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Biochem. J. (1961) **80**, 616

A Direct Quantitative Paper Chromatography of Amino Acids and its Application to the Urinary Excretions of some Human Ethnic Groups

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(Received 2 January 1961)

The work of Dent & Harris (1951), Harris (1953), Harris & Warren (1953) and Datta & Harris (1953) has indicated the existence of characteristic patterns of urinary amino acid excretion in human and other mammalian species. As an item in the study of biochemical aspects of human racial differentiation it was planned to examine the excretions among physiologically normal members of ethnologically distinct human groups on Singapore Island and in the Federation of Malaya.

With so potentially extensive a programme it was proposed to employ direct transmission photometry ('minimum transmission' type) of ninhydrin-treated two-dimensional paper partition chromatograms for most of the assays. Established procedures were found to lack delicacy and accuracy, more in respect of colour development and photometry than of chromatography. A brief report of the preliminary work and details of the photometry refinements, which alone render the means of triplicate assays as reliable as those of some twelve replicates otherwise obtained, have been provided elsewhere (Lugg & McEvoy-Bowe, 1957, 1961; McEvoy-Bowe & Lugg, 1958, 1959). This article describes the chromatography, the closely controlled colour-development procedure (which, like the refined photometry, greatly reduces variability of results) and the preliminary treatment of urine samples, and records the results of the exploratory ethnic-group survey. Attention was directed principally to the excretion rates of glycine, taurine, β -aminosobutyric acid, alanine and glutamine, but those of cystine (determined polarographically) have also been included.

EXPERIMENTAL

Chromatography. Square sheets (20 cm. $\times$ 20 cm.) of Whatman no. 1 filter paper were used in two-dimensional ascending-front separations. Perforated at the corners, they were fitted usually 12 at a time with glass spacing sleeves on the rails of a stainless-steel frame resting in a trough of this alloy (cf. Datta, Dent & Harris, 1950), at the bottom of a glass tank (36.5 cm. $\times$ 26.5 cm. base and 24.5 cm. height; as supplied by Shandon Scientific Co. Ltd., London, W. 10) fitted with a glass sealing lid. Aluminium-alloy frames and troughs (as used by Datta *et al.* 1950) were found unsatisfactory. Climatic conditions made it necessary to control, thermostatically, the temperature of the tank enclosure (at $29.5 \pm 0.5^\circ$).

The initial spot was a 5.0 mm. disk with centre 4.5 cm. diagonally inward from a corner, applied as evenly as possible from an Agla (Burroughs Wellcome and Co. Ltd., London, N.W. 1) micrometer syringe, and dried with warm air from a hair-drier. The spot was briefly exposed to ammonia gas and excess blown away.

So-called 'single-phase' solvent systems, claimed in the literature to be advantageous, were used for each 'run'. They appear to function as conventional double-phase systems in association with sheet fibres (Martin, 1949). For the first run, which was stopped with the front approx. 1 cm. from the top, the solvent was prepared from water-saturated phenol by dissolving in it 10% (w/v) phenol, 0.04% (w/v) 8-hydroxyquinoline as heavy-metal chelating agent (Block, 1950), and 0.5% (w/v) 2:6-lutidine. With this solvent the sheets were used as supplied, as prior washing conferred no benefit. For the second run, stopped with the front almost at the top, the solvent (Block, Durrum & Zweig, 1955) was prepared by mixing 55 vol. of redistilled 2:6-lutidine with 25 vol. of 95% ethanol and 20 vol. of water. Diethylamine (1.5 ml.) was placed in the bottom of the tank, as direct addition to the solvent appreciably increased the 'background'.

Solvents were removed by blowing air at room temperature (28–30°) over the sheets until characteristic odours were dispelled (approx. 12 hr. for the first and 1 hr. for the second solvent).

Reversing the order of solvents improved band-shape regularity but capriciously affected the tints of the final colour bands. Flow rates and band-shape regularity varied with batches of paper (supplied by H. Reeve Angel and Co. Ltd., London, E.C. 4). Batches of paper requiring approx. 7 hr. for the first run were selected.

Use of the ninhydrin reagent. The acidity or basicity of materials on the paper affects the absorption spectra of the final bands (Thompson, Zacharius & Steward, 1951; Lewis, 1952). If solubilities of band substances in the reagent are not very low they may flow on immersion of sheets and even be dissolved off.

Stock solutions of ninhydrin (3%, w/v, in 95% ethanol) were kept at 3° in the dark for not more than a few weeks. The final reagent was prepared immediately before use by mixing 5 vol. of stock solution with 5 vol. of 95% ethanol, 39 vol. of redistilled chloroform and 1 vol. of 2:6-lutidine. Sheets were immersed for 2 sec., the bulk of solvents evaporating during withdrawal. They retained approx. 0.05 mg. of ninhydrin/cm.² of sheet, a quantity well in excess of requirements. They were then exposed, on a frame, to a vigorous current of air at room temperature for 1–2 min. before heat treatment (see below).

Colour development. The humidity of the reaction space, the temperature, and the time allowed, are all of great importance, as suggested by the work of Redfield & Barron (1952) and of Wellington (1953). Many experiments have been carried out to determine optimum conditions for assay and only the crucial ones are recorded (see Results). The optimum conditions were found to be as follows.

Room air was blown at 21 l./min. by a rotary blower [supplied by Baird and Tatlock (London) Ltd.] through 1 l. of acidulated water maintained at a definite temperature (43° recommended) in a 2 l. flask and thence, via a steam-jacketed condenser tube, to the bottom of a thermostatically regulated oven (90° recommended), vented at the top, and of internal dimensions 40.5 cm. × 30.5 cm. base and 43 cm. height, accommodating the chromatograph frame fitted with only three assay and three alternating standard sheets to facilitate air flow. The recommended period of treatment is 30 min. In the experiments leading to these recommendations steam was admitted to the jacket sufficient to raise the entering air to within a few degrees of that of the oven, and the lowest relative humidity, virtually zero except for sorbed moisture on the sheets, was provided by substituting for the 2 l. flask a column of anhydrous calcium chloride pellets.

Photometry. Substance bands were identified primarily by their R_F values as compared with those of known substances on standard sheets run simultaneously, and secondarily by the tints, which were somewhat characteristic. Developed sheets and excised portions thereof, or both, were kept in the dark (and for no more than several hours) before photometry.

Transmission photometry of the 'minimum transmission' type was employed, the densitometer (McEvoy-Bowe & Lugg, 1958) permitting precise positional replacement of the excised sheet portions after bleaching, and thus correcting for textural irregularities in determinations of band

and background transmittances. Bleaching was carried out by exposing the excised portions to chlorine gas from moist bleaching powder until they appeared the same as unused sheet similarly exposed, the optical properties of unused sheet actually being unaffected by this treatment. Galvanometer deflexions were sensibly proportional to the intensity of monochromatic light impinging on the selenium barrier-layer photocell. If R is the deflexion for sheet with band and background, B that for sheet alone there, R' for the sheet with background and B' for sheet alone there, then, if all light reflected back is restored to the sheet, R/B is the transmittance of band and background and R'/B' is the background transmittance, and to close approximation $RB'/R'B$ is the transmittance (T) of band alone where measured (Lugg & McEvoy-Bowe, 1961).

With the simple optical system actually employed in this work (with near-white light, with no restoration of reflected-back light) the above relationships do not strictly obtain, but the terms may be computed as if they do and a simple function of the computed band transmittance, T , is found to be related linearly to the quantity, Q , of a substance applied in the initial spot (see Results and Discussion).

Collection, preparation and assay of urine samples. A 24 hr. collection of urine with toluene as preservative was obtained from each subject. Storage (at 4°) was kept as short as possible to minimize decomposition, especially of glutamine (see Stein, 1953). Portions were taken for the estimation of cystine (polarographic method of Reed, 1942), and for desalting (to avoid distortion of bands) before chromatography.

Clarkson & Kench (1956) found desalting with ion-exchange resins satisfactory for many amino acids. Losses of taurine and of glutamine (by deamidation) may be substantial and variable, but complete recovery of glutamine has been claimed for the modification of Thompson, Morris & Gering (1959). For assay of taurine, β -aminoisobutyric acid, alanine and glutamine, electrolytic desalting by the method of Astrup, Stage & Olsen (1951) was adopted, but the cathode compartment of the desalter was fitted with a water jacket. A 10 ml. urine portion mixed with 10 ml. of distilled water was electrodialysed with a starting current of usually 1.0–1.2 A. After the current had fallen to 0.6 A (approx. 30 min.) the process was continued for a further 15 min., the current having then fallen to 0.25–0.5 A. The fluid was withdrawn and, with washings, diluted to 25 ml., and of this volume a suitable portion was applied as initial spot to a sheet. The portion was usually in the range 0.0005–0.001 (represented by 10–20 μ l.) and depended on the volume of 24 hr. urine and the size of the subject. As hippuric acid is partially hydrolysed to glycine during the electrolytic desalting process (see Results), ion-exchange desalting by the method of Clarkson & Kench (1956) was employed in estimating the glycine contents of urines.

Subjects. Ten subjects were taken at random from somewhat restricted categories of healthy male members in the 17–50 year age range of each of the European (university teaching staff), Southern Chinese (resident university students) and Sēnoi (transferred several years before from a jungle to a reserve environment) ethnic groups living on Singapore Island or in the Federation of Malaya. The European sample included one Maltese. The Sēnoi sample included members of the Sēmai and Temiar divisions (see Williams-Hunt, 1952).

RESULTS

The function of T to which Q was found to bear a linear relationship is $(1/T) - 1 = a$. However, a may also be taken as a measure of band colour intensity under various conditions of colour development, and $(B'/R') - 1$ may be taken as the corresponding measure of background colour intensity.

Variation of colour intensity with conditions of colour development. In testing the effects upon colour development of varying the heating period, the relative humidity and the temperature, mixtures of the five substances were taken for chromatography in such quantities as would not confuse a graphical presentation of results. The effects of varying the heating period at 90° and 12.3% relative humidity, are shown in Fig. 1. The effects of varying the relative humidity at 90° and for a 30 min. heating period, are shown in Fig. 2, but values of a at 'zero' relative humidity are undoubtedly too high because of the sorbed moisture introduced with the sheets.

The general effects of reducing the temperature, from 90° to 80° to 70°, upon the conditions for optimum colour development, were to increase the

period required at constant relative humidity and, with reasonably lengthy constant period, to increase the relative humidity required.

Relationships between Q and T ; variability of results. It has been shown (Lugg & McEvoy-Bowe, 1961) that even if the parent substances behave ideally in the chromatography, and sheet and colour substances on it absorb 'entering' light (namely incident light less that reflected back and lost) in a manner conforming with Lambert's and Beer's laws, the computed absorbance ($D = -\log T$) of the band when incident light-intensity is constant can be proportional to Q only if the reflected light is restored. The proof presumes that colour substance density is proportional to parent substance density (see Discussion). Standard chromatograms were run together in triplicate. Seven such experiments were made at the 2 and 20 $\mu\text{m-mole}$ and five at the 4 and 10 $\mu\text{m-mole}$ quantities. Overall mean D values at each quantity were examined, and for all five substances were found to increase with Q but disproportionately slowly. This is to be expected with the optical system used if the above-mentioned circumstances actually obtain.

Fig. 1. Relationships between band colour intensities (as a values) for five substances chromatographed at unspecified but constant quantities and of 'background' colour intensities (as $B'/R' - 1$ values) and time, the ninhydrin reaction being conducted at 90° in air maintained at 12.3% relative humidity. $\circ$, Glycine; Δ , taurine; $\bullet$, β -aminoisobutyric acid; $\square$, alanine; $\blacksquare$, glutamine; $\blacktriangle$, 'background'.

Fig. 2. Relationships between band colour intensities (as a values) for five substances (chromatographed at unspecified but constant quantities) and relative humidity, the ninhydrin reaction being conducted in air at 90° for a period of 30 min. $\circ$, Glycine; Δ , taurine; $\bullet$, β -aminoisobutyric acid; $\square$, alanine; $\blacksquare$, glutamine.

An exhaustive statistical analysis of the original data for β -aminoisobutyric acid at the 2 and 20 μ m-mole quantities is given elsewhere (Lugg & McEvoy-Bowe, 1961) together with a discussion of the physical significance of the linear relationship between Q and a . In Table 1 is a digest of the original data which established the semiempirical equation:

$$Q = k'''a = k'''[(1/T) - 1], \quad (1)$$

where k''' is a constant for the substance concerned, in the range 2–20 μ m-moles under the prevailing conditions.

This equation is considered further in the Discussion together with the variabilities of a . Therein is stressed the advisability of making 'within-experiment' assays, to which recourse was had [by using equation (2), derived from equation (1)] in the urine analyses:

$$Q_{(\text{assay})} = Q_{(\text{std.})} a_{(\text{assay})}/a_{(\text{std.})}, \quad (2)$$

where $a_{(\text{assay})}$ and $a_{(\text{std.})}$ are closely similar means from triplicate chromatograms run together, the % standard error (s.e.) of $Q_{(\text{assay})}$ being $\sqrt{2}$ times the appropriate value obtained by interpolation from the final column of Table 1.

Effects of desalting upon recoveries. Experiments were made to test recovery (a) from four simulated urines containing (in addition to these five substances), urea, creatinine, uric acid, Na^+ , K^+ , NH_4^+ , Ca^{2+} , Cl^- , SO_4^{2-} , H_2PO_4^- and HPO_4^{2-} ions, all at normally occurring concentrations, and (b)

from ten actual urines in which additional quantities of the five substances had been dissolved.

To within the standard errors of the means the recoveries in the (a) series were complete, except for taurine with a mean recovery of 92 % (s.e. 1 %) which agrees closely with the 93 % recovery found by Astrup *et al.* (1951) after desalting in a 0.15M-sodium chloride medium. In the (b) series the mean recoveries of glycine, alanine and glutamine were complete, but the mean recovery of β -aminoisobutyric acid was only 80 % (s.e. 2 %) and that of taurine appeared excessive, 107 % (s.e. 5 %).

Work on the Snoi and some European urine samples was completed early in the survey, the urines being desalted only electrolytically. The mean 24 hr. excretion of glycine by the European subjects, 3.43 m-moles, was about twice that reported by Stein (1953), who used ion-exchange chromatographic analysis. This and other evidence suggested that urinary glycine conjugates, such as hippuric and glycocholic acids, might undergo some hydrolysis in the desalter and lead to spuriously high values.

In pure solution hippuric acid was not affected in the electrolytic desalter, but in the presence of glycine, the effect increasing with the initial salt concentration and with time in the desalter. As fresh collections from the Snoi subjects could not be obtained their glycine values were discarded, but in repeated work with European subjects and

Table 1. Relationship between quantity (Q) of applied substance and the measure (a) of band colour intensity, with information derived from analyses of variance

Triplicate chromatograms of all five substances were run together in each experiment: seven experiments at the 2 and 20 μ m-mole and five experiments at the 4 and 10 μ m-mole quantities. c-v, Coefficient of variation.

Substance	Q (μ m-moles)	Mean a	Within-expt. c-v (%)	Total c-v (%)	s.e.m. of triplicate within expt. (%)
Glycine	2	0.1147	16	17	9.2
	4	0.2146	10	11	5.8
	10	0.5425	8.4	12	4.8
	20	1.0868	5.7	8.1	3.3
Taurine	2	0.1187	8.4	12	4.9
	4	0.2412	4.5	15	2.6
	10	0.6014	7.0	14	4.0
	20	1.2477	5.6	11	3.2
β -Aminoisobutyric acid	2	0.1171	13	13	7.5
	4	0.2052	7.4	9.6	4.3
	10	0.5591	6.2	10	3.6
	20	1.1556	5.0	5.3	2.9
Alanine	2	0.2128	10	11	5.8
	4	0.3829	8.5	9.4	4.9
	10	0.9327	5.2	9.0	3.0
	20	1.8861	5.0	7.8	2.9
Glutamine	2	0.1476	9.2	12	5.3
	4	0.2556	8.9	9.1	5.1
	10	0.6688	5.6	13	3.2
	20	1.3297	5.6	5.9	3.2

Table 2. *Rates of excretion of substances by individual members of the three ethnic groups, Chinese (C), European (E) and Sēnoi (S), with means and estimates of group standard deviations (S.D.)*

Subject	Body wt. (kg.)	Excretion rates (m-moles/24 hr.)					
		Glycine	Taurine	β -Aminoiso- butyric acid	Alanine	Glutamine	Cystine
C1	55.4	1.52	0.90	0.30	0.60	0.67	0.17
C2	54.4	1.49	1.03	0.36	0.49	0.67	0.12
C3	58.1	0.91	0.91	0.14	0.37	0.46	0.09
C4	61.7	2.31	1.37	0.82	0.68	0.71	0.08
C5	81.7	0.91	2.45	0.36	0.34	0.70	0.19
C6	56.3	1.06	1.24	0.10	0.37	0.69	0.17
C7	50.8	2.02	1.25	0.10	0.24	0.49	0.06
C8	54.4	1.23	1.19	1.54	0.34	0.52	0.06
C9	56.7	1.66	1.80	0.48	0.41	0.57	0.14
C10	60.8	1.12	0.94	0.38	0.47	0.22	0.09
Mean	59.03	1.423	1.308	0.458	0.431	0.570	0.117
S.D.	8.575	0.4715	0.4804	0.4359	0.1318	0.1543	0.0481
E1	83.9	1.05	1.02	0.25	0.30	0.68	0.26
E2	63.5	1.40	0.22	0.30	0.17	0.54	0.20
E3	69.0	1.70	0.65	0.19	0.37	0.81	0.18
E4	73.5	1.44	0.69	0.18	0.32	0.61	0.22
E5	60.3	2.96	0.93	0.15	0.34	0.97	0.24
E6	62.6	1.04	0.76	0.18	0.28	0.32	0.24
E7	71.2	1.26	1.17	0.03	0.36	0.52	0.21
E8	70.8	2.20	1.88	0.27	0.49	0.84	0.33
E9	64.0	3.16	0.92	0.05	0.29	0.55	0.15
E10	74.4	1.43	1.64	0.11	0.50	0.93	0.25
Mean	69.32	1.764	0.988	0.171	0.342	0.677	0.228
S.D.	7.074	0.7609	0.4839	0.0894	0.0980	0.2070	0.0492
S1	49.5	—	0.61	0.79	0.08	0.12	0.13
S2	49.9	—	1.14	0.36	0.16	0.25	0.33
S3	43.6	—	1.06	1.02	0.11	0.18	0.12
S4	62.6	—	1.42	1.54	0.28	0.35	0.20
S5	49.5	—	0.74	0.42	0.09	0.19	0.15
S6	61.7	—	0.97	0.40	0.17	0.19	0.15
S7	55.4	—	0.29	0.04	0.05	0.10	0.07
S8	50.8	—	0.55	0.25	0.19	0.19	0.14
S9	52.2	—	1.04	1.25	0.11	0.25	0.15
S10	51.3	—	0.38	1.96	0.13	0.22	0.18
Mean	52.65	—	0.820	0.803	0.137	0.204	0.162
S.D.	5.804	—	0.3634	0.6256	0.0662	0.0706	0.0684

in the work with Chinese subjects, glycine was estimated only after ion-exchange desalting (as previously described).

Ethnic-group data. Table 2 records the rates of excretion of the six substances by members of the three ethnic groups. β -Aminoisobutyric acid values have been corrected for 20% loss (see Discussion).

DISCUSSION

However closely the ninhydrin reaction may come to yielding diketohydrindylidene-diketohydrindamine stoichiometrically in suitably constituted solutions (Troll & Cannan, 1953; Moore & Stein, 1954), the requisite conditions cannot be provided satisfactorily on paper sheets. The reaction is customarily carried out by heating the ninhydrin-impregnated sheets at a definite temperature for a definite period with usually, but not always, some deliberate control of humidity.

Even among the α -amino- α -carboxylic acids the absorption spectra of the colour substances differ slightly and the molecular extinction indices at any chosen light-frequency may vary appreciably. However, whatever the reaction(s) may be, there is evidence (Moore & Stein, 1948) that the quantities of colour substance(s) produced are proportional to the quantities of parent substance reacting with the excess of ninhydrin.

Results of the experiments reported here are seriously in conflict with those of Redfield & Barron (1952) that led them to conclude that the optimum relative humidity is $50 \pm 10\%$ at 90° , but they may be in harmony with those of Wellington (1953) which indicated an optimum relative humidity of $40 \pm 3\%$ at $20 \pm 1^\circ$. It is apparent from Fig. 2 and cognate data that at any given temperature both the magnitude and degree of control of the relative humidity for satisfactory colour development are critical.

The electrolytic desalting loss of β -aminoisobutyric acid which occurred in the (b), but not the (a), series of experiments has been attributed to reaction with one or more substances present in normal but not in simulated urines, and the correction for 20% loss in urine assays was based upon this interpretation. The presence of taurocholic acid in urines might lead to spuriously high taurine assays after electrolytic desalting, but this possibility cannot explain the apparent 7% 'gain' of taurine in the (b) as compared with the 8% loss in the (a) series of experiments: in the circumstances no correction was applied to the urine assay values.

Standard curves for assay purposes may be constructed from the Q and $-\log T (= D)$ values. Linear regression equations based upon the Q and the $1/T$ or the a values may similarly be used, though there are certain theoretical objections to deriving and using them when either variate (here Q) has values confined within controlled experimental limits. Both approaches involve acceptance of the usually substantial 'between-experiment' contributions to total variance (see Table 1). An alternative approach (adopted in the present work), which excludes these contributions, is to accept equation (1) as adequately expressing the relationship between Q and a and to employ it, as equation (2), in 'within-experiment' estimations. Incidentally, with chromatograms of sugars on the same type of paper and similar densitometry of the colour bands produced by aniline hydrogen phthalate treatment, equation (1) is equally valid (E. McEvoy-Bowe, unpublished work, 1957).

As was indicated earlier, failure to employ the refined photometry used in this work would require some 12 'within-experiment' replicates to furnish means with percentage standard errors as low as those shown in Table 1. Yet these errors (range 2.6–9.2% with 4.5% mean) are only about one-third of those reported by Block *et al.* (1955) for the mean of 12 replicates, namely 10–15%. This further gain in reliability is attributed partly to better control of the chromatography but mostly to rigorous control of colour development. All factors combined make the mean of triplicates as reliable as the mean of about 100 replicates in the procedure recommended by Block *et al.* (1955).

The purpose of the survey was to disclose likely differences in excretion patterns for more extensive future studies. As in other work with larger samples from these three groups (Lugg & Bowness, 1957) the European subjects were usually the heaviest and the Snoi the lightest. More subtle differences than those suggested by Table 2 might be revealed by relating the excretions to (for example) creatinine excretions and by working with larger samples.

Individual values, means and standard devia-

tions, considered as they stand and also with some reference to body weight, suggest that significance might be attached (i) to the low cystine excretions in the Chinese sample and to the low alanine and glutamine excretions in the Snoi, (ii) to the incidence of notably higher glycine:taurine ratios in the European than in the Chinese sample, and (iii) to several aspects of the differences in distributions of β -aminoisobutyric acid values.

In respect of (i) the cystine values would include any other disulphides and any thiol compounds (unlikely to be present), or both, in the urines. The European values correspond closely with those found by Reed (1942) and, like them, are considerably greater than those found by Stein (1953) employing an ion-exchange chromatographic method. The chemical significance of this discrepancy is uncertain (Evered, 1956). The statistical significance of the difference between the European and Chinese means is very high (Student's $t = 4.95$, $P = < 0.001$), nor would it be diminished vastly by referring excretion rates to a weight basis. There is no instance of 'cystinuria', classical or otherwise (see Dent & Harris, 1951), in any of the samples. The generally low alanine and glutamine values in the Snoi sample may be merely a reflexion of the relatively protein-poor (though not inadequate) diet of the group.

No indication of (ii) has emerged from previous studies. High glycine excretion rates appear to be frequent among Europeans (Dent & Harris, 1951). But, whereas Evered (1956) was inclined to associate elevated taurine excretion rates with protein-rich diets, it is noteworthy that (especially in relation to body weight) the mean rate in the Snoi sample is not low, and in the Chinese sample relatively high, compared with that in the European. The evidence, viewed as a whole, strongly suggests the involvement of endogenous, racial factors in the excretion of taurine (such factors operate in populations of mice; Harris & Searle, 1952) as well as of glycine.

With reference to (iii), although there is evidence (Fink, Henderson & Fink, 1951) that the excretion of β -aminoisobutyric acid by the rat is subject to the effects of endogenous (dietary) factors, these are believed to play a negligible role in normal human subjects maintained on normal diets. Within-sample distributions of values recorded in Table 2 are shown in Fig. 3. In constructing the frequency polygons the disturbing effects of chance in sampling with such small samples have been reduced by distributing the observations (as fractions mostly) between single-valued classes (Lugg & Bowness, 1957) instead of conventionally placing each wholly within a class span.

A bimodal distribution of human subjects into 'low' and 'higher' excretors of β -aminoisobutyric

Fig. 3. Frequency distributions of β -aminoisobutyric acid excretion rates in the three ethnic groups. $\circ$, Chinese; Δ , European; $\square$, Sēnoi.

acid was suggested by the original work of Crumpler Dent, Harris & Westall (1951) and supported by that of Harris (1953). If, from the appearance of Fig. 3, the 'low' excretors are accepted as those excreting less than 0.6 m-mole/24 hr. then, reverting to the original data, the European sample contained no 'higher' excretors, whereas the Chinese contained two (C4 and C8) and the Sēnoi five (S1, S3, S4, S9 and S10). The statistical significance of the differences in incidence of 'low' and 'higher' excretors is appreciable: χ^2 (2 degrees of freedom) = 7.07, $P = 0.03$. Probabilities of encountered or greater differences (in either direction) in the pair comparisons, computed by the 'exact' method (see Fisher, 1954), are: $P = 0.47$, for Chinese with European; $P = 0.35$, for Chinese with Sēnoi; and $P = 0.032$, for European with Sēnoi.

Only in the European-Sēnoi comparison is the statistical significance fairly high. Apart, however, from this type of differentiation, the European 'low' excretors (especially in relation to body weight) appear to be differentiated somewhat from the 'low' excretors in the other two ethnic groups.

Estimates, by varying criteria, of the incidence of higher excretors among populations of basically Caucasoid origin have varied: 4.8% (Crumpler *et al.* 1951), 8% (Fink *et al.* 1951), 9.6% (Harris, 1953) and 10.2% (Allison, Blumberg & Gartler, 1959). The incidence is clearly low, and the lack of a 'higher' excretor in the present small European sample is not contrary to expectations.

Estimates by Allison *et al.* (1959) of the incidence of 'higher' excretors among populations generally recognized as of basically Mongoloid origin are, 56 and 59% for two 'Amerind' groups and 23% for Alaskan Eskimos. Sutton & Clark's (1955) findings from their comparative study of excretion rates of β -aminoisobutyric acid and other substances in

European and Chinese groups are open to question because of the divergence of their assay values from those of other investigators.

Closer examination of Fig. 3 suggests that the 'higher' excretors themselves may not form a unimodally disperse fraction, a possibility already implicit in the results of Fink *et al.* (1951). The Chinese sample might well be regarded as containing one 'high' and one 'very high' excretor, and the Sēnoi as containing two or three 'high' and (correspondingly) three or two 'very high' excretors, depending upon where the division might be made.

All in all, the β -aminoisobutyric acid data, if not the rest, are in conformity with earlier evidence (for brief review see Lugg, 1957) that the Sēnoi are, like the Southern Chinese, of basically Mongoloid origin.

Departures from unimodality of distributions of β -aminoisobutyric acid excretion rates in human subjects have been attributed (Crumpler *et al.* 1951; Harris, 1953) to inherited variability in the renal threshold levels. The evidence supporting this interpretation is substantial; but the actual pattern of inheritance would now seem to be far more complex than has formerly been supposed.

SUMMARY

1. Conditions of running two-dimensional paper-sheet partition chromatograms of mixtures of amino acids (and derivatives), and of developing band colorations with ninhydrin, have been studied with a view to furnishing sheets suitable for direct transmission photometry with a refined type of densitometer.

2. The recommended conditions, coupled with the simplest form of photometry afforded by the densitometer, make the mean of triplicate assays as reliable as the mean of about one hundred replicates in the procedure recommended by Block *et al.* (1955).

3. By the refined methods an exploratory survey has been made of the rates of urinary excretion of glycine, taurine, β -aminoisobutyric acid, alanine and glutamine by members of one aboriginal (Sēnoi) and two civilized (European and Southern Chinese) ethnic groups living on Singapore Island and in the Federation of Malaya. The work involved a preliminary study of 'desalting' techniques. Cystine excretion rates, estimated polarographically, were included in the survey.

4. Attention has been drawn to the more salient aspects of ethnic-group differentiation and correspondence suggested by the results of the survey.

We wish to express our indebtedness to the subjects for their co-operation, to Mr Lim Yen Heng, Department of Biochemistry, University of Malaya, for much technical

assistance, and to Mr R. O. D. Noone, Adviser on Aborigines, Federation of Malaya, for facilitating our access to the aborigines congregated at Gombak. We are indebted also to Mr N. Stenhouse, Mr C. Boundy, and staff, C.S.I.R.O. Regional Laboratories, Western Australia, for assistance with statistical calculations.

This work embodies part of a thesis submitted by E. McEvoy-Bowe for the degree of Ph.D. in the University of Malaya.

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Flight-Muscle Mitochondria of *Lucilia cuprina* and *Musca domestica*

ESTIMATION OF THE PYRIDINE NUCLEOTIDE CONTENT AND OF THE RESPONSE OF RESPIRATION TO ADENOSINE DIPHOSPHATE

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(Received 16 January 1961)

The ability of many species of flying insects to increase their consumption of oxygen during flight and the factors controlling respiration during rest and activity have been the subject of much research (for a recent summary, see Sacktor, 1958; Bucher, Klingenberg & Zebe, 1958). The production of adenosine triphosphate by oxidative phosphorylation in the flight-muscle mitochondria is a most important aspect of energy transformation in such animals (Sacktor, 1954; Slater & Lewis, 1954), as in many other organisms. However, Sacktor and co-workers have described certain unusual

features of both the control of respiration with α -glycerophosphate, which is oxidized most readily by mitochondria from insect muscles (e.g. Chance & Sacktor, 1958), and in the composition of the electron-transport chain of particles isolated from thoraces of the fly *Musca domestica* L. With preparations from mature flies (3–7 days old) they found that the oxidation of α -glycerophosphate was independent of a supply of adenosine diphosphate (Sacktor, 1958; Sacktor & Cochran, 1958; Estabrook & Sacktor, 1958) and that pyridine nucleotide coenzymes were not detectable by the