

Similarly, non-specific seed agglutinins can be classified on the basis of their capacity to be inhibited by various simple sugars<sup>5</sup>; thus, for example, the agglutinins of *Pisum sativum* and *Lathyrus latifolius* are inhibited by D-glucose and N-acetylglucosamine but not by D-galactose or lactose, the converse being true of the agglutinins of *Ricinus communis* and *Abrus precatorius*. There is close correspondence between the classification based on the agglutination of animal erythrocytes and that based on inhibition by sugars; for example, the agglutinins of *Ricinus communis* and *Abrus precatorius* belong to the same class when either method of distinction is adopted.

I have shown<sup>6</sup> that the action of the *Ricinus communis* precipitin, which is identical with the agglutinin, is apparently directed towards the basic chemical framework of the human A, B, H and Le<sup>a</sup> blood group specific substances. This structure is so closely similar to that of the specific polysaccharide of type XIV pneumococcus that the *Ricinus* precipitin may be considered specific for this polysaccharide; this view has some experimental support<sup>7</sup>.

Because *Abrus precatorius* and *Ricinus communis* agglutinins are classified together on the results of both animal erythrocyte agglutination and sugar inhibition reactions, it seemed likely that the activity of the *Abrus precatorius* principle is also essentially directed towards type XIV pneumococcus polysaccharide; *Abrus precatorius* seed extract was therefore studied in this light.

The extract was prepared by the method of BOYD and REGUERA<sup>8</sup>, filtered through Whatman No. 42 filter paper and sterilised by Seitz filtration. It formed precipitates with the salivas of persons of the CEPPELLINI genotypes SSLL, ssLL, SSll, and ssll<sup>9</sup>, and with purified A, B, H, and Le<sup>a</sup> blood group specific substances, a purified ovarian cyst mucopolysaccharide 'F1' devoid of ABH or Le<sup>a</sup> activity, and purified type XIV pneumococcus polysaccharide. Titration of 1/1000 (w/v) aqueous solutions of the purified blood group specific substances, the material 'F1' and purified pneumococcus polysaccharides of types II, III, IX, XII, XIV, and XVII against *Abrus precatorius* extract gave the results shown in the Table. The precipitation titres correspond closely with those previously obtained with *Ricinus communis* extract<sup>6,7</sup>; of the pneumococcus polysaccharides, only type XIV was precipitated.

OUCHTERLONY agar gel precipitation tests<sup>10</sup> showed that the *Abrus* precipitin, like that of *Ricinus*<sup>6</sup>, reacts with a single substance common to the various salivas,

the blood group specific substances, the material 'F1' and type XIV pneumococcus polysaccharide. Agar gel tests also showed that *Abrus* extract, *Ricinus* extract and horse antiserum to type XIV pneumococcus give a 'reaction of identity'<sup>11</sup> when allowed to diffuse either against type XIV polysaccharide or the chemically similar material 'F1', which is believed to represent the unmodified basic substrate of the human blood group specific substances<sup>12</sup>. Thus the activity of the *Abrus precatorius* precipitin, like that of *Ricinus communis*, is essentially directed towards the chemical configuration of type XIV pneumococcus polysaccharide.

It is noteworthy that extracts of other seeds, such as those of various species of *Datura*, which also contain powerful non-specific agglutinins for human erythrocytes but are placed in a different class to that of *Ricinus communis* and *Abrus precatorius* on the basis of animal erythrocyte agglutination and sugar inhibition characteristics, fail to precipitate any of the polysaccharides used in this investigation; the basis of action of these extracts remains to be determined.

**Zusammenfassung.** Präzipitine aus *Abrus precatorius*-Samen scheinen folgende Spezifität zu besitzen: 1. gegen Pneumococcus-Polysaccharide des Typus XIV; 2. gegen die menschlichen blutgruppenspezifischen Substanzen A, B, H und Le<sup>a</sup>.

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<sup>5</sup> A. ENSGRABER, Ber. Dtsch. Bot. Ges. 71, 349 (1958).

<sup>6</sup> G. W. G. BIRD, Vox Sanguinis 4, 313 (1959).

<sup>7</sup> G. W. G. BIRD, Nature, in press (1960).

<sup>8</sup> W. C. BOYD and R. M. REGUERA, J. Immunol. 62, 333 (1949).

<sup>9</sup> R. CEPPELLINI, Proc. IVth Int. Congr. Blood Transfusion, p. 207 (1955).

<sup>10</sup> O. OUCHTERLONY, Proc. VIth Int. Congr. Microbiol., p. 276 (1953).

<sup>11</sup> The 'reaction of identity' obtained in this investigation requires elaboration; this will form the subject of a separate communication.

<sup>12</sup> W. M. WATKINS and W. T. J. MORGAN, Vox Sanguinis 4, 97 (1959).

## The Anthocyanins of Roses. Occurrence of Peonin

Although the garden rose contains a great range of colour varieties, only two anthocyanins have so far been identified in the petals of cyanic forms. Cyanin (cyanidin 3:5-diglucoside) was isolated from *Rosa gallica* by WILLSTÄTTER and NOLAN in 1915<sup>1</sup> and a pelargonidin 3:5-dimonoside (presumably the 3:5-diglucoside, pelargonin) was reported in the scarlet polyantha varieties 'Gloria Mundi', 'Prince of Orange' and 'Paul Crampel'<sup>2,3</sup>. The related flavonols, quercetin and kampferol were also known to occur in glycosidic form in rose petals. A third flavonol, myricetin, was recently described as occurring in about 20 Hybrid Tea varieties by SESHADRI et al.<sup>4</sup>. Since current work in this laboratory has shown that delphinidin and its methylated derivatives occur in association with myricetin in purple or mauve petals of a number of garden

Precipitation reactions of *Abrus precatorius* seed extract

Polysaccharide	Dilution of extract			Controls
	1/1000	1/10000	1/100000	
A-substance	2	—	—	—
B-substance	1	—	—	—
H-substance	3	1	—	—
Le <sup>a</sup> -substance	3	1	—	—
'F1'	3	1	tr	—
Pn II	—	—	—	—
Pn VII	—	—	—	—
Pn IX	—	—	—	—
Pn XII	—	—	—	—
Pn XIV	3	1	tr	—
Pn XVII	—	—	—	—

3:2:1:tr—degrees of precipitation; controls—extract and saline; polysaccharide and saline.

<sup>1</sup> R. WILLSTÄTTER and T. J. NOLAN, Liebig's Ann. 408, 1 (1915).

<sup>2</sup> G. M. ROBINSON and R. ROBINSON, Biochem. J. 28, 1712 (1934).

<sup>3</sup> R. SCOTT-MONCRIEFF, J. Genet. 32, 117 (1936).

<sup>4</sup> S. R. GUPTA, K. S. PANKAJAMINI, and T. R. SESHADRI, J. sci. Indian Res. B. 16, 154 (1957).

flowers<sup>5,6</sup>, a search for a delphinidin derivative among the anthocyanins of roses was undertaken. In particular, blooms of well established purple and mauve varieties as well as those of the latest and bluest breeding lines were examined.

No delphinidin was found and a re-examination of the rose varieties reported to contain some myricetin<sup>4</sup> showed that only kampferol and quercetin were present. In the course of this survey, however, a third major anthocyanin

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<sup>5</sup> J. B. HARBORNE, *Biochem. J.* **68**, 12P (1958).

<sup>6</sup> J. B. HARBORNE, *Biochem. J.* **74**, 262 (1960).

<sup>7</sup> R. WILLSTÄTTER and T. J. NOLAN, *Liebigs Ann.* **408**, 136 (1915).

<sup>8</sup> J. B. HARBORNE, *Nature, Lond.* **187**, 240 (1960).

Tab. I. Identification of peonin and pelargonin

Pigment and source		Spectral max in MeOH/HCl (in mμ)		Spectral ratio 440 mμ/max (as %)		Rf values in			Hydrolysis Products*
						BAW	BuHCl	1% HCl	
Peonin	authentic	274	523	13	0.31	0.10	0.17	{	peonidin, the 3- and 5-glucoside and glucose
	from <i>Rosa</i>	273	524	14	0.31	0.10	0.17		
	from <i>Pelargonium</i>	274	523	13	0.31	0.10	0.17		
Pelargonin	authentic	269	505	21	0.31	0.14	0.22	{	pelargonidin, the 3- and 5-glucoside and glucose
	from <i>Rosa</i>	266	507	20	0.31	0.14	0.23		

\* For details of their identification see <sup>6</sup>.

was discovered in *Rosa rugosa* and derived varieties, e.g. 'Roseaie de L'Hay'. The pinkish red petals of these plants contain cyanin and the new pigment, which was readily identified as peonin (Tab. I). Since peonin is rare and has only previously been found in quantity in peony blooms<sup>7</sup>, its presence in pink roses provides a valuable alternative source. Other new sources are the pink flowered garden geranium (Tab. I), a plant already known to contain pelargonin and malvin<sup>2</sup>, and dark red varieties of *Lathyrus odoratus*<sup>8</sup>.

Of the two previously known anthocyanins of roses cyanin is the most widely distributed, being present in all but two of the hundred or so varieties examined. The pelargonidin derivative, whose identity with pelargonin has now been confirmed, occurs in a number of scarlet varieties (e.g. 'Radar' and 'Will Scarlet') besides those already mentioned. Colour in the rose is therefore mainly due to pelargonin, cyanin or peonin or to mixtures of these pigments. Traces of the related 3-glucosides accompany these 3:5-diglucosides in some varieties. Purple or mauve colours are produced by co-pigmentation of cyanin; a fact which has been established by spectral measurements of aqueous acid extracts of the appropriate varieties (Tab. II).

**Zusammenfassung.** Im Gegensatz zu einer früheren Mitteilung konnten wir in den Blumenblättern der Hybriden-Tee-Rose kein Myricetin finden. Delphinidin kam in keiner der geprüften Rosenblüten vor. In roten Varianten erhält man violette Farben bei der «Copigmentation» von Cyanin. Pelargonin ist in gelbten Rosen vorhanden und Peonin wurde erstmals in der rosa Variante gefunden.

Tab. II. Co-pigmentation in mauve roses

Variety	Petal colour	Visual max in 1% aq. HCl (in mμ)	Anthocyanidin formed on acid hydrolysis
'Belle Poitevine'	red	507	cyanidin
'Reine des Violettes'	violet	509	cyanidin
McGredy 56/944	violet blue	510	cyanidin
McGredy 55/1965	mauve	512	cyanidin

### Rate of Respiration in Relation to Autogamy in *Paramecium aurelia*

It is well known that cultures of paramecia in which autogamy (self-fertilisation) and conjugation (cross-fertilisation) are prevented by daily re-isolation into fresh medium undergo a progressive process of aging. Rejuvenescence can be obtained by allowing autogamy (or conjugation) to take place. A point is finally reached, however, when rejuvenescence can no longer be obtained by this procedure and the clone is doomed to extinction<sup>1</sup>. The theoretical basis of this phenomenon is as yet obscure—rejuvenescence is dependent on a reproductive process involving reconstitution of the nuclear apparatus of the organism by a meiotic process (autogamy or conjugation) in which there is no segregation of genetic material since homozygous lines can be used with equal facility.

In the present investigation measurements have been made of the rate of respiration of single paramecia from serial isolation cultures before and after the occurrence of autogamy in order to elucidate the nature of the metabolic changes occurring as a consequence of autogamy.

The stocks of paramecia used were selected at random and belong to two syngens (varieties) of the species *Paramecium aurelia*; namely stock 60, syngen 1 (collected originally in Burlington, Virginia), and stock 39, syngen 9 (isolated originally from the river Eure, France).

Respiration was measured by Cartesian diver micro-respirometry and the apparatus and methods employed were similar to those developed by LINDERSTRØM-LANG<sup>2,3</sup> and HOLTER<sup>4</sup> with only minor modification. In order to facilitate comparison two divers only were used throughout. A single paramecium of known physiological status was loaded into the diver under sterile conditions. The charge containing the paramecium consisted of standard culture medium (an infusion of dried lettuce inoculated with a strain of *Aerobacter aerogenes*) passed through a

<sup>1</sup> T. M. SONNEBORN, *J. Protozool.* **1**, 38 (1954).

<sup>2</sup> K. LINDERSTRØM-LANG, *Nature, Lond.* **140**, 108 (1937).

<sup>3</sup> K. LINDERSTRØM-LANG, *C. R. Lab. Carlsberg, Sér. Chim.* **24**, 333 (1943).

<sup>4</sup> H. HOLTER, *C. R. Lab. Carlsberg, Sér. Chim.* **24**, 399 (1943).