fact that since both steps in the reaction, tyrosine to DOPA and DOPA to o-quinone, are under the influence of the single enzyme, tyrosinase, there is apparently double inhibition when the primary substrate, tyrosine, is used.

When the two copper methods used previously with serum and purified ceruloplasmin (50%) were employed on the samples containing the tyrosinase and uricase preparations to determine if copper to protein linkages were ruptured, clear cut results were not obtained. This is now understandable since MAHLER et al.[9] have shown that in the case of uricase, the complexing or color reagent diethyldithiocarbamate will react with copper in this system regardless of its state in the protein molecule. This is probably also true for tyrosinase. Therefore, the irradiated uricase and tyrosinase samples were dialyzed in order to see if any 'unbound' copper would pass through the membrane. As seen from the data on Table I, copper ions could pass through the membrane used in the dialysis procedure. However, the uricase activity was lost when the preparation was subjected to this treatment. Further attempts to correlate the loss of enzymatic activity with any change in copper content or state were unsuccessful.

In the case of plant tyrosinase, the dilemma could be circumvented since the enzyme was only slightly inactivated after dialysis. Therefore, after irradiation, samples subjected to different amounts of UV radiation, as well as a control, were dialyzed against copper-free distilled water at 4°C. If copper was removed or released from the bound site in the protein molecule during UV irradiation, this amount of copper would pass through the membrane and into the copper-free water on the outside. Therefore, after 24 h, the dialyzed material in the bags was removed and again assayed for oxidase activity and total copper content. These data are given in Table II and are corrected for the slight increase in volume due to dialysis. It was found that the copper concentration did not decrease proportionately as the UV dose increased. There was a difference of approximately 40 μg/100 ml of copper between the non-dialyzed sample and all the dialyzed samples. These data suggested that there must have been 40 μg of free copper/100 ml present in the original material and this was removed in each sample regardless of the amount of UV irradiation. The fall in enzymatic activity was characteristic of non-dialyzed samples subjected to similar amounts of UV irradiation.

Furthermore, it was not possible to demonstrate a change in the absorption curves (240–900 mμ) of the irradiated plant tyrosinase samples as was the case with the copper protein, ceruloplasmin. The latter had an absorption peak at 600 mμ which decreased as the oxidase activity fell and as the UV irradiation dose increased[1].

Finally, in the case of the mouse tumor extract, the difficulties encountered were such that again a direct conclusion was impossible. The optical density of this crude extract was too large to enable us to determine if slight changes in the direct-reading copper occurred after irradiation.

Tab. I. Effect of dialysis on a solution of $CuSO_4$[a] (unbound copper)

Time of dialysis (h)	Copper concentration (μg/100 ml)	% lost due to dialysis
0	200	0
5.5	14	93
24.0	3	99

[a] 5 ml samples of copper sulfate solution were used. Dialysis was done at 4°C against copper free water.

Tab. II. Effect of dialysis on copper content of UV irradiated plant tyrosinase solutions[a]

UV dose (ergs/mm²)	Dialysis time (h)	Enzymatic activity (OD/min)	Copper content (μg/100 ml)
0	0	0.0876	170
0	24	0.0825	125
48.9×10^4	24	0.0535	136
97.8×10^4	24	0.0381	122
146.7×10^4	24	0.0274	136

[a] 5 ml samples of plant tyrosinase (4 mg/ml protein) were irradiated and subsequently dialyzed against copper-free distilled water (4°C). Approximately 40 μg/100 ml of direct reading or 'unbound' copper is present; copper content of the samples does not increase with UV irradiation.

Résumé. L'activité de l'uricase et des tyrosinases d'origine végétale et animale est diminuée par irradiation UV (2537 Å). Il existe une relation exponentielle entre l'activité enzymatique relative et la quantité de rayonnement. Il n'a pas été possible de mettre en évidence une relation entre la perte d'activité de la tyrosinase végétale des échantillons irradiés et la teneur en cuivre de ces derniers.

M. H. APRISON and K. M. HANSON

The Institute of Psychiatric Research and Departments of Biochemistry and Psychiatry, Indiana University Medical Center, Indianapolis, October 24, 1960.

Specific Precipitins for Type XIV Pneumococcus Polysaccharide from *Abrus precatorius* Seeds

Several seed haemagglutinins, specific for certain human blood group characters, have been described[1]. However, many others fail to differentiate human erythrocytes[2]; the precise basis of action of these non-specific agglutinins is generally obscure. Attempts to ascribe specificity for certain 'high-incidence' human blood group antigens to some non-specific seed agglutinins were unsuccessful[3]. However, many seed agglutinins, which fail to make individual distinctions among human erythrocytes, are not altogether devoid of specificity in that they can make species distinctions among the bloods of various animals[4]. Thus various non-specific seed agglutinins can be divided into distinct classes, each class having a characteristic pattern of reactions; for example, the agglutinins of *Lens esculenta* and *Vicia faba* agglutinate rabbit and guinea pig erythrocytes but not those of several other species.

[1] G. W. G. BIRD, Brit. Med. Bull. *15*, 165 (1959).
[2] M. KRÜPE, *Blutgruppenspezifische pflanzliche Eiweisskörper (Phytagglutinine)* (Ferdinand Enke Verlag, Stuttgart 1956).
[3] G. W. G. BIRD, Vox Sanguinis *4*, 318 (1959).
[4] G. W. G. BIRD, Brit. J. exp. Path. *35*, 252 (1954).

Similarly, non-specific seed agglutinins can be classified on the basis of their capacity to be inhibited by various simple sugars[5]; 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[6] 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^a 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[7].

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[8], 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*[9], and with purified A, B, H, and Le^a blood group specific substances, a purified ovarian cyst mucopolysaccharide 'Fl' devoid of ABH or Le^a activity, and purified type XIV pneumococcus polysaccharide. Titration of 1/1000 (w/v) aqueous solutions of the purified blood group specific substances, the material 'Fl' 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[6,7]; of the pneumococcus polysaccharides, only type XIV was precipitated.

OUCHTERLONY agar gel precipitation tests[10] showed that the *Abrus* precipitin, like that of *Ricinus*[6], reacts with a single substance common to the various salivas, the blood group specific substances, the material 'Fl' 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'[11] when allowed to diffuse either against type XIV polysaccharide or the chemically similar material 'Fl', which is believed to represent the unmodified basic substrate of the human blood group specific substances[12]. 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.

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^a-substance	3	1	—	—
'Fl'	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.

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^a.

G. W. G. BIRD

Armed Forces Medical College, Poona (India), August 21, 1960.

[5] A. ENSGRABER, Ber. Dtsch. Bot. Ges. *71*, 349 (1958).
[6] G. W. G. BIRD, Vox Sanguinis *4*, 313 (1959).
[7] G. W. G. BIRD, Nature, in press (1960).
[8] W. C. BOYD and R. M. REGUERA, J. Immunol. *62*, 333 (1949).
[9] R. CEPPELLINI, Proc. IVth Int. Congr. Blood Transfusion, p. 207 (1955).
[10] O. OUCHTERLONY, Proc. VIth Int. Congr. Microbiol., p. 276 (1953).
[11] The 'reaction of identity' obtained in this investigation requires elaboration; this will form the subject of a separate communication.
[12] 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[1] 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'[2,3]. 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.[4]. 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

[1] R. WILLSTÄTTER and T. J. NOLAN, Liebigs Ann. *408*, 1 (1915).
[2] G. M. ROBINSON and R. ROBINSON, Biochem. J. *28*, 1712 (1934).
[3] R. SCOTT-MONCRIEFF, J. Genet. *32*, 117 (1936).
[4] S. R. GUPTA, K. S. PANKAJAMINI, and T. R. SESHADRI, J. sci. Indian Res. B. *16*, 154 (1957).