ISSN 0300—9254

JOURNAL OF THE NATIONAL SCIENCE COUNCIL OF SRI LANKA

VOLUME 3 No. 1

JUNE 1975

Journal of the National Science Council of Sri Lanka

PUBLICATION: One volume of two issues (June and December) is published annually by the National Science Council of Sri Lanka.

Subscription Annual subscription—Foreign $ 10.00 ; Local Rs. 25.00. Accepted on a calendar year basis. Rates include postage.

Single issues — Foreign $ 5.00; Local Rs. 12.50 each
Back issues — Foreign $ 4.00; Local Rs. 7.50 each
Rates include postage.

Payment must accompany all orders. Remittances in favour of National Science Council of Sri Lanka.

Change of address notice is required 4 weeks before issue date.

Orders and all correspondence relating to them should be sent to the **Accountant,** National Science Council of Sri Lanka at the address given below.

Manuscripts Research Papers, Reviews and Short Communications in all fields of Science and Technology in Sinhala, Tamil and English may be submitted for editorial consideration. Manuscripts should conform to the style adopted in this issue. For instructions as to preparation of papers see inset at back of this issue. Separates of General Instructions and Special Instructions in Chemical, Physical and Medical Sciences may be had on application to the Secretary, Editorial Board at the address given below.

No responsibility is assumed by the National Science Council of Sri Lanka for statements and opinions expressed by the contributors to this Journal.

Manuscripts and all correspondence relating to them should be sent to the **Secretary,** Editorial Board, Journal of the National Science Council of Sri Lanka, 47/5 Maitland Place, Colombo 7, SRI LANKA.

Printed at Sri Lanka University Press, Katubedde. 14069 (10/75)

J. Natn. Sci. Coun. Sri Lanka 1975 3(1) : 1-10

Control of Sulphide Formation in Coconut Toddy

E. R. JANSZ, E. E. JEYARAJ, D. J. ABEYRATNE AND I. G. PREMARATNE
Industrial Microbiology Section, Ceylon Institute of Scientific and Industrial Research (CISIR), P. O. Box 787, Colombo 7, Sri Lanka.

(*Paper accepted*: 4 *February* 1975)

Abstract : Toddy, fermented with wild yeast, contains volatile sulphide.[3] The total sulphide content of toddy fermented under different conditions was quantitatively estimated spectrophotometrically (after aspiration from acid medium and trapping H_2S as an insoluble sulphide) by a method modified from Budd and Bewick[1] which is specific for inorganic sulphide. Results showed that 0.01% NH_4^+ was, in most cases, sufficient to eliminate sulphide formed during wild fermentation. In a few instances, however, much higher concentrations were necessary. Urea did not inhibit at the same order of nitrogen concentration. Sulphide formation could also be controlled by choice of strain of yeast ; several strains isolated from toddy gave ferments nearly free of sulphide. Strains capable of forming high levels of sulphide do not produce this compound in the presence of 0.05%NH_4^+.

Volatile sulphide in ferments can be trapped as CuS by addition of Cu turnings. Addition of Fe or steel to the ferments increased sulphide formation several fold ; NH_4^+ (0.1%) only partly inhibited this.

Studies have shown that SO_4^{2-} does not increase sulphide formation, but that cysteine and (to a lesser extent) methionine appear to provide the sulphur for sulphide formation.

1. Introduction

Coconut toddy, the fermentation product of coconut sap (an exudate of the tender floral spathe[2]) is used directly as an alcoholic beverage[4] and also as the starting material for coconut arrack (a distilled product). Both products have been exploited extensively on a commercial scale for a number of years. More recently the unfermented material (sweet toddy) has been introduced in the bottled form.[7] Under some conditions all these products contain volatile sulphide[3,8,10] which contribute to the objectionable odour of toddy.

This paper describes the method by which inorganic sulphide formation in fermenting toddy can be prevented. The paper also describes in detail a method of determining the sulphide content of toddy.

2. Experimental

2.1. Materials

2.1.1. *Toddy*

Sweet toddy was obtained by collecting the sap after the normal tapping procedure[2] in clean vessels (free of microorganisms). Tapping commenced at 6 p.m. and samples were collected at 6 a.m. the following morning. Analysed at 8 a.m. the

sweet toddy contained 14 to 16% sugar. Samples were used directly for studies of fermentation with wild yeast and generally contained 10^6 to 10^7 cells/ml. For studies with pure cultures, toddy was either sterilized by autoclaving or by flash heating as in the sterilization procedure for bottled coconut water[5]; the toddy was used immediately in the first case and after bottling for future use when the second procedure was adopted. Toddy samples stored in this way gave only traces ($< 4\,\mu$ moles/l) of sulphide when tested after a period of weeks, showing that there was negligible formation of sulphide by non-microbiological pathways.

2.1.2. *Yeast cultures*

Strains of yeast isolated from toddy and other sources, as well as commercial yeast strains were used. Yeast cultures were single cell isolates, free of bacteria and were maintained on glucose-peptone-yeast extract-agar slants. Cells for experiments were grown aerobically on a shaker on standard glucose-peptone-yeast extract medium and used when physiologically active (showed a high rate of fermentation).

2.1.3. *Synthetic medium for sulphide formation*

The basal synthetic medium used for H_2S studies consisted of glucose, 12%; KCl, 0.05%; KH_2PO_4, 0.1%; $MgSO_4.7H_2O$, 0.5%and $FeSO_4$ trace, 0.001%. To this medium was added NH_4Cl, methionine and cysteine as given in section **2.5** The final pH of the medium adjusted to 4.5.

2.2. The fermentation process

Sweet toddy (200 to 250 ml) was introduced into a sterilized gas washing bottle (500 ml) and the required variables added. Studies with wild yeast were performed at a cell density of about 10^6 to 10^7 cells/ml while in studies with pure yeast strains, a cell density of 2×10^7 to 5×10^7 cells/ml was used. In the latter case cells were washed once with sterilized water before use. CO_2 evolved was led into a trap of cadmium acetate (200 ml of 0.3% w/v) in another similar gas washing bottle. The completion of fermentation was judged by observing the bubbles formed per min in the trap. The fermentation is over in 20 to 30 h (wild yeast) or 40 to 60 h (pure cultures). Sulphide formation occured mainly during the middle part of the fermentation and stopped when the fermentation ended.

2.3. Recovery of sulphide from toddy

The toddy sample was acidified with 2 ml of 98% H_2SO_4 (w.w)/100 ml toddy and gassed (> 250 ml/min) with CO_2 for 45 min. Recovery experiments showed that the gas used was suitable for the purpose (section **3.1.**). In initial experiments a double trap containing zinc acetate (200 ml of 1.2% w/v) followed by Cadmium acetate (200 ml of 0.3% w/v) was employed. In subsequent experiments a special device was used to break up the gas bubbles (the gas was forced through small pores in an adaptor that was attached). This greatly facilitated sulphide absorption enabling quantitative recovery of $> 95\%$ of H_2S using the Cadmium acetate trap.

Free sulphide was also determined by gassing without the addition of acid. This method of estimation was used only in the studies using metals and was not as reliable as acid conditions because increased period of bubbling gave rise to slight increases in free sulphide content. Generally the free sulphide content of toddy was 3 to 13 μmoles/l less than the total inorganic sulphide content.

2.4. Estimation of sulphide

Sulphide was estimated using NN diethyl p-phenylenediamine sulphate. The colour development technique was similar to that described in standard methods.[11] However, the strength of NN di-ethyl p.phenylene-diamine sulphate used was 0.81 g per 100 ml of 50% H_2SO_4 (w/v) and the intensity of colour formed was determined using a U.V. Spectrophotometer (UNICAM SP 500) at 660 nm. The estimation was done using a standard curve plotting S^{2-} content (Na_2S precipitated as ZnS and estimated by the I_2/ $Na_2S_2O_3$ titration) versus optical density. The test was most reliable over the range of 0.06 to 0.23 μmoles S^{2-} per sample. The standard curve had a gradient of 0.35 O.D./0.1 μmoles and a standard error of > 5% in this range. The test is specific for inorganic sulphide ; methionine and cysteine sent through the recovery procedure produced no colouration with the reagent.

2.5. Qualitative scoring system for volatile sulphide in toddy

This method was found to be useful for qualitative studies. Toddy (35 ml) was contained in a boiling tube (100 ml) plugged with cotton wool. A strip of filter paper of constant size, dipped in lead acetate, was affixed to the cotton wool plug. The extent of blackening of the paper was scored on a system of + signs from one to four.

Comparative tests with the quantitative method indicated that +, ++, +++ and ++++ as given in the text of this paper were in the ranges of 10, 10-30, 30-60 and > 60 μmoles/l respectively.

3. Results

3.1. Recovery of sulphide from toddy

Recovery of H_2S was tested by adding sulphide at concentrations in the range of 8 to 160 μmoles sulphide/l (as ZnS) into test solutions of (*a*) water (*b*) fermented H_2S free toddy. Solutions were gassed with CO_2 before addition of sulphide. Results showed that at least 90% of the sulphide could be recovered within 45 minutes using the traps, acid concentrations and rate of bubbling as described in section **2.3.** Sulphide-free toddy was obtained by fermenting toddy in the presence of 0.5% NH_4^+; aspiration in acid medium confirmed that no inorganic sulphide was present in this toddy.

3.2. Reproducibility

Reproducibility of the combined operations viz. the fermentation process, recovery of sulphide and quantitation of sulphide is reflected in the results in Table 1, which show that the method adopted was adequate to illustrate the effects described in this paper. However, it must be mentioned that extreme care must be taken in the fermentation stage with respect to : (1) volume of toddy used[3] (2) yeast cell density (3) the state of fermentation (Table 2) and (4) contamination. The first two factors must be kept constant within an experiment and the fourth scrupulously avoided.

TABLE 1. Reproducibility of assays

Sample	Inorganic sulphide formed (μmoles/l)		
	Experiment A	Experiment B	Experiment C
1	35.3	15.0	5.3
2	33.8	15.9	5.9
3	39.7		

In the three experiments above, parallel samples (triplicate in expt A and duplicate in expts B and C) were fermented. Reproducibility as above is a reflection of the combined effect of errors of the fermentation, recovery and detection stages of the process.

TABLE 2. A time course for sulphide formation in relation to sugar utilization.

Time (h)	Sulphide formed (μmoles/l)	Residual sugar (%)
0	0	16.4
10.5	1.0	12.5
21.5	128	9.0
27.5	172	7.9
32.5	225	7.2
50	228	5.0
70	228	3.3

Toddy was fermented in a series of bottles under identical conditions. Determinations were done on a separate ferment at the times given above. Total sugar was determined with Fehlings solution after inversion with HCI (sp. gr. 1.1029). Yeast culture No. 2 was used in this experiment.

3.3. Studies with wild yeast

Toddy samples showed a wide variation with respect to inorganic sulphide content ; of the 12 samples quantitatively studied, 7 produced > 30 μmoles H_2S/l. The largest sulphide content observed was 150 μmoles/l.

The ammonium ion had a marked effect on sulphide formation.[3] In most cases, 0.01% ammonium was sufficient to eliminate H_2S formation (Table 3). However, there was a marked sample variation and in some instances 0.3% NH_4^+ was necessary for the purpose. However, in all cases 0.06% NH_4^+ was sufficient to reduce the H_2S level to < 10 μmoles/l.

TABLE 3. Effect of NH_4^+ on sulphide formation during wild yeast fermentation.

Conc. of NH_4^+ added (%)	Sulphide formed (μmoles/1)		
	Expt A	Expt B	Expt C
0	116	150	16.0
0.005	91	19	4.1
0.01	—	<1.0	<1.0
0.02	N.D.	—	—
0.03	—	<1.0	N.D.
0.05	N.D.	—	—
0.1	N.D.	<1.0	—
0.2	N.D.	—	—

NH_4^+ added in the form of NH_4Cl to the same sweet toddy sample.

N.D. not detected.
— no experiment.

The source of NH_4^+ ion does not appear to be important but urea is not an effective inhibitor of sulphide formation (Table 4). Other experiments have shown that urea will inhibit at about 10 fold higher concentrations that NH_4^+. It was also found that addition of SO_4^{2-} (0.1%) did not affect the extent of H_2S formation.

TABLE 4. Effect of N sources on sulphide formation.

N source	Conc. added (as NH_4^+) (%)	Sulphide formed (μmoles/1)
NH_4Cl	0.02	<1.0
$(NH_4)_2SO_4$	0.02	<1.0
$(NH_4)_2HPO_4$	0.02	<1.0
$CO(NH_2)_2$	0.04	28.1
Control	0	29.7

The above sources of nitrogen were added to the same sweet toddy sample.

3.4. Studies with pure strains of yeast

Of the 34 strains of yeast tested 18 were able to produce > 30 μmoles H_2S/l. Generally top yeasts produced less H_2S than bottom yeasts. Of the 11 top yeasts used, only 1 produced > 30 μmoles H_2S/l. Results using some of these strains are shown in Table 5.

TABLE 5. Effect of yeast strain on sulphide formation.

Yeast culture No.	Source of yeast	Sulphide formed (μmoles/l)
2	Coconut toddy	59
15	Coconut toddy	128
17	Coconut toddy	<1
19	Coconut toddy	5.6
20	Coconut toddy	106
21	Coconut toddy	116
28	Palmyrah toddy	59
32	Mysore wine yeast	<1
40	Coconut toddy	N.D

N.D. not detected

The experiment was done with the same sweet toddy sample for all strains.

Different toddy samples with the same strain of yeast gave some difference in total sulphide content.

Two strains of yeast were chosen for further study. It was found that 0.05 % NH_4^+ was necessary to eliminate H_2S formation (Table 6).

TABLE 6. Effect of NH_4^+ on sulphide formation by high H_2S producing cultures.

Conc. of NH_4^+ added (%)	Sulphide formed (μmoles/l)		
	Culture 20	Culture 2	*Culture 20 and 2 mixed
0	112	125	185
0.005	78	112	—
0.01	59	69	69
0.03	—	—	5
0.05	—	—	<1
0.1	—	—	<1

Sweet toddy was fermented with the above strains of yeast.

*A different sample of toddy was used in this case.

— no experiment.

3.5. Studies using synthetic media

Addition of methionine and cysteine to a synthetic medium showed that : (1) of the two amino acids, cysteine produced H_2S more readily (2) the strain producing more H_2S from toddy produced more H_3S from the amino acids and (3) NH_4^+ inhibited H_2S formation (Tables 7, 8). This is consistent with H_2S being formed via the route of cysteine desulphydrase, the two latter points indicating a similar mechanism in toddy.

TABLE 7. Effect of methionine and cysteine on sulphide formation.

Yeast culture	Met. (μmoles/1)	Cys. (μmoles/1)	NH_4^+ (%)	Sulphide formed (μmoles/1)
32	1677	0	0	<1.0
32	1677	0	0.7	N.D.
32	1677	0	0.2	N.D.
20	1677	0	0	11.8
20	1677	0	0.2	6.0
32	0	2067	0	122
32	0	2067	0.7	4.1
20	0	2067	0	209
20	0	2067	0.7	<1.0
20	0	0	0	N.D.

Methionine, Cysteine and NH_4^+ were added in the above quantities to sweet toddy and the fermentation done with pure strains of yeast.

N.D. not detected.

TABLE 8. Effect of NH_4^+ on H_2S formation from cysteine and methionine.

NH_4 (%)	Methionine (μmoles)	Cysteine (μmoles)	Yeast culture no.	Volatile sulphide* formed 24 h	48 h
0	6.7	0	32	—	+
0.2	6.7	0	32	—	+
0.4	0	0	32	—	—
0.4	6.7	0	32	—	—
0.1	6.7	0	32	—	—
0	6.7	0	20	++	+++
0.2	6.7	0	20	—	+++
0.4	0	0	20	—	—
0.4	6.7	0	20	—	+
1.0	6.7	0	20	—	—
0.2	0	8.2	20	++++	++++
0.4	0	—	20	—	—
0.4	0	8.2	20	++++	++++
1.0	0	8.2	20	++++	++++
0	0	8.2	32	++++	++++
0.2	0	8.2	32	++++	++++
0.4	0	8.2	32	—	—
0.4	0	8.2	32	++	++++
1.0	0	8.2	32	++	+++

*Sulphide was detected by lead acetate paper (section 2.5). On a synthetic medium (section 2.1.3), other details as in Table 7.

3.6. The effect of metals on free H_2S content

Addition of metals to the ferment produced varying results (Table 9). Whereas Sn, Al and stainless steel produced no significant difference, copper (turnings) reduced free H_2S content, and mild steels increased free sulphide. The effect of copper appears to be due to the formation of CuS. The effect of the Fe is only partly inhibited by the NH_4^+ ion.

TABLE 9. Effect of metals on H_2S formation

Conditions	Sulphide liberated (μmoles/l)			
	Experiment 1	Experiment 2		Experiment 3
		Aspiratable	Acid liberatable	
+ Cu	4.4	—	—	<1.0
+ Al	13.1	—	—	—
+ Fe*	81	53	8.7	—
+ Sn	9.7	—	—	—
+ Mild steel	30.0	—	—	—
+ Bright steel	—	114	234	—
+ Stainless steel	—	17.5	24.7	—
+ Fe* + 0.1% NH_4^+	—	1.9	20.6	—
Control	9.4	21.9	25.6	54.4

Mass of metals, Experiment 1, 3g ; Experiment 2, 10g ; and Experiment 3, 20g.

* B.D.H. electrolytically deposited iron

— no experiment.

3.7. The effect of NH_4^+ on the fermentation

Addition of NH_4^+ ion increases the rate of fermentation slightly but there is no significant effect on the efficiency of alcohol formation from sugar (Table 10). Flavour is altered and the distillate has no typical toddy odour, the odour being closer to that of a purer alcohol.

TABLE 10. Effect of NH_4^+ on the fermentation.

	NH_4^+ (0.05%) added		Control	
	21 h	48 h	21 h	48 h
Residual sugar (% w/v)	8.5	0.6	11.9	3.9
Alcohol (% w/v)	3.96	7.52	2.52	5.64
Ratio of alcohol formed to sugar utilized	0.50	0.47	0.52	0.45

Initial sugar, 16.6%. Alcohol was determined by specific gravity after distillation. Residual sugar was determined with Fehlings solution after inversion. Percentages are expressed as weight by volume. Yeast strain No. 20 was used in this experiment.

4. Discussion

The results clearly show that the inorganic sulphide produced in toddy varies markedly primarily due to differences in the characteristics of yeast in toddy. It is highly suggestive that the immediate source of H_2S is cysteine arising from the sweet toddy. The final enzyme in the pathway, cysteine desulphhydrase, has been isolated in bacteria.[6] The difference in extent of H_2S formed, using a single yeast strain with different samples of toddy could be due to variations in the sulphoamino acid content or concentrations of inhibitors in the different toddy samples.

Results show that even cultures that do not give H_2S with toddy (e.g. no. 32) may produce the enzyme in other media (as shown in the studies with the synthetic medium). The tendency to produce sulphide also varies from strain to strain, which is not surprising.

The inhibitory effect of ammonia could be due to either repression of synthesis of the enzyme cysteine desulphhydrase or to NH_4^+ acting as an inhibitor of the yeast enzyme (unlike the bacterial enzyme[6]).

The inhibition of inorganic sulphide formation as well as the reduction in intensity of flavour indicates a general 'sparing effect' of amino acids, well known in many alcoholic fermentations, which is probably connected with repression of synthesis of the enzymes concerned with amino acid degradation. A detailed study of the mechanism of inhibition by ammonia using cell-free extracts is in progress.

Another interesting point is that bottled sweet toddy (sterilized) produces small amounts of H_2S[8]. This may be due to incomplete deactivation of cysteine desulphhydrase or due to the participation of pyridoxal phosphate (a cofactor in the reaction) in a non-enzymic conversion of cysteine to H_2S, (remembering that the above cofactor can catalyse transaminase reactions in the presence of Fe^{3+}, Al^{3+} and Cu^{2+} at temperatures around 100°). Is this the mechanism for the enhancement of H_2S formation by Fe filings? Work is also proceeding on this aspect.

The study shows that H_2S and the consequent odour can be removed by : (1) use of selected yeast strains, (2) addition of small amounts of NH_4^+ and (3) addition of copper turnings or wire into the collection tank or even the still.

The fourth solution is only feasible for distilled products and may be applicable in the coconut arrack industry when methods of removing H_2S during the distillation procedure are not available.

Addition of NH_4^+ (0.01—0.03%) at collection points will prevent the formation of offensive odour of volatile sulphides. So will the use of selected strains of yeast on sweet toddy. The product will be a more pleasant sweet or fermented toddy, provided the increased salt content in the former case is acceptable. Distillation products of

NH_4^+ containing fermentation liquors would not have the characteristic flavour associated with coconut arrack and may be used as a spirit base for more selective purposes. Use of selected strains of yeast will be of use not only for the purpose of obtaining new flavours but also should result in increased yield of alcohol.

This study has not dealt with any aspect concerning organic sulphides in toddy ferments ; this line of study is worth investigating.

Acknowledgements

The authors thank the Director, CISIR for the facilities provided, Miss S. Paranawithane for the data on the sweet toddy used in these experiments, Mr. S. Mohanadas for valuable discussions and for bottled sweet toddy samples, the State Distilleries Corporation for allowing us to visit the Seeduwa Distillery and Mrs. I. Bandara for secretarial assistance.

References

1. Budd, M. S. & Bewick, H. A. (1954) *Anal. Chem.* **24** : 1536.
2. Coconut Research Institute, Ceylon (1967) *Leaflet no.* 48 : *Toddy tapping*, 10 p.
3. Jansz, E. R., JeyaRaj, E. E., Premaratne, I. G. & Abeyratne, D. J. (1974) *Proc. Ceylon Ass. Advmt Sci.* **30**(1) : 110.
4. JeyaRaj, E. E. (1961) *Proc. Ceylon Ass. Advmt Sci.* **17**(2) : 32.
5. JeyaRaj, E. E. & Jansz, E. R. (1969) *Ceylon Patent Application*. No. 6365.
6. Metaxas, M. A. & Delwiche, E. A. (1955) *J. Bacteriol.* **70** : 735-737.
7. Mohanadas, S. (1974) *Proc. Ceylon Ass. Advmt Sci.* **30**(1) : 89.
8. Paranawithane, S. (1974) Unpublished results.
9. Snell, E. E. (1969) *Proc. Int. Cong. Pharmacol.* **4**(5) : 210-226.
10. State Distilleries Corporation, Sri Lanka (1974) Private communication, 'H_2S from the patent still'.
11. Standard Methods for the Examination of Water, Sewage and Industrial Wastes, 10th edn., 1955, p. 277. New York : American Public Health Association.

J. Natn. Sci. Coun. Sri Lanka 1975 3(1): 11-17

Conditions for Steady Circulation in Rotating Magnetic Stars with Finite Electrical Conductivity

M. MAHESWARAN

Department of Mathematics, University of Sri Lanka, Peradeniya Campus, Peradeniya, Sri Lanka.

(*Paper accepted*: 26 *February* 1975)

Abstract : It is shown that the conditions for steady circulation derived for perfectly conducting rotating magnetic stars by Chandrasekhar and Mestel may be extended to fluids with finite electrical conductivity provided that the magnetic flux density belongs to the class of solutions of the vector wave equation. When the field becomes force-free it is shown that the stellar fluid and the electric currents must flow along the same streamlines in a meridian plane, and that in the case of slow meridian circulations in stars a steady state might not be achieved with a force-free field unless the azimuthal component of the field is much stronger than the meridional component.

1. Introduction

The study of stellar structure is difficult when rotation and magnetic fields are present. It becomes complicated when large scale circulations are set up in the interior, which may react back on the rotation and magnetic fields that drive them. The easiest approach to this problem is to study structures in which a steady state has been reached. In such a state we would expect the rotation, circulation and magnetic field to be related to each other in some way. The conditions for steady circulation in an axisymmetric star, when its material is treated as a perfect electrical conductor have been obtained by Chandrasekhar[1] and Mestel.[2] The basic equations for a perfectly conducting system in a steady state are :

$$\text{curl}\ (\mathbf{v} \wedge \mathbf{B}) = 0 \tag{1}$$

$$\text{div}\ \mathbf{B} = 0 \tag{2}$$

and

$$\text{div}\ (\rho \mathbf{v}) = 0 \tag{3}$$

where $\mathbf{B}$ denotes the flux density, ρ the material density and $\mathbf{v}$ the velocity. If we denote the meridional and azimuthal components using subscripts m and a respectively, the steady state conditions in the axisymmetric case may be written (Mestel[4]) in Gaussian units as

$$\mathbf{v}_m = k\,\mathbf{B}_m, \tag{4}$$

$$\Omega - \frac{kB_a}{\varpi} = \alpha, \tag{5}$$

$$\rho k = \frac{\rho v_m}{B_m} = \gamma, \tag{6}$$

and

$$-\frac{\varpi B_a}{4\pi\mu}+\rho k\Omega\varpi^2=-\frac{\beta}{4\pi}, \tag{7}$$

where Ω is the angular velocity, ϖ the axial distance, k a scalar, μ the permeability and α, β, γ are constants on the streamlines of circulation. Equations (4), (5) and (6) follow from equations (1), (2) and (3). Equation (7) is derived from the azimuthal component of the equation of motion, which says that the convection of angular momentum by the circulation is balanced by its transport by magnetic stresses. We note that for consistency B_a should not vanish.

We note that, though perfect electrical conductivity is a good first approximation for stars, the actual electrical conductivities are such that the magnetic Reynolds number Rm is finite and not very large when we consider the Eddington-Vogt circulations (Maheswaran[3]). Hence, it would be useful to consider the extension of the Chandrasekhar-Mestel problem for stars where the electrical conductivity is finite. In this paper we confine our attention to axisymmetric stars with constant conductivity.

2. The Equations

The Magnetohydrodynamic induction equation, in Gaussian units, reads

$$\frac{\partial \mathbf{B}}{\partial t}=\operatorname{curl}(\mathbf{v}\wedge\mathbf{B})-\operatorname{curl}(\eta\operatorname{curl}\mathbf{B}) \tag{8}$$

where $\eta=c^2/4\pi\mu\sigma$ is the magnetic resistivity ; c being the speed of light and σ the electrical conductivity.

We shall consider systems in which η is constant and the magnetic fields decays like $\exp(-\eta t/l^2)$, where l denotes some decay length scale. Writing

$$\mathbf{B}=\mathbf{B}_0\exp(-\eta t/l^2) \tag{9}$$

in equation (8) we obtain

$$\operatorname{curl}(\mathbf{v}\wedge\mathbf{B}_0)=\eta\left(\operatorname{curl}\operatorname{curl}\mathbf{B}_0-\frac{1}{l^2}\mathbf{B}_0\right). \tag{10}$$

We shall refer to the state in which $\mathbf{v}$ is steady and $\mathbf{B}$ decays as described by equation (9) as the quasi-steady state.

The problem of finding general solutions of equation (10) is a difficult one, but a particular solution which is of interest may be obtained easily. We shall consider a system in which equation (10) reduces to

$$\operatorname{curl}(\mathbf{v}\wedge\mathbf{B}_0)=0=\operatorname{curl}\operatorname{curl}\mathbf{B}_0-\frac{1}{l^2}\mathbf{B}_0 \tag{11}$$

This gives us

$$\nabla^2 \mathbf{B}_o + \frac{1}{l^2} \mathbf{B}_o = 0 \tag{12}$$

and

$$\mathbf{v} \wedge \mathbf{B}_o = \nabla f, \tag{13}$$

where f is a scalar function.

Equation (12) states that $\mathbf{B}_o$ is a solution of the vector wave equation. Hence, the most general form of $\mathbf{B}_o$ may be written (e.g. vide Ferraro and Plumpton[2], p. 62)

$$\mathbf{B}_o = \lambda_1 \operatorname{curl} (\mathbf{a}\psi) + \lambda_2 l \operatorname{curl} \operatorname{curl} (\mathbf{a}\psi) \tag{14}$$

where $\mathbf{a}$ is a constant unit vector, ψ is a scalar satisfying the wave equation

$$\nabla^2 \psi + \frac{1}{l^2} \psi = 0$$

and λ_1, λ_2, are arbitrary constants.

We shall now investigate the conditions for steady circulation that will result from equations (2), (3), (12) and (13).

3. Conditions for Steady Circulation

In the case of a three dimensional system it is not easy to obtain conditions any simpler than those expressed in equations (12) and (13). However, considerable simplification is possible when we look at axisymmetric systems.

3.1. Axisymmetric systems

In an axisymmetric system equation (13) simplifies to

$$\mathbf{v}_m \wedge \mathbf{B}_{om} = 0 \tag{15}$$

and

$$\mathbf{v}_a \wedge \mathbf{B}_{om} + \mathbf{v}_m \wedge \mathbf{B}_{oa} = \nabla f \tag{16}$$

These equations are the same as for $\mathbf{B}$ in the perfect conductor case and therefore equations (15) and (16) together with equations (2) and (3) yield (vide Mestel[4]).

$$\mathbf{v}_m = k\mathbf{B}_{om} \tag{17}$$

$$\Omega - \frac{kB_{oa}}{\varpi} = \alpha \tag{18}$$

and

$$\rho k = \frac{\rho v_m}{B_{om}} = \gamma \tag{19}$$

where α, γ are constant on the streamlines of circulation and k is a scalar.

If we take the equation of motion in the azimuthal sense we have

$$\left(\frac{\text{curl } \mathbf{B} \wedge \mathbf{B}}{4\pi\mu}\right)_a = \rho \frac{1}{\varpi} \mathbf{v}_m \cdot \nabla (\Omega \varpi^2) \tag{20}$$

which may be written

$$\exp(-2\eta t/l^2) \left(\frac{\text{curl } \mathbf{B}_0 \wedge \mathbf{B}_0}{4\pi\mu}\right)_a = \frac{\rho}{\varpi} \mathbf{v}_m \cdot \nabla (\Omega \varpi^2). \tag{21}$$

The presence of the time dependent term complicates the problem. However, if we are interested only in a time scale short compared with the decay time scale i.e $t << l^2/\eta$, we can approximate equation (21) to

$$\varpi \left(\frac{\text{curl } \mathbf{B}_0 \wedge \mathbf{B}_0}{4\pi\mu}\right)_a = \rho \mathbf{v}_m \cdot \nabla (\Omega \varpi^2). \tag{22}$$

As in the perfect conductor case we assume that $\mathbf{B}_{0a}$ does not vanish. Further, we shall assume that the field is not force-free, for otherwise the left hand side of equation (22) will vanish. We shall consider the force-free case separately in the ensuing section. Equation (22) together with equations (17) and (19) yield (as in Mestel[4])

$$-\frac{\varpi B_{0a}}{4\pi\mu} + \rho k \Omega \varpi^2 = -\beta/4\pi. \tag{23}$$

Hence, a set of conditions for steady circulation over time scales short compared with the decay time scale are given by equations (17), (18), (19), (23) and (12) together with equation (9); i.e. a set of conditions for the finite conductor case may be obtained by replacing $\mathbf{B}$ by $\mathbf{B}_0$ in the conditions for the perfect conductor case with the additional condition that $\mathbf{B}_0$ should belong to the class of solutions of the vector wave equation.

3.2. Force-free fields

In the preceding section we noted that if the field was force-free, the left hand side of equation (22) vanishes. Also, we know that a class of solutions of equations (12) for $\mathbf{B}_0$ is that of the force-free fields, which will be given by

$$\text{curl } \mathbf{B}_0 = \frac{1}{l} \mathbf{B}_0. \tag{24}$$

It is known that a field cannot be everywhere force-free in an isolated system in which the field vanishes at the surface (e.g. vide Ferraro & Plumpton[2]). However, we may apply our results to a portion of a larger system (e.g. like the radiative region of an axisymmetric star).

We shall now discuss the consequences of supposing that $\mathbf{B}_o$ satisfies (24). The electric current vector $\mathbf{j}$ is given by

$$\mathbf{j} = \frac{1}{4\pi\mu} \text{curl } \mathbf{B} \tag{25}$$

If we put $\mathbf{j} = \mathbf{j}_o \exp(-\eta t/l^2)$ we have from equations (24) and (25) that

$$\mathbf{j}_o = \mathbf{B}_o/4\pi\mu l. \tag{26}$$

Hence, we may write, using equations (17), (18) and (19), which remain unaltered,

$$\mathbf{v}_m = k'\mathbf{j}_{om}, \tag{27}$$

$$\Omega - \frac{k'}{\varpi} j_{o\phi} = \alpha' \tag{28}$$

and

$$\rho k' = \frac{\rho v_m}{J_{om}} = \gamma', \tag{29}$$

where k' is a scalar and α', γ' are constants along the streamlines.
Equations (28) and (29) together yield

$$\mathbf{v} = k'\mathbf{j}_o + \alpha' \varpi \mathbf{e}_\phi \tag{30}$$

where $\mathbf{e}_\phi$ is the unit azimuthal vector. This equation tells us that the motion consists of an arbitrary uniform rotation of each poloidal loop superimposed on a velocity $k'\mathbf{j}_o$.

When we come to the azimuthal component of the equations of motion we find that the earlier condition (23) is altered. The equation of motion now reads

$$\mathbf{v}_m \cdot \nabla(\Omega \varpi^2) = 0, \tag{31}$$

which requires that either $\mathbf{v}_m$ vanish or the angular momentum per unit mass be constant along the streamlines. i.e.

$$v_m = 0 \quad \text{or} \quad \Omega\varpi^2 = \beta', \tag{32}$$

where β' is constant on streamlines.

Hence, a set of conditions that may be used to obtain systems with steady circulation is given by equations (27), (28), (29), (32) and (24) together with equation (9).

4. Discussion

Since the form of the steady state equations discussed here are the same as those for the perfect conductor case their consequences will remain essentially the same except for the restrictions on the choice of the magnetic field **B**. Hence, the discussion provided by Mestel[4,5] will be valid in this special case too.

The following cases are worth noting here :

(i) $v_m = 0$.

When $v_m = o$ we have no circulation. So $k = 0 = k'$ and the angular velocity will be constant on the field lines which is Ferraro's law of isorotation.

(ii) $v_m << v_a = \Omega\bar{\omega}$

This is true for slow circulations (as in the case of the Eddington-Vogt circulations in stars). In this situation

(*a*) If $B_a \lesssim B_m$, we have from either equations (17) and (18) or equations (27) and (28) that

$$\Omega = \text{constant on streamlines.} \tag{33}$$

Now, if the field is not force-free this will be possible. However, if the field is force-free, equations (31) and (33) will be inconsistent. So, in this case, in order to maintain a steady state the field must not be force-free. We might note that this applies equally to the perfect conductor case.

(*b*) If $B_a >> B_m$ such that $v_m B_a \sim v_a B_m$, then equations (18) and (28) retain their forms and there is no apparent inconsistency.

Hence, we might conclude that in a case where the circulation speed is small compared with the rotation speed, the field must either be not force-free or, if it is force-free then $B_a/B_m \sim v_a/v_m$.

What we have shown in this paper is that the equations of Chandrasekhar and Mestel for steady circulation in an axisymmetric perfectly conducting rotating magnetic star may be extended to a fluid of finite conductivity with the additional condition that the magnetic field **B** belongs to the class of solutions of the vector wave equation and has a uniform exponential decay. However, though these equations are convenient for application to models, they represent a situation in which a characteristic feature of material with finite conductivity is absent. Whereas

in the perfect conductor the field lines and the streamlines of circulation must coincide, we know that in material of finite conductivity the field lines may cross the streamlines. Therefore, it is likely that this set of conditions will be relevant only for special cases of rotating magnetic stars. Besides, we should note that in the radiative regions of real stars the electrical conductivity will change with position.

Acknowledgements

This work commenced while the author was a Postdoctoral Research Fellow at the Institut d'Astrophysique de l'Universite de Liege. He is grateful to the Institute and Prof. R. Simon for their kind hospitality.

References

1. CHANDRASEKHAR, S. (1956) *Astrophys. J.* **124** : 232.
2. FERRARO, V.C. A. & PLUMPTON, C. (1966) *An introduction to magneto fluid mechanics*, London : Oxford University Press.
3. MAHESWARAN, M. (1969) *Mon. Not. R. Astr. Soc.* **145** : 197.
4. MESTEL, L. (1961) *Mon. Not. R. Astr. Soc.* **122** : 473.
5. MESTEL, L. (1965) *Stars and stellar systems*. Vol. 7, edited by L.H. Aller and D.B. McLaughlin. Chicago University Press.

J. Natn. Sci. Coun. Sri Lanka 1975 3(1) : 19-29

Myocardial Infarction in Young Ceylonese

N. J. WALLOOPPILLAI AND D. P. ATUKORALE
Cardiology Unit, General Hospital, Colombo, Sri Lanka

(*Paper accepted* : 11 *March* 1975)

Abstract : Forty patients below the age of 40 years with myocardial infarction were studied with a view to finding the aetiological factors of the disease. The cholesterol values of the young patients with myocardial infarction were found to be significantly higher than those of the controls. The lipoprotein profile of young infarcts using nephelometry showed that the M fraction (= VLDL) in the patients was significantly higher than that of the controls. Hyperlipoproteinaemia occured in 45% of the young patients with myocardial infarction, W.H.O. type IV being the commonest. Another interesting feature was that arcus cornea occured in 50% of the young patients with myocardial infarction. 40% of the patients gave a family history of myocardial infarction in first degree relatives under the age of 60 years. Obesity, hypertension and diabetes mellitus are uncommon risk factors in our series. 90% of the patients had one or more of the risk factors for C.H.D. the major risk factors being smoking and abnormal lipoprotein patterns.

The cholesterol, triglycerides and "M" values (VLDL) of the relatives of the patients with myocardial infarction were significantly higher than those of the controls. Elevated VLDL appears to be a risk factor in the young patients with myocardial infarction.

1. Introduction

Coronary heart disease (C.H.D.) appears to have become a serious problem in Sri Lanka. For many years it was considered a disease of middle and old age; more recently there appears to be an increasing incidence in younger individuals.[14,17] This paper therefore focusses attention on the risk factors present in those individuals below the age of 40 years with a definite myocardial infarction.

2. Subjects, Methods and Terminology

The subjects comprised a group of 40 patients below the age of 40 years at the time of the first myocardial infarction, investigated in the Cardiology Unit, General Hospital, Colombo, between January and November 1974. Out of the 40 patients, 32 were admitted in the acute stage of the illness, while 8 patients had well documented myocardial infarction prior to January 1974. Those who failed to survive following the infarction were not included in this study.

All the patients were questioned with regard to (1) occupation and income, (2) any past history of ischaemic heart disease, hypertension, diabetes mellitus or cerebrovascular accidents, (3) smoking habits and (4) history of ischaemic heart disease, diabetes mellitus, cerebrovascular accidents or hypertension among the first degree relatives. In addition to the above, the females were questioned regarding the use of oral contraceptives.

The physical examination included : (1) a full examination of the cardiovascular system, (2) measurement of height and weight and (3) examination for clinical signs of hyperlipoproteinaemia such as arcus corneae, xanthomata, yellow deposits over palmar creases, yellow papules on skin and mucose, lipaemia retinalis and hepatosplenomegaly.

In every patient the following investigations were done routinely : (1) fasting blood sugar and if this is above 100 mg% or if there was a family history of diabetes mellitus in the first degree relatives, a glucose tolerance test 3 weeks following the ischaemic episode, (2) haemoglobin and packed cell volumes, (3) white blood count and differential count, (4) erythrocyte sedimentation rate, (5) plasma proteins, (4) serum cholesterol by Sackett's method, (7) serum triglyceride using method described by Laurell and Scan, after a 14 hour fast, (8) serial E.C.G's and (9) teleradiogram of the chest. The investigations (1)—(5) and (9) were done 2 weeks following the infarction. If the E.S.R. was persistently high, other laboratory tests to exclude collagenosis were performed.

Lipoprotein patterns of the young patients with myocardial infarction, their relatives, and controls were estimated by membrane filtration and nephelometry.[20,21] This is a simple and an inexpensive method of lipoprotein analysis. Blood was drawn after a 14 h fast at least 3 months after the attack of myocardial infarction. All the patients were advised to stop all the lipid lowering agents for at least one month prior to lipoprotein estimation. In this method L particles (chylomicrons, Sf > 400), were separated from M particles (VLDL, Sf 20—400) by membrane filtration of diluted serum and the concentration of particles in each fraction quantified by measurement of light scattering intensity (LSI) using the nephelometer described by Thorp *et al.*[23] The concentrations of the three lipoprotein fractions S (beta fraction, Sf 0—20), M(prebeta, Sf 20—400) and L(chylomicrons, Sf > 400), were calculated from the nephelometric measurements of LSI and the value of serum cholesterol, using the equation described by Stone *et al.*[22] These authors have shown a high degree of correlation between these estimated values and those obtained by analytical ultracentrifugation.

Twenty patients admitted to Cardiology Unit, suffering from noncardiac chest pain, congenital heart disease, chronic rheumatic heart disease and hypertrophic cardiomyopathy between ages of 20 and 40 years were used as a control group. Ten first degree relatives of the subjects were also studied.

Glucose tolerance was assessed by conventional criteria. Patients were considered overweight if their weight was 20% more than normal weight for the respective age, sex and height.[16]

The criteria for the diagnosis of myocardial infarction were the presence of 2 or more of the following : (1) characteristic clinical presentation (2) pathological Q waves, ST elevation or T wave inversion in the electrocardiogram with evolutional changes and (3) rise in serum aspartate aminotransferase (SGOT) and/or rise in total level of lactic acid dehydrogense (LDH) as well as a rise over 70% of the heat stable fraction of the LDH.

A person was considered to be suffering from hypertension when there was sustained diastolic pressure of more than 90 mmHg. In each set of cholesterol estimations made, the laboratory standards and I.C.I. reference standards were estimated as a check on accuracy. A person was considered to be hypercholesterolaemic if the serum cholesterol level was higher than 240% and hypertriglyceridaemic if the serum triglyceride level was higher than 140% respectively. The upper limit of normal for S fraction was taken as 47[illegible] mg% ; the upper limit of normal M fraction was taken as 470 mg%.

3. Results

3.1. Age

The age distribution of the patients with myocardial infarction is given in Table 1, which shows that myocardial infarction is rare before the age 30. The mean age of the group is 34.0 (± 4.89) years.

TABLE 1. Age distribution of young patients with myocardial infarction.

Age	No. of cases	Percentage
20 — 24	1	2.5
25 — 29	5	12.5
30 — 34	11	27.5
35 — 39	23	57.5
Mean age 34.0 (± 4.89)	40	100.0

3.2. Sex

There were only 4 females. Male : Female ratio in our group was 9 : 1.

3.3. Occupation

Table 2 shows that the majority of the patients with myocardial infarction belong to the middle and low income groups. The clerical staff was most commonly affected, followed by technicians.

TABLE 2. Occupations of young patients with myocardial infarction.

Occupation	No. of subjects
Doctors	4
Executives	3
Businessmen	4
Engineers	1
Planters	1
Army Personnel	2
Priests	1
Technical Staff	5
Clerical	10
Teachers	2
Stenographers	1
Housewives	2
Sailors	1
Rubber Tappers	1
Unemployed	2
	40

3.4. E.C.G. Appearances

Seventeen patients developed inferior transmural infarctions and 14 had anterior transmural infarctions. There was one patient who had two infarctions.

3.5. Risk Factors

3.5.1. *Hypercholesterolaemia and Hypertriglycerideamia*

Twelve patients had hypercholesterolaemia and 12 had hypertriglyceridaemia (Table 3). Of these, 5 patients had both hypercholesterolaemia and hypertriglyceridaemia. The cholesterol values of young patients with myocardial infarction were observed to be significantly higher than those of the controls (Table 4).

TABLE 3. Risk factors in young patients with myocardial infarction.

Risk factors	No. of patients	Percentage
Hypercholesterolaemia*	12	30.0
Hypertriglyceridaemia*	12	30.0
Hypertension	5	12.5
Smoking	27	67.5
Abnormal glucose tolerance	2	5.0
Obesity	9	22.5
F.H. of I.H.D.	16	40.0

*5 patients had both hypercholesterolaemia and hypertriglyceridaemia.

TABLE 4. Comparison of serum lipid values among patients with myocardial infarction and controls.

	Young Infarcts n = 40		Control n = 20		
	Mean	S. D.	Mean	S. D.	
Age	34.0	4.89	30.0	5.38	P > 0.1
B.P. Systolic	125.0	13.81	120.0	9.43	P > 0.1
B.P. Diastolic	78.0	4.58	78.0	7.35	P > 0.1
Serum Cholesterol	231.0	50.01	204.0	31.37	0.05 > P > 0.02
Serum Triglycerides	108.0	106.10	64.0	37.38	0.1 > P > 0.05

3.5.2. *Lipoprotein estimation*

Lipoprotein estimation showed that L(Sf > 400) and S(=Sf 0—20), values of patients showed no significant difference when compared to controls (Table 5); the M values (=Sf 20—400) of patients were significantly higher than those of the controls. In case of relatives of patients with myocardial infarction, cholesterol, triglyceride and M values were significantly higher than those of the controls (Table 6). Table 7 shows that out of 40 patients, 2 had type IIa pattern, 3 had IIb pattern, and 13 had type IV pattern indicating that 45% of the young patients with myocardial infarction had some type of hyperlipoproteinaemia.

TABLE 5. Comparison of serum lipoprotein values of patients and controls.

	Young Infarcts n = 40		Controls n = 20		
	Mean	S.D.	Mean	S.D.	
Age	34	4.89	30	5.38	p > 0.1
L(Sf over 400)	20	11.75	15	6.48	0.1 > P > 0.03
M(Sf 20 — 400)	450	246.12	306	81.01	0.02 > P > 0.01
S(Sf 0 — 20)	392	101.54	344	66.46	P > 0.1

3.5.3. *Hypertension*

The mean systolic blood pressure for the young infarcts (Table 4) was 125 mmHg (S.D. 13.81) and diastolic 78 mmHg (S.D.4.58) and there were 5 patients who had been treated for diastolic hypertension in our series. In three of them, blood pressure returned to normal following the infarction. In the remaining 2, persistent diastolic pressures of 95 to 100 mmHg and 105 to 110 mmHg respectively were recorded following the myocardial infarction.

TABLE 6. Comparison of serum lipid and lipoprotein values of relatives of patients and controls.

	Relatives of Young Infarcts n — 10		Controls n — 20		
	Mean	S.D.	Mean	S.D.	
Age	33.0	4.36	30.0	5.38	
B.P. Systolic	124.0	11.49	120.0	9.43	
B.P. Diastolic	84.0	5.91	78.0	7.35	
Serum Cholesterol	235.0	20.53	204.0	31.37	0.05 > P > 0.02
Serum Triglycerides	132.0	51.18	64.0	37.38	0.001 > P
L(Sf > 400)	18.0	10.72	15.0	6.48	P > 0.1
M(Sf 20 — 400)	550.0	29.43	307.0	81.07	0.001 > P
S(Sf 0 — 20)	374.0	54.55	344.0	66.46	P > 0.1

TABLE 7. Frequency distribution of different types of hyperlipoproteinaemia.

Type of Hyperlipoproteinaemia		Frequency distribution Young myocardial infarction	
Classification		Number	Percentage
SML	*WHO recommended*		
S	IIa	2	5.0
SM + MS	IIb	3	7.5
M + ML	IV	14	32.5
ML*	V	0	0

*ML patterns in which the large particles (Sf > 400) concentration exceeded 100mg/100ml.

3.5.4. *Cigarette Smoking*

There were 27 smokers and 13 non-smokers (Table 8). There were no patients who gave a history of cigar or pipe-smoking. Sixteen patients gave a past history of smoking less than 20 cigarettes a day and 7 had smoked over 3 packets (30 cigarettes) a day for several years.

3.5.5. *Diabetes Mellitus*

There were only 2 cases of diabetes mellitus confirmed by glucose tolerance test. Both were mild diabetics and were controlled on diet alone.

TABLE 8. Prevalence of cigarette smoking in young patients with myocardial infarction.

No. of cigarettes smoked per day.	No. of subjects
Less than 20	16
20 — 30	4
Over 30	7
	27

TABLE 9. Clustering of risk factors in patients with myocardial infarction.

No. of risk factors present	No. of subjects	Percentage
0	4	10.0
1	12	30.0
2	11	27.5
3	10	25.0
4	3	7.5

3.5.6. *Family History*

Sixteen patients gave a history of myocardial infarction in first degree relatives under the age of 60 years. Of these, 8 patients gave a history of myocardial infarction in 2 or more of their first degree relatives. Six patients gave a family history of diabetes mellitus among first degree relatives ; there were no cases with a family history of hypertension.

3.5.7. *Obesity*

Nine of the 40 patients had obesity.

3.5.8. *Haemotocrit, Sedimentation and Plasma Proteins*

Estimations of haemoglobin and packed cell volumes were done in all the cases. These values were found to be within normal limits. Erythrocyte sedimentation rate and plasma proteins were within normal limits.

3.5.9. *Arcus Cornea and Xanthomaia*

Twenty patients had arcus corneae and 5 patients had xanthelasma. Tuberous xanthomata were seen only in 3 patients. There were no patients with yellow papules over the skin or yellow palmer creases or with lipemia retinalis. Two patients had slight hepatomegaly but they gave a past history of alcohol consumption.

4. Discussion

It has been recognised for many years that ischaemic heart disease is an uncommon condition under the age of 40. A review of recent literature emphasies that more and more frequently coronary heart disease is diagnosed in young adults.[14,17,24]

In Sri Lanka myocardial infarction occurs on a background of atherosclerosis.[5] Table 3 shows that well known risk factors such as hypercholesterolaemia, hypertriglyceridaemia, hypertension, smoking, obesity, abnormal glucose tolerance and family history of ischaemic heart disease in first degree relatives were quite common in this group of young infarcts.

Table 9 shows that a majority of patients had more than one risk factor. In 4 patients there was no obvious risk factor and in 12 patients, only one risk factor was present.

Diabetes mellitus has often been suggested as an important explanation for the premature development of ischaemic heart disease.[7] In our series only 5% of the patients had evidence of abnormal glucose tolerance. In the series reported by Oliver,[12] only 4 out of 94 patients had abnormal glucose tolerance. In our series only 12.5% of patients gave a past history of hypertension. Thus hypertension and diabetes mellitus are uncommon risk factors in our series.

The effect of obesity on the incidence of coronary heart disease remains uncertain. French and Dock[6] analysing the clinical and pathological features of 80 fatal cases of young soldiers between 20 and 36 years revealed that the most striking presumable predisposing factor was overweight which was present in 91% of the cases and the basis of the coronary occlusion was found to be arteriosclerosis in all cases. Ancel Keys and his colleagues[8] suggested that neither relative weight nor obesity assessed by skinfold thickness had any significant effect on future coronary heart disease if the effects of increased age, serum cholesterol blood pressure and smoking were discarded. Authors of the Framingham study predict that for each 10% increase in weight, there is a 30% increase in the incidence of coronary heart disease. 9 (22.5%) in our series were obese and the incidence of obesity is higher than in an earlier series reported from Sri Lanka.[11]

In our series, 12 patients (30%) had hypercholesterolaemia and 12 (30%) had hypertriglyceridaemia (Table 3). Of these, 5 had both hyperchloesterolaemia and hypertriglyceridaemia. The cholesterol values of the young patients with myocardial infarction were observed to be significantly higher than those of the controls ($P<.05$) whereas comparison of the triglyceride levels of patients with controls showed no significant difference ($P>05$). Lewis and colleagues[10] too, found a similar incidence of hypercholesterolaemia and hypertriglyceridaemia in their coronary patients of the age group 26 to 39 years. There can be little doubt that an increase in cholesterol

and triglycerides levels predispose to overt and early appearance of clinical coronary disease.[10] There is evidence that in younger patients with I. H. D. hypercholesterolaemia is a commoner abnormality than in older patients.[13] A prospective study in Stockholm found that the occurrence of new events in coronary artery disease were linearly related to increased triglycerides, cholesterol and smoking and not to an increase in weight/height index.[1] Valentine *et al.*[24] investigated the angiographic appearances of coronary arteries in 40 patients below the age of 40 years with documented cardiac infarction and found an association between elevation of triglyceride levels with the more severe and more diffuse type of occlusive process. Raised triglyceride values were interpreted to be an independant risk factor for I.H.D. in 2 recent prospective surveys[1,15] but not in others.[2]

Estimation of the lipoprotein profile of young infarcts using nephelometry showed that the M fraction (VLDL, Sf 20—400) in the patients was significantly higher than that of the controls ($P < .02$) In the series reported by Lewis *et al.*[10] increase in the VLDL (M) fraction was the most pronounced abnormality detected in the young coronary patients and VLDL cholesterol contributed substantially in some patients to the total serum cholesterol. There is controversy regarding the changes in M fraction following an acute myocardial infarction. Smith[18] reported a rise in triglyceride rich prebeta (M) fraction reaching a maximum in 3 to 5 weeks after cardiac infarction. Dodds and Mills[3] confirmed these findings and showed that return to normal levels occured after the 8th week. The lipoprotein estimation in our cases was performed at least 3 months after the attack of cardiac infarction. The significantly high M(Sf 20—400) levels in our cases suggest that elevated M fraction may be a risk factor for coronary heart disease in young Ceylonese.

Another interesting feature in our series is that the triglyceride values and the M fraction of the relatives of the young infarcts were significantly higher than those of the controls, ($P < .001$) whereas the triglycerides of the patients were not significantly different from those of the controls (Tables 4 and 6). It is probable that the patients had modified their diet during the first 3 months following infarction to alter their triglyceride values from what prevailed before the infarct. If we assume that the dietary habits of the young patients with myocardial infarction and their relatives are similar, then the patients might have made drastic changes in their diet, since their "attack" to lower their triglyceride values in particular, whereas the relatives who had not been adequately motivated to alter their diet would show triglyceride values higher than those of the controls.

Table 7 shows that there is a very high prevalence of hyperlipoproteinaemia as assessed by membrane filtration and nephelometry.[20] Type IV hyperlipoproteinaemia appears to be the commonest type, and 45% of our patients showed some lipoprotein abnormality. Our series is similar to the series reported by Stone

and Dick[19] who using the same method of lipoprotein estimation found that 20% of his control group and 40% of the ischaemic heart disease patients had hyperlipoproteinaemia, type IV (W.H.O.) being the commonest. The method of membrane filtration and nephelometry has been shown to produce reproducible analysis in different laboratories.[21] The same authors have compared the results of analytical ultracentrifugation with those obtained by membrane filtration and nephelomtery and have demonstrated a high correlation ($r = 0.96$) between ultracentrifugation and nephelometric measurements. In the series reported by Lewis *et al.*[10], out of 15 patients under the age of 40 years 11 had hyperlipoproteinaemia, the commonest abnormality being type IV. The high incidence of hyperlipoproteinaemia in young infarcts has not been reported previously from Sri Lanka.

Paul and Siegel[14] in a study of 19 cases of myocardial infarcts below the age of 40 years found that 95% of the patients were cigarette smokers. Serum cholesterol was higher in the patients compared to the controls. The blood pressures were not very different although the diastolic pressures were higher in the coronary group. A family history of cardiovascular disease and diabetes occurring before the age of 65 years was found in approximately half the coronary group. Our group is similar to the series reported by Paul and Siegel[14] in that cigarette smoking, hypercholesterolaemia and positive family history are common findings in both series (Table 3).

Arcus corneas below the age of 40 years is associated with premature atherosclerosis. In our series 20 (50%) had arcus corneae. Three patients with type II hyperlipoproteinaemia had xanthomata in addition to arcus corneae.

The most striking feature in our series is that 90% of the patients had one or more of the risk factors for coronary artery disease. The major risk factors in our group were smoking and abnormal lipoprotein patterns.

Acknowledgements

We are grateful to the National Science Council for the grant which enabled this research project to be carried out. We wish to thank Dr. A. V. B. Perera, the Clinical Biochemist, General Hospital, Colombo., Dr. U. D. Wijayawardhane, Registrar, the house physicians and nursing staff of the Cardiology Unit, Mr. O. Harridge and Mr. C. M. Anthony of the Lipid Research Laboratory of the Cardiology Unit, and all the physicians and general practitioners who referred in the cases. A special word of thanks to I.C.I. Ltd. for donating the Nephelometer. We cannot conclude without thanking Dr. J. Jayaratnam, Senior Lecturer, Department of Public Health and Community Medicine, Colombo Medical Faculty for the statistical analysis of the data.

References

1. Carlson, L. A. & Bottiger, L. E. (1972) *Lancet* **1** : 865.
2. Coronary Drug Project (1970) *J. Am. Med. Ass.* **220** : 996.
3. Dodds, C. & Mills, G. L. (1959) *Lancet* **1** : 1160.
4. Doyle, J. T., Dawber, T. R., Kannel, W. B., Kinch, S. H. & Kahn, H. A. (1964) *J. Am. Med. Ass.* **190** : 886.
5. Fernando, P. B., Kanagarajah, M., De Silva, W. S. S. & Rajasuriya, K. (1965) *J. Trop. Med. Hyg.* **68** : 69.
6. French, A.J. & Dock, W. (1944) *J. Am. Med. Ass.* **124** : 1233.
7. Herman, M. V. & Gorlin, R. (1965) *Am. J. Med.* **38** : 481.
8. Keys, A., Aravanis, C., Blackburn, H., Van, B. F. S. P., Buzina, R., Djordjenic, B. S, B. S., Findazo, R., Karvonen, M. J., Merotti, A., Puddu, V. & Taylor, H. L. (1972). *Ann. Internal Med.* 77 : 15.
9. Laurell, S. & Scan, J. (1966) *Clin. & Lab. Invest.* **18** : 680.
10. Lewis, B., Chait, A., Oakley, C. M. C., Wooton, I. D. B., Krikler, D. M., Onitri, A. Sigurdssom, S., & February, A. (1974) *Br. Med. J.* **3** : 489.
11. Nagaratnam, N., Gunawardena, K. R. W. & De Silva, S. P. (1973) *Indian J. Med. Res*, **61** : 1173.
12. Oliver, M. F. (1974) *Br. Med. J.* **4** : 253.
13. Patterson, D. & Slack, J. (1972) *Lancet* **1** : 393.
14. Paul, O. & Siegel, R. (1973) *Singapore Med. J.* **14** : 339.
15. Rosenman, R. H. *et al.* (1970) *J. Chronic Dis.* **23** : 173.
16. Sargent, D. W. (1963) *Am. J. Clin. Nutr.* **13** : 318.
17. Schettler, G. (1973) *Singapore Med. J.* **14** : 334.
18. Smith, E. B. (1957) *Lancet* **2** : 910.
19. Stone, M. C. & Dick, T. B. S. (1973) *Br. Heart J.* **35** : 954.
20. Stone, M. C. & Thorp, J. M. (1966) *Clin. Chim. Acta* **14** : 812.
21. Stone, M. C., Thorp, J. M., Mills, G. L. & Dick, T. B. S. (1970) *Clin. Chim. Acta* **30** : 809.
22. Stone, M. C., Thorp, J. M., Mills, G. L. & Dick, T. B. S. (1971) *Clin. Chim. Acta* **31**: 333.
23. Thorp, J. M., Horsfall, C. B., & Stone, M. C. (1967) *Med. & Biol. Engng* **5** : 51.
24. Valentine, P. A., Hare, W. S. C. & Sloman, J. G. (1973) *Singapore Med. J.* **14** : 341.

J. Natn. Sci. Coun. Sri Lanka 1975 3(1) : 31-39

On Examinations

T. D. M. A. SAMUEL
Department of Engineering Mathematics, University of Sri Lanka, Peradeniya Campus, Peradeniya, Sri Lanka.

(*Paper accepted :* 17 *March* 1975)

Abstract : The parameters possibly influencing the performance at examinations are discussed. After a simple analysis, the results of a particular examination are discussed in terms of these parameters. This discussion reveals the need for the definition of a 'Standard Performance Range'.

1. Introduction

The main purpose of this paper is to increase the understanding of the machinery of examinations. In order to achieve this, the parameters possibly influencing the 'performance' at an examination are postulated. This is followed by a general discussion of these parameters and a discussion of general remedial measures against unfavourable influences of these parameters at examinations. A need for the introduction of definitions of 'performance', etc. is immediately felt and these are provided in the Appendix.

This is followed by a very simple analysis of the Final Part I Examination in Engineering of the University of Sri Lanka, Peradeniya Campus held immediately after a change in Regulations and Syllabus. The analysis reveals certain inconsistencies. Guide lines are therefore suggested for the maintenance of a certain degree of consistency.

2. Parameters influencing end of year Examination

2.1.

In this paper, examination is viewed in its role in ranking students belonging to a particular batch and in deciding on pass/fail. Feed back information for teaching, etc. are not considered. The rank and pass/fail of candidates depend on their performance in individual subjects and also on the examination as a whole. The performance in a particular subject at an examination depends largely on :—

(i) standard of paper
(ii) standard of marking
(iii) attitude of examiner towards candidates
(iv) examination temperament of candidates
(v) attitude of student towards subject
(vi) nature of the subject
(vii) foundation in allied subjects in the previous years.

The first three are in the domain of influence of the examiner, the fourth and fifth in that of the candidate and the last two depend on the subjects. At this stage, it does not appear to be possible to determine the exact degree to which each of the above factors influence performance. Factors like (vi) which are not totally independent of the other factors would make this kind of analysis extremely difficult if not impossible. But an analysis of the performance at an examination, influenced to varying degrees by these factors, can increase our understanding to enable us to formulate in the future a reasonable Mathematical model.

In this context, if it is assumed that (i) – (v) and (vii) are conducive to a 'standard performance', any unusual performance (see Appendix) in any particular subject or subjects *may be attributed* to the difference in the nature of the subject or subjects. The existence of this kind of difference is seen from the example of distribution curves for subjects in a School Certificate Examination obtained by Crofts and Jones[1] as shown in Figure 1.

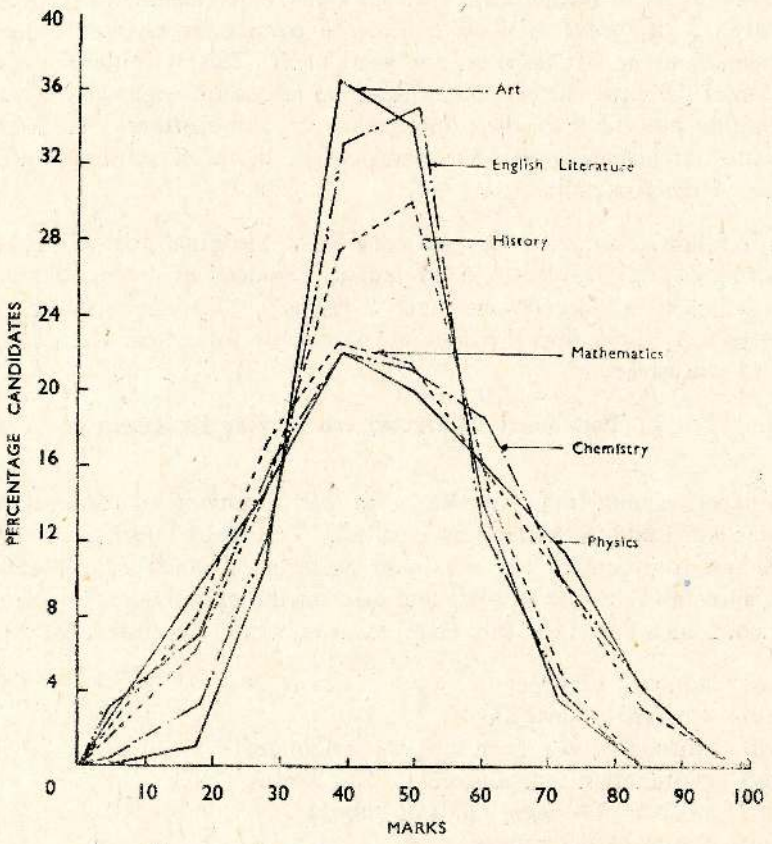

Figure 1. Shows two distinct groups of subjects.

In this example, the subjects fall into two distinct groups. The curves are flatter for subjects like Mathematics and Science and they are peaked towards the centre for subjects like History and Art. In the latter case a very large number of candidates get marks in a range about 45. In the former, there is a wider spread with the possibility of very high and very low marks. These differences in curves are reported by Crofts and Jones to be persistent. Furthermore, it is found that the coefficient of rank correlation (see Appendix) between examination and class performance (continual assessment) is as small as 0.75 compared with 0.85 for the former (flatter curves). These are due to inherent differences in the subjects.

In the event of the existence of two distinct subject groups as described above, the following difficulties arise :—

(i) Assume that in an examination only one subject—subject A (say)—belongs to that group where it is difficult to score high marks. If this examination is a competitive one, in which case the rank of candidates is important, then those not offering A will be at a definite advantage over the others.

(ii) This can also affect the 'doubtful cases' because subject A with only low marks will reduce the average which has an influence on the decision of the board of examiners.

A conscious effort to remove these difficulties must be evident in any examination system. Having established the nature of the subjects in an examination we can lay guide lines for consistency in (i)—(v).

2.2. Examiner

(i) and (ii) are dependent on the examiners. Assuming that (iii)—(vii) do not have any adverse influence on the examination, it is reasonable to expect a fair degree of consistency in performance in a particular subject. This would be true provided the different batches are statistically equivalent and that the standard of the subject has not changed drastically. If this position is granted, a useful guide would be to compare the performance in any examination against a 'model performance range'. This is discussed in the sections to follow.

2.3. Candidates

Poor performance due to (iv) can be alleviated by continual assessment of the students in the different subjects. It can take the form of tests in course units or its equivalent in the form of tutorials. On the results of this, and the end of year examination, it is possible to determine the coefficient of rank correlation between continual assessment and the final examination. If in a particular examination this 'coefficient' is large enough, then a student who has just failed to obtain the pass mark can be pushed up on the strength of the continual assessment marks.

It is difficult at this stage to formulate guide lines against failings in (iii) and (v), but it is hoped that the elimination of discrepencies in (i), (ii), (iv), (vi) and (vii) will alleviate failings in (iii) and (v).

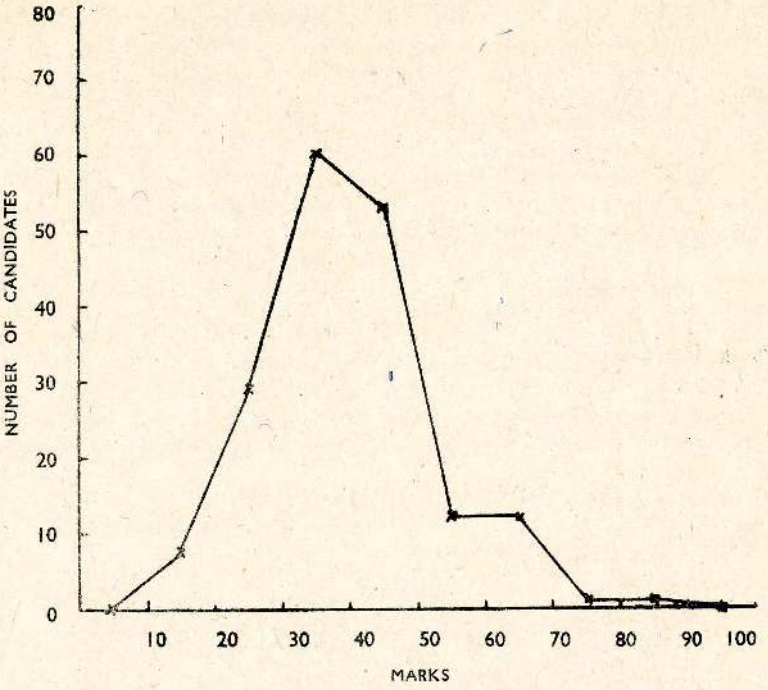

Figure 2. Frequency distribution for July 1969 Examination.

3. Analysis of Part I (New Regulations) results

Figure 2 gives the frequency distribution curve for the averages obtained by the students in the July 1969 examination. This was the first time that this examination was held. The parameters for the averages are as follows :

Arithmetic Mean = 39.0	UQ-Median = 7
Median = 38.0	Interquartile Range = 14
Mode = 34.5	Quartile Deviation = 7
Lower Quartile (LQ) = 31	Standard Deviation = 12.45
Upper Quartile (UQ) = 45	Third Moment = 0.99*
Median-LQ = 7	Fourth Moment = 3.69*

*Third and Fourth Moments for a Normal Distribution are 0 and 3 respectively.

These show that the average representative of the group is 39% and the average most frequently awarded is 34.5%. But the distribution is fairly symmetric about 38%. Thus a symmetric distribution has been obtained at the cost of an unusually

very poor performance. The performance at this examination as measured by the averages is shown by curve A of Figure 3. The performance in the August 1970 examination, of only those who failed the July 1969 examination, is shown by curve B. It is seen that the performance is better in the whole range of the averages. This is very unusual assuming consistency in (i)—(vii). A slightly better performance in a small range about 40%, would have been a reasonable result. This is seen in the case of the August 1970 first time failures repeating the examination in March 1971 as shown by curves C and D of Figure 3.

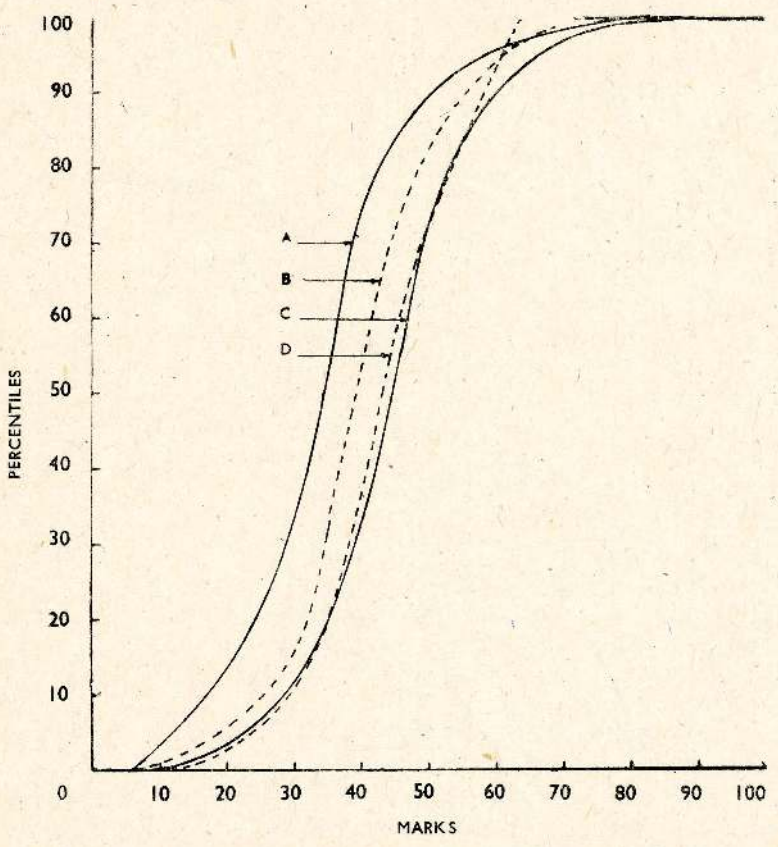

Figure 3. A—Part I, July 1969 ; B—Failures of Part I July 1969 sitting examination in August 1970 ; C—Part I, August 1970 first attempt only ; D—Part I Repeat Examination March 1971.

The performance at the August 1970 examination is a big improvement on the July 1969 examination. The performance in each of the five subjects at the July 1969 examination is shown in Figure 4. All except subject 4 have comparable performance. The general performance of the batch determined by the percentile

curve of the averages AV as shown in the figure has been greatly influenced by subject 4. Figure 5 shows the performance curves in the different subjects of the August 1970 examination. The performance range of the subjects is fairly wide and there is no substantial agreement in performance in any two subjects. There are extreme qualities in performance like subject 4 at one end and subjects 1 and 5 at the other. With this wide variety in quality of performance, it is very unlikely that tests in all subjects have been successful in the just fulfilment of their roles. This shows the need to define a 'Standard Performance Range'.

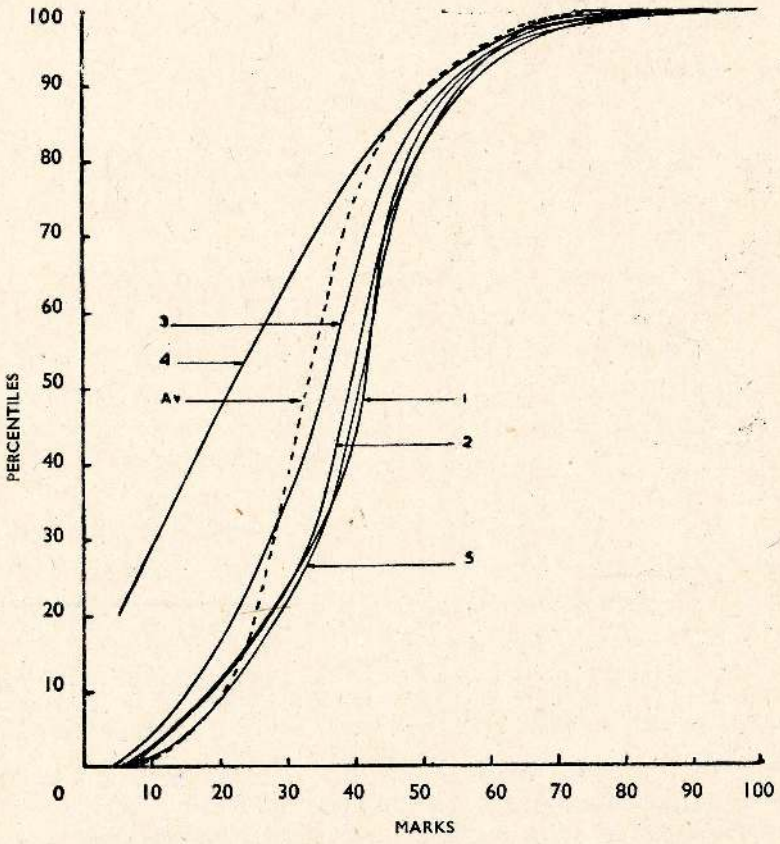

Figure 4. The percentile curves for subjects 1 to 5 and average AV of the July 1969 Examination.

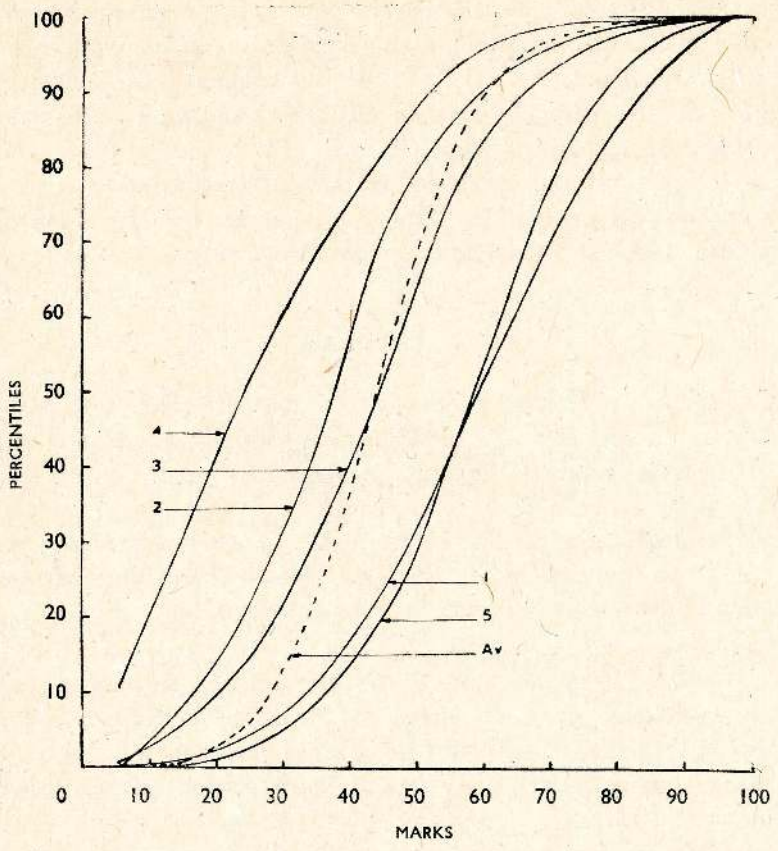

Figure 5. The percentile curves for subjects 1 to 5 and average AV of the August 1970 Examination.

4. Suggestions

A 'Standard Performance Range' for the Part I New Regulations examination as a whole may be obtained from the analysis of the averages of an 'equivalent examination' the results of which over a long period of time, are already available. When any examination performance falls outside this range i.e. when the performance is unusual, it is advisable to analyse the results in order to ascertain which of the parameters has influenced it. If it is possible to establish this, then a method of bringing the performance to within the 'Standard Performance Range' — *standardisation* — may be found. This may also be achieved by determining the 'Standard Performance Range' for each subject ; and ensuring — if possible, after analysis of results — that the performance in each subject falls within its subject performance range. This in itself will not ensure that the performance in the whole examination will fall within

the Performance Range of the whole examination, if one of the subjects is influenced by parameter (vi). In this case, in order to surmount difficulties (i) and (ii) discussed in section 2, the marks in this subject should be weighted. The weightage can be determined using the percentile curve of this subject and a percentile curve—to be chosen— in the 'Standard Performance Range'. If it is found that it is not possible to do any of these in order to correct an unusual examination performance and therefore since the examination has failed in its role, then the only course open is to repeat the examination. This defines a *repeat examination.*

5. Conclusions

The traditional end of year examination is still the only method of assessment in many institutions in Sri Lanka. Even if other methods of assessment are introduced, there are compelling reasons for the continued existance of the traditional examination as a major assessment method. It is therefore essential that it is made more reliable. The gradual evolution of a 'Standard Performance Range' is therefore of paramount importance to any educational institution. The author expects the 'Standard Performance Range' to be different for different institutions.

A major assumption in this paper is that the different batches of students are statistically equivalent. In this context, the 'Standard Performance Range' can indicate any sudden change in quality of students entering the institution. The author hopes to show a method of determining a 'Standard Performance Range' in a subsequent article.

Appendix

Definitions

The mth *percentile*, Pm, of a frequency distribution is the value of the variable x, that corresponds to the cumulative frequency, m per cent of N.

In the problem considered, x = percentage marks/average, m = percentage of students obtaining a certain percentage of marks/average, N = total number of students.

Ranked Data

Data arranged in order of relative magnitude or importance is called Ranked Data.

Coefficient of rank correlation

This is defined by the formula (Spearman formula)

$$r_R = 1 - \frac{6 \Sigma d^2}{N(N^2 - 1)}$$

where d is the difference between any paired ranks. The value is restricted to the range $-1 \leq r_R \leq +1$. No assumption concerning underlying distribution of variables is made.

New Definitions

The *performance* of a group of students in any subject or in the whole examination is defined in terms of the Percentile Curve (Orgive Curve). Two such curves can define the boundaries for a range of performance. Performance in a subject/examination is said to be *Standard* if the Percentile Curve falls within this range. If it falls outside this range then performance is said to be *Unusual.*

Acknowledgements

The author is grateful to Mr. V. Sanmuganayagam of the Faculty of Engineering, University of Sri Lanka, Peradeniya Campus for spending his valuable time in drawing the figures.

References

1. CROFTS, J. M. & JONES, D. C. (1927) *Secondary school examination statistics*, p. 49, London : Longmans. Green & Co. Ltd.

J. Natn. Sci. Coun. Sri Lanka 1975 3(1): 41-50

Cyanogenic Glucoside Content of Manioc

III. Fate of Bound Cyanide on Processing and Cooking

NIRMALA PIERIS* AND E. R. JANSZ

Industrial Microbiology Section, Ceylon Institute of Scientific and Industrial Research (CISIR), P.O. Box 787, Colombo 7, Sri Lanka.

(*Paper accepted : 2 May* 1975)

Abstract : On preparation of manioc starch, bound cyanide is converted to free cyanide and removed with the wash water. Whereas, aging of moist manioc chips leads to a decrease in total cyanide content, aging of whole tubers results in increased total cyanide content of the flours prepared by these processes. The cyanogenic glucosides of manioc flour are partly lost when bread and 'roti' are prepared. However, 'roti' contains free cyanide. Preparation of 'pittu' from manioc flour and fried manioc chips from fresh manioc gave products with negligible free cyanide but nearly the full amount of bound cyanide. The above results are discussed in terms of the linamarin-linamarase reaction.

1. Introduction

Manioc contains cyanogenic glucosides and also the enzyme (linamarase) which causes its hydrolysis. In the plant material the enzyme and glucosides are localised such that they do not interact unless the cells are damaged. When this occurs, reactions take place which result in the liberation of cyanide.[2]

Traditionally manioc has been consumed in this country in the form of the boiled product (the cyanogenic glucoside content of boiled manioc has been reported previously[4]). However, the manioc tuber has poor storage characteristics and in addition to quick microbial spoilage, it has also been reported that the cyanide content of the tuber increases on keeping.[5] In this study we present results that are consistent with the above report. As a result, it is clear that if manioc is to be used extensively, it must be processed to a form with better keeping qualities.

The two main forms of processed manioc are starch and flour. Starch is the product of a wet extraction process of manioc and contains very little cyanide,[4] while flour prepared by a dry-milling process contains considerable quantities of cyanogenic glucosides when no special detoxification process is used.[3] Manioc flour would be used in preference to manioc starch as a substitute for wheat flour in several bakery products and other traditional types of food such as ' roti ', ' pittu ', etc. because of its lower cost. Although methods of detoxifying flour were available,[3] the flour used for cooking purposes usually contains sizeable amounts of cyanogenic glucosides.

*This paper forms a part of the Ph.D. thesis (University of Sri Lanka, Colombo Campus) of Nirmala Pieris.

In this study, the effect of cooking processes such as frying, roasting, baking and steaming on the cyanogenic glucosides of the manioc flour used has been investigated. The fate of bound cyanide when starch is prepared and also when manioc is processed after aging of the moist chips has been studied.

All assays have been done after enzymic hydrolysis of cyanogenic glucosides using excess linamarase to ensure complete hydrolysis.[4]

2. Experimental

2.1. Sampling

2.1.1. *Tubers for aging experiments (for section* 3.3)

Manioc (50 to 60 lbs) of the same variety was harvested with supervision and divided at random into 10 batches each containing 5 to 6 lbs. Each batch contained about 10 tubers. Two batches were processed for each experimental point.

2.1.2. *Sampling of chips (for section* 3.2)

Method (1) : Manioc was sampled by the method of quartering described earlier[4] which were uniformly chipped to give 2 samples of similar cyanide content.

Method (2) : Manioc (about 20 lbs) was chipped and sampled into batches as described.[3]

2.2. Preparation of manioc flour

The manioc chips (spread out as much as possible to give quick drying) were dried in a forced draft oven at 55° C. For aging experiments, each experimental point was done in duplicate. The dried chips (of each sample) were powdered separately and the total cyanide content determined. Flours for the preparation of 'pittu', 'roti' and bread were assayed and used after sieving (600μ).

2.3. Preparation of manioc starch

Manioc starch was prepared by homogenisation of the peeled, washed manioc tuber (approx. 600 g) with 1 litre of water in a Waring blendor. The starch milk was sampled for total cyanide determination and separated from the pulp by sieving to give the 1st pulp wash. The pulp was washed twice with similar volumes of water and sieved each time to give the 2nd and 3rd pulp washes. The remaining material which does not pass through the sieve (unbroken cellular material) is termed the pulp waste. After the specified time, the wash water of each pulp wash was separated from the starch cake to give the 1st starch wash waters. Each starch cake was resuspended in water and left to settle to give the three 2nd starch washes. Free and bound cyanide were determined on each of the starch washes, the pulp waste and starch (on the former directly and on the other two after drying in a forced-draft oven for 24 h).

2.4. Preparation and sampling of cooked products

Manioc bread (30% manioc flour and 70% wheat flour) was prepared by incubating the mixture (containing yeast and other requirements) for 3 h and 5 h and baking at 220°C for 25 min. A wheat flour bread control was used as a blank. The bread crust ($\sim$ 1 cm) was separated from the rest of the loaf and both types of sample were cut into pieces about 1 cm cube. Random samples containing about 10—15 g dry weight were withdrawn for cyanide and moisture determination.

Manioc 'roti' was prepared by mixing manioc flour and scraped coconut in the ratio of 2 : 1. A small amount of water was added to give the correct consistency and the mixture made into a mass of approximately $\frac{1}{2}$ cm thick and 15 cm diameter and left to stand (for the given time) before roasting. Roasting was done on an aluminium plate heated by a direct flame, a little coconut oil being smeared to prevent sticking. After the roasting process, the roti was cut up into pieces of about 1 cm square and random samples were withdrawn for analysis. Parallel wheat flour samples gave no blank values on assaying for total cyanide.

Manioc pittu was prepared using a 1 : 1 mixture of scraped coconut and manioc flour. Water was added to the mixture until discrete pellets formed and the mixture was immediately introduced into a steamer of about 7 cm diameter and 20 cm long and the pittu steamed for 15 min over boiling water. The pittu was then crushed and sampled for analysis.

Fried manioc was prepared by frying chips approximately 2 mm thick in coconut oil until slightly brown in colour (moisture level of 1—2%). The fried chips were powdered for the determination of free and bound cyanide, moisture and linamarase activity.

2.5. Determination of free and bound cyanide

This was done by the methods described.[4] However, for studies on cooked manioc products, about 300 units of linamarase were used on samples of about 15 g dry weight.

2.6. Linamarase activity

In this study we have attempted to gauge the relative linamarase activity of samples of flour by estimating the amount of cyanide released at fixed intervals after incubating 10 g dry weight of flour with 100 ml of water. This is not the absolute linamarase activity as the substrate linamarin (in the material) is not saturating. There also appeared to be a time-lag for this reaction possibly for the absorption of water by the flour. We would prefer to call this set of data an 'enzyme activity index' and it is felt that this index is more significant than absolute linamarase activity in this instance.

3. Results

3.1. Fate of cyanide in the preparation of starch

Results showed that more than 80% of the glucoside was converted to cyanide and removed in the wash water (Table 1). Results of other experiments showed that complete conversion of the glucoside to cyanide may occur as early as 4 h after disintegration of the cells (depending on the material used). It was also found that both the pulp waste and the starch, after drying in a forced-draft oven, contained only small quantities of total cyanide.

TABLE 1. Fate of cyanide during processing of manioc to starch.

		Cyanide mg/kg Tuber (fresh wt)	
Pulp wash	Starch wash	Free	Bound
1st	1st	117	<3
2nd	1st	13	<3
3rd	1st	<3	<3
1st	2nd	4	—
2nd	2nd	<3	—
3rd	2nd	<3	—

The original manioc sample contained 163 mg CN^-/kg fresh weight. The pulp was washed 3 times (1st, 2nd and 3rd pulp washes) and sieved and left to settle for 24 h. The supernatant (1st starch wash) was tested for free and bound cyanide and the starch cake resuspended in water and left to settle for a further 24 h for the 2nd starch wash.

— not determined.

3.2. Effect of aging of moist chips

These experiments were done to check the commonly believed hypothesis that moist chips on aging followed by drying gave reduced total cyanide levels in the flour prepared from it. The experiments were done with two methods of sampling. Both methods resulted in the duplicate samples of flours giving total cyanide levels that tallied to within 15% error. Table 2 shows the results obtained using sampling method 1. Each batch originated from different manioc samples and a strict comparison on the effect of aging cannot be made from one batch to another due to differences in enzyme levels from batch to batch. The results show that there is a progressive loss of cyanide with aging. A similar conclusion was reached with method 2 of sampling (Table 3) where all points were obtained from the same batch of manioc. It must be noted, however, that although 16 to 24 h aging results in the loss of a large part of the cyanide, the chips frequently showed sliminess and odour associated with bacteriological spoilage.

TABLE 2. Effect of aging moist manioc chips.

Batch No.	Time of aging (h)	Total cyanide mg/kg (dry wt) Initial (no aging)	Final
1	4	233	246
2	4	134	114
3	8	288	134
4	8	349	154
5	16	334	176
6	16	267	86
7	24	350	74
8	24	157	42

Manioc was sampled by method 1, chipped, dried at 55°C for 24 h in a forced draft oven and powdered after which total cyanide was determined.

TABLE 3. Effect of aging moist manioc chips.

Time of aging (h)	Total cyanide mg/kg (dry wt) A	B
0	179	153
4	148	151
8	118	134
16	75	73
24	33	32

Manioc was sampled as in method 2, chipped, dried at 55°C for 24 h and powdered. Duplicate samples (A and B) were tested for total cyanide after aging (room temperature).

3.3. Aging of manioc tubers

This set of experiments was done in order to determine if the total cyanide content of manioc flour was affected by the aging of the tubers. Results obtained showed that duplicate preparations of flour showed deviations on some occasions. It was felt that this was due to inadequate sampling of tubers (the cyanide content of tubers varies very widely and therefore a representative sample of tubers can be obtained by using a very large number of tubers per sample). However, the results obtained (Table 4) show a definite trend of increased cyanide content, the magnitude of which tallied with the findings of Rajaguru.[5]

TABLE 4. Effect of aging manioc tubers.

Batch	Time of aging (h)	Total cyanide mg/kg (dry wt) A	B
1	1	102	106
	6	147	104
	11	106	129
	24	136	151
	31	137	157
2	1	70	92
	24	134	159
	48	150	121
	72	152	216
	96	187	198

Duplicate batches of manioc tubers (A and B) sampled as in methods (Section **2.1.1**) were chipped, dried at 55° C for 24 h, powdered and total cyanide determined after aging for the above periods.

TABLE 5. Cyanogenic glucoside content of manioc 'roti'.

Batch	Incubation time (min)	Cyanide in 'roti' mg/kg manioc flour (dry wt) Free	Bound
A	10	24	44
	20	16	35
	30	24	14
B	0	15	63
	30	13	48
	60	21	36
C	0	29	29
	30	38	08
	60	25	06
	120	32	08

Original flour for Batches A, B and C were 138, 106 and 106 mg CN^-/kg (dry weight) respectively. 'Roti' was roasted after incubating the flour—coconut mixture for the above times.

3.4. Cyanogenic glucoside content of manioc 'roti'

Preparation of 'roti' (roasting) using samples of manioc flour of known total cyanide content showed (Table 5) that there was: (1) a loss in bound cyanide and (2) an increase in free cyanide. The loss of bound cyanide increased with (i) time of incubation before roasting and (ii) linamarase activity of flour (Table 6). The prepared roti (after roasting) contained no linamarase activity.

TABLE 6. Enzyme activity index of manioc flours.

Batch	Time of incubation (min)	Cyanide liberated mg/kg (dry wt)
A	0	14
	15	15
	30	49
	60	73
B	0	18
	15	31
	30	49
	60	56
C	0	08
	15	38
	30	71
	60	92

Batches A, B and C were the same as those given in Table 5. Cyanide liberated by linamarase present in the flour was determined after incubation for the above times.

3.5. Cyanogenic glucoside content of manioc bread

A similar loss of total cyanide was observed on baking. The loss in the crust was more than that in the inner portion of the bread (Table 7). This reduction is possibly due to the decomposition of the glucoside (which undergoes thermal decomposition at $\sim$ 150°C) ; the temperature of the surface of the bread being higher than the inner portion by more than 100°C.

3.6. Cyanogenic glucoside content of manioc 'pittu'

'Pittu' (which is prepared by a process of steaming) was found to lose very little total cyanide (Table 8). The preparation was steamed immediately after mixing. Pittu samples had, when present, relatively small amounts of free cyanide and no linamarase activity.

TABLE 7. Cyanogenic glucoside content of manioc bread.

Time of fermentation (h)	Part of bread analysed	Total cyanide mg/kg manioc flour (dry wt)
3	Crust	47
		53
5	Crust	40
		30
3	Inner portion	97
		103
5	Inner portion	104
		106

Manioc flour sample used contained 196 mg total CN^-/kg (dry weight). Wheat flour blanks were equivalent to 5 and 7 mg CN^-/kg for the crust and inner portion of the bread respectively.

TABLE 8. Cyanogenic glucoside content of manioc 'pittu'.

	Cyanide, mg/kg manioc flour (dry wt)		
Batch	Original flour	Free cyanide in 'pittu'	Bound cyanide 'pittu'
B	106	N.D.	101
C	106	N.D.	89
D	178	09	124

'Pittu' was prepared without an incubation period. The product did not have linamarase activity.

N.D. Not detected.

3.7. Cyanogenic glucoside content of fried manioc chips

Manioc chips (50 to 60% moisture) on frying also did not lose significant amounts of total cyanide when the final moisture content was 1 to 2%. However, on overfrying some loss of cyanide was observed probably due to the thermal decomposition of the glucoside (Table 9). In this case too, very small amounts of free cyanide were present in the product. Linamarase activity was absent in the fried chips.

TABLE 9. Cyanogenic glucoside content of fried manioc chips.

	Total original cyanide mg/kg (dry wt)	Free cyanide mg/kg (dry wt)	Bound cyanide mg/kg (dry wt)
1.	105	03	103
2.	107	03	71*
3.	454	12	407

Sampling was done by method 1. Fried chips were tested for free and bound cyanide. Fried chips did not contain linamarase activity.

*Chips were charred.

4. Discussion

The fate of bound cyanide in manioc and its products depends mainly on one factor, *viz.* the linamarin-linamarase reaction. The reaction is dependent on : (1) enzyme substrate contact, (2) enzyme activity of material and (3) rate and extent of deactivation of enzyme (reversible deactivation by drying of material or irreversible deactivation by denaturation heating in liquid medium). In specialized instances, as in the soaking of the dried chip, permeability of the tissue to the glucoside is also significant.[3] The absence of free cyanide in products depends mainly on how much the system is heated, not only for removing free HCN but also for the thermal decomposition of the cyanohydrins.

Results of experiments described in this paper and those described previously[3] show that fast drying prevents enzyme activity. This results in no significant hydrolysis of the glucoside and therefore a product (dried chip or flour) with the full complement of bound cyanide as well as an enzyme system that can be reactivated by moistening. It is this type of flour which is generally used for cooking purposes.

Preparation of flour by the method of keeping in the shade for 24 h causes the loss of 60 to 80% of the bound cyanide (increased enzyme substrate contact due to autolysis of the tissue) but levels of total cyanide are greater than that observed by the process described earlier;[3] in addition flour obtained by this method may not be bacteriologically suitable for human consumption.

Use of flours containing linamarase (that can be activated) in addition to bound cyanide is not desirable for cooking purposes unless either the enzyme is deactivated before or during cooking and any free cyanide is driven off by the cooking process.

This appears to be the case with manioc ' pittu ' and with fried manioc chips which contain high levels of bound cyanide (quick deactivation of linamarase) but no significant amounts of free cyanide (any free cyanide being driven off by cooking). Although the relative absence of free cyanide is encouraging it is felt that it would be far more desirable if the bound cyanide is also eliminated before the flour is used.[1]

The situation with manioc 'roti' is more serious as the final roasting does not remove the free cyanide formed. The experiments on 'roti' clearly illustrate that the factor involved is the linamarin-linamarase reaction. The enzyme activity index of the flour used was C > A > B. This tallied with residual bound cyanide in the 'roti' which was in the order of B > A > C. The experiments also clearly illustrate the effect of incubation time on the system. Free cyanide levels in the 'roti' do not have any fixed trend. This is understandable as the level of free cyanide is dependent on the amount of heat the material is subjected to, which, in these experiments, cannot be effectively controlled.

The experiment with manioc bread, in addition, shows the effect of temperature on total cyanide content. The outer crust of the bread is subject to a very high temperature which no doubt causes the lower total cyanide content of the crust in comparison to that of the inner part of the bread. Whether this is due to thermal decomposition of the glucoside or of the cyanohydrin or both is still under investigation.

The results concerning the aging of tubers is consistent with the general idea of old manioc being more toxic than fresh manioc ; the total cyanide levels of the flour show a general trend of increased cyanide content with aging. Inadequate sampling reduces the values of these results, but the trend closely resembles that reported by Rajaguru[5] recently. Increased cyanide in the unpeeled tuber could be caused by either a synthesis of glucoside in the edible part of the tuber or a migration of glucoside from the rind (which is rich in glucoside) to the edible part of the tuber.

Acknowledgements

We thank the Director, CISIR for the facilities provided ; Mr. S. F. Laurentius (Deputy Director Research, CISIR) and Mr. E. E. JeyaRaj, Head, Industrial Microbiology Section for encouragement ; the laboratory staff of the Industrial Microbiology and Analytical Section, CISIR for technical assistance ; Mrs. R. Curtis for preparation of the bread in the CISIR Bakery and Mrs. I. Bandara for secretarial assistance.

References

1. JANSZ, E. R., JEYARAJ, E. E., PIERIS, N. & ABEYRATNE, D. J. (1974), *J. Natn. Sci. Coun. Sri Lanka* **2** (1): 57-66.
2. JANSZ, E. R., & NETHSINGHA, C. (1973) *J. Natn. Sci. Coun. Sri Lanka* **1** (2): 83-96.
3. JANSZ, E. R., PIERIS, N., JEYARAJ, E. E. & ABEYRATNE, D. J. (1974) *J. Natn. Sci. Coun. Sri Lanka* **2** (2) 129-134.
4. PIERIS, N., JANSZ, E. R. & KANDAGE, R. (1974) *J. Natn. Sci. Coun. Sri Lanka* **2** (1): 67-76.
5. RAJAGURU, A. S. B. (1973) *Proc. Ceylon Ass. Advamt. Sci.* **29** (1) : 70-71.

J. Natn. Sci. Coun. Sri Lanka 1975 3(1) : 51-63

In Vitro Studies on Human Blood Cholinesterase and its Action Towards Organophosphate Insecticides

S. SENTHESHANMUGANATHAN AND M. RAJARATNAM
Department of Biochemistry, Medical Research Institute (MRI), Colombo, Sri Lanka.

(*Paper accepted* : 2 *May* 1975)

Abstract : Inhibition studies conducted with various concentrations of baytex and malathion indicated that at low levels malathion did not show any inhibitory effect but baytex at low levels and malathion at higher concentrations inhibited the blood pseudocholinesterase activity (ChE). The ChE activities which were inhibited by these insecticides were found to be reactivated slowly by antidote, pyridine-2-aldoxime-N-methiodide (PAM). PAM alone did not produce any inhibitory effect.

The clinical norm of ChE activities for Ceylonese subjects as determined by the Rapid Field Test method is 80% with a SD of 15.3%. The screening of personnel engaged in the handling and spraying of these insecticides for blood ChE activities was carried out by the rapid field test method. Such studies carried out on more than 80 personnel attached to 5 different Filaria units of the Department of Health, indicated that about 25% of them had blood ChE level 50% of the normal. Some of these low values may be due to congenital defects but this aspect was not investigated further.

1. Introduction

In vector control programmes D.D.T. (2,2-bis (p-chlorophenyl)—1,1,1-trichloroethane) and dieldrin (1,2,3,4,10,10—hexachloro 6-7-epoxy—1,4,4a,5,6,7,8,8a—octahydro-1,4-endo-exo 5-8-dimethanonaphthalene), have been in use for several years and no reports of any serious harmful effects have been recorded among the thousands of people who use them daily in malaria and filaria eradication campaigns. It has become necessary to introduce alternative insecticides since vectors have developed resistance to DDT and dieldrin ; such insecticides which are in use in Sri Lanka today are the organophosphates, viz. baytex (0-0-dimethyl-0-4 (methylthio)—m-tolyl-phosphorothioate) and malathion, (S—1,2-bis (ethoxy carbonyl) ethyl 0-0-dimethyl phosphorodithioate).

These organophosphates are generally toxic to animals since they interfere with the mechanism of the transmission of nerve impulse by inhibiting the hydrolysis of acetyl choline. These compounds inhibit the cholinesterase action by phosphorylating the active site of the enzyme.[5] The reactivation of the inactivated enzyme can be accelerated *in vivo* and *in vitro* by oximes, which are used as antidotes for poisoning by organophosphorus compounds.[5]

Considerable interest has developed in recent years in connection with the use of the muscle relaxant suxamethonium (succinyl choline or scoline). This substance is rapidly hydrolysed by pseudocholinesterase (acyl choline-acyl hydrolase, ChE) but

in individuals with low serum ChE, hydrolysis of suxamethonium takes place slowly so that apnoea due to it is considerably prolonged and may threaten life. This occurs in individuals whose ChE activity is low due to pathological conditions such as in liver disease ; gradual poisoning is due to exposure to organophosphates or is due to a congenital defect.

The present investigation was therefore undertaken with a view

1. to determining the inhibitory effects of baytex and malathion on human ChE *in vitro* since such data is not available,

2. to study the antidote effect of PAM (pyridine-2-aldoxime-N-methiodide) on cholinesterase, inactivated by baytex and malathion *in vitro*,

3. to establish the clinical norm of ChE-activity for Ceylonese subjects, and

4. to screen the personnel, who are engaged in the handling of organophosphorus compounds in vector control programmes with a view to determining the inhibitory effect of baytex and malathion on human ChE *in vivo*.

Some of the findings have been reported earlier.[10]

2. Materials and Methods

Baytex and malathion used were commercially available solutions obtained from the Filaria campaign of the Department of Health, Sri Lanka ; they were used without any further purification. Antidote PAM was obtained from Farbenfabriken Bayer A. G., Leverkusen, Germany, and benzoycholine and acetyl choline were obtained from British Drugs House Ltd. Poole, U.K. The blood serum used for the inhibition experiments and for the effect of antidotes was that of one of the authors (S. Sentheshanmuganathan) and was obtained by venepunture. The blood was allowed to clot at 37°C and the clear serum separated by centrifugation. The serum, when stored at -20°C, retained its ChE activity without any change for a month.

For the establishment of the clinical norm, capillary blood samples were collected from volunteers of the various grades of officers working at the Medical Research Institute, Colombo, Sri Lanka. Capillary samples of blood for testing were collected from the workers attached to the Filaria units in the country.

2.1. Assay of cholinesterase activity

Spectrophotometric[9] and colorimetric[4] methods were used in the assay of serum and whole blood cholinesterase activities respectively.

2.2. Spectrophotometric assay of ChE

A Unicam SP 500 spectrophotometer was used. All experiments were conducted at 37°C and changes of temperature of the solution during the mixing were kept to a minimum. This was achieved by keeping the reagent tubes in the water bath which was feeding the thermospacers. The cell holder with the absorption cells was always kept in the cell compartment in contact with the thermospacers except for a few seconds for filling.

The decrease of absorbance during the first 3 min was called ΔA_3,[8] if obtained under standard conditions (Serum 1 : 200, Benzoyl choline 5×10^{-5}M 26°C, 10 mm light path, pH = 7·4, wave length 240 mu). Then $0{\cdot}165 \times \Delta A A_3 = 2{\cdot}5 \times 10^{5}$–M. A decrease of absorbance of 0·165 corresponds to a hydrolysis of 0·025 μmoles benzoyl choline per ml. Then $606 \Delta A_3$ = μmoles benzoyl choline hydrolysed by 1·0 ml in 1 h at 26°C. In order to convert the values to 37°C, a factor of 1·74 is used, e.g. from 26 to 37°C it is $1057 \Delta A_3$.[9] Since the experiments were conducted at 37°C, it was not necessary to use a temperature correction factor.

2.3. Colorimetric method

For the colorimetric method of the assay of whole blood ChE, the Lovibond Tintometer developed by the Tintometer Ltd., Waterloo Road, Salisbury, U.K. was used as described by Edson.[4] The standard Lovibond comparator disc 5/30 covers the range 0 – 100% normal activity in steps of 12·5%. The acetic acid liberated from the substrate, acetyl choline, by the cholinesterase lowers the pH of a weak buffer solution and changes the colour of the indicator bromothymol blue. The change in colour is proportional to a degree of enzyme activity, which is matched against the standardised discs of the Lovibond comparator.

3. Results

3.1. Inhibition of cholinesterase activity by baytex and malathion

When the serum was incubated with benzoyl choline, the decrease in absorbancy at 240 mμ at 37°C was 0·225 over the first 3 min of incubation (Figure 1). But when baytex and malathion were incubated singly with the same serum under identical conditions, the corresponding decreases in absorbancies were 0·070 and 0·170 respectively. The final concentration in the mixture was : baytex, 11.25 μM and malathion, 19·23 μM. Baytex thus produced an inhibition of ChE activity by 68% of the total activity as compared to malathion which gave an inhibition of only 24%. The results indicate that baytex is a stronger inhibitor of ChE activity than malathion. The above concentrations of baytex and malathion were chosen since concentrations higher than these amounts produced turbidity which interfered in the spectrophotometric measurements.

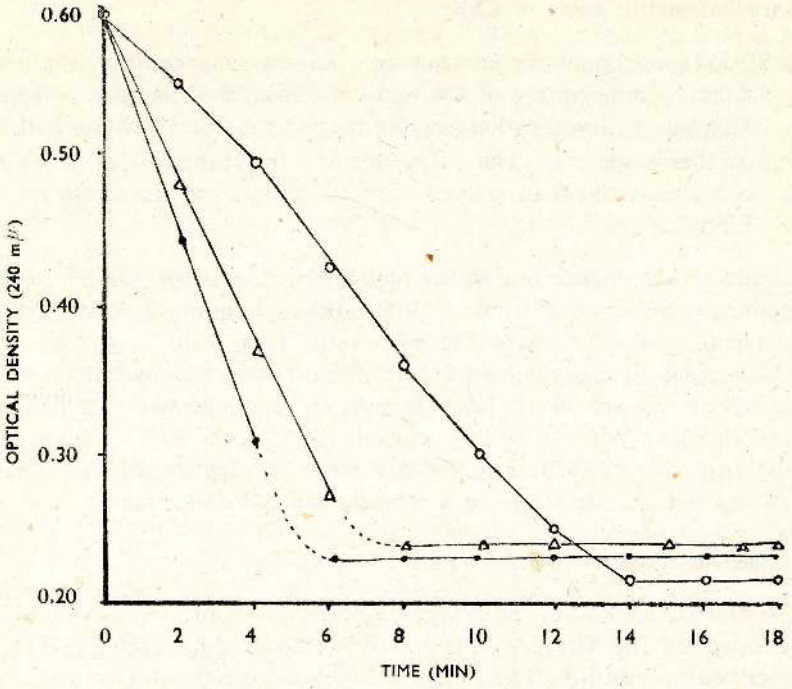

FIG. 1. Inhibition of cholinesterase activity by baytex and malathion.

The enzyme activity was followed by observing the decrease in absorbancy at 240 mμ. The incubation mixture (4.0 ml) contained serum (2.0 ml of 1 : 100 dilution in phosphate buffer pH. 7.4, 2.0 ml benzoyl choline in phosphate buffer, conc. 5×10^{-5} M), organophosphate (0.1 ml = 11.25 μm baytex or 19.23 μm, malathion) Incubation temp., 37°

•—• No inhibitor
o—o Baytex
Δ—Δ Malathion

3.2. Effect of insecticide concentration on ChE activity

In this experiment, the concentration of the substrate and enzyme (serum) was kept constant while the amounts of baytex and malathion were increased. The conditions of assay used were as described (Figure 1). This was studied over the concentration range of 0.3 to 3 μM. The percentage inhibition of ChE activity by malathion increased linearly with increasing concentration of the organophosphate up to a final concentration of 0·75 μM while that of baytex increased to 0·45 μM (Figure 2). At these two concentrations of organophosphates the inhibitions observed were 55% and 45% respectively. Thereafter, increasing the concentration of either baytex or malathion did not show the same linearity. At the final concentration of 3.0 μM of the organophosphate, baytex produced an inhibition of 83% of the total activity while malathion showed an inhibition of 63%.

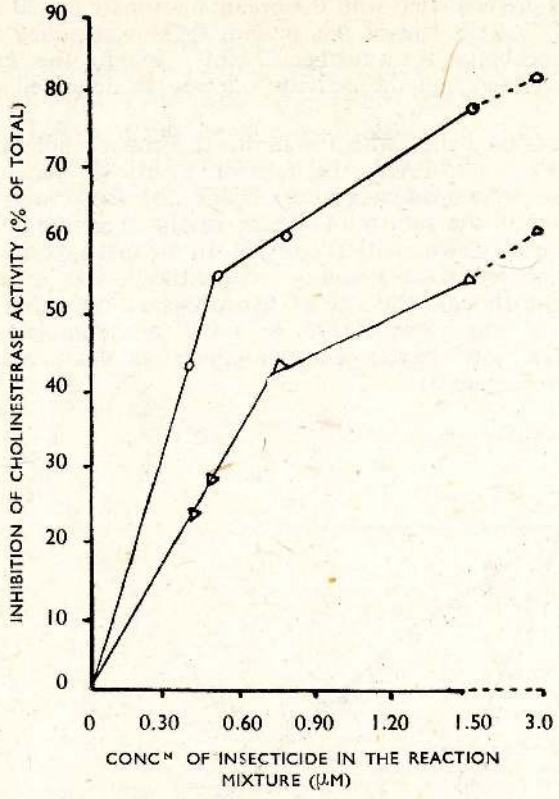

FIG. 2. Inhibition of serum cholinesterase activity by increasing amounts of baytex and malathion. The experimental conditions were as described under Fig I.

o—o Baytex

Δ–Δ Malathion

3.3. Antidote effect of PAM on the inactivated serum cholinesterase by baytex and malathion, *in vitro*

In order to establish that the antidote PAM could reactivate the ChE activity inhibited by baytex or malathion, it was thought desirable to carry out this study in the following manner :—

(1) Enzyme (serum) was incubated simultaneously with the organophosphate, antidote PAM and the substrate, benzoyl choline.

(2) Enzyme was first preincubated with the organophosphate for 20 min to inactivate the enzyme. Then the substrate, benzoyl choline was added and the hydrolysis followed until it ceased. Subsequently, PAM was added and the hydrolysis was followed again until the reaction reached a stationary stage.

(3) Enzyme was preincubated with the organophosphate for 20 min to inactivate the enzyme. At the end of this period, PAM was added to reactivate the enzyme by incubating for a further 20 min. Finally the substrate, benzoyl choline was added and the activity followed as described in Section 2.

When the serum was incubated with the substrate, benzoyl choline with or without the addition of the antidote PAM, the rate of hydrolysis remained unchanged. Similar observations were made when the enzyme and PAM were incubated first before the addition of the substrate. These results therefore indicate that PAM has no action on the enzyme activity (Figure 3). In the first experiment, enzyme and substrate were incubated simultaneously with either baytex or malathion. Such studies showed that though the rate of hydrolysis as measured within the first 3 min of incubation was low, (0·175 to 0·05) yet complete hydrolysis was achieved in 16 min with baytex and malathion as compared to 5 min in the absence of inhibitor (Figure 3).

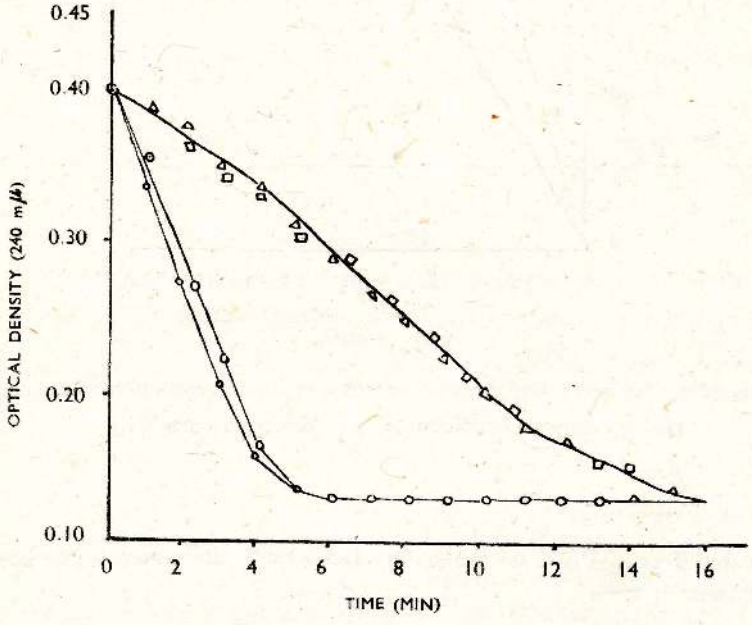

FIG. 3. Inhibition of serum cholinesterase activity by baytex and malathion and the subsequent effects by PAM (Pyridine-2-aldoxime-N-methyl iodide).

The experimental conditions were as described under Fig. 1. PAM and baytex or malathion were added in equimolecular amounts.

•—• Serum + Benzoyl choline.

o—o Serum was preincubated with PAM for 20 min then the substrate, benzoyl choline was added.

Δ—Δ Serum + malathion or baytex + benzoyl choline were added simultaneously.

□—□ Serum preincubated with baytex or malathion for 20 min then PAM and benzoyl choline were added simultaneously.

In the second series of experiments, when the substrate was incubated with the malathion inactivated enzyme, a slight change in the absorption was observed (from 0·425 to 0.413) but when PAM was added, the hydrolysis proceeded gradually reaching a value of 0.30 in 20 min which is equivalent to a hydrolysis of 40% of the total (Figure 4). With baytex, however, a hydrolysis of 60% was achieved over the same period. In all experiments, PAM and malathion or baytex were present in equimolecular amounts in the incubation mixture.

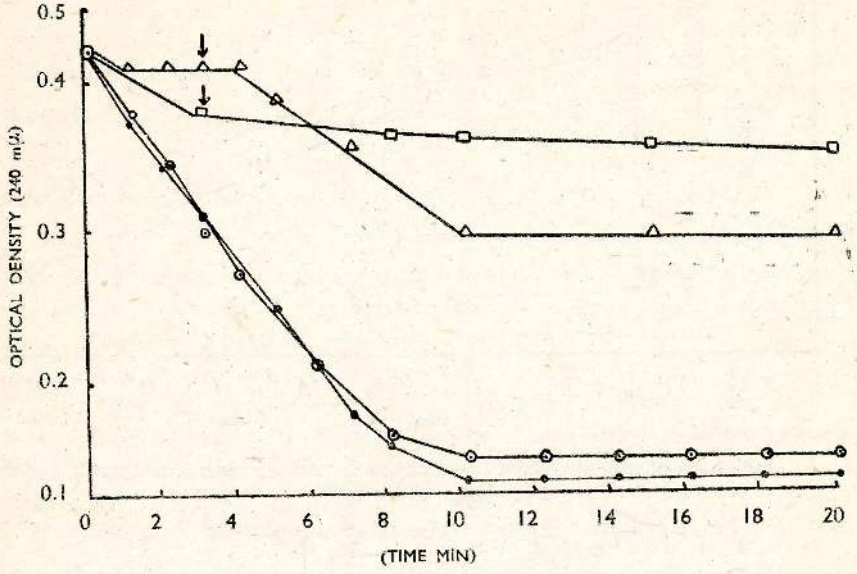

FIG. 4. Inhibition of serum cholinesterase activity by malathion and reactivation of the inactivated enzyme by PAM.

The experimental conditions were as described under Fig 1. Arrows indicate time of addition of either PAM or Benzoyl choline (BC).

•—• Serum + Benzoyl choline

o—o Serum + Benzoyl choline + PAM.

Δ..Δ Serum was incubated with malathion (for 20 min), then benzoyl choline was added and the reaction was followed until there was no change in the optical density at 240 mμ.

PAM was added and the reaction was followed again.

▫—▫ Serum + malathion were incubated (20 min). PAM was added and incubated further for 20 min Benzoyl choline was added and the reaction was followed again.

(PAM and malathion were added in equimolecular amounts, 76.9 μm.).

In the third series of investigations, when the substrate was added after reactivating the inhibited enzyme by PAM, the amounts of hydrolysis achieved with malathion was 16% of the total as compared to 80% with baytex (Figures 4 & 5).

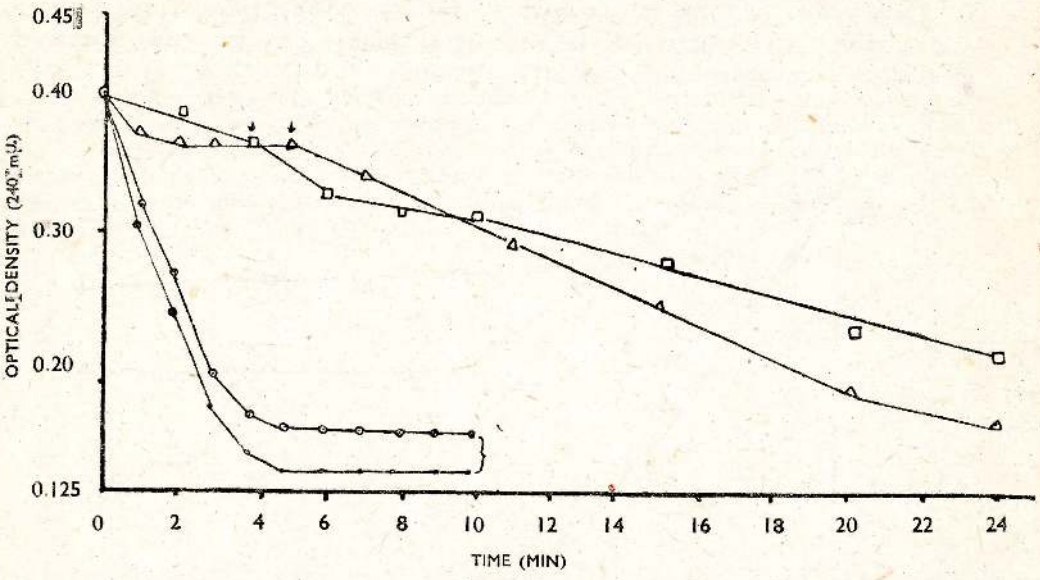

FIG. 5. Inhibition of serum cholinesterase activity by baytex and reactivation of the inactivated enzyme by PAM.

The experimental conditions were as described under Fig. 1. Arrows indicate time of addition of either PAM or Benzoyl choline (BC).

•—• Serum + Benzoyl choline

o—o Serum + Benzoyl choline + PAM

Δ-Δ Serum was incubated with baytex (20 min) Benzoyl choline was added and the reaction was followed until there was no change in the optical density at 240 mμ. PAM was added and the OD was measured.

□—□ Serum + Baytex were incubated (20 min.): PAM was added and incubated further for 20 min Benzoyl choline was added and the reaction was followed again.

(PAM & Baytex were present in equimolecular quantities, 45 μ-moles).

3.4. Clinical norm for Ceylonese subjects by the Rapid Field Test (Colorimetric)

More than 100 samples of capillary blood collected from normal subjects have been estimated for cholinesterase activity (Table 1). The clinical norm for Ceylonese subjects by this method was 80 ± 15·28 with a range of 50 to 100%. 101 workers attached to 6 Filaria units in the country, who had been handling baytex and malathion for about 3 years were screened for their ChE levels (Table 1). This survey revealed that 25% of the workers had their ChE levels lowered by 50% of the normal (95%).

TABLE 1. Blood cholinesterase levels of normal subjects and personnel, involved in the spraying of insecticides, attached to the various Filaria Units of the Department of Health, Sri Lanka.

Subjects	No. of Subjects	Blood cholinesterase levels 0	12.5	25	37.5	50	62.5	75	87.5	100
Volunteers (MRI Staff)	101	00	00	00	00	00	18	36	17	24
Filaria Units										
1. Dehiwela	40	00	00	01	06	06	07	13	05	02
2. Kolonnawa	12	00	00	00	01	00	01	03	05	02
3. Kolonnawa Installation ...	11	00	00	00	00	01	02	02	05	01
4. Kotte	19	00	00	00	01	03	02	09	03	01
5. Peliyagoda	12	00	00	00	01	01	04	04	01	01
6. Wattala	07	00	00	00	00	01	03	02	00	01

The cholinesterase levels were determined by the Rapid Field Test method.[2]
Clinical norms = mean = 80% ; SD = ± 15.3%.

In another study, 4 workers attached to the blending plant of the Ceylon Petroleum Corporation (CPC) were tested for their ChE levels at the time of recruitment for employment (pre-exposure level) and subsequently at monthly intervals for 12 months. None of the 4 showed any change in the ChE levels (Table 2 a) over the 12 months of handling the organophosphates. Seven other workers of the same plant were screened before coming into contact (pre exposure level) with the organophosphates and subsequently for another 2 months of handling the insecticides. Of the 7, 1 had a drop of 50% (87·5 to 37·5%) and another worker of 25% (62·5 to 37·5% Table 2 b) ; while the remaining 5 showed no change.

TABLE 2. (a) Blood cholinesterase levels of workers attached to the Blending Plant, Ceylon Petroleum Corporation. (Batch No. 1 : 4 workers).

Dates on which tests were carried out	(1)	(2)	(3)	(4)
21.09.71* ...	100	87.5	100	87.5
09.11.71 ...	100	87.5	100	87.5
09.12.71 ...	100	87.5	100	87.5
11.01.72 ...	100	100	100	100
16.02.72 ...	100	100	100	87.5
23.05.72 ...	100	100	100	100
31.07.72 ...	—	—	—	100
29.09.72 ...	—	—	—	87.5

*Pre-exposure values.

Blood cholinesterase levels were estimated by the Rapid Field Test method.[4]

TABLE 2(b). Blood cholinesterase levels of workers attached to the Blending Plant, Ceylon Petroleum Corporation. (Batch No. 2 : 7 workers).

Dates on which tests were carried out	(1)	(2)	(3)	(4)	(5)	(6)	(7)
31.07.72*	100	87.5	75	87.5	87.5	62.5	62.5
29.09.72	75	37.5	62.5	100	75	37.5	62.5

*Pre-exposure values.

Blood cholinesterase levels were estimated by the Rapid Field Test method.[4]

4. Discussion

There are 2 types of cholinesterases present in man. One of them is true cholinesterase (acetylcholine-acyl hydrolase) which is specific for acetylcholine and present mainly in erythrocytes and in the region of cholinergic nerve endings. In various tissues especially plasma, there are other cholinesterase which are non specific for acetylcholine, called pseudocholinesterase (ChE). It is the latter type which we were interested in, since they are inhibited by organophosphates, eg. baytex, malathion, fenthion and sumithion.

Most of the data available on the inhibitory effect on blood ChE have been obtained with rats, rabbits and insect blood.[6] The only data available on human blood have been derived with the ChE obtained from human erythrocytes and serum using Parathion, Systex, Systox Thinoisomer and Dithiono-pyrophosphate *in vivo* and *in vitro*.[6] In view of the use of baytex and malathion in Sri Lanka in vector control programmes and the non availability of data for anti-ChE activity in human blood, we investigated the effect of these compounds on human serum.

The present investigation has shown that both baytex and malathion are inhibitors of pseudocholinesterase activity of human blood serum. In this study we followed the enzyme activity using benzoyl choline as substrate as described.[9] The rate of hydrolysis was observed by following the decrease in absorbance at 240 mμ over the first 3 min of adding the substrate. It is seen from Figure I, that the rate of hydrolysis of benzoyl choline by the serum corresponds to a decrease in absorbance of 0·225 ; when baytex (11·25 μm) was also added to a system, the decrease in absorbancy was 0·07 while with malathion (19·23 μm), it was 0·170. Thus baytex and malathion inhibited the enzymic activities by 68% and 24% of the original activity respectively. These findings indicate that baytex is a stronger inhibitor of ChE activity than malathion. It has been reported[11] that malathion has the lowest toxicity of all organophosphorus compounds. Our findings are in agreement. The WHO report, however, states that in practice malathion at lower concentration does not depress the cholinesterase level. In the presence of baytex and malathion, a given amount of enzyme will hydrolyse the same amount of substrate in 14 and 8 min respectively,

while without any inhibitor it would take 6 min to bring about an equal degree of hydrolysis. When the concentration of baytex was increased from 0 to 0·45 μm, the percentage inhibition of ChE activity also increased in a linear manner and above that the inhibition attained a maximum value of 78% at a concentration of 1·5 μm ; with malathion a similar pattern was obtained. Malathion produced a linear relationship up to a concentration of 0·75 μm of the inhibitor and produced a maximum inhibition value of 55% at a concentration of 1·5 μm. Above a concentration of 1·5 μm of either of the organophosphates, the inhibitions produced were not very marked. Increasing the concentration 10 fold (3·0 μm) increased the inhibition by only 5% and 9% (Figure 2). We may therefore assume that at these concentrations, the active sites of the enzymes are saturated by phosphorylation and this prevents the entry of the substrate for action. It could be inferred from Figure 2, that baytex is a stronger inhibitor of ChE activity than malathion. The high toxicity of the organophosphates, which have been developed as insecticides for agricultural use, depends largely upon the blockage of esterases such as the cholinesterases. These enzymes are phosphorylated and the inhibition that results is practically irreversible.[1,2,3] Recovery from such poisons depends on the formation of fresh enzyme which takes weeks. The usual symptomatic treatment for the endogenous acetylcholine intoxication that is produced by these drugs has hitherto consisted of the administration of high doses of atropine, artificial ventilation of the lung and correction of dehydration. It has now become possible to supplement these measures with the use of specific antidotes, *viz.* oximes whose chemical proporties enable them to displace the phosphate radical from the phosphorylated esterases and thus restore the activity of the enzyme. The most widely used oxime is PAM (Pyridine-2-aldoxime methiodide). Several cases of parathion poisoning treated successfully with antidote PAM have been reported.[5] Though the poisoning by parathion and its successful management have been reported,[5] there are no reports of successful management of either baytex or malathion poisoning. In view of this, we studied the effect of antidote PAM on the enzyme inhibited by baytex and malathion. Such studies showed that PAM alone has no anticholinesterase activity on human serum (Figure 4). When PAM was added to a serum sample inhibited by either baytex or malathion and incubated further, there was a release of the phosphorylated esterase, which hydrolysed benzoyl choline gradually. Even though the amount of PAM used in the present study was in equimolecular amounts to that of baytex or malathion, yet it could not release all of the activity originally present. With malathion only 16% of the total activity was restored. This may have been due to some denaturation caused during the long period of incubation or of some other factor ; this aspect was not investigated. With baytex, however, 60% of the original activity was restored in 20 min. Even though baytex is a stronger inhibitor of ChE activity than malathion (Figures 2 & 3), yet the inactivation caused by baytex seems to be of a milder or weaker type than that of malathion. It might be that the affinity of the esterase is more towards PAM than baytex, or that the bond which operates in the phosphorylated intermediate

between baytex and enzyme is weaker than that which operates between the malathion enzyme complex. This is the only explanation we could provide with the existing data. It has been observed[5] that PAM should be given by injection within 24 to 48 h of parathion, poisoning since after this period, intravenous injection of PAM no longer leads to a reactivation of the enzyme. This is also true *in vitro*, when PAM was added to a sample of blood from the patient poisoned with parathion;[5] at this time PAM even showed anticholinesterase activity of its own. From the present studies it could be inferred that the failure of PAM to liberate the lost activity may be a phenomenon similar to that observed with parathion, suggesting that poisoning by malathion management may need a different approach altogether. With baytex poisoning, the treatment with PAM could yield rapid results.

The clinical norm of ChE activities for Ceylonese subjects was determined by the Rapid Field Test method[4]. This method was chosen so that it could be used by any trained technician in the field, factory or any hospital with the use of simple portable equipment. More than 100 samples of capillary blood collected from normal subjects have been estimated for ChE activities by this method (Table 1). The clinical norm for Ceylon subjects is 80% with a standard deviation of 15·3% thus giving a range of 64·7 to 95.3%. For the western countries, the normal value was found to be 100%.[11] Diagnosis of poisoning by organophosphorus compounds can be confirmed by demonstrating a much reduced ChE activity in the whole blood, plasma or serum. A reduced level of ChE activity in the blood indicates that an anti-cholinesterase chemical has been absorbed, or the individual is having some pathological conditions affecting the liver or a congenital defect. The latter can be confirmed by determination of the dibucaine and fluoride numbers.[7]

If the depression of ChE is marked, the patient will be more susceptible to the effects of further exposure to an organophosphate. Inhibition of cholinesterase only causes inequivocal symptoms when the enzyme activity has fallen to less than 25 to 30% of the pre-exposure value of that individual.[11] When this degree of inhibition is reached the symptoms of poisoning may progress so rapidly as to threaten life ; immediate treatment then becomes imperative. In view of the above observations made[11] and the normal values established by us, we suggest that any person who has a ChE value of 60% or less should not be employed in the handling of organophosphorous compounds either in a blending plant, in vector control programmes or in agriculture. All personnel handling these insecticides are therefore advised to have their ChE levels examined every 7 to 14 days. If the ChE value is found to be less than 60%, the individual should not be allowed to return to work, or to come into contact with organophosphates until his ChE has returned to at least 70% of the normal.[11]

The ChE levels of 4 workers were tested before assigning them to the Ceylon Petroleum Corporation blending plant of organophosphorus compounds. These were taken as their pre-exposure values. They were subsequently screened for ChE levels at regular intervals for 12 months (Table 2 a). In all the 4 cases tested, pre-exposure values were 87.5 to 100%. None of them showed any change in their ChE levels. In another study, with 7 other workers over a period of 2 months, one had a decrease in the ChE value of 50% (87.5 to 37.5%) and another one by 25% (62.5 to 37.5%); the value of 37.5 is very much lower than the limiting value of 60%. Even with this value of 37.5% both the workers did not show any positive symptoms of organophosphorous compounds poisoning. This is expalained in the earlier findings[5] in which it has been established that symptoms appear only if the value falls below 25% of the pre-exposure value which in these 2 cases is 16.8%.

Acknowledgements

The authors are indebted to some officers of the Medical Research Institute for voluntary blood donations, to Mr. D. D. Jayatillake for carrying out the statistical analysis, to Dr. T. W. Goonewardena for his advice in the preparation of this paper, to Hayleys Ltd. for the gift of a sample of pyridine-2-aldoxime-N-methyl iodide (PAM), to Mr. N. E. W. Perera for drawing the diagrams and to Mr. A. P. D. G. Fonseka for all technical assistance.

References

1. EDITORIAL (1960) *Br. Med. J.* **2** : 215-228.
2. EDITORIAL (1953) *Lancet* **1** : 1036-1039.
3. EDITORIAL (1960) *Lancet* **2** : 201-208.
4. EDSON, E. F. (1958) *World Crops* **10** : 49-52.
5. ERDMAN, W. D. (1960) *German Med. Mon.* **5**(9) : 304:306.
6. HANDBOOK OF TOXICOLOGY (1959) Vol. 3 : Insecticides ; edited by the National Academy of Sciences, National Research Council. Philadelphia : Saunders.
7. HARRIS, H. & WHITTAKER, M. (1961) *Nature* **191** : 496-498.
8. KALOW, W. & GENEST, K. (1957) *Can. J. Biochem.* **35** : 339-347.
9. KALOW, W. & LINDSAY, N. A. (1955) *Can. J. Biochem. Physiol.* **33** : 568-574.
10. SENTHESHANMUGANATHAN, S. & RAJARATNAM, M. (1970) *Proc. Ceylon Ass. Advmt Sci.* **26**(1): 94-96.
11. WORLD HEALTH ORGANIZATION (1967) *Tech. Rep. Ser.* no. 356 : 34-46.

J. Natn. Sci. Coun. Sri Lanka 1975 3(1):

ලිපිවල සාරාංශ - සිංහල පරිවර්තන

පොල් රා වල සල්පයිඩ සෑදීම පාලනය කිරීම

ජෑන්ස්, ඊ. ආර්., ජෙයරාජ්, ඊ. ඊ., අබේරත්න, ඩී. ජේ. සහ ශ්‍රේමරත්න, අයි. පී.

J. Natn. Sci. Coun. Sri Lanka 1975 3(1): 1 – 10

වල් යීස්ට් යොදා පැසවූ රා වල වාෂ්පශීල සල්පයිඩ අන්තර්ගතය. අකාබනික සල්පයිඩ සඳහා විශේෂ වූ බඩ හා බෙච්ක් ක්‍රමය ඇසුරෙන් විකරණය කර ගන්නා ලද ක්‍රමයක් මගින් (අම්ල මාධ්‍යයෙන් චූෂණය කරගෙන ඉක්බිති අදාවය්‍ය සල්පයිඩයක් වශයෙන් H_2S අල්ලා ගැනීමෙන්), විවිධානත්ත්ව යටතේ පැසවන ලද රා වල අන්තර්ගත මුළු සල්පයිඩ ප්‍රමාණය වර්ණාවලී-දීප්තිමානයක් ඇසුරෙන් ප්‍රමාණාත්මක ලෙස අන්වේෂණය කරන ලදී. අමු පැසැවුමේ දී සෑදෙන සල්පයිඩ ඉවත් කිරීමට බොහෝ අවස්ථාවල දී 0.01 % NH_4^+ ප්‍රමාණවත් වූ බව ප්‍රති-ඵලවලින් පෙනිණි. එහෙත් අවස්ථා කිහිපයෙක දී පමණක් මීට වැඩි සාන්ද්‍රණ අවශ්‍ය විය. නයිට්‍රජන් වල සාන්ද්‍රණයේ ගණයෙන් ම යුරියා නිෂේධනය වූයේ නැත. යීස්ට් මාදිලිය නෝරාගැනීම අනුව ද සල්පයිඩ සෑදීම පාලනය කළ හැකිය. රා වලින් වෙන් කර ගත් මාදිලි කිහිපයෙකින් ම බොහෝ දුරට සල්පයිඩ් තොර කින්වයක් ලැබිණි. ඉහළ මට්ටමින් සල්පයිඩ් සාදලිය හැකි මාදිලි, 0.05 % NH_4 හමුයේ දී මේ සංයෝගය උපදවන්නේ නැත.

කින්වවල ඇති වාෂ්පශීල සල්පයිඩය Cu සුරුන්ඩු එක් කිරීමෙන් CuS වශයෙන් අල්ලා ගන්නට පුළුවනි. කින්ව වලට Fe හෝ වානේ හෝ එක් කිරීමෙන් සල්පයිඩ සෑදීම කිහිප ගුණයෙකින්ම වැඩි විය. NH_4^+ (0.1 %) මෙය නිෂේධනය කළේ අඩ වශයෙනි.

SO_4^{2-}, සල්පයිඩ සෑදීම වැඩි නොකරන නමුදු සිස්ටීන් ද (තරමක් දුරට) මෙතයනින් ද සල්පයිඩ සෑදීම සඳහා අවශ්‍ය සල්පර් සපයන වගක් පෙනෙන බව අධ්‍යයන වලින් පෙනීයයි.

පරිමිත විද්‍යුත් ඝන්නායකතාවෙකින්යුත් භ්‍රමණ චුම්බක තාරකාවල සතත සංසරණය පිළිබඳ අවශ්‍යතා

මහේස්වරන්, එම්.

J. Natn. Sci. Coun. Sri Lanka 1975 3(1) : 11 - 17.

චුම්බක ස්‍රාව ඝනත්වය දෛශික තරංග සමීකරණයේ විසඳුම් පන්තියට අයත් වෙතොත්, පරිපූර්ණ ඝන්නයන භ්‍රමණ චුම්බක තාරකා සඳහා චන්ද්‍රාසේකර් හා මෙස්ටල් යන දෙදෙනා විසින් අපෝහනය කරන ලද යතත සංසරණය පිළිබඳ අවශ්‍යතා, පරිමිත විද්‍යුත් ඝන්නායකතා-වෙකින් යුත් තරල සඳහා ද විස්තීරණය කළ හැකි බව මින් පෙන්නුම් කැරෙයි. ක්ෂේත්‍රය බල මුක්ත බවට පත්වූ විට තාරිය තරලයක් විද්‍යුත් ධාරාත් ආධ්‍රැවික තලයෙක එකම අනාකුල රේඛා ඔස්සේ ගලා යා යුතු බවද මෙහි දැක්වෙයි. එසේම තාරකාවල ලැයි ආධ්‍රැවික සංසරණ පිළිබඳව සලකන විට ක්ෂේත්‍රයෙහි උද්දිගංශ සංරවකය දාධ්‍රැවික සංරවකයට වඩා බෙහෙවින් ප්‍රබල නම් මීස බල මුක්ත ක්ෂේත්‍රයෙකින් සතත අවස්ථාවක් ලබාගත නොහැකි වනු ඇත.

ලාංකික තරුණයන් අතර වහිරුකන්තුක ඉන්පාකිහවනය

වාලුසිල්ලේ, එන්. ජේ. සහ අතුකෝරල, ඩී. ජී.

J. Natn. Sci. Coun. Sri Lanka 1975 **3**(1): 19 – 29

වහිරුකන්තුක ඉන්පාකිහවනයේ නිදන සාධක සෙවීමේ අටියෙන් මේ රෝගයෙන් පෙළෙන වයස අවුරුදු 40 ට අඩු රෝගීන් හතලිස් දෙනකු අධ්‍යයනයට ලක් කැරිණි. වහිරුකන්තුක ඉන්පාකිහවනයෙන් පෙළෙන තරුණ රෝගීන්ගේ කොලෙස්ටරෝල් අගය පැලන කාණ්ඩයේ, එම අගයට වඩා සෑහෙන ලෙස වැඩි බව පෙනී ගියේය. ආමිලතාමිතිය භාවිතයෙන් ලබාගත් තරුණ ඉන්පාකයන්ගේ ලයිපොප්‍රෝටීන් පැතිකඩ අනුව මේ රෝගීන්ගේ M භාගය (=VLDL) පාලන කාණ්ඩයේ M භාගයට වඩා සෑහෙන ලෙස වැඩි විය. වහිරුකන්තුක ඉන්පාකිහවනයෙන් පෙළෙන තරුණ රෝගීන්ගෙන් 45% දෙනකුටම හයිපර් ලයිපොප්‍රෝටීනමියාව ඇති විය: W.H.O. type IV (ලෝ. සෞ. සං. IV වර්ගය) මෙහි ඉහාම සුලබ ස්වරූපය විය. තවත් සිත් ගන්නා සුල ලක්ෂණයක් වූයේ වහිරුකන්තුක ඉන්පාකිහවනයෙන් පෙළෙන තරුණ රෝගීන්ගෙන් 50% කට චාප ස්වච්ඡය (Arws Commea) ඇතිවන බවයි. රෝගීන් ගෙන් 40% ක වයස අවුරුදු 60 ට අඩු පළමු වන ඥාතීන්ව ඥාතීන් අතර වහිරුකන්තුක ඉන්පාකිහවනය ඇති පවුල් ඉතිහාසයක් දක්විණි. ස්ථූලභාවයද අත්‍යාතතිය, ද මධ්‍ය මේහයද මේ ශ්‍රේණියෙහි දැනි අසාමාන්‍ය අවදානම් සාධක වෙයි. මේ රෝගීන්ගෙන් 90% ක් කුළුම C.H.D. සඳහා අවදානම් සාධක එකක් හෝ වැඩි ගණනක් හෝ තිබිණි. දුම්බීම ද අසාමාන්‍ය ලයිපොප්‍රෝටීන් රටාද ප්‍රධාන අවදානම් සාධක විය.

වහිරුකන්තුක ඉන්පාකිහවනයෙන් පෙළෙන රෝගීන්ගේ ඥාතීන්ගේ කොලෙස්ටරෝල් ට්‍රයිග්ලිසරයිඩ හා 'M' අගය (VLDL) පාලන කාණ්ඩයේ අයට වඩා සෑහෙන ලෙස අධික විය. දාරෝහිත VLDL, වහිරුකන්තුක ඉන්පාකිහවනයෙන් පෙළෙන තරුණ රෝගීන් අතර අවදානම් සාධකයක් බව පෙනේ.

විභාග පිළිබඳව

සැමුවෙල්, වී. ඩී. එම්. ඒ.

J. Natn. Sci. Coun. Sri Lanka 1975 **3**(1): 31 – 39

විභාගවලදී සිසුන්ගේ දක්ෂතා කෙරෙහි බලපෑමට ඉඩ ඇති පරාමිති ගැන මින් සාකච්ඡා කැරෙයි. සරල විශ්ලේෂණයකට පසුව එක්තරා විභාගයෙක ප්‍රතිඵල ගැන මෙකී පරාමිති ඇසුරෙන් සාකච්ඡා කැරෙයි. 'සම්මත කරණ පරාසය' ක් පිළිබඳව අර්ථ දක්වීමේ අවශ්‍යතාව මේ සාකච්ඡාවෙන් අනාවරණය වෙයි.

මයියොක්කාවල අන්තර්ගත සයනජනික ග්ලූකොසයිඩ ප්‍රමාණය

III යැකඩිමේ දී හා පිසිමේ දී බැදුණු සයනයිඩවලට සිදුවන දෙය

පීරිස්, නිර්මලා සහ ජැන්ස්, ඊ. ආර්.

J. Natn. Sci. Coun. Sri Lanka 1975 **3**(1): 41 – 50

මයියොක්කා පිටි පිළියෙළ කිරීමේදී බැඳුණු සයනයිඩය මුක්ත සයනයිඩයට පරිවර්තනය වී දෙවුම් ජලය සමග ඉවත්ව යයි. එහෙත් වියැලීම නිසා තෙත මයියොක්කා පෙතිවල මුළු සයනයිඩ ප්‍රමාණය අඩු වෙයි. සම්පූර්ණ ආකන්ද වියැලීම නිසා මේ යැකඩිම මගින් පිළියෙළ කරනු ලබන පිටිවල අන්තර්ගත මුළු සයනයිඩ ප්‍රමාණය වැඩි කැරෙයි. මයියොක්කා පිටි වලින් පාන් හා රොටි පිළියෙළ කරන විට ඒ පිටිවල ඇති සයනජනික ග්ලූකොසයිඩ තරමක් දුරට හානි වී යයි. එසේ වුවත් රොටිවල මුක්ත සයනයිඩ ඇත. මයියොක්කා පිටිවලින් පිළියෙළ කළ පිටිවලද අලුත් මයියොක්කා වලින් පිළියෙළ කළ මයියොක්කා පෙතිවල ද අන්තර්ගත මුක්ත සයනයිඩ ප්‍රමාණය නොගිණිය යුතු තරම් ය; එහෙත් ඒවායෙහි බැඳුණු සයනයිඩ ප්‍රමාණය සම්පූර්ණයෙන්ම පාහේ තිබිණි. ඉහත කී ප්‍රතිඵල ලිනමරින්-ලිනමරේස් ප්‍රතික්‍රියාව ඇසුරෙන් මෙහි දී සාකච්ඡා කැරෙයි.

මිනිස් රුධිර කෝලිනෙස්ටරේස් ද කාබනිකපොස්පේට කෘමිනාශක කෙරෙහි එහි ක්‍රියාව ද පිළිබඳ තාලස්ථ අධ්‍යයන

යෙන්තිමන්මුගනාදන්, එස්. සහ රාජරත්නම්, එම්.

J. Natn. Sci. Coun. Sri Lanka 1975 3(1): 51 – 63

බේටෙක්ස් (baytex), මැලතියොන් (malathion) යන කෘමිනාශකවල විවිධ සාන්ද්‍රණ ඇසුරෙන් කරන ලද නිමේඛන අධ්‍යයන අනුව පහත් සාන්ද්‍රණ මට්ටම්වල දී මැලතියොන් කිසිදු නිමේඛන ඵලයක් නොදක්වූ නමුදු පහත් සාන්ද්‍රණ මට්ටම්වල දී බේටෙක්ස් ද ඉහළ සාන්ද්‍රණවලදී මැලතියොන් ද රුධිර සිව්ඩොකෝලිනෙස්ටරේස් සක්‍රියතාව (ChE) නිමේඛනය කළ බව දක්වීනි. මේ කෘමිනාශක මගින් නිමේඛනය කැරුණු ChE සක්‍රියතා පිරිධීන් – 2 – ඇල්ඩොක්සිම් – N – මෙතයිඩයිඩ (PAM) ප්‍රතිගරලය මගින් මෙමෙන් ප්‍රතිසක්‍රිය කැරුණු බව දක්නට ලැබිණි. තුදෙක් PAM මගින් පමණක් කිසිදු නිමේඛක ඵලයක් ඇති නොකැරිණි.

ගීඝ්‍ර ක්ෂේත්‍ර පරීක්ෂා ක්‍රමය මගින් නිර්ණය කර ගත්තා ලද පරිදි ලාංකික වැසියන් සඳහා ChE සක්‍රියතාවල ශාස්ත්‍රීය ප්‍රමාණය 15.3 % ක SD යක් සහිතව 80 % ක් වෙයි. මේ කෘමිනාශක පරිහරණය කිරීමේ හා ඉයිමේ කාර්යයන්හි නිරත වන පුද්ගලයන් ගේ රුධිර ChE සක්‍රියතා පිළිබඳව ගීඝ්‍ර ක්ෂේත්‍ර පරීක්ෂා ක්‍රමය මගින් සොයා බලන ලදී. සෞඛ්‍ය දෙපාර්තමේන්තුවේ විවිධ පයිලේරියා (=බරවා රෝග) ඒකක 5 කට සම්බන්ධ පුද්ගලයන් 80 දෙනකුට වැඩි ගණනාක් පිළිබඳව කරන ලද එබඳු අධ්‍යයනවල දී ඔවුන් අතුරෙන් 25 % දෙනකුගේ පමණ රුධිර ChE මට්ටම සාමාන්‍ය ප්‍රමාණයෙන් 50 % ක් බව පෙනී ගියේය. මින් ඇතැම් පහත් අගයවලට හේතුව යහජ දෝෂ විය හැකි නමුදු මේ අංශය ගැන තවදුරටත් අන්වේෂණය කළේ නැත.

J. Natn. Sci. Coun. Sri Lanka 1975 3(1):

இந்த இதழின் கட்டுரைகளின் சுருக்கங்கள்

தென்னங்கள்ளின் சல்பைட்டு உற்பத்திக் கட்டுப்பாடு

ஜான்ஸ் E. R.; ஜெயராஜ், E. E.; அபயரத்தினே, D. J.; பிரேமரத்தினே, I. G.

J. Natn. Sci. Coun. Sri Lanka 1975 3 (1): 1 – 10

காட்டு நொதியப் பொருள் இட்டு புளிக்கப்பெற்ற கள்ளில் ஆவிப்பறப்புள்ள சல்பைட்டு[3] உள்ளது. பல்வகைப்பட்ட சூழ்நிலைகளின்கீழ் நொதிக்கப் பெற்ற கள்ளின் சல்பைட்டு மொத்தமானது அளவறிதற்குரிய வகையில் நிறமாலையொளிமானியால் மதிப்பீடு செய்யப்பட்டது. இம்மதிப்பீடானது, அசேதனவுறுப்புச் சல்பைட்டுகளை மதிப்பீடு செய்ய விசேடமாகப் பயன் படுத்தப்படும், பட்டு (Budd) பெவிக்கு (Bewik[1]) முறையினைத் தழுவி சீராக்கப்பட்ட புதிய முறையொன்றின் அடிப்படையிலேயே (அமில ஊட கம் வாயிலாக உறிஞ்சியெடுத்தலாலும் கரையாத்தகவுள்ள சல்பைட்டாக H_2S சை அடைப்பு செய்தலாலும்) செய்யப்பட்டது. காட்டுப் பொருள் இட்டு நொதிக்கும்போது உற்பத்தியாகும் சல்பைட்டை நீக்குவதற்குப் பல சோதனைகளின்போது $0.01\%HN_4^+$ என்பது போதுமானதெனப் பெறுபேறுகளால் புலனுயிற்று. ஆயினும் சில சோதனைகளின்போது இதைவிட அதிகமான செறிவுகள் உபயோகிக்கப்பட வேண்டியதாயிற்று. ஒரே மாதிரியான நைதரசன் செறிவு மட்டங்களிலே யூரியாவுக்குப் பயிர்ப் புத்தடை ஏற்படவில்லை. தக்க நொதிப்பொருள் மரபுக் கூறுகளைத் தேர்ந்தெடுப்பதனாலும் சல்பைட்டு உற்பத்தியைக் கட்டுப்படுத்தலாம்; கள்ளிலிருந்து சிலவகை மரபுக் கூறுகளைப் பிரித்தெடுக்கப்பட்ட போது நொதிகளில் சல்பைட்டு இல்லவே இல்லை என்று கூறத்தக்க நிலை ஏற்பட்டது. உயர்மட்ட சல்பைட்டு நிலைகளை உருவாக்கக்கூடிய மரபியற் கூறுகள் $0.05\%NH_4^+$ இருக்கும்போது இக்கலவையை உற்பத்தி செய்யமாட்டா.

நொதிப்புள்ள ஆவிப்பறப்புள்ள சல்பைட்டை CuS ஆக Cu அடை தல்களைக் கூட்டுவதனால் அடைக்கலாம். நொதிப்புகளுக்கு இரும்பு அல்லது உருக்குப் பொருள்களைக் கூட்டுதனால் சல்பைட்டு உற்பத்தியானது பன் மடங்கு அதிகரிக்கப்படலாம். NH_4^+ (0.1%) என்பது இத்தொழிற்பாட் டுப் பயிர்ப்புத் தடை ஏற்படுத்துவதில் பாதியாகவே வெற்றிகண்டது.

SO_4^{2-} என்பது சல்பைட்டு உற்பத்தி அதிகரிக்கவில்லை என்றும், சிஸ் தீன், (ஆகக் குறைந்த அளவுக்கு) மீதியோனீன் ஆகியவை சல்பைட்டு உற்பத்திக்குத் தேவையான சல்பரைக் கொடுக்கும் தன்மையன என்றும் இக்கல்விகள் மூலம் அறியக்கிடக்கின்றன.

முடிவுள்ள மின்கடத்தத்திறனுடு சுழல் காந்த உடுக்களின் உறுதி சுற்றுவட்டத்திற் குரிய வரையறைகள்

மகேஸ்வரன், M.

J. Natn. Sci. Coun. Sri Lanka 1975 3 (1): 11-17

பூரணமாகக் கடத்தல், சுழல் திறனுடு விளங்கும் காந்த உடுக்கள் தொடர்பாக சந்திரசேகர், மெஸ்தல் ஆகிய முறைகள் மூலம் ஆக்கப்பெற்ற உறுதி சுற்றுவட்டத்திற்குரிய வரையறைகள் முடிவுள்ள மின்கடத்துகையுடைய பாய்பொருள்களுக்கும் விரிவுபடுத்தப்படலாம். அவ்வாறு செய்வதற்குக் காந்தபாயவடர்த்தியானது குறிப்பிட்ட காவி அலைச்சமன்பாட்டின் கரைசல் வகுப்புக்குரியதாதல் வேண்டும். மண்டலம் விசையின் பிடிப்பு அற்றதாகிவிடும்போது உடுபாய்பொருளும் மின்னுட்டங்களும் உச்ச நெடுங்கோட்டுத்தளத்தின் அதே அருவிக் கோடுகளைத் தழுவிப் பாயவேண்டுமென்பதையும் மண்டலத்தின் உச்ச நெடுங்கோட்டுக் கூற்றினும் பார்க்க திசைவில் கூற்றானது அதிசக்தி வாய்ந்ததாகவிருந்தாலொழிய, உடுக்களின் மந்த உச்ச நெடுங்கோட்டுச் சுற்றுவட்டம் விசையின் பிடிப்பற்ற மண்டலத்தில் உறுதிநிலை அடையமாட்டாது என்பதையும் இக்கட்டுரையில் விளக்கியுள்ளார்.

இலங்கையின் இளைய தலைமுறையினருள் காணப்படும் இதயத்தசை இன்பாற்றுநிலை

வல்லோப்பிள்ளை, N. J.; அத்துக்கோரளை, D. P.

J. Natn. Sci. Coun. Sri Lanka 1975 3 (1): 19-29

இதயத்தசை இன்பாற்று நிலையால் பீடிக்கப்பட்ட 40 வயதுக்குக் குறைந்த நாற்பது நோயாளிகள், இந்நோயின் காரணங்களை அறியும் நோக்குடன், பரிசோதிக்கப்பட்டனர். இதயத்தசை இன்பாற்று நிலையால் பீடிக்கப்பட்ட இளைய நோயாளிகளின் கொலெத்தரோல் பெறுமானங்கள் ஒப்புநோக்கற் கட்டளைக்குரியவர்களது பெறுமானங்களினின்றும் உயர்ந்ததாகவே உள்ளன வென்பது குறிப்பிடத்தக்கது. புகையுருமானத்தைப் பயன்படுத்தி இளைய இன்பாற்று நோயாளிகளின் இலிப்போபுரதப் பக்கப் பார்வையினைச் சோதித்தபோது நோயாளிகளின் M பின்னம் (=VLDL) நிலையானது ஒப்பு நோக்கற் கட்டளைக்குரியவர்களது நிலையிலும் பார்க்க உயர்ந்ததாகவே இருந்தது. இளந்நோயாளிகளின் 45% வீதமானோர் இதயத்தசை இன்பாற்று நிலையோடு ஐபர்லிப்போபுரோட்டேனேமியா (Hyperlipoproteinaemia) நோயாலும் தாக்கப்பட்டிருந்தனர். அதிலும் W.H.O. மாதிரி IV என்னும் வகைதான் பரவலாகக் காணப்பட்டது. இதயத்தசை இன்பாற்று நிலைக்கு ஆளான இளந்நோயாளிகளின் 50% வீதத்தினர் ஆர்குஸ் கோர்ணியா (விழிவெண்மண்டல வளையம்) எனப்படும் நோயாலும் பாதிக்கப்பட்டிருந்தனர் என்பது கவனிக்கத்தக்க வேறொரு விடயமாகும். இந்நோயாளிகளின் 40% வீதத்தினர் தம் குடும்பங்களின் 60 வயதுக்குக் குறைந்த

நெருங்கிய உறவினர்களிடையில் இதயத்தசை இன்பாற்றுநிலை நோய் பற்றிய வரலாறுகள் கொண்டவராகவிருந்தனர். மட்டுமீறிய தூலிப்பு, மட்டுமீறிய குருதி அமுக்கம், மதுநீரிழிவு ஆகிய நோய்கள் இந்த வரிசையில் ஏற்படக்கூடிய இடர்பாடுகளாயினும் அவை அரிதேதோன்றுகின்றன. நோயாளிகளின் 90% வீதத்தினர் C.H.D. வுக்குரிய இடர்க்காரணிகளுள் ஒன்று அல்லது அதற்கு மேற்பட்டவை கொண்டவர்களாகவிருந்தனர். புகைத்தலும் இயற்கைக்கு மாறான இலிப்போபுரத இன மாதிரிகளும் பிரதான இடர்க் காரணிகளாக விருந்தன.

இதயத்தசை இன்பாற்றுநிலையால் பீடிக்கப்பட்ட நோயாளிகளின் உறவினர்களது கொலெத்தரோல், திரிகிளிசரைட்டுக்கள், "M" பெறுமானங்கள் (VLDL) ஆகியன ஒப்புநோக்கற் கட்டளைக்குரியவர்களின் பெறுமானங்களிலின்றும் உயர்ந்ததாகவுள்ளமை குறிப்பிடத்தக்கது. இதயத்தசை இன்பாற்றுநிலையுள்ள இளந்நோயாளிகளின் பிரதான இடர்க் காரணியாக அதிகரிக்கப்பட்ட VLDL நிலை தோன்றுகின்றது.

பரீட்சைகள் பற்றி

சாமுவல், T. D. M. A.

J. Natn. Sci. Coun. Sri Lanka 1975 3 (1): 31–39

பரீட்சைகளின் நிறைவேற்றுத் தரங்களைப் பாதிக்கக்கூடியதாகவுள்ள சாராமாறிகளைப் பற்றி ஆராய்ந்துள்ளார். சரளமான ஒரு பகுப்பாய்வுக்குப் பின் குறிப்பிட்டவொரு பரீட்சையின் பெறுபேறுகளை இச்சாரமாறிகளை ஓர் அளவுக் கோலாகக் கொண்டு ஆராந்துள்ளார். "நியம நிறைவேற்றுகை விச்சு" என்பது யாது என்பதற்கு இலக்கணம் கூறுவதன் அவசியத்தை இந்த ஆராய்ச்சி மூலம் எடுத்துக்காட்டியுள்ளார்.

மரவள்ளிக் கிழங்கின் சயனசன் குளுகோசைட்டு

III பதனிடல், சமைத்தல் ஆகியவற்றின்போது கட்டுச் சயனைட்டின் தன்மை

நிர்மலா பீரிஸ், P. R. ஜான்ஸ் E. R.

J. Natn. Sci. Coun. Sri Lanka 1975 3 (1): 41–50

மரவள்ளிக் கிழங்கைக் கொண்டு கஞ்சிப்பசைகளை ஆக்கும்போது கட்டுச் சயனைட்டு, கட்டில்லாச் சயனைட்டாக மாறி கழுவுத் தண்ணீருடன் நீக்கப்படுகின்றது. ஈரமுள்ள மரவள்ளிக் கிழங்கு வட்டுக்களை முதுமையடைந்த போது ஆக்கப்படும் மாவின் மொத்த சயனைட்டு அளவு குறைந்துபோகுமெனினும் முழுக்கிழங்குகளாக முதுமையடைந்தபின் ஆக்கப்படும் மாவில் மொத்த சயனைட்டு அளவு அதிகரித்துவிடும். மரவள்ளிக் கிழங்கு மாவிலுள்ள சயனசன் குளுகோசைட்டுக்கள், அம்மாவைக்கொண்டு பாண், றொட்டி அல்லது பிட்டு ஆக்கப்பட்டவிடத்து இல்லாமற் போகும். எவ்வாயிருனும்,

ரொட்டியில் கட்டில்லாச் சயனேட்டு உண்டு. மரவள்ளிக் கிழங்கு மாவால் பிட்டு தயாரிக்கப்பட்டபோதும் பச்சை மரவள்ளிக் கிழங்கை வெட்டி வறுக்கப்பட்ட மரவள்ளி வட்டுக்களிலும் கட்டில்லாச் சயனேட்டின் சிறிய அளவும் கட்டுச் சயனேட்டின் ஏறத்தாழ முழு அளவும் உண்டு. மேற்போந்த பெறுபேறுகள் இனமரின்—லினமருசுத் தாக்கத்தின் பிரகாரம் ஆராயப்படுகின்றன.

மனித இரத்தக் கொலீனெத்தராசும் சேதனவுறுப்புப் பொசுப்பேற்றுப் பூச்சிக்கொல்லிகளின்பால் அதன் தாக்கமும் பற்றிய சோதனைக்குழாய்க் கல்வி

செந்திசண்முகநாதன், S.; இராசரத்தினம், M.

J. Natn. Sci. Coun. Sri Lanka 1975 3 (1); 51–63

பேதட்சு, மலதியோன் ஆகியவற்றின் பல்வகைப்பட்ட செறிவுகளை உபயோகித்து மேற்கொள்ளப்பட்ட பயிர்ப்புத் தடைக்கல்விகளின் பெறுபேறுகள். சிறிய அளவுகளில் பயன்படுத்தப்பட்ட மலதியோன், பயிர்ப்புத் தடையேதும் காட்டவில்லையாயினும் குறைந்த மட்டத்தில் பேதெட்சினையும் மிக உயர்ந்த செறிவுகளில் மலதியோனையும் பயன்படுத்தும்போது இரத்தப் போலிக் கொலீனெத்தராசுத் தொழிற்பாட்டில் (ChE) பயிர்ப்புத் தடை ஏற்படுவதைக் காணமுடிந்தது. இப்பூச்சிக்கொல்லிகளால் தடைக்கப்பட்ட ChE தொழிற்பாடுகள் பிரிடீன்—2—அதொக்சீம்—N—மேதியோடைட்டு (PAM) என்னும் மாற்று மருந்தைப் பயன்படுத்த பின்னர் மெதுவாக மீண்டும் இயங்கத் தொடங்கின. பயிர்ப்புத் தடை விளைவுயேதும் PAM மட்டுமே உண்டுபண்ணவில்லை.

விரைவுக் களச் சோதனை முறையினைக்கொண்டு, இலங்கையின் நோயாளிகள் தொடர்பில் தீர்மானிக்கப்பட்ட ChE தொழிற்பாட்டின் ஆய்வுக் கட்டளை மாதிரியானது 15.3% வீத SD உடன் 80% வீதமாகும். இப் பூச்சிக்கொல்லிகளைக் கையாளுவதிலும் தெளிப்பதிலும் ஈடுபட்டுள்ள ஆளணியினரை, ChE தொழிற்பாடுகள் தொடர்பாகச் சோதனையிடுதல் விரைவுக்களச் சோதனை முறையினைப் பின்பற்றி மேற்கொள்ளப்பட்டது. சுகாதாரத் திணைக்களத்தின் 5 மாறுபட்ட யானைக்கால் நோய் தடுப்புப் பிரிவுகளைச் சேர்ந்த 80 பேருக்கு அதிகமானவர்களைச் சோதனையிட்டு மேற்கொள்ளப்பட்ட கல்விகள் மூலம் அவர்களின் 25% வீதமானோர் மேற்கூறிய கட்டளை மாதிரியின் 50% வீத இரத்த ChE மட்டத்தில் இருந்தார்கள் என்பது புலனுயிற்று. இவர்களுட் சிலரில் காணப்பெற்ற குறைந்த மட்டங்கள் பிறவியோடு தொடர்புபட்ட குறைபாடுகளால் ஏற்பட்டிருக்கலாம் எனினும் அந்த அமிசத்தைப் பற்றி நுண்ணுய்வுகள் மேற்கொள்ளப்படவில்லை.

Journal
of the
National
Science
Council of
Sri Lanka

Instructions to Contributors

Aims and Scope

The purpose of this Journal is to provide a medium for the quick dissemination of the results of research in all fields of Science and Technology. Published material will range from original contributions to review articles describing the state of the art in specific areas, together with short communications.

Manuscripts and all correspondence relating to them should be sent to : The Secretary, Editorial Board, Journal of the National Science Council of Sri Lanka, 47/5 Maitland Place, Colombo 7, Sri Lanka.

Published by The National Science Council of Sri Lanka and Printed at Sri Lanka University Press, Katubedde

JOURNAL OF THE NATIONAL SCIENCE COUNCIL OF SRI LANKA

INSTRUCTIONS TO CONTRIBUTORS

Manuscripts and all correspondence relating to them should be sent to :
The Secretary, Editorial Board,
Journal of the National Science Council of Sri Lanka,
47/5 Maitland Place, Colombo 7,
SRI LANKA.

EDITORIAL POLICIES

Submission of Papers : Papers are accepted for editorial consideration with the understanding that they have not been published, submitted or accepted for publication elsewhere. Papers accepted for publication may not be published elsewhere in the same form, either in the language of the paper or any other language, without the consent of the Editorial Board.

Research papers, Papers read at Symposia and Reviews may be submitted to the Editorial Board. Research papers should describe original investigations or technological achievements. Reviews should be critical evaluations of existing knowledge in a specialised field. The Journal also accepts Short Communications. They should be submitted if the results are of sufficient importance to merit publication in advance of a full paper.

Languages of Publication : Sinhala, Tamil and English.

Refereeing and Editing : All material submitted is examined by two or more referees prior to publication. Papers are edited to increase clarity and ease of communication. In preparation for the press, particular attention is paid to grammar and the conventions of the Journal with regard to symbols, illustrations, tables, references and nomenclature.

Manuscripts submitted for editorial consideration can be processed expeditiously if they conform from the outset to the style of the Journal. Authors are therefore advised to follow closely the form described in these instructions.

PRESENTATION OF MANUSCRIPTS

No maximum length of contributions is prescribed but papers should be written clearly and concisely. All unnecessary textual matter, figures and tables must be eliminated. In general, the impersonal form should be used.

Supplementary material of a detailed nature, which is not essential in the printed paper, but may be useful to other workers, may be deposited with the Secretary. Such material will be made available to other scientists on request and a note to this effect should be included in the paper.

The paper should be reasonably subdivided into sections, and if necessary, sub-sections. The following pattern is suggested for Research Papers : (a) Introduction (b) Experimental (c) Results (d) Discussion (e) Conclusions (f) Acknowledgements (g) References. In many cases, two of sections (b), (c) and (d) can be combined. When a separate Discussion is used, it should not recapitulate the results but discuss their significance and relation to the object of the work and to the work of other people. Conclusions should not merely repeat preceding sections.

Special care must be taken in citing references correctly. Responsibility for the accuracy of these rests entirely with the authors. It is the authors' responsibility to obtain written permission to reproduce material which has appeared in another publication.

FORM OF MANUSCRIPTS

Manuscripts should be submitted **in triplicate** — including the original typewritten copy — typed throughout in double spacing on one side of the paper only. Adequate margins should be left (4 cm) with liberal spacing at the top and bottom of each page. The typescript should be free of corrections.

Headings of major sections should be centred and sub-section headings should be placed on the left of the page. The complete set of headings and sub-headings in an article should be numbered following the style adopted in this Journal and the set should reflect the logical development of ideas.

Paging : Each page of the manuscript should be numbered and the name of the first author and page number indicated in the upper right-hand corner of the page.

The *first* page should contain the article title, the name(s) of the author(s) and name and address of the establishment where the work was carried out. In the case of co-authors, respective addresses should be clearly indicated. Female authors should include one of their given names. The title should be concise but informative. The first word of the title should preferably be one useful in indexing and information retrieval. Where a series of related papers is submitted, each individual paper should have the same general heading, followed by a series number and title of the part. Any footnote to the title should be given at the bottom of this page.

The *second* page should contain an abstract (of not more than 250 words) which should be a summary of the entire paper, not of the conclusions alone and intelligible without reference to the paper itself. The text should begin on page three and each subsequent major section — references, figure legends and table legends should begin on a new sheet.

The *last* page should contain (a) a note as to the number of manuscript pages, figures and tables, (b) proposed running title of less than 42 characters (letters and spaces) and (c) the name and mailing address of the person to whom the proofs should be sent.

Illustrations : All illustrations are considered as figures and each graph, drawing or photograph should be numbered in sequence with Arabic numerals. Authors must submit the original and two duplicates of each figure. Figures should be planned to fit the proportions of the printed page (12 x 17 cm).

Figures must be drawn in Indian ink on plain white paper or board or tracing paper, not larger than 20 x 30 cm. Drawings should be lettered with a lettering set; lettering should be kept large enough to be legible after a reduction of 50 to 60%. If this is not possible, all letters and numerals must be inserted clearly and lightly in blue pencil and not in ink.

Each figure should carry a legend so written that the general meaning of each illustration can be understood without reference to the text. The amount of lettering on a drawing should be reduced as far as possible by transferring it to the legend. Figure legends should be typed on a separate sheet and placed at the end of the manuscript.

Graphs should be plotted on white or blue-lined graph paper or tracing cloth; grid lines that are to be shown in the engraving should be inked in black. The caption of each axis should be lettered parallel to its axis. Each figure should be identified in the margin with author's name and figure number. The preferred position of all illustrations should be indicated in pencil in the manuscript.

Photographs : Half-tone illustrations should be included only when essential. Good glossy prints with sharp contrasts between black and white areas should accompany the manuscripts; they should not be attached to manuscript pages. The size should be such that when the print is reduced to the normal size for reproduction (12 x 17 cm maximum), the detail is still clear. Magnification should be indicated with a scale line on the photograph. The author's name and figure number should be given on the back of each photograph.

Tables should not repeat data which are available elsewhere in the paper. Each table should be typed on a separate sheet with due regard for the proportions of the printed page. They should be numbered consecutively with Arabic numerals. Tabulated matter should be clearly set out and the number of columns in each table should be kept as low as possible. Tables should have legends which make their general meaning clear without reference to the text and all table columns should have explanatory headings. Units of measure should be indicated in parentheses in the heading of each column. Vertical lines should not be used and horizontal rules used only in the heading and at the bottom. A one-column table may be up to 42 characters (letters and spaces) wide. A two-column table may be 90 characters wide. Footnotes to the tables are to be

placed directly below the table and should be indicated by superscript lower-case italic letters (*a*, *b*, *c*, etc.). Each table should carry on the back of the sheet the author's name and figure number. The preferred position of tables should be indicated in pencil in the manuscript.

References to the literature must be indicated in the text by a small superior number referring to the list of references which must be inserted on a separate sheet at the end of the paper. The list should be arranged in alphabetical order by author and numbered in Arabic numerals. All authors' initials must be given after surnames. The year of publication should follow in parentheses. When journal articles are listed, the journal name should be abbreviated in accordance with the *World List of Scientific Periodicals* 1900—1960, 1972, 4th edn, London : Butterworths Scientific Publications. If the journal is not in this list, the name should be given in full. The abbreviated journal title should be underlined to indicate italic type and followed by the volume number underlined with a wavy line to indicate bold type, the issue number in parentheses and then the inclusive pages. When books are listed, the order should be : author(s), year, book title, volume number, edition, pagination/inclusive pages, place of publication and publisher. When sections of a book are listed the order should be : author (s) of section, year, the word *In* followed by author of book, book title, volume number, edition, inclusive pages, place of publication and publisher. The series title of a book should be given in parentheses after the publisher.

Examples :

Journal — ANGMOR, J.E., DICKS, D. M., EVANS, W. C. & SANTRA, D.K. (1972) *Planta Med.* **21**(4) : 46-420.

Book — SCHOKMAN, D. (1966) *Vegetable growing : local and exotic varieties*, 29p. Colombo: Agriculture Department.

Section of Book — ZITNAK, A. (1973) *In Chronic cassava toxicity : proceedings of an interdisciplinary workshop, London, England, 29-30 January* 1973, pp. 89-95. Ottawa: International Development Research Centre. (IDRC-00e).

Footnotes which are *indispensable* should be indicated in the text by small superior figures and listed on a separate page in the manuscript.

Abbreviations and Symbols recommended in the various parts of British Standard 1991 : *Letter symbols, signs and abbreviations* should be used. Authors are encouraged to use the S.I. System of units (see description in British Standard PD 5686 : *The use of S. I. Units*).

Authors whose papers contain mathematical expressions should submit a list of the symbols used carefully and clearly indicated for the guidance of the printer. This list will not appear in print.

Formulae and Equations : Equations should be typewritten and *quadruple* spaced. They should be started on the left margin and the number placed in parentheses to the right of the equation.

Nomenclature : Scientific names of plants and animals will be printed in italics, and should be underlined in the manuscript. In the first citation, genus, species and authority must be given. e. g. *Tylenchorhynchus claytoni* Steiner. In later citations, the generic name may be abbreviated to its initial letter. e.g. *T. claytoni*.

Special instructions in the fields of Physical, Chemical and Medical Sciences are available on application to the Secretary.

Short Communications : The Journal may include a limited number of short communications. Authors should submit short communications only when they believe that rapid publication of their results is of the utmost importance. A short communication must not exceed 1,200 words, i.e. 4 pages of copy inclusive of illustrations and tables. Short communications should be complete in their own right and suitable for citation. The title should indicate the content clearly as these papers do not carry abstracts.

Proofs : Corrected galley proofs must be returned to the Secretary without delay as directed. Failure to do so will result in delay in publication. Correction of proofs by authors must be restricted to printer's and similar errors. They should be marked in pencil. Any modification of the original text is to be avoided. Responsibility for correcting proofs rests entirely on the authors though editorial assistance will be provided.

Reprints : 50 reprints will be supplied free of charge for each article. Additional reprints can be ordered on the reprint order form which will accompany the proofs.

CONTENTS OF PREVIOUS VOLUME

Journal of the
National Science Council
of Sri Lanka

Vol. 3 No. 1 June 1975

Contents

Published by
The National Science Council of Sri Lanka
and Printed at Sri Lanka University Press, Katubedda.