the presence of a small quantity of citric acid (which is very slightly dissociated in alcohol), the colour does not entirely disappear, and the solution remains pink. Transference to water restores the colour, which is at first red, owing to the increased ionisation of the citric acid by the water that soaks into the petal. After a time the acid diffuses away into the surrounding water, leaving the liquid in the petals practically neutral, when the pigment becomes purple.

Finally Keeble, Armstrong, and Jones note that when the colour is restored to petals by immersion in water, and the colour is allowed to diffuse out of them, coloration is again restored by transferring them to hot water, and this process may be repeated two or three times.

They hold that the successive restorations of colour are due to fresh supplies of chromogen being produced by the plant under the influence of the hot water, and that each fresh amount is then oxidised to anthocyanin.

We suggest that these phenomena are explained by the fact that though a certain amount of pigment diffuses out into the water, a large proportion of that which was originally present is retained by the coagulated proteins of the petals, of course in the colourless state. It is the successive liberation of fractions of this retained pigment that accounts for the fresh production of colour in hot water, and not a new formation of chromogen.

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*On the Heat Production Associated with Muscular Work.**

By R. T. Glazebrook, M.A., F.R.S., and D. W. Dye, B.Sc.

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On reading Prof. Macdonald's paper it appeared that it might be interesting to see if his results connecting the heat production and muscular work could be expressed graphically or by means of some simple formula. The tables in his paper give the heat production in calories per hour of a number of individuals when doing a carefully measured amount of mechanical work on a kind of treadmill or cycle. This amount of work is kept constant for each group of observations in the paper. Table I gives his average results.

Table I.

	Mechanical work.	Heat production.	
		From observation.	From formula.
Group A	13	182	179
B	19	199	202
C	43	297	296
D	56	346	347

On plotting these as is done in fig. 1, it is clear that the points lie very approximately on a straight line, and it is easily seen that the equation to this line may be written

$$H = 128 + \frac{W}{0{\cdot}256}; \qquad (1)$$

* A Note on Prof. J. S. Macdonald's paper, *supra*, p. 96.