considerable inhibitor activity occurred, as has been reported by GUPTA et al.[2].

Both acid and alkaline RNAses were present in plasma, and 18 h after whole-body irradiation the alkaline RNAse activity was increased by a factor of 2 in guinea-pig plasma, and the acid RNAse activity by a factor of 3.1 (Table). Present work on the radiation-induced variations in the RNAses of guinea-pig tissues may enable the relationships between the tissue and plasma enzyme levels to be clarified.

In rat plasma, the acid and alkaline RNAse activities were increased by factors of 1.6 and 7 respectively after irradiation (Table): in rat tissues, the acid RNAse levels also show smaller increases than alkaline RNAse levels[3-5]. The changes in plasma enzyme levels were presumably caused by a combination of (1) increased RNAse synthesis and (2) the release of the enzyme from damaged cells. It is possible to separate these 2 factors to some extent in rats, for work on spleen, thymus and intestine has shown that in these organs there appears to be increased alkaline RNAse synthesis and comparatively little cell damage after local-irradiation of the head (with the trunk shielded), while after local-irradiation of the trunk (with the head shielded) there is little change in RNAse synthesis but extensive cell damage[4-6]. Irradiation of the head (Table) caused the plasma alkaline RNAse level in rats to increase by a factor of 3.3, while irradiation of the trunk produced an increase by a factor of 1.8. The increase by a factor of 7 in the alkaline RNAse level of rat plasma after whole-body irradiation may therefore be the result of 2 effects: the increased rate of synthesis of the enzyme from damaged tissues into the blood, more than trebling the plasma RNAse level; and the increased rate of release of the enzyme from damaged tissues into the blood, doubling the plasma RNAse level.

Irradiation of the trunk and of the head of rats produced increases by factors of 1.2 and 1.3 respectively in the plasma acid RNAse level in rats (Table), but the significance of this cannot be determined, as the effects of localized irradiation on the acid RNAse levels in different tissues are not yet known.

Effects of whole-body and local-irradiation on plasma RNAse levels

Animal	Treatment	Acid RNAse activity[a]	Alkaline RNAse activity[a]
Guinea-pig	Control	4.95 ± 0.2[b]	7.4 ± 0.5[b]
Guinea-pig	700r whole-body irradiation	14.9 ± 1.0	14.8 ± 0.8
Rat	Control	2.9 ± 0.25	4.35 ± 0.3
Rat	700r whole-body irradiation	4.6 ± 0.2	30.4 ± 3.7
Rat	700r to head	3.8 ± 0.3	15.0 ± 1.3
Rat	700r to trunk	3.6 ± 0.2	8.3 ± 0.7

[a] μmole ribonucleotide liberated/h/ml of plasma. [b] Each value is the mean of the enzyme levels in 6 animals, the standard deviation of the mean is also shown.

Zusammenfassung. Unter Bestrahlung wurde die RNAse-Aktivität im Blut untersucht. Es zeigte sich, dass Bestrahlung des Kopfes allein zu einer starken Erhöhung der alkalischen RNAse im Serum führt.

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Anthocyanins in *Dioscorea alata* L.

Because of the recent paper of the same title by RAŠPER and COURSEY[1], we were prompted to publish our own results on the near total structure of the anthocyanins which make up the pigmentation of the West Indian variety 'St. Vincent Red' of *Dioscorea alata* L.

The standard methods of HARBORNE[2] were used to extract the pigments from the tubers of 'St. Vincent Red' yams so as to minimize the possibility of deacylation or other degradation of the dissolved anthocyanins. The pigments were then precipitated from the concentrated extract with dry ether, redissolved in methanol containing 0.5% HCl, and purified by repeated chromatography on Whatman 3 MM. paper. Banding in BAW – *n*-butanol-acetic acid-water (4:1:5 v/v) – produced 1 main pigment A, and 2 minor pigments B and C. The 3 pigments were rebanded in BAW and then in 15% HA – acetic acid-water (85:15 v/v) – to remove free sugars[3]. The minor pigment C, which was present in very small amount, faded in the aqueous solvent and could only be successfully rebanded in alcoholic solvents. The pigments were then eluted for structural analysis.

All 3 anthocyanins yielded a single anthocyanidin as the result of exhaustive acid hydrolysis. This anthocyanidin was magenta in visible light and bright pink in UV-light, and when spotted on Whatman No. 1 paper together with reference anthocyanidins, it had Rf values similar to cyanidin. This was confirmed by co-chromatography with an authentic sample of cyanidin and by a spectral analysis.

The UV-spectrum of the anthocyanidin in 0.01% HCl in methanol showed maxima at 535 and 280 nm and was identical with that of a solution of cyanidin prepared in the same way.

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The sugar components remaining after the extraction of the anthocyanidin material were treated with amberlite ion exchange resin IR–45 (OH) to remove excess cations. It was then spotted on Whatman No. 1 paper together with standard sugar markers of glucose, galactose, xylose, rhamnose and arabinose. Aniline hydrogen phthalate was used as a spray reagent[4]. The chromatograms were developed in the 2 solvent systems BAW and BBPW – *n*-butanol-benzene-pyridine-water (5:1:3:3 v/v). Only 1 sugar spot was produced by the exhaustive acid hydrolysis of the 3 anthocyanins, and Rf values and co-chromatography with an authentic sample confirmed that the unknown sugar was glucose. To distinguish between glucose and galactose, the chromatogram in BAW was allowed to run for 30 h.

Traces of arabinose were detected on some chromatograms but this may have been an artifact[5].

A spectral analysis of the pigments A, B and C is shown in Table III. Maxima at 328 nm indicate that the 3 pigments are acylated. The analysis of pigment C is on partially purified material, due to the small quantity of this pigment available at the present time. Acylation was confirmed by a mild alkaline hydrolysis of the total *D. alata* pigments. This produced 2 new pigments, D and E, and an acid, whose Rf values in various solvents were identical with those of ferulic acid. The spectral data for the main deacylated pigment D is included in Table III. The E_{440}/E_{max} ratios correspond closely to the data of HARBORNE for 3-glycosides[6].

Acylation was also indicated by the production of ferulic acid and fluorescent compounds resembling acylated sugars[7] as the result of a partial acid hydrolysis of the acylated pigments. The partial acid hydrolysis of the main deacylated pigment D produces only 1 intermediate glycoside which was shown, by co-chromatography and spectral comparison with an authentic sample, to be cy-3-glucoside. Glucose and a disaccharide, which separated from cellobiose and sophorose but which had Rf values similar to those of gentiobiose, were also produced during the partial acid hydrolysis of the deacylated pigment D. Therefore although D has values similar to both cy-3, 5-diglucoside and cy-3-gentiobioside in certain solvents, the absence of cy-5-glucoside during partial acid hydrolysis and the spectral data of D suggest that it is cy-3-gentiobioside. Cy-5-glucoside was readily obtained during the partial acid hydrolysis of cy-3, 5-diglucoside which was performed at the same time. Chromatographic data for pigments A, B, C, D and E are shown in Table IV together with the data for reference compounds.

The fluorescent compounds shown in Table V were readily produced during the partial acid hydrolysis of pigment B in 1% HCl, but they were very slowly produced during a similar hydrolysis of pigment A which was not fully hydrolysed after 45 min. Ferulic acid was the only definite fluorescent compound produced during the partial acid hydrolysis of pigment C.

The purified total acylated pigments were oxidized with H_2O_2 in methanolic solution using the procedure developed by CHANDLER and HARPER[8]. This produced 2 fluorescent compounds of Rf values 0.21 and 0.62 in BAW, but it has not yet been possible to isolate a sufficient quantity of either compound for structural analysis.

Table III. Spectral analysis

Pigment	λ_{max} in MeOH-HCl nm			Ratios as % E_{440}/E_{max}	E_{328}/E_{max}
A	278	328	531	28	67
B	278	328	530	29	120
C	278	328	530	29?	125?
D	280		525	29	

Table I. Chromatographic analysis of the anthocyanidin in *D. alata* with reference compounds

Anthocyanidin	Rf values in[a]			
	Bu/HCl	Forestal	Formic/HCl	BAW
Petunidin	0.49	0.43	0.16	0.50
Pelargonidin	0.83	0.63	–	0.80
Cyanidin	0.75	0.45	0.17	0.68
Malvidin	0.61	0.55	–	0.53
Anthocyanidin of *D. alata*	0.75	0.45	0.17	0.67

[a] Solvent abbreviations at the bottom of Table IV.

Table II. Sugar identification

Sugar	Rf values in	
	BAW	BBPW
Glucose	0.10	0.28
Galactose	0.08	0.25
Arabinose	0.16	0.35
Rhamnose	0.30	0.50
Unknown	0.10	0.28

Table V. Fluorescent compounds produced during the partial acid hydrolysis of *D. alata* pigments

Compound	Rf values in BAW	1% HCl	Colour in UV-light + NH_3	
A	0.23	0.48	blue	green
	0.46	0.36		
	0.64			
	0.78	0.22	blue	turquoise
B	0.12?	0.48		
	0.23	0.36	blue	green
	0.46	0.31?		
	0.64			
	0.78	0.22	blue	turquoise
Ferulic acid	0.78	0.22	blue	turquoise

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Table IV. Properties of the anthocyanins of *D. alata* and reference compounds

Pigment	Rf values in BAW	Bu HCl	1% HCl	Products of alkaline hydrolysis	Products of[a] partial hydrolysis
A	0.23	0.29	0.08	D, Ferulic acid	D, cy-3-glucoside
B	0.18	–	0.13	E, Ferulic acid	E, D, A, cy-3-glucoside
C	0.10	0.20	0.18	E, Ferulic acid	E, D, B, A, cy-3-glucoside
D	0.19	0.11	0.14	–	cy-3-glucoside
E	0.06	0.04	0.60	–	D, cy-3-glucoside
cy-3-glucoside	0.28	0.20	0.07	–	–
cy-3-sophoroside [Loganberry]	0.19	0.17	0.40	–	cy-3-glucoside
cy-3-cellobioside [Red poppy]	0.25	–	0.40	–	cy-3-glucoside
cy-3-triglycoside [Loganberry]	0.23	0.21	0.60	–	cy-3-glucoside
cy-3-gentiobioside[b] [*Primula sinensis*	0.20	–	0.14	–	cy-3-glucoside
cy-3,5-diglucoside	0.20	0.08	0.16	–	cy-5-glucoside cy-3-glucoside

[a] The fluorescent compounds obtained during partial hydrolysis are shown in Table V. [b] Published data. Solvent abbreviations: Bu HCl: *n* butanol – 2 N HCl (1:1 v/v top layer). The paper was equilibrated with the lower aqueous phase for 24 h before use. 1% HCl: 12 N HCl-water (97:3 v/v). Forestal: water-acetic acid-conc. HCl (10:30:3 v/v). Formic/HCl: Formic acid-12 *N* HCl-water (5:2:3 v/v).

A similar H_2O_2 oxidation of the deacylated pigment D produced a disaccharide with Rf values similar to those of gentiobiose[9].

Résumé. Trois arthocyanidines acylées ont été extraits de tubercules d'une variété de *Dioscorea alata* L. des Antilles. Tous 3 sont des glycosides de la cyanidine: le pigment principal est la cyanidine-3-gentiobioside acylée d'acide ferrulique; les 2 autres pigments secondaires sont semblables aux cyanidine-3-glycosides acylées d'acide ferrulique.

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University of the West Indies, Trinidad, 15 October 1967.

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Concerning Amino Acids in Human Saliva

In our search for a simple, yet sensitive biochemical assay which might reflect the activity of the pyridoxine-dependent enzyme glutamic decarboxylase in man, we attempted to estimate one of its end products, γ-aminobutyric acid (GABA), in a number of readily available body fluids, including saliva, in which it had allegedly been found[1-4]. Standard bidimensional paper chromatography of freshly expectorated saliva revealed a spot which seemed to correspond to GABA. More careful techniques, however, have revealed that saliva does not contain any detectable GABA and that the compound identified as such in other laboratories most likely represents δ-aminovaleric acid (DAVA). This had been suspected by other investigators[5,6].

Saliva was expectorated into a clean beaker after the mouth had been rinsed several times with plain tap water. A portion of the samples was immediately treated with an equal volume of 95% ethanol and 1 ml aliquots were used for a bidimensional paper chromatographic separation. A second portion of the untreated sample was kept at room temperature for 2 days and subsequently treated with 95% ethanol and chromatographed in the same manner. The first separation consisted of high voltage electrophoresis (3500 V, 260 mA) in an 8% formic acid buffer (pH 1.6) for 45 min. This was followed by ascending chromatography in a butanol acetic acid water (12:3:5) solvent. The amino acids were stained with 0.2% ninhydrin. The third portion of the original samples was deproteinized with solid sulfosalicylic acid and 3 ml aliquots were subsequently applied to a Technicon amino acid analyzer[7] (50 cm spherix xx 8-60-0 ion exchange column).

The amino acid which had been thought to represent GABA had the chromatographic properties of DAVA, an amino acid containing an additional methyl group. None of the saliva samples contained any detectable GABA, either by bidimensional paper chromatography or by ion-exchange chromatography. In the butanol acetic acid water solvent system, a standard solution of DAVA had

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