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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an Rf value of 50, compared to GABA which had an Rf value of 45. In the high-voltage electrophoresis system, DAVA had a mobility equal to that of glycine, whereas GABA migrated halfway between glycine and lycine. By ion-exchange chromatography using a Technicon amino acid analyzer, GABA was eluted after 8 h and 40 min while DAVA was eluted after 10 h and 10 min. In all of the samples the latter appeared as a very sizable peak between ammonia and ornithine. Five saliva samples contained an average of 0.131 μmoles of DAVA/ml. This was equaled only by the amino acid proline and exceeded the quantity of any other amino acid in saliva. Average quantities of all of the free amino acids are listed in the Table. The portions of saliva which had been kept at room temperature for 2 days contained much more DAVA and proline than did the specimens chromatographed immediately after expectoration. This suggests that bacterial contamination and the process of putrefaction may be largely responsible for the presence of free DAVA and proline in human saliva, as had been suspected by FOSDICK and PIEZ[6]. Our experience indicates that meaningful biochemical data on human saliva must be obtained from specimens collected by sterile catheterization of the salivary gland ducts. Finally, we must conclude that GABA is not present in human saliva[8].

Quantitative values of free amino acids in human saliva as determined by ion-exchange chromatography

Amino acid	Average of 5 samples (μmoles/ml)	Amino acid	Average of 5 samples (μmoles/ml)
Taurine	0.071	α-amino butyric acid	trace
Aspartic acid	0.026	Valine	0.080
Threonine	0.005	Isoleucine	0.006
Serine	0.005	Leucine	0.008
Glutamine	0.006	Tyrosine	0.015
Proline	0.088	Phenylalanine	0.012
Glutamic acid	0.037	Lysine	0.032
Citrulline	0.013	Histidine	0.018
Glycine	0.144	Arginine	0.010
Alanine	0.032	δ-amino valeric acid	0.131

Résumé. Dans les travaux de divers laboratoires on peut lire que l'acide γ-amino-butyrique (GABA) existe dans la salive humaine. Nos récents examens chromatographiques ont mis en évidence que cet acide avait été identifié par erreur et qu'il représente en fait l'acide δ-amino-valérique (DAVA), un constituant de la salive provenant de l'action bacterienne.

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The Influence of Lethal X-Irradiation on Trace Metal Uptake by the Mitochondrion

Marked variation has been demonstrated in the concentrations of trace elements in tissues of lethally X-irradiated rats (HEGGEN, OLSON, EDWARDS, CLARK and MAISEL[1]). Determinations of the post-irradiation dynamic picture of trace metal levels in a small intracellular functional entity, the mitochondrion, was undertaken in the present study by means of radioisotopic tracers. The spleen, one of the more radiosensitive tissues, was chosen as a source of mitochondria. The trace metals selected for study (zinc, manganese, cobalt, iron, chromium, selenium, and nickel) are coenzymatic in nature (DIXON and WEBB[2]).

Material and methods. The principles of laboratory animal care as promulgated by the National Society of Medical Research were observed.

Adult female albino rats were fed standard laboratory food ab libitum. Irradiation was carried out with a 300 kVP X-ray therapy unit, 900 rads total body (300 kV, 20 mA), target distance 85 cm, at a rate of 49.13 r/min, inherent filtration 4.75 mm Be, added filtration 2.0 mm Cu. One, three, and six days post-irradiation, 4 rats were sacrificed by neck fracture; their spleens were immediately removed and processed to isolate mitochondria (CLELAND and SLATER[3]). One washing with a solution consisting of sucrose (0.05 *M*), KCl (0.02 *M*), and Sorensen phosphate buffer, pH 7.45 (0.02 *M*) was performed. The mitochondrial pellet was resuspended in the sucrose-KCl-buffer solution and distributed among several polypropylene centrifuge tubes; about 0.2 mg nitrogen equivalent of protein per tube. 1 μc of carrier-free radioisotope (^{58}Co, ^{63}Ni, ^{51}Cr, ^{59}Fe, ^{54}Mn, ^{63}N, ^{75}Se or ^{65}Zn) in sucrose-KCl-buffer solution, was added to each tube (final volume 2 ml) and incubated at 4 °C for 1 h (SPECTOR[4]). Analyses were performed in triplicate. After centrifugation at 8000 *g*, the mitochondria were washed once with 10 ml of 0.001 *M* non-radioactive isotope in isotonic saline, recentrifuged, and transferred to screw-capped test tubes. Mitochondria from control (non-irradiated) rats were carried through the same procedure concurrently. Results were expressed in terms of c.p.m. of each specimen divided by the product of mg nitrogen (microkjeldahl assay) and c.p.m. of standard radioisotope (exactly $^1/_{100}$ of the amount added to the mitochondria). This expression standardizes amount of mitochondria, amount of radioactivity, and decay of the radioisotope in all specimens (ANDERSON[5]).

Results. The mean results of the experiment are presented in Table I. The pattern of change in cation uptake

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