Volume 2, Number 2
June 1987

ISSN: 0127-7065

JOURNAL OF NATURAL RUBBER RESEARCH

Continuation of the Journal of the Rubber Research Institute of Malaysia

Price: Malaysia: 30 Ringgit Per Issue
100 Ringgit Per Volume
Overseas: US$15 Per Issue
US$50 Per Volume

JOURNAL OF NATURAL RUBBER RESEARCH

Rubber Research Institute of Malaysia (RRIM)
Malaysian Rubber Research and Development Board (MRRDB)
Malaysian Rubber Producers' Research Association (MRPRA)

Each volume of the Journal of Natural Rubber Research constitutes four issues published quarterly in March, June, September and December each year.

Published by the Rubber Research Institute of Malaysia
(A Statutory Agency under the Ministry of Primary Industries)
Printed by Percetakan Sinar Suria
June 1987

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Direct Bonding of Natural Rubber to Nitrile Rubber

R.P. CAMPION*

Strong bonds can form between natural rubber (NR) and nitrile rubber compounds when held together during vulcanisation if the former is modified by the addition of liquid polybutene as a compatible extender. Successful bonding is also dependent on the vulcanisation systems employed.

It is suggested that the phenomenon be regarded as one of liquid-solid contact. A NR compound thus modified is sufficiently fluid to wet, under moulding pressure, a high-viscosity nitrile rubber surface (notionally a solid) before vulcanisation is well-established. As the NR compound possesses the lower surface tension (or energy), the system is thermodynamically favourable.

Regarding the vulcanisation systems, a fast-curing nitrile compound is required to maintain the 'notional solid' role. Bond strengths decrease progressively as the NR vulcanisation system is changed from conventional through semi-EV to EV; high strengths are apparently associated with the many polysulphidic crosslinks which form, for conventional systems, during early stages of vulcanisation. It may require the relatively long lengths of polysulphidic crosslinks to traverse the bond interface. An alternative suggestion is that bond formation arises from the maturation reactions of polysulphidic crosslinks near the interface.

Bonds were not affected by immersion in either sea water or ASTM No. 3 oil for thirty days at room temperature. The polybutene levels used did not reduce the NR's high tearing energy characteristics and reduced hardness and modulus by only 10%.

The direct bonding of natural rubber (NR) and oil-resistant rubber compounds could benefit the manufacture of oil-transporting hoses. Oil-resistant rubbers such as acrylonitrile-butadiene copolymer (NBR) tend to be deficient in fatigue and tear properties, and for this reason these hoses are constructed as composites with layers of other, more durable, rubbers (together with textile and steel wire plies as reinforcements) bonded outside an oil-resistant lining rubber. When NR is used in the outer region of the hose wall construction to utilise its good fatigue resistance, bondability or low water uptake properties, it is usual to build outwards from the liner with several intermediate compound layers, each successive layer (frequently a blend) being nearer to NR in compatibility. In this way, components at each interface are sufficiently compatible to bond together and oil resistance decreases gradually outwards through the hose wall construction.

As hose manufacturing techniques improve through the use of one-piece extruded liners and better bonding to end-fittings, the chance of oil leakage through 'micro-corridors' in butt joints or wrapped interfaces is reduced. Natural rubber could be employed next to the liner, provided that good bonding between NR and NBR could be achieved; this is not normally possible because the two rubbers are incompatible. This paper describes a technique whereby NBR compounds can be bonded directly to NR-based compounds; the early stages of the bonding process are regarded as the wetting of one component by the other rather than as a mutual

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interaction occurring for reasons of compatibility.

THEORY

Successful bonding under pressure between adjacent surfaces of two elastomers requires initial intimate interfacial contact (termed *Stage 1* of the bonding process). For good vulcanised inter-elastomer bonding, bonds similar to crosslinks must then form between the elastomers during vulcanisation *(Stage 2).* Bonding is normally achieved by using 'compatible' rubbers of sufficient tack for *Stage 1* to take place. Compatibility can be defined in terms of the solubility parameter δ *i.e.* the square root of the cohesive energy density[1-4]. The requirement for tack is that polymer chain packing gives sufficient free volume, suitably distributed between chains, to allow two-way diffusion of chain portions across the interface into large enough holes[5-8].

When two tacky rubbers which satisfy the conditions for compatibility are brought into contact by viscoelastic flow due to moulding pressure, spontaneous interdiffusion ensures that the contact is maintained *(Figure 1a).* In addition, the presence in the bulk of the second elastomer of diffused chain portions of the first elastomer will facilitate *Stage 2:* normal crosslinking reactions can link these portions with chains of the second elastomer. The high tack of NR, apparently related to its chain structural features[8], makes it particularly suitable for bonding in this way to a similar rubber.

The question then arises as to when two elastomers are compatible or not. An estimation *(Appendix A)* of the thermodynamic requirements at equilibrium conditions for compatibility *i.e.* for a reasonable amount (say 0.1 mole %) of interdiffusion (molecular interfacial mixing) to occur between two polymers 1 and 2 of molecular mass $\sim 10^5$ at a pre-vulcanisation moulding temperature of 110°C is that

$$(\delta_1 - \delta_2) < ca.\ 0.25\ \text{cal}^{1/2}\text{cm}^{-3/2} \quad \ldots 1^*$$

In reality, this value is probably an underestimate, especially when considering compounds of rubbers *(Appendices A* and *B).* For elastomer pairs which do not satisfy *Equation 1,* little interdiffusion can occur, and these elastomers can be termed incompatible. Measurements of δ obtained by solubility parameter spectroscopy[2,9,10] for typical compounds of NR and NBR are 8.6 $\text{cal}^{1/2}\text{cm}^{-3/2}$ and 10.3 $\text{cal}^{1/2}\text{cm}^{-3/2}$ respectively, values well outside the limits imposed by *Equation 1.*

However, the unique material properties of polymers provide an alternative approach to the bonding of incompatible pairs by considering *Stage 1* as a liquid/solid wetting phenomenon rather than liquid/liquid mixing. Although the outline of the suggested approach given here is probably an oversimplification of the actual processes involved, it has led to successful bonding being achieved between compounds of NR and NBR.

The critical surface tension γ_c of a solid surface is an empirically-based term pioneered by Zisman[11]. Only liquids with a surface tension $\gamma_1 < \gamma_c$ will wet that solid *i.e.* spontaneously spread over its surface indicating that the solid/liquid inter-molecular forces are higher than the liquid/liquid forces. This phenomenon is well utilised in adhesives technology[12]. It is proposed that, for two incompatible elastomers to make such contact, the elastomer of lower viscosity is termed a 'notional liquid' and that of higher viscosity a 'notional solid'; if the surface tension of the former is less than that of the latter, intimate contact by wetting is permitted thermodynamically.

As γ_1 and γ_c are generally accepted as being similar for the same material[13], tabulated values of the latter[14] can be employed. In practice, these thermodynamic considerations are over-ruled in the short term by the high viscosity of the notional liquid compared with normal liquids: the time required to achieve wetting will be very long, even when favourable thermodynamic conditions are augmented by

*The units $\text{cal}^{1/2}\text{cm}^{-3/2}$ are equivalent to $(\text{MPa}/4.184)^{1/2}$ in the S.I. system.

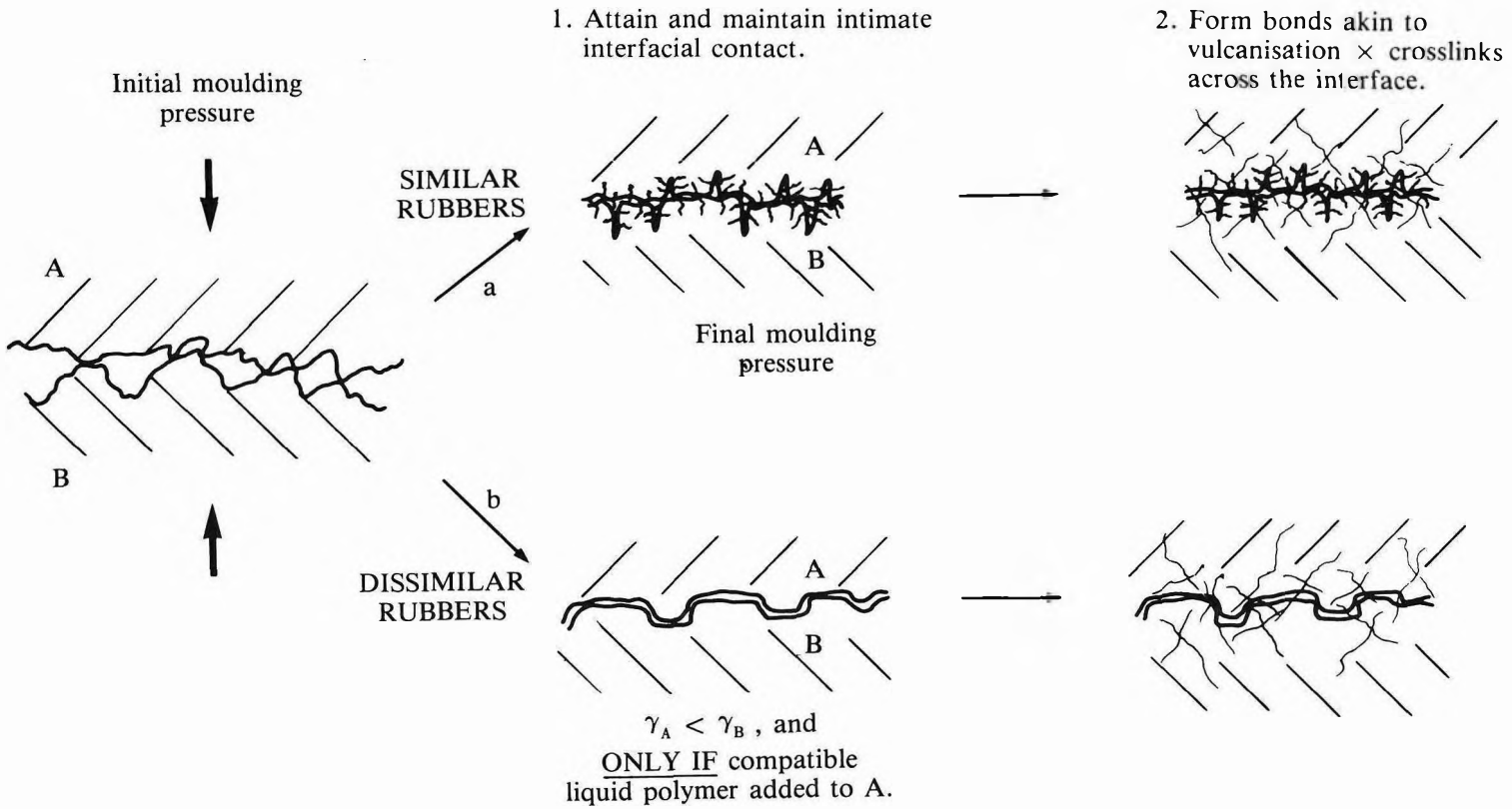

Figure 1. Schematic illustration of inter-elastomer bonding.

the considerable moulding pressures normally met during rubber processing. A reasonable means of overcoming the problem *(Figure 1b)* is by increasing the fluidity of the notional liquid by incorporating a compatible low molecular-mass polymer, *i.e.* a true liquid. (In this context, compatibility is defined as a solubility parameter difference of less than ~0.8 $cal^{1/2}cm^{-3/2}$ when using reasonable proportions of rubber and low molecular-mass polymer. The calculation *(Appendix B)* is considerably influenced by molecular mass.

Literature values[14] of γ_c for NR and NBR are 31 $dyne.cm^{-1}$ and 37 $dyne.cm^{-1}$ (or mNm^{-1}) respectively; the NR compound must, therefore, be chosen as the notional liquid in the wetting process. The low molecular-mass polymer employed to increase fluidity was a polybutene of $M_n = 1300$ (designated PB1300), with a δ value[10] of 8.0 $cal^{1/2}cm^{-3/2}$, so that

$$\delta_{NR} - \delta_{PB1300} = 0.6\ cal^{1/2}cm^{-3/2}$$

This difference satisfies the condition for compatibility noted above so that NR and PB1300 can be considered to be compatible. The value of γ_c for PB1300 may be taken as near to 27 $dyne.cm^{-1}$, the value for butyl rubber[14], so that the nitrile surface should be wetted by either NR or PB1300. The work described in this paper has shown that a NR compound containing PB1300 is sufficiently fluid to allow this thermodynamically predicted wetting of NBR to occur under moulding conditions well within practical vulcanisation times, giving good bonding between the elastomeric compounds.

The development of significant bond strength during vulcanisation — *Stage 2* of the bonding process — suggests that crosslinks must form across the interface despite the absence in this system of significant interfacial interdiffusion. Such crosslinking is presumably possible because of the closeness of contact between the NBR and PB-extended NR surfaces. Experimental data on this point are also given below.

EXPERIMENTAL

Materials

Natural rubber-based formulations are shown in *Table 1. Compounds 1*–3 contained no polybutene and differed only in vulcanisation systems: *1* contained a conventional sulphur system, *2* a semi-EV and *3* an EV system. *1* and *2* differed only in minor detail from formulations fully described in *Natural Rubber Engineering Data Sheets EDS18* and

TABLE 1. NATURAL RUBBER-BASED COMPOUNDS

Formulation	Parts by weight						
	1	2	3	4	5	6	7
NR (SMR 10)	100	100	100	80	75	75	75
N330, HAF black	50	50	50	50	50	50	50
Process oil[a]	5	5	5	—	—	—	—
Zinc oxide	5	5	5	5	5	5	5
Stearic acid	2	2	2	2	2	2	2
Sulphur	2.5	1.5	0.4	2.5	2.5	1.5	0.4
CBS	0.6	1.5	6	0.6	0.6	1.5	6
Antioxidant/antiozonant[b]	2	2	2	2	2	2	2
Polybutene (Mn = 1300)[c]	—	—	—	20	25	25	25

[a]Low viscosity naphthenic, Petrofina 2059

[b]Santoflex 13 (Monsanto)

[c]Hyvis 30, kindly supplied by BP Chemicals Ltd

EDS40, respectively. *Compounds 4–7* contained some PB1300 and no process oil: *4* and *5* resembled *1* with 20 p.p.h.r. and 25 p.p.h.r. respectively of NR replaced by PB1300; similarly *6* and *7* resembled *2* and *3* with 25 p.p.h.r. NR replaced by the polybutene.

The NBR formulation used is shown in *Table 2.* The accelerated sulphur-donor vulcanisation system was much faster than those in the NR-based formulations. This ensured that during bonding the NBR remained notionally 'solid' relative to the notionally 'liquid' extended NR compounds until completion of cure.

TABLE 2. ACRYLONITRILE-BUTADIENE COPOLYMER COMPOUND

Formulation	Parts by weight
NBR[a]	100
Plasticiser[b]	10
N330, HAF black	50
Zinc oxide	5
Stearic acid	2
Sulphur donor[c]	2.9
CBS	1
Antioxidant/antiozonant[d]	1

[a]Acrylonitrile-butadiene copolymer, Krynac 34-50 (Polysar)

[b]Diallylphthalate

[c]Dipentamethylenethiuram tetrasulphide Robac P25, oiled (5% oil) (Robinson Brothers Ltd)

[d]Santoflex 13 (Monsanto)

Bonding and Test Details

Bond strengths were measured by a reinforced 180° peel test. The reinforcing material was rubberised tyre fabric supplied by Dunlop Aviation Division, Birmingham, United Kingdom. Tests were carried out on specimens vulcanised in a long plunger (follow-on) mould. A suitably-sized slab (4 mm) of each compound was compression moulded for 5 min at 100°C–110°C between sheets of polyester film, then well cooled. Two rectangles of tyre fabric were cut to the same shape with the cords running lengthways: when bonding to NBR, one surface of one rectangle was treated with consecutive brush-coatings of *Chemlok 220* and *205* with suitable inter-coat drying. The polyester sheets were removed and the slabs each backed with the tyre fabric pieces, the *Chemlok*-treated fabric surface contacting the NBR. The other two faces of the slabs were brought into contact, with a small metal-foil insert at each end to form tabs for gripping when testing. The composite structures were press-cured, using the plunger mould, at an actual pressure of 11.4 MPa (1660 p.s.i.) for 45 min at 150°C; a shorter cure would have sufficed.

Two 25-mm wide testpieces were cut from the vulcanised composite slab so as to avoid edge effects. The 180° peel test was carried out using a Zwick tensile tester with a jaw separation speed of 50 mm per minute.

Durability Tests

Several testpieces were submerged in either ASTM No. 3 oil or (synthetic) sea water for thirty days at room temperature before testing.

RESULTS AND DISCUSSION

The form of the trace obtained from the peel test depends on the magnitude of the separative force. *Figure 2* shows typical traces depicting low bond strength (unextended NR to NBR) and high bond strength (NR/PB1300, *Compound 5,* to NBR). In the former case, the peel load rises to the point where adhesive separation occurs at a constant value. In the latter the recorded force is variable, reflecting 'stick/slip' behaviour when the separative peel force is close to the rubbers' bulk strength in this mode. To quantify this behaviour a representative value *(F)* was taken as indicated by the broken line in *Figure 2.*

As with the fracture of rubbers generally, adhesion failure is best represented energetically. The ideal case of increasing (by peeling) an adhesive fracture by a small length for a flexible (rubber) strip bonded to an unyielding metal strip was considered by Lindley[15]. For a rein-

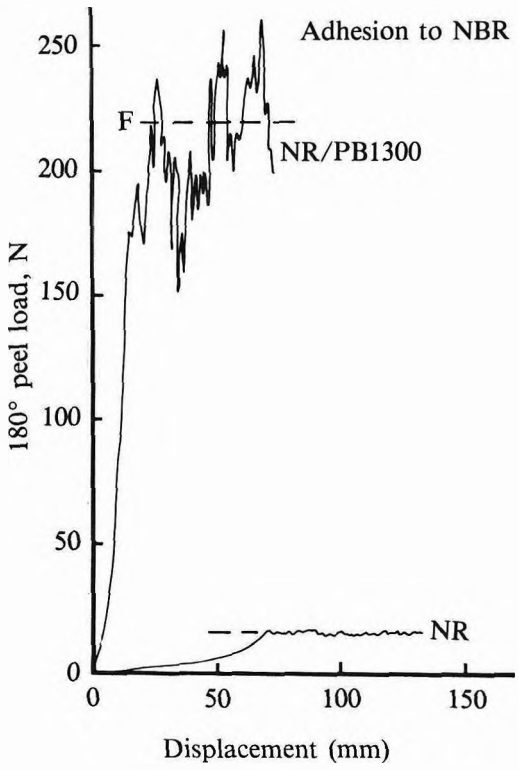

Figure 2. Typical peel adhesion strength traces.

forced testpiece peeled at 180°, the equation derived reduces to

$$P = 2F/w \qquad ...2$$

where P is the peel adhesion energy, F the peel force and w the testpiece width. On applying Lindley's approach to the situation of two flexible, bonded strips under peel test, the general equation differs slightly from the original. However, the modified equation also reduces to the form of *Equation 2* for reinforced testpieces peeled at 180°, and this equation was used to convert peel forces to peel energies.

The peel energies obtained are shown logarithmically as histograms in *Figure 3,* each histogram being the mean of four measurements. The NR-NR bond (*Compound 1* to itself) gave a peel adhesion energy of 100 kJm^{-2}. *Compound 1* bonded to NBR gave only 1½kJm^{-2}; adhesion was non-existent. *Compound 5,* NR:PB1300 (75:25) to NBR, gave a value of about 15 kJm^{-2}. Photographs of the testpieces *(Figures 4–6)* confirm that NR-NBR adhesion is non-existent, but suggest that both NR-NR and NR/PB1300-NBR are high-adhesion systems. The latter conclusion arises from the shape of the separated surfaces and the obvious occurrence of cohesive failure in the rubber in both cases, but especially for NR-NR bonds. *Compound 4,* NR:PB1300 (80:20) to NBR, also gave a peel adhesion energy of 15 kJm^{-2} *(Figure 3).*

Use of *Equation 2* for very low adhesion systems with the testpieces employed is an over-simplification. The textile reinforcement used to prevent extension of the gripping tabs during testing gave rise to strips relatively stiff in the peeling mode. Thus a minimum interfacial bond strength was necessary for the testpiece to be pulled into the position shown in *Figure 4* (180° bond separation) at the start of each test. For adhesion levels below this minimum, bond separation occurred during the early motion so that 180° separation was never achieved (as shown in *Figure 5*) and therefore *Equation 2,* based on 180°, did not apply. As the present work was concerned with achieving good adhesion, the refining of low adhesion measurements by recording actual separation angles and producing a more suitable equation, was not performed.

Furthermore, with the highest adhesion systems, the energy dissipated as rubber fracture normal to the direction of separation (*i.e.* branching) is not accounted for in the original analysis: the value of 100 kJm^{-2} for the NR-NR bond *(Figure 4)* includes contributions from many such fractures into the strips and is thus an over-estimate of the interfacial peel adhesion energy. This conclusion is supported by the observation by Stevenson[16] that the tearing energy of bulk NR (measured by crack growth fatigue testing) is only about 40 kJm^{-2}. Nevertheless, *Equation 2* provides a convenient, reasonable, means of expressing bond strengths, as most results are away from these extremes.

The bond strengths to NBR obtained with NR/PB1300 compounds containing semi-EV

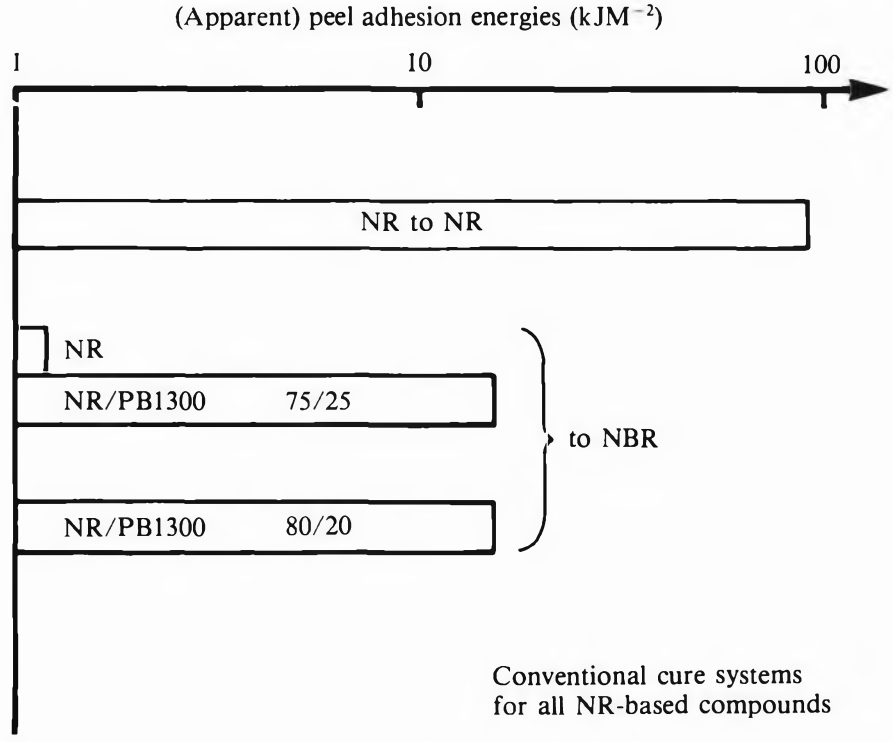

Figure 3. Apparent peel adhesion energies for NR compounds with conventional vulcanisation systems bonded to NR or NBR.

and EV systems are shown in *Figure 7.* Successful bonding occurred for all NR-NR testpieces irrespective of curing system. However, a clear loss in bond strength to NBR was observed as the curing system was changed from conventional through semi-EV to EV.

Mechanistic Aspects

Porter *et al.*[17] have demonstrated that the lengths of crosslinks after vulcanisation, *i.e.* in mature networks, decrease as curing systems increase in efficiency. In the model proposed for bonding dissimilar rubbers shown schematically in *Figure 1b, Stage 2* requires bonds similar to crosslinks to form across the interface. It is suggested that the possibilities of such bonds forming are greater if the crosslinks (bonds) are long: hence the decrease in adhesion level between NR/PB1300 and NBR as curing system changes from conventional to EV agrees broadly with this simple model. An alternative suggestion is that bond formation arises from the maturation reactions of polysulphidic crosslinks near the interface and to their labile nature. These reactions, which continue throughout vulcanisation[17], could also reasonably be involved in links across the surface. In either case, the independence of the NR-NR bond with regard to curing system would also be expected, as the diffusion of chain molecular portions from one interfacial surface into the other before cure means that crosslinking during vulcanisation will 'tie-in' diffused portions, whatever the detail of vulcanisation chemistry.

As already mentioned, successful bonding requires a fast vulcanisation system for the NBR compound. This system might also be involved in the bond development stage, e.g. by forming active species which could migrate across the interface and diffuse into the NR/PB compound. This suggestion is not supported by

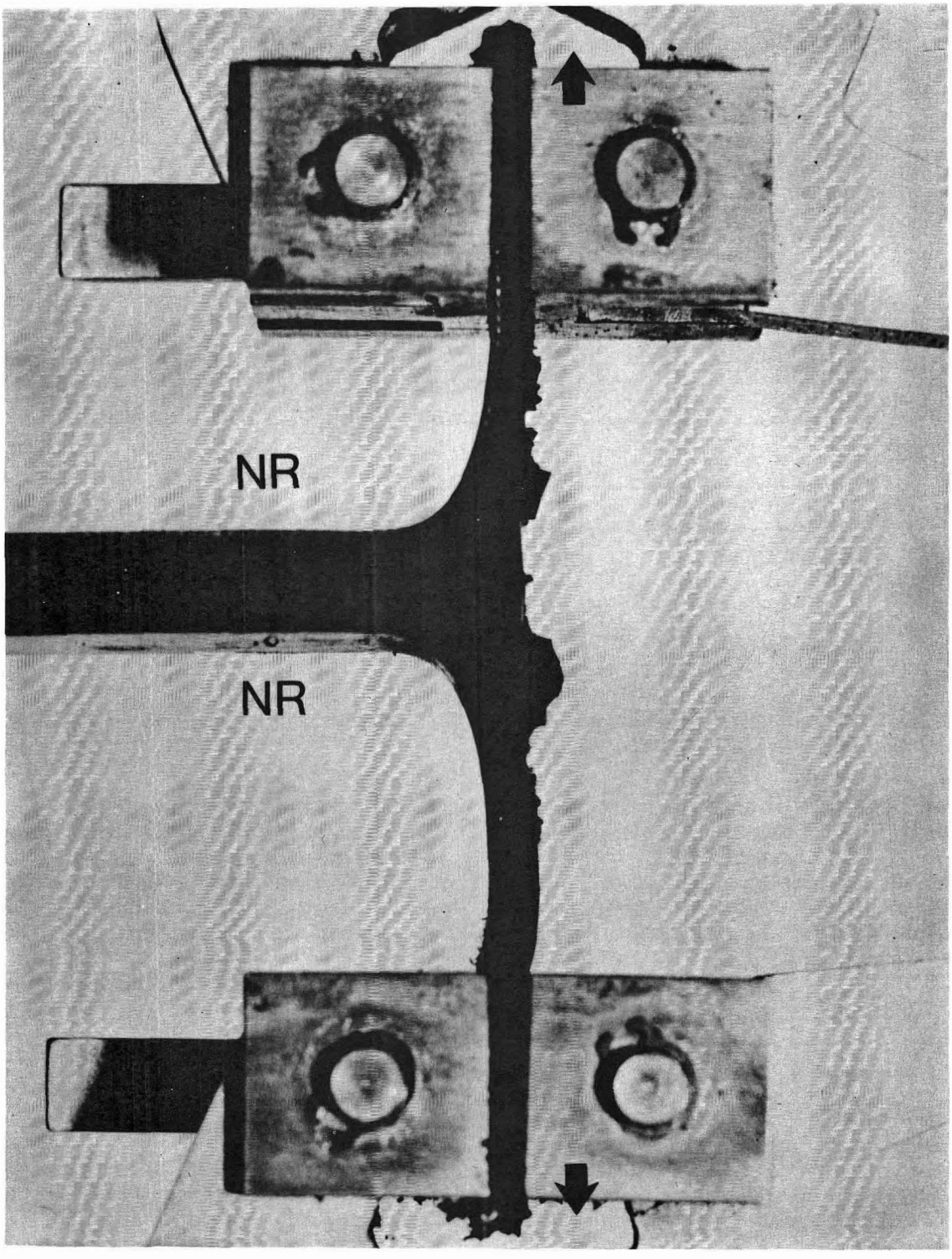

Figure 4. Peeled testpiece showing cohesive failure of NR-NR bond.

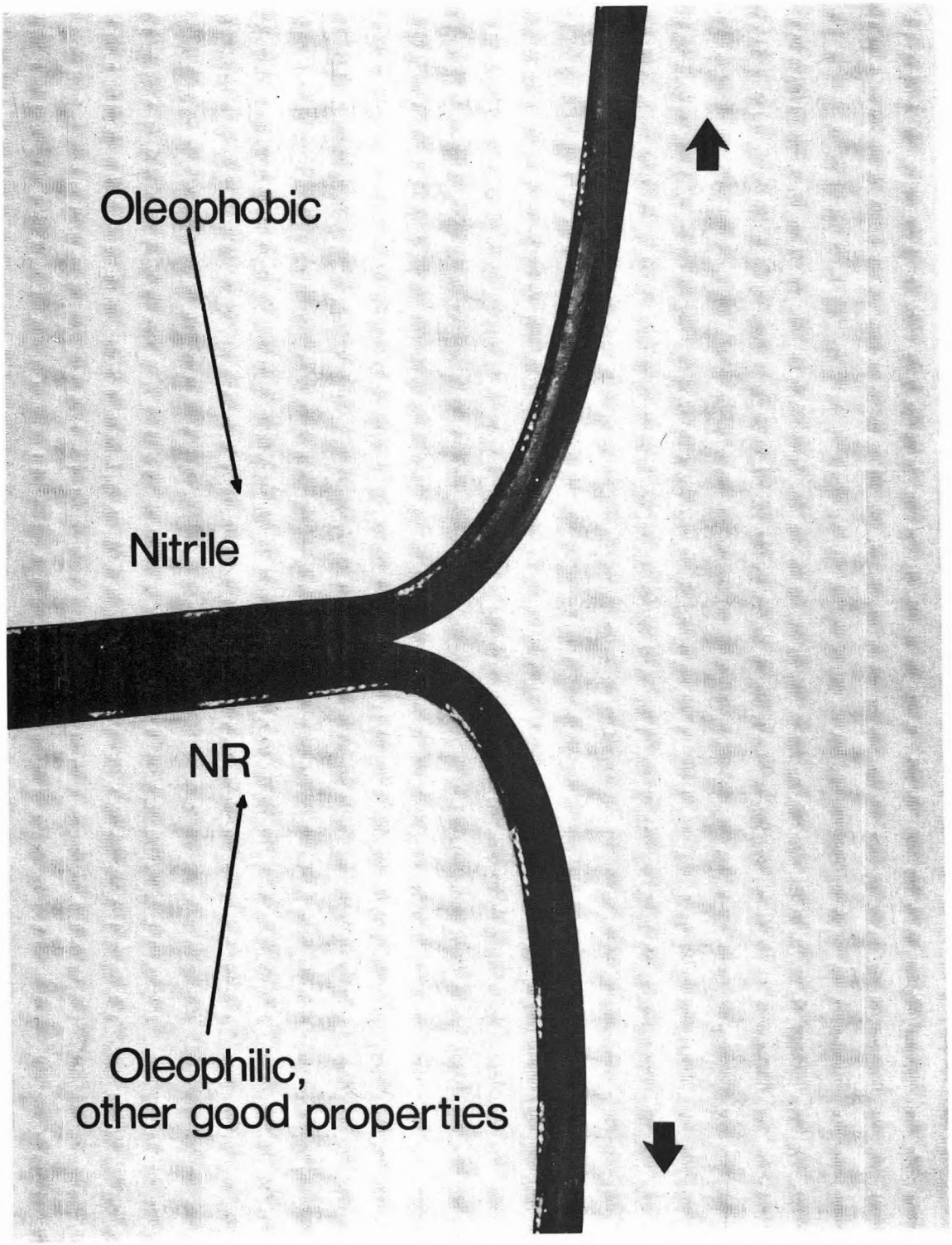

Figure 5. Peeled testpiece showing adhesive failure of NR-NBR bond.

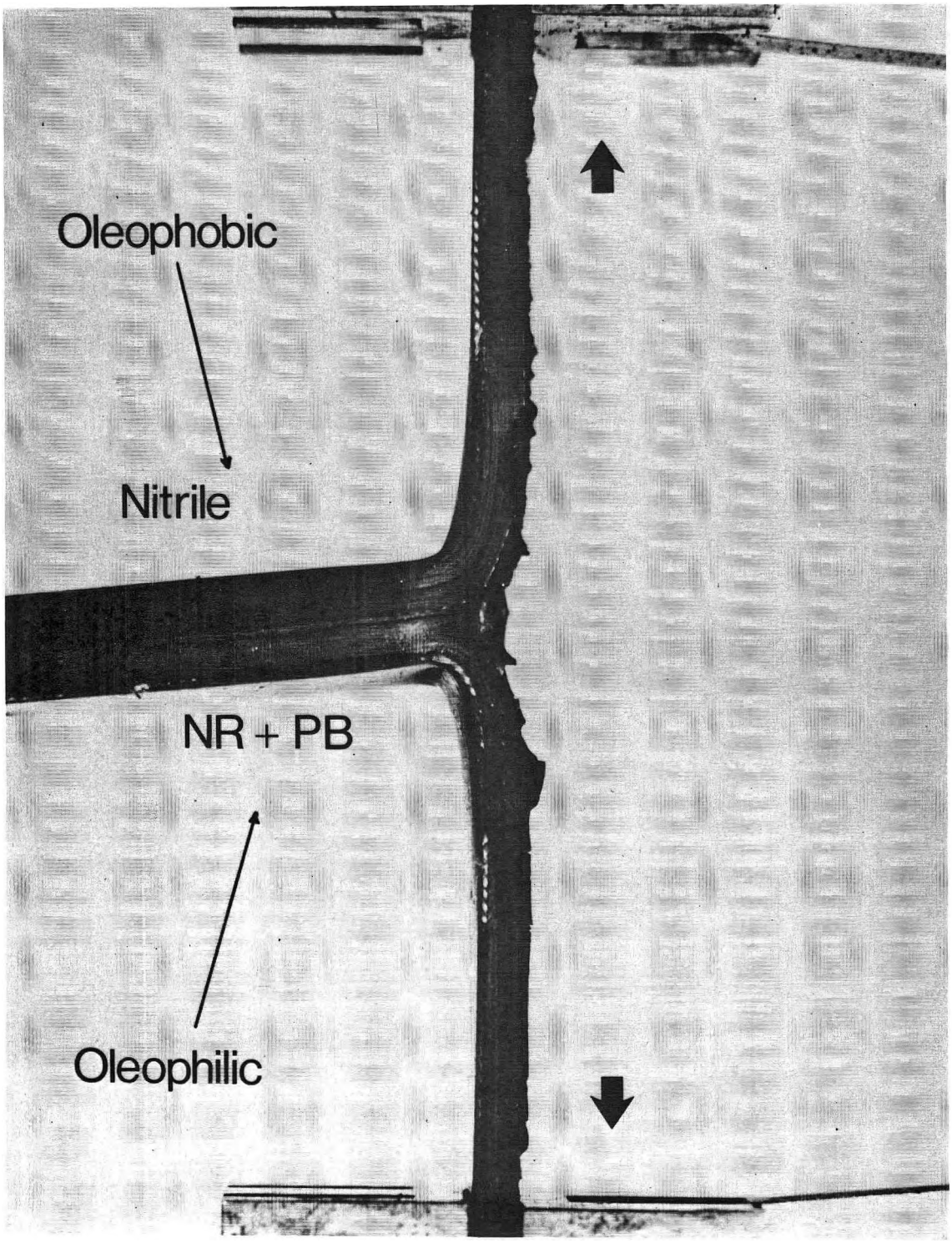

Figure 6. Peeled testpiece showing cohesive failure of NR/PB-NBR bond.

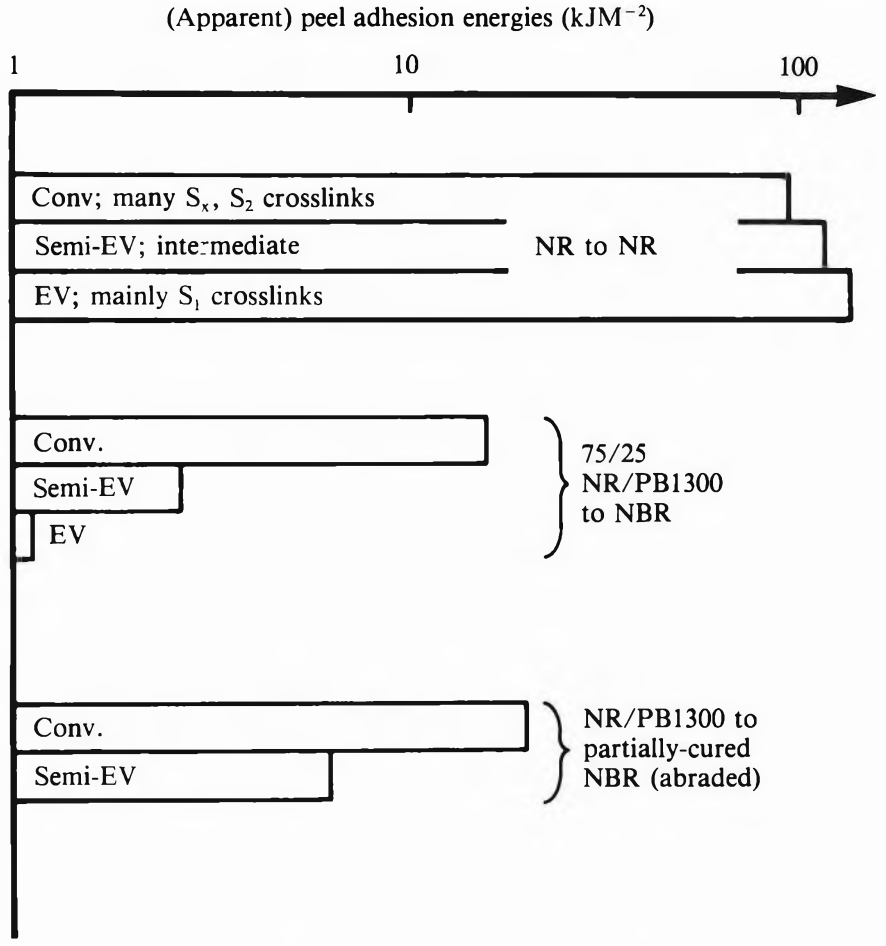

Figure 7. Apparent peel adhesion energies for NR compounds with varying cure systems bonded to NR or NBR.

the data in *Figure 7* for two pressed slabs of NBR pre-cured for 20 min at 150°C (long enough for all active species to be used up in the early stages of crosslinking[18]), surface-abraded and then bonded to NR/PB1300 by the normal procedure. In one case the NR/PB1300 curing system was conventional, in the second case the semi-EV system was used. Results were at least as good as the two corresponding results for bonding to uncured NBR. This suggests that active species formed during early stages of NBR vulcanisation do not become involved in bond formation.

A more rigorous study of the interfacial region could reveal other aspects of wetting and crosslinking relevant to successful bonding between NR/PB1300 and NBR; for instance, PB may conglomerate at the surface and extra sulphur may be involved locally in the bonding.

Durability and Fatigue

The bond strengths of samples immersed in water or oil for thirty days before testing are compared with untreated samples in *Figure 8* as percentage loss of adhesion. Immersion in sea water caused very little change in the NR-NR bond strengths. As the peel failure was still cohesive, little deterioration in bulk strength had occurred. Oil caused swelling and excessive loss in bulk strength, giving completely

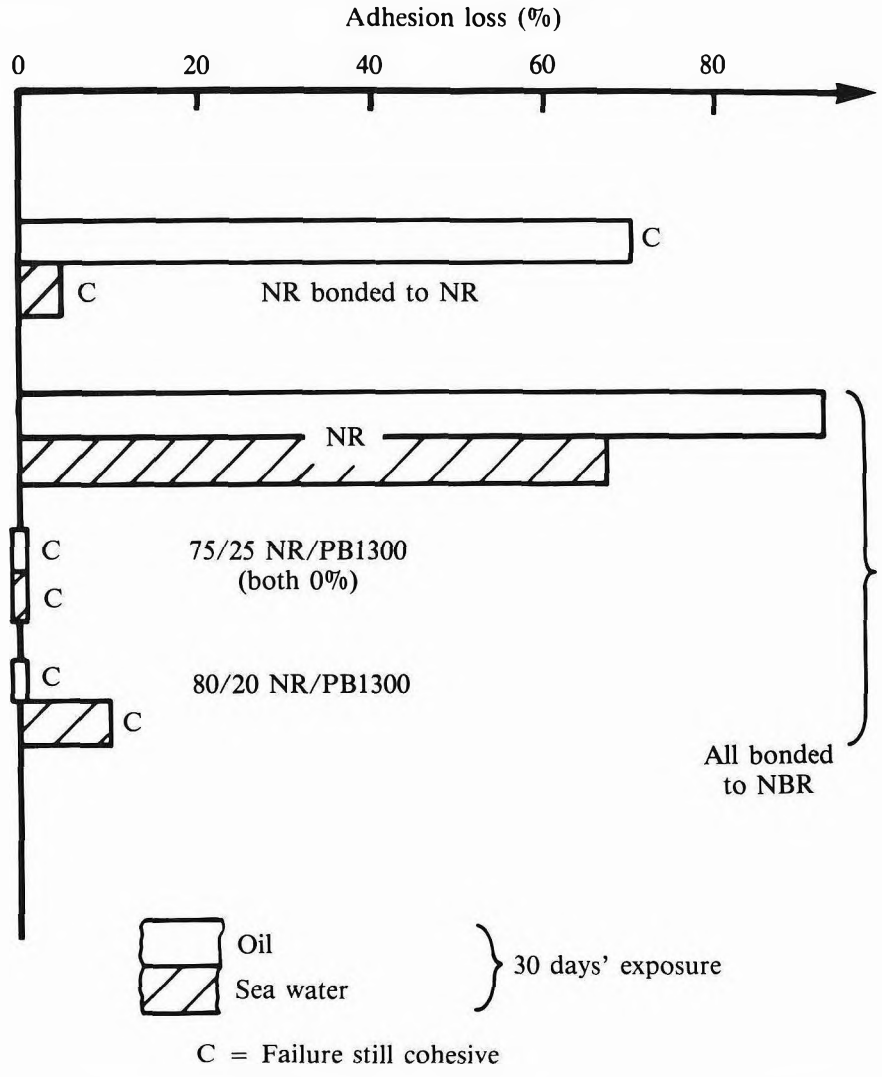

Figure 8. Effect of exposure to oil or sea water on bond strengths.

cohesive failure: the NR-NR bond was still intact.

The NR/PB1300-NBR testpieces gave < 10% (and sometimes zero) adhesion loss at both NR/PB ratios, failure again being cohesive. The bond between the two rubbers remained intact in both oil and sea water and, perhaps surprisingly, swelling was restricted within the NR component so that its cohesive strength was never less than that of the NBR. The restricted swelling was presumably due to the influence of the well-bonded NBR which itself was only slightly swollen.

In contrast, the poorly-bonded NR/NBR testpieces immersed in these liquids essentially lost any adhesion which the system originally possessed. The exercise as a whole suggests that the greater the initial bond strength, the greater the chance of retaining the bond when immersed in oil or sea water.

Although not detailed here, data have been obtained[19] from cut growth fatigue

measurements which led to the tearing energy for most of the compounds discussed in this paper. Briefly, whereas at any tearing energy crack growth rate in the NBR compound was up to ten times faster than in NR, the rate in the 75/25 NR/PB1300 compound was the same as in NR. In addition, the critical tearing energy was 40 kJm^{-2} for both NR and NR/PB1300 whilst that for NBR was only 10 kJm^{-2}. Hence the presence of 33% of PB1300 in NR does not weaken the rubber in fracture energy terms. The main effects on general physical properties are losses in tensile strength and modulus and in hardness of about 10%.

ACKNOWLEDGEMENT

The author thanks the Board of the Malaysian Rubber Producers' Research Association for permission to publish this paper, Drs A. Stevenson and M. Porter for helpful discussions, and a referee for suggested refinements, especially concerning mechanistic aspects.

REFERENCES

1. HILDEBRAND, J.H., PRAUSNITZ, J.M. AND SCOTT, R.L. (1970) *Regular and Related Solutions.* New York: Van Nostrand Reinhold Co.
2. GEE, G. (1943) Interaction between Rubber and Liquids. *Trans Inst. Rubb. Ind.*, **18(6)**, 266.
3. BURRELL, H. (1975) Solubility Parameter Values. *Polymer Handbook (Brandrup, J. and Immergut, E.H. ed),* 2nd ed, Chap. 3. IV-337. New York: John Wiley and Sons Inc.
4. BRYDSON, J.A. (1961) Selection of Compounding Ingredients. *Plastics,* 107.
5. VOYUTSKII, S.S. (1963) *Autohesion and Adhesion of High Polymers (Transl Kaganoff, S.).* New York: Interscience.
6. VASENIN, R.M. (1961) The Sticking Pressure in the Diffusion Theory of Adhesion of Polymers. *Vys. Soed.*, **3**, 679. RAPRA translation 1010.
7. SKEWIS, J.D. (1966) Self-diffusion Coefficients and Tack of Some Rubbery Polymers. *Rubb. Chem. Technol.*, **39**, 217.
8. CAMPION, R.P. (1975) The Influence of Structure on Autohesion (Self-tack) and Other Forms of Diffusion into Polymers. *J. Adhesion,* **7**, 1; also (1977) Microstructure and Autohesive Tack. *Adhesion 1 (Allen, K.W., ed),* Chap. 5. London: Applied Science Publishers Ltd.
9. HALE, P.T. (1981) Solubility Parameter Spectroscopy *Int. Rubb. Conf.* (Poster Session) Harrogate; also private communication.
10. CAMPION, R.P. AND STEVENSON, A. To be published.
11. AMERICAN CHEMICAL SOCIETY (1964) *Commemorative Volume on the Work of W.A. Zisman,* Advances in Chemistry series No. 43, Washington DC.
12. WAKE, W.C. (1976) *Adhesion and the Formulation of Adhesives.* London: Applied Science Publishers Ltd.
13. GARDON, J.L. (1976) Cohesive Energy Density and Adhesion. *Proc. Chem. Inst. Can. Symp. Polym. Interfaces: Focus Adhes. Montreal,* 33.
14. LEE, L.H. (1967) Wettability of Elastomers. *J. Polym. Sci. A-2,* **5**, 1103; (1968) Relationships between Surface Wettability and T_gs of High Polymers, *J. Appl. Polym. Sci.*, **12**, 719.
15. LINDLEY, P.B. (1971) Ozone Attack at a Rubber-Metal Bond. *J. Inst. Rubb. Ind.*, **5**, 243.
16. STEVENSON, A. (1984) *Rubber in Offshore Engineering (Stevenson, ed),* p. 23. Bristol: Adam Hilger Ltd.
17. BATEMAN, L., MOORE, C.G., PORTER, M. AND SAVILLE, B. (1963) Chemistry of Vulcanisation. *The Chemistry and Physics of Rubber-like Substances (Bateman, ed),* Chap. 15. London: Maclaren & Sons Ltd.
18. PORTER, M. Private communication.
19. CAMPION, R.P. AND STEVENSON, A. (1985) The Role of Liquid Polybutenes in Enhancing the Adhesion of NR to NBR and Improving Durability. *Polym. Phys. Group Meet. Univ. of Reading;* abstract only.

APPENDIX A

THE (INTERFACIAL) MIXING OF TWO SIMILAR RUBBERS

The thermodynamic requirement for the mixing of two components is that the Gibbs free energy of mixing, ΔG, must be negative. The relation is:

$$\Delta G_m = \Delta H_m - T\Delta S_m \qquad \ldots 3$$

From regular solution theory[1], the molar entropy of mixing molecules of unequal size is given by:

$$\Delta S_m = -R\,(x_1 ln\phi_1 + x_2 ln\phi_2) \qquad \ldots 4$$

where x_1, x_2 and ϕ_1, ϕ_2 are the mole fractions and volume fractions of the two components. Applying *Equation 4* to an interface of polymers of molecular mass 10^5 and density 0.95 g/cm^3 we can estimate the requirements for achieving a 50/50 mixture as follows:

$$\begin{aligned} \Delta S_m &= -(1.9872 \text{ cal/mole/deg})\,[2\,(0.5\ ln\ 0.5)] \\ &= -\,1.9872\,(ln\ 0.5)/(10^5/0.95)\ \text{cal/cm}^3\text{/deg.} \\ &= \quad 1.309 \times 10^{-5}\ \text{cal/cm}^3\text{/deg.} \end{aligned}$$

Therefore at moulding temperature (383°K)

$$T\Delta S_m = 0.005\ \text{cal/cm}^3$$

$$\text{Also } \Delta H_m = (\delta_1 - \delta_2)^2\phi_1\phi_2\ \text{cal/cm}^3 \qquad \ldots 5$$

$$= 0.25(\delta_1 - \delta_2)^2\ \text{cal/cm}^3$$

Therefore from *Equation 3,* for ΔG_m to be negative,

$$0.25(\delta_1 - \delta_2)^2 < 0.005$$

$$\text{or } \delta_1 - \delta_2 < 0.14\ \text{cal}^{1/2}\text{cm}^{-3/2} \qquad \ldots 6$$

However, if we assume that the mixing of as little as 0.1 mole % of one polymer into the surface layer of the other is sufficient to maintain contact, then the solubility parameter difference which can be tolerated (estimated as above) is given by

$$\delta_1 - \delta_2 < 0.24\ \text{cal}^{1/2}\text{cm}^{-3/2} \qquad \ldots 7$$

NB. For rubber compounds, *Equations 6* and *7* might be underestimates for various reasons — the presence of low molecular mass rubber at the surface — local concentration variations — (for copolymers) favourable chain segment co-alignment.

APPENDIX B

THE MIXING OF NR AND LOW MOLECULAR WEIGHT PB

From *Equation 4,* the blend proportions affect the estimate. Taking the 75/25 proportion often employed, for NR (molecular mass 10^5) and PB1300 (molecular mass 1300):

x_1, the mole fraction of NR, $= \dfrac{0.75/10^5}{0.75/10^5 + 0.25/(1.3 \times 10^3)}$

$= 0.038$

Similarly x_2 (for PB1300) $= 0.962$
(for PB1300)

Therefore, assuming a representative density of 0.9 g/cm^3,

$\Delta S_m = -1.9872\ (0.038\ 1n\ 0.75 + 0.962\ 1n\ 0.25)$

$= 2.672$ cal/mole/deg.

Representative blend molecular mass

$= (0.038 \times 10^5 + 0.962 \times 1.3 \times 10^3)$

$= 5051$

Therefore $\Delta S_m = 2.672/(5051/0.9) = 4.76 \times 10^{-4}$ cal/cm^3/deg.

For the PB to remain compatible during storage, take $T = 273°K$.

Therefore $T\Delta S_m = 0.13$ cal/cm^3

From *Equation 5,* $\Delta H_m = (0.75)\ (0.25)\ (\delta_1 - \delta_2)^2 = 0.1875\ (\delta_1 - \delta_2)^2$

Therefore from *Equation 3,* for ΔG_m to be negative,

$0.1875\ (\delta_1 - \delta_2)^2 < 0.13$

or $\delta_1 - \delta_2 < 0.83$ cal$^{1/2}$/cm$^{3/2}$ ...8

NB. *Equation 8* might be an underestimate for the reasons in *Appendix A* and because of tortuosity effects of fillers.

J. nat. Rubb. Res., 2(2), 82-93

Thermal Analysis of Rubberwood

A.G. TAN* AND J.B. STOTT**

The thermal behaviour of rubberwood in an inert environment was examined using differential thermal analysis (DTA). The resultant DTA trace contains a number of peaks, both endothermic and exothermic, attributed to degradation of the individual wood components. The effects of particle size of the wood, the gas flow rate, the heating rate and fungal infestation on the thermal response of the material were investigated. Reducing the gas flow rate has almost the same effect as increasing the particle size of the wood. A comparison was also made between the thermal behaviour of rubberwood and those of other wood species.

Rubberwood is perhaps the most common fuelwood in Malaysia. It is widely used as a domestic fuel in the rural areas and as a source of energy for the drying of agricultural commodities (including rubber), bricks and related products, sawn timber and other materials. It is also converted into charcoal for use in steel production and in certain areas, as a source of fuel.

Although rubberwood has long been used as fuel, very little information was available on its thermal degradation characteristics. These were investigated in a study using differential thermal analysis (DTA) and thermogravimetry (TG), in both oxidative and non-oxidative environments. The analyses were carried out in a self-designed thermal analyser, which allows testpieces of up to around 20 mm in diameter to be used, compared with only a few millimetres in the case of most of the ready-made apparatus. For comparison, a few local wood species were included in the study. Results of DTA and TG analyses in an oxidative environment have been reported[1]. In this paper, the results of DTA of rubberwood in an inert atmosphere are presented and discussed.

EXPERIMENTAL

Apparatus

This has been described in an earlier publication[1].

Materials

The wood of four old rubber trees, one each from clones Tjir 1, PR 107, RRIM 605 and RRIM 623 were used in the investigation. They were obtained from the RRIM Experiment Station at Sungai Buloh, in the form of trunk sections of approximately 50 mm thick. These trunk sections were divided into two similar lots, one of which was immediately oven-dried at around 80°C while the other lot was kept in a poorly ventilated and humid place for two weeks (during which time the wood became mouldy) before being oven-dried. For ease of reference, the first lot is referred to as 'fresh' wood and the second lot as 'mouldy' wood. The densities of the various wood pieces at 8% moisture content are shown in *Table 1*.

For particle sizes below 4 mm in diameter, the test samples were prepared by passing chips through a hammermill and separating the wood particles into various fractions with a set of Endecotts test sieves. Testpieces of larger diameters were cut out directly from a disc of around 13 mm thick using the appropriate hole saws and were cylindrical in shape.

The wood from the Tjir 1 tree was used in the main investigation while those from the other trees were used for comparison. Unless otherwise stated, the test samples were derived from the fresh wood sections and were oven-dried at 105°C overnight before use.

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TABLE 1. DENSITIES OF FRESH AND MOULDY WOOD

Tree	Density (kg/m^3) Fresh wood	Mouldy wood
Tjir 1	647	619
PR 107	694	624
RRIM 605	655	581
RRIM 623	627	571

The local hardwood species used for comparison were obtained from the Forest Research Institute of Malaysia (FRIM) at Kepong, in the form of hand samples measuring 75 × 100 × 18 millimetres.

Procedure

The experimental set-up is shown in *Figure 1*. The reference material consisted of 5 g of aluminium oxide of particle size −100 + 240 mesh. After the test sample had been dropped onto the sample bed, nitrogen (of 99.995% purity) from a gas cylinder was passed through the two tubes with a combined flow rate of 0.8 litre per minute. Following this, the reactor was heated at a rate of 5.5°C per minute. While the reference material was fluidised from the outset, the test sample remained stationary throughout the run. The reference bed temperature and the temperature difference between the two beds were measured using 1-mm diameter chromel/alumel thermocouples and were recorded continuously on a hot-pen recorder. The experiment was stopped when pyrolysis of the wood had been completed, as indicated by the DTA trace levelling off. This usually occurred above 500°C.

RESULTS AND DISCUSSION

Shape of a Differential Thermal Analysis Curve

Figure 2 shows the DTA curve of the fresh wood of Tjir 1 of particle size −36 + 72 mesh. The sample size used was 3 grammes. The curve exhibits the following features:

- A weak endotherm between 130°C and 155°C *(A)*
- Part of an endotherm from around 155°C to 240°C *(B)*
- An exotherm peaking at 320°C *(C)*
- An endotherm peaking at 360°C *(D)*
- An exotherm peaking at 400°C *(E)*
- A week endotherm at around 490°C *(F)*.

Only *Endotherms A* and *F* exist as individual peaks; the rest overlap to a certain degree with the adjacent peaks.

Interpretation of the DTA curve had been made by Tan[2], based on the results of further investigations and also on the information available in the literature[3-7]. *Endotherm D* and *Exotherm E* were shown to be due to the thermal decomposition of cellulose and lignin respectively. *Endotherms A* and *F* seemed to be attributed to the extractives while *Endotherm B* and *Exotherm C* appeared to be due to a combination of the extractives and the hemicelluloses.

Visual observations of the sample were made in a repeat run. It was noted that:

- An aromatic odour was emitted from the sample tube from about 145°C.
- Smoke began to be emitted from around 190°C and its intensity increased with increasing temperature.
- At 220°C, the sample had turned slightly brownish and the aromatic odour was still present.
- The sample became darker with increasing temperature. At around 290°C, its view was totally blocked by the smoke.
- Visibility was partially restored at 390°C, by which temperature the sample had turned into char.

Thus, it is quite clear that charring of the wood took place mainly between 290°C and 390°C, which corresponds to degradation of cellulose and part of lignin.

Effect of Particle Size

The effect of particle size on the thermal behaviour of rubberwood was examined using

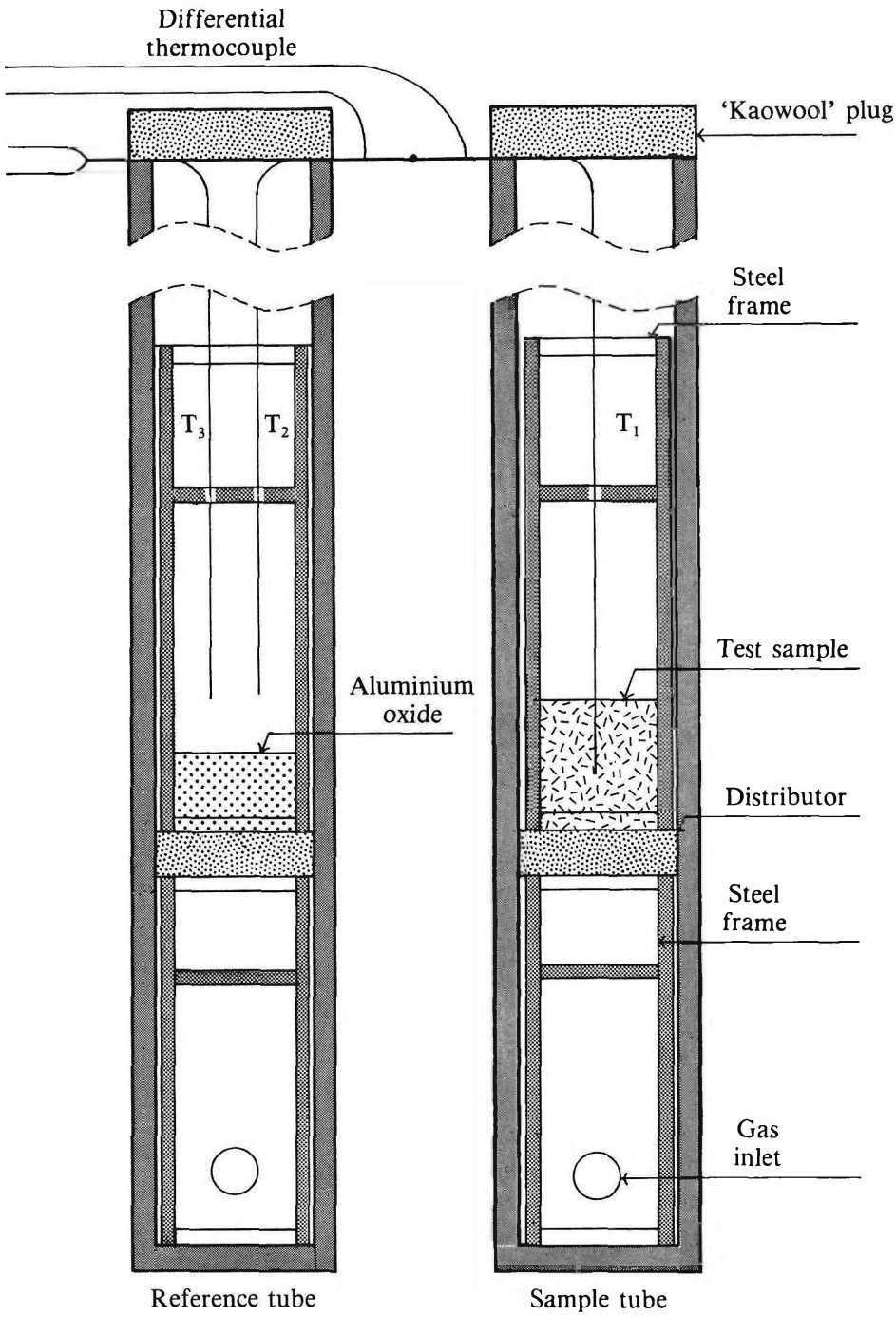


Figure 1. Experimental set-up.

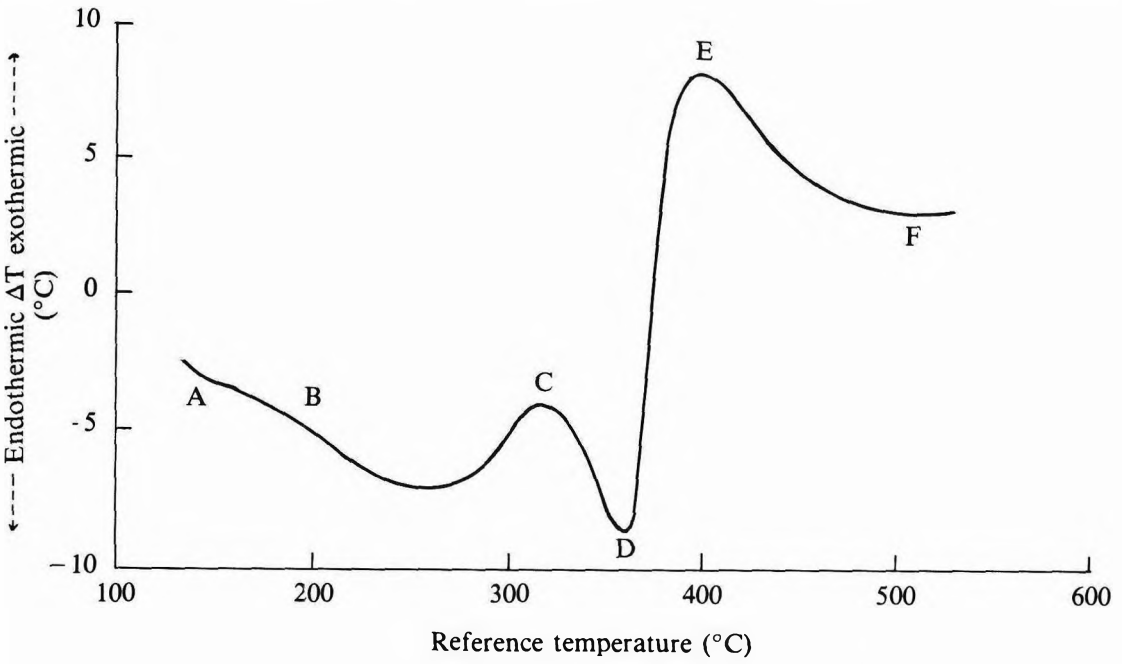


Figure 2. DTA curve of rubberwood of particle size −36+72 mesh.

3-g samples. The particle sizes used ranged from <150 mesh to 10 mm in diameter. As shown in *Figure 3,* the DTA curves of the <150 mesh, −36+72 mesh and −10+18 mesh particles are almost similar in shape, except for the magnitude of *Endotherms A* and *F.* They are, however, quite different from those of the 5 mm and 10 mm diameter fractions, which contain an extra exotherm between 340°C and 380°C *(G)* and whose *Exotherm C* is relatively large in size.

Additional runs were carried out using a single testpiece with the thermocouple's sensing tip being placed within the wood through a 1.5-2 mm diameter hole of depth 7 millimetres. The DTA traces of 5 mm, 10 mm and 19 mm diameter pieces are fairly similar in shape *(Figure 4);* the most glaring difference being in the relative size of *Peak G* which increases with particle size, apparently at the expense of the exotherm to its right, *i.e. Peak E.* There is hardly any difference between the DTA curve of the 5 mm diameter piece and the one obtained earlier using a 3-g sample. In the case of the 10 mm diameter size group, the resolution of the reaction peaks is much better using this technique that the one used earlier (in which the thermocouple is surrounded by the testpieces), although there are no basic differences between the two curves obtained. Likewise, the reaction peaks of the 19 mm diameter piece are well-defined. Thus, in the DTA of wood of larger particle sizes, there is a clear advantage in using a single testpiece with the thermocouple tip placed inside it.

Further analyses were performed on 10 mm and 19 mm diameter single testpieces with a portion of their water-soluble extractives removed by boiling or soaking in water. From the DTA curves obtained *(Figure 5),* it is evident that *Exotherm G* is attributed to the water-soluble extractives, directly or indirectly. In other words, the peak could either result from the thermal degradation of the extractives or reactions between the pyrolysis products of the extractives and those of the other components. What is clear is that it masks part of

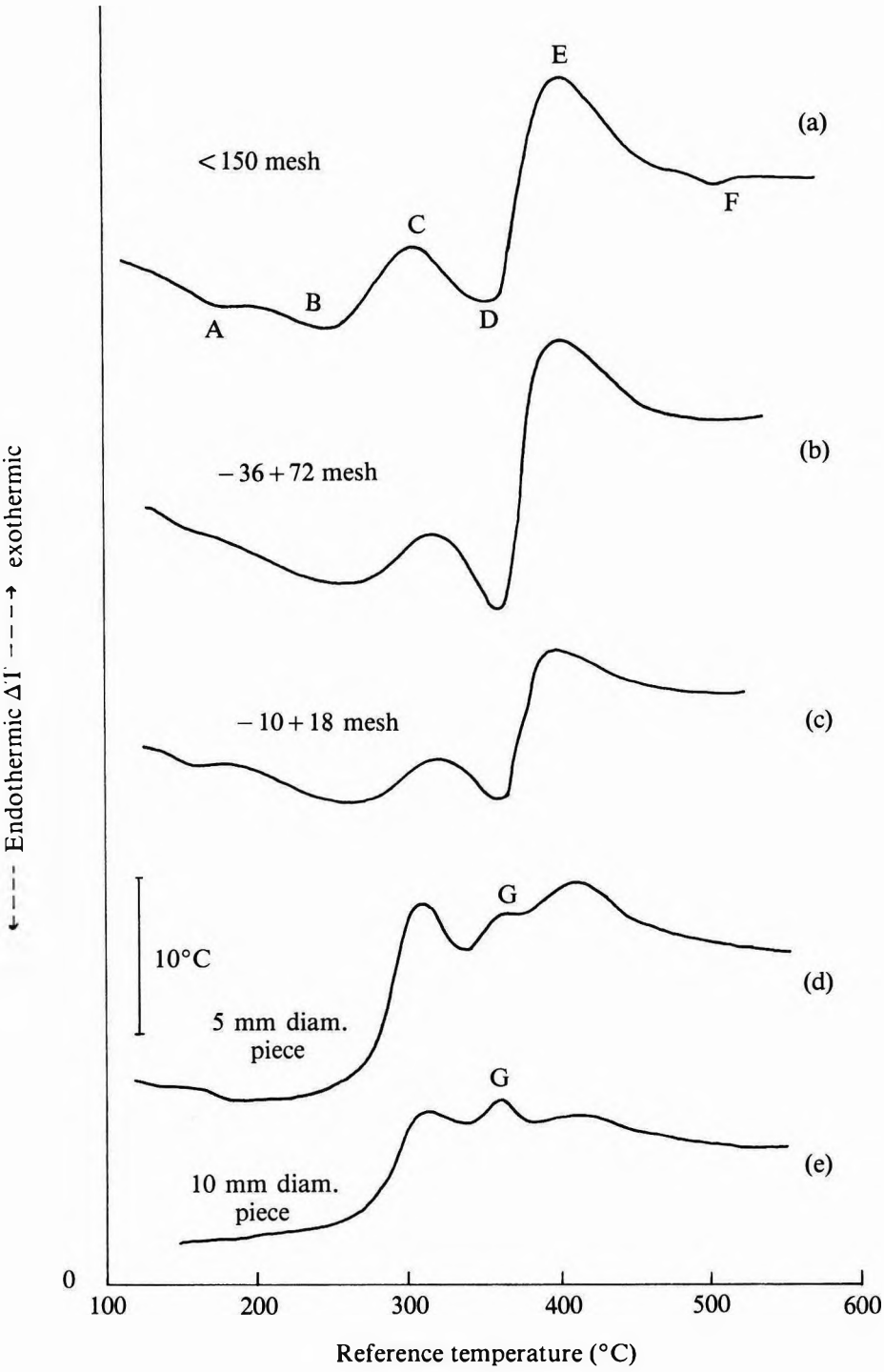


Figure 3. DTA curves of rubberwood of different particle sizes.

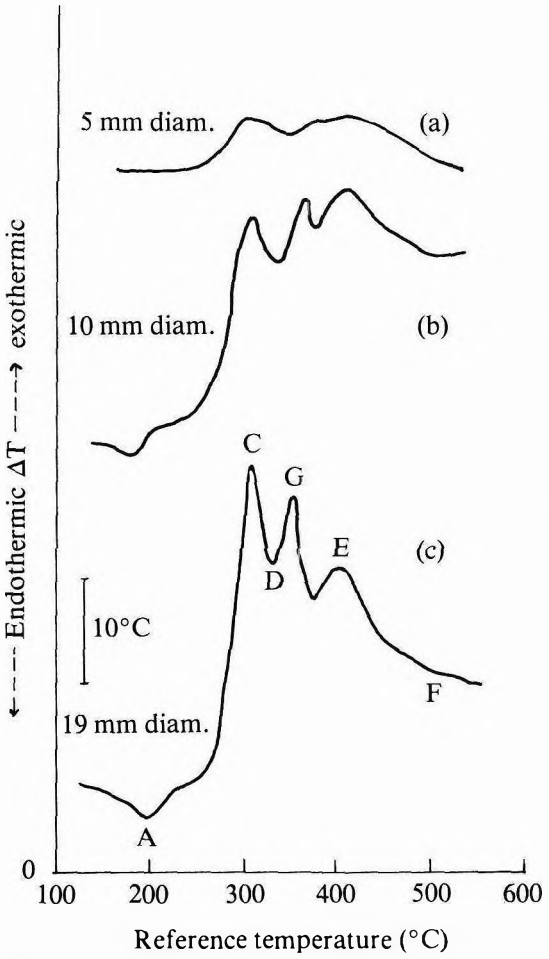


Figure 4. DTA curves of rubberwood of various diameters (single piece).

the cellulose endotherm in the DTA curves of the larger wood pieces.

The above experiments show that the thermal response of rubberwood is dependent on particle size. From the shapes of the DTA curves obtained, it appears that the pyrolysis of the larger wood pieces has an overall exothermic effect. The picture is not so clear in the case of wood of smaller particle sizes.

Effect of Gas Flow Rate

The effect of gas flow rate on the shape of the DTA curve was investigated using −36+72 mesh particles with a sample size of 3 grammes. As shown in *Figure 6,* reducing the gas flow rate from 0.8 litre per minute to 0.05 litre per minute resulted in (a) the formation of an extra peak between 350°C and 390°C, similar to the one present in the DTA curves of the larger wood pieces and (b) pyrolysis of the wood being more exothermic. In essence, lowering the gas flow rate has a similar effect as increasing the particle size of the wood. This could perhaps be explained by the fact that with a lower gas flow rate, the retention time of the pyrolysis products is longer, thus giving them more time to react, as happened within the larger testpieces. It is interesting to note that *Curve (c)* has almost the same shape as the DTA curves of the 5 mm diameter size group obtained earlier.

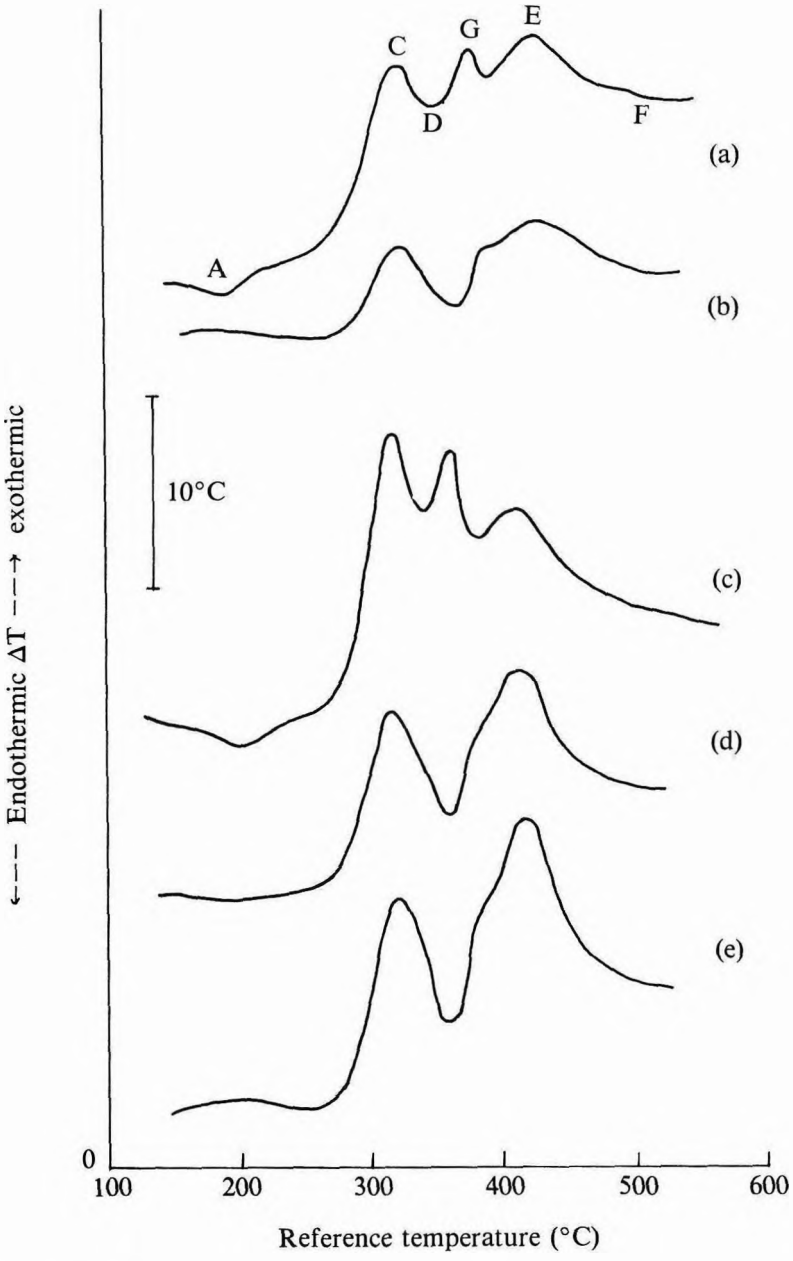
Effect of Heating Rate

To examine the effect of heating rate on the thermal response of rubberwood, runs were carried out on 19 mm diameter single testpieces using a heating rate of 3.6°C per minute. Owing to the relatively large thermal capacity of the reactor (around 20 kJ/K), the study could not be extended to heating rates above 6°C per minute. As demonstrated in *Figure 7,* reducing the heating rate from 5.5°C per minute to 3.6°C per minute resulted in the second exotherm, *i.e. Peak G,* increasing in size. This appears to be the only noticeable difference, as there was very little change in the individual peak temperatures.

Comparison of Wood from Different Trees

The thermal behaviours of rubberwood from the four trees were compared using three particle sizes, *viz.* −36+72 mesh fraction, 5 mm diameter pieces and 19 mm diameter single testpieces. A sample size of 3 g was used for the first two particle sizes while the weights of the single testpieces varied from 2.4 g to 2.7 grammes.

As shown in *Figure 8,* there is no noticeable difference between the DTA curves of rubberwood from the four trees for the −36+72 mesh fraction. In the DTA curves of the 5 mm diameter size group *(Figure 9), Exotherm G* is more conspicuous for the wood from the PR 107 tree than for those from the other three trees. This is also the case for the 19 mm diameter



(a) 10 mm diameter piece
(b) 10 mm diameter piece, boiled in water for 8 h
(c) 19 mm diameter piece
(d) 19 mm diameter piece, boiled in water for 14 h
(e) 19 mm diameter piece, soaked in water for four days

Figure 5. Comparison of DTA curves of rubberwood with and without a portion of its extractives removed.

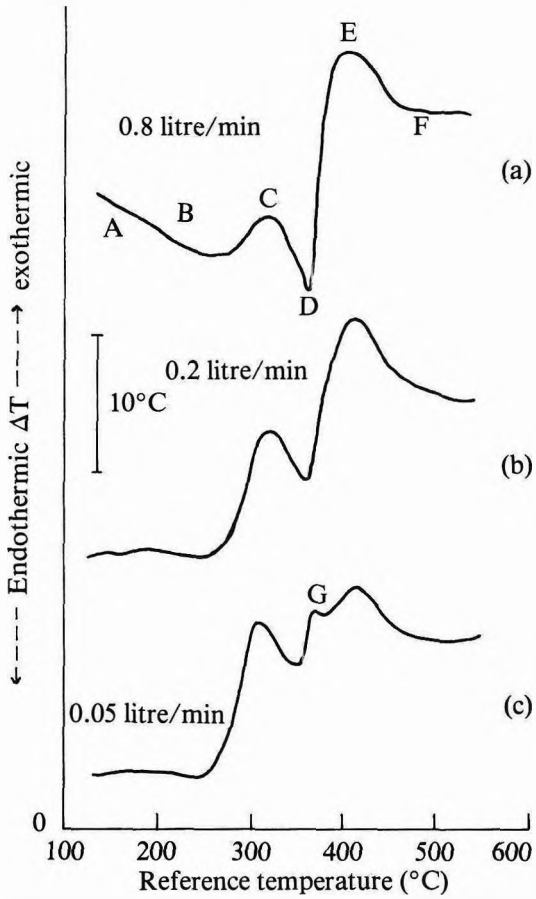


Figure 6. DTA curves of rubberwood (−36+72 mesh) from runs using different gas flow rates.

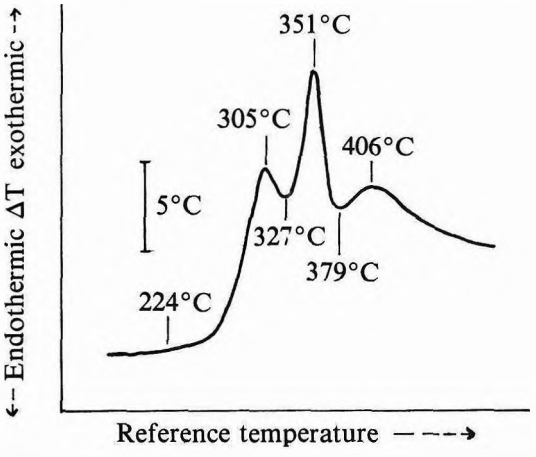


Figure 7. DTA curve from a run using a heating rate of 3.6°C per minute.

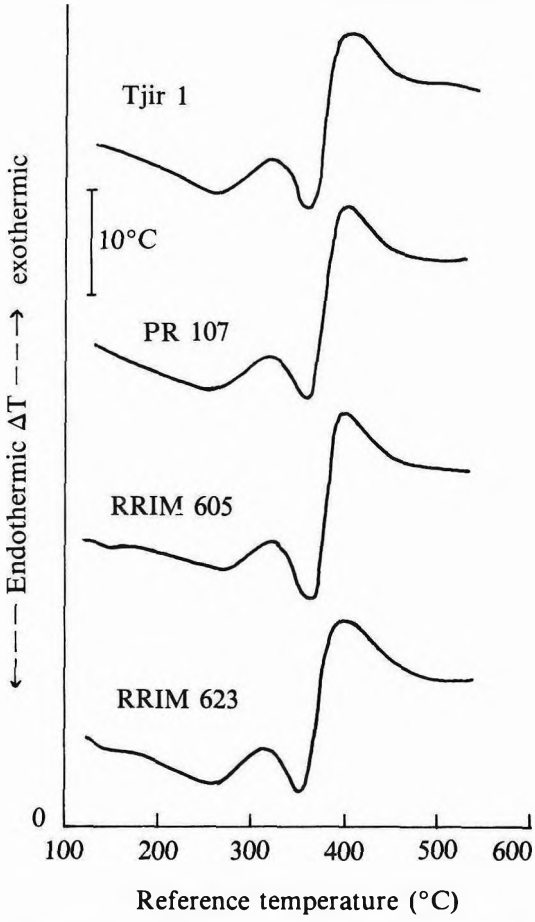


Figure 8. DTA curves of rubberwood from different trees (−36+72 mesh particles).

single testpieces *(Figure 10)*. The results obtained indicate that there is a slight difference in the thermal behaviour of wood from the four trees and that the difference is only brought about by the use of larger testpieces. As *Exotherm G* is mainly attributed to the water-soluble extractives, it would appear that the wood from the PR 107 tree contains a higher proportion of these materials than those from the other three trees. This view is supported by the fact that the PR 107 wood is 6%–11% denser than the wood from the other trees.

Comparison between Fresh and Mouldy Wood

A comparison was made between the thermal behaviours of the fresh and the mouldy wood

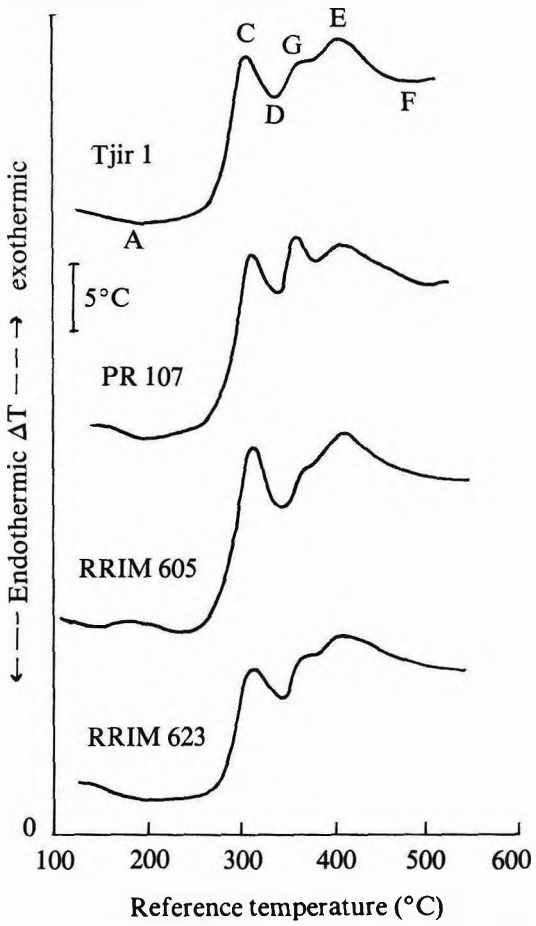


Figure 9. DTA curves of rubberwood from different trees (5 mm diameter pieces).

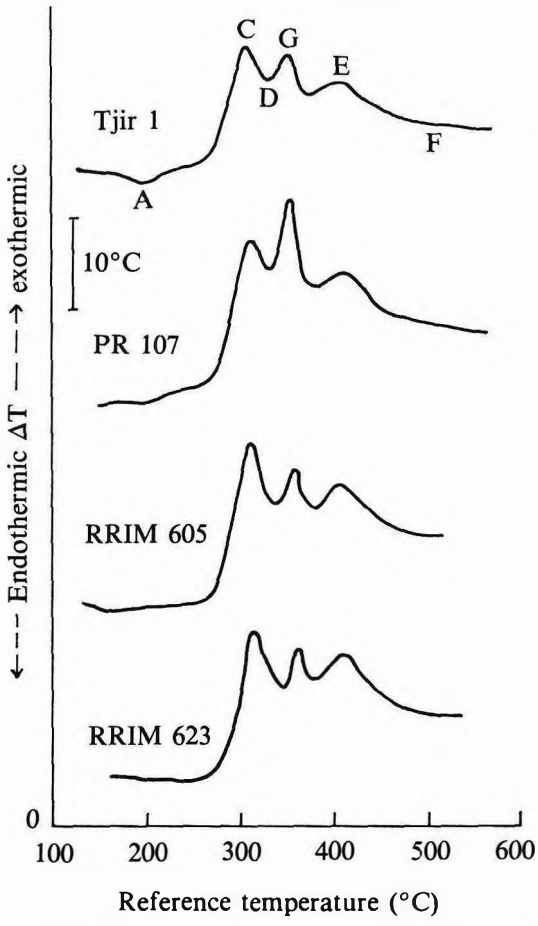


Figure 10. DTA curves of rubberwood from different trees (19 mm diameter pieces).

from each tree using 19 mm diameter single testpieces. As demonstrated in *Figures 11* and *12, Peak C* is smaller while *Peak G* is larger in the DTA curve of the mouldy wood than in that of the fresh wood from the same tree. This could be due to a change in the chemical composition or physical properties of the wood as a result of fungal infestation. The fact that the density of the mouldy wood is 5%–11% lower than that of the corresponding fresh wood indicates that some changes have taken place within the wood.

Comparison with Other Wood Species

The comparison of the thermal behaviours of rubberwood and other hardwood species was made using 19 mm diameter single testpieces. The resultant DTA curves are shown in *Figure 13*. With the exception of *Endotherms A* and *F*, all the reaction peaks which are present in the DTA curve of rubberwood are also present in those of the other wood species. There was only a slight variation in the individual peak temperatures; *Peak C*, for example, varied from 293°C to 316°C, *Peak G* from 351°C to 367°C and *Peak E* from 406°C to 429°C. The difference was greater in the relative sizes of the individual peaks. It will be noted that *Peaks E* and *G* are more prominent in the DTA curves of the other wood species than in that of rubberwood. It thus appears that pyrolysis of the other wood species above 320°C is more exothermic than that of rubberwood. If the

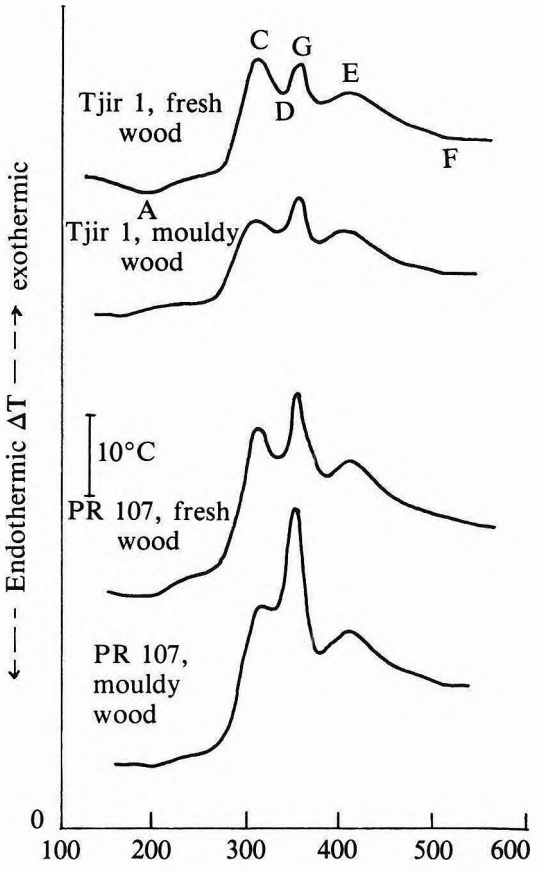


Figure 11. Comparison of DTA curves of fresh and mouldy wood (from Tjir 1 and PR 107 trees).

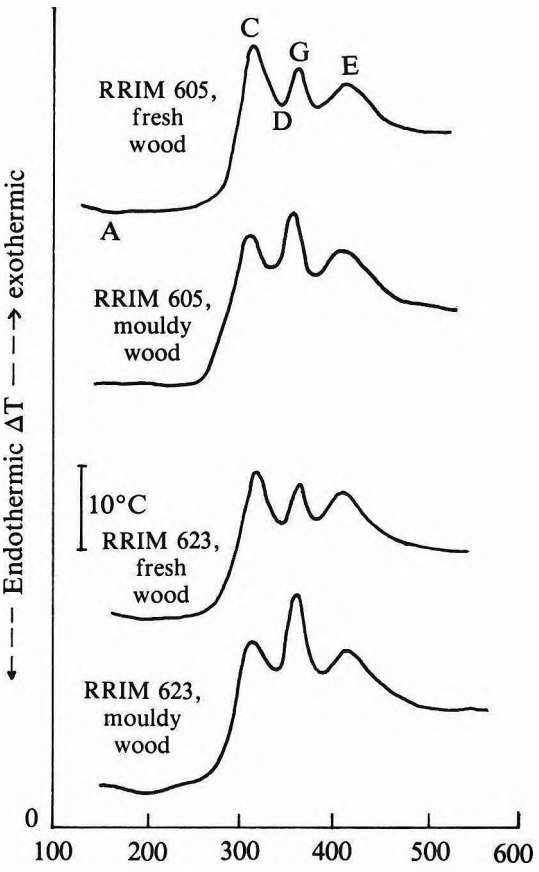


Figure 12. Comparison of DTA curves of fresh and mouldy wood (from RRIM 605 and RRIM 623 trees).

wood components which are responsible for *Peaks E* and *G* are the same for all the wood species, an inference which may be made from the results obtained is that the other wood species contain a higher percentage of lignin and the extractives than rubberwood, which is not surprising since the rubber trees from which the test samples were obtained were only between twenty to twenty-five years old compared with the forest trees which were over fifty years old at the time of felling.

Besides the tropical hardwoods, comparison of the thermal behaviour of rubberwood was also made with those of its bark, coconut wood and oil palm wood. Again 19 mm diameter single testpieces were used, except for the bark whose test sample consisted of a square piece of dimensions 16 × 16 × 6 millimetres. As shown in *Figure 14,* the DTA curves of coconut wood and oil palm wood are fairly similar in shape. Each of them appears to contain two strong and one weak exotherms, the first peaking at around 275°C (compared with around 300°C in the case of rubberwood), the second at around 340°C and the third at around 400°C. As the third exotherm is attributed to lignin, the results obtained indicate that the lignin contents of coconut wood and oil palm wood are lower than that of rubberwood. The DTA curve of the bark sample differs considerably from that of rubberwood but both of them

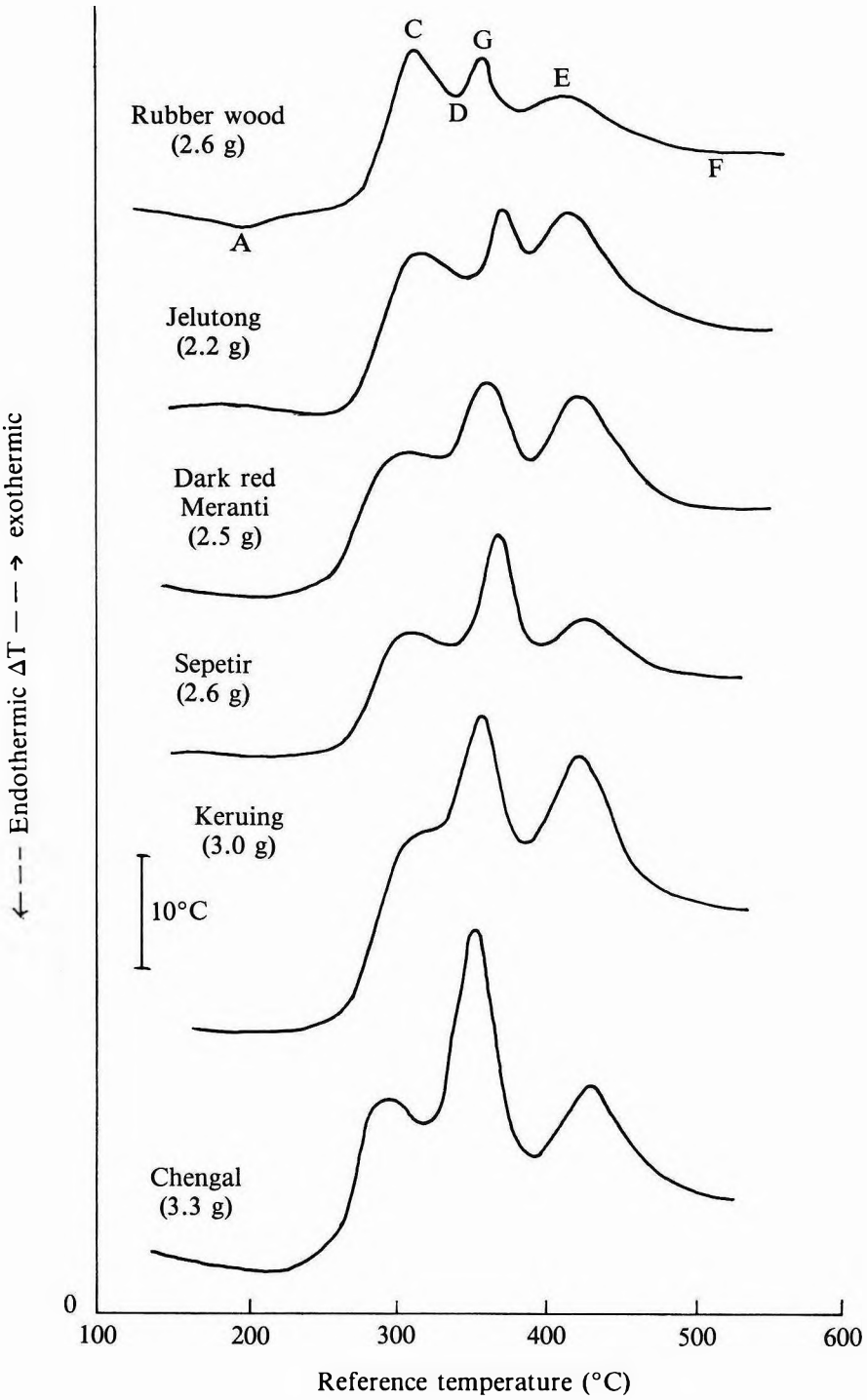


Figure 13. Comparison of DTA curves of rubberwood and some hardwood species.

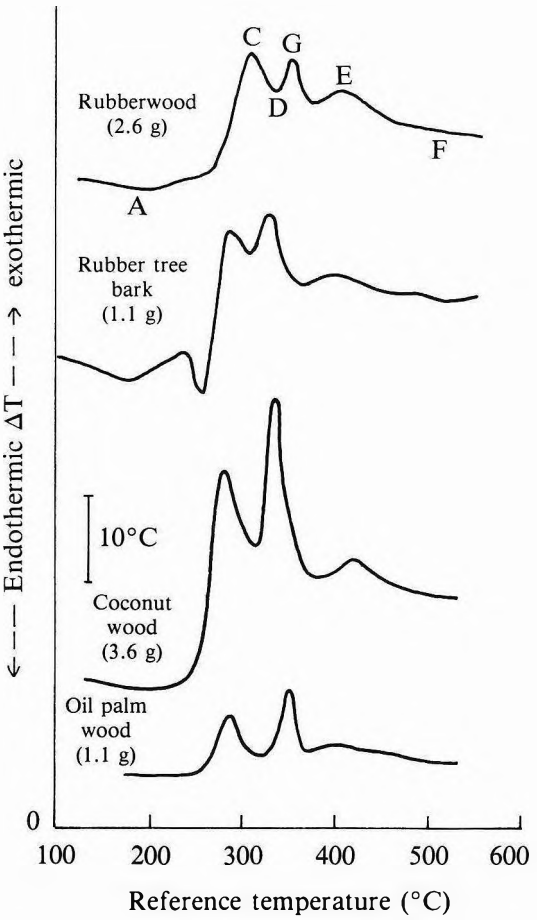


Figure 14. Comparison of DTA curves of rubberwood and some agricultural by-products.

contain certain common features, namely an endotherm below 200°C, an exotherm peaking at around 400°C and a weak exotherm at around 500°C. The lignin content of rubber tree bark also appears to be relatively low.

CONCLUSIONS

The pyrolysis of rubberwood in nitrogen begins at around 130°C and continues until around 500°C, with the major reactions occurring between 250°C and 400°C. The shape of the DTA trace is influenced by the particle size of the wood, the gas flow rate, the heating rate and fungal infestation. Thus, for comparison of the thermal behaviours of different wood species, it is important that the same experimental conditions are used. The same reaction peaks which are present in the DTA curve of rubberwood are also present in those of the tropical hardwoods, except for a difference in size which is believed to be due to a difference in the chemical compositions of the various wood species.

ACKNOWLEDGEMENT

The authors acknowledge the assistance of the technical staff of the Department of Chemical and Process Engineering, University of Canterbury, in fabricating the thermal analyser. Thanks are also due to the Rubber Research Institute of Malaysia for providing most of the test materials.

REFERENCES

1. TAN, A.G. AND STOTT, J.B. (1987) A Comparative Study of the Combustion Characteristics of Rubberwood and Some Malaysian Wood Species. *J. nat. Rubb. Res.*, **2(1)**, 27.
2. TAN, A.G. (1986) The Combustion of Wood, Mainly as Assessed by Fluidised-bed Differential Thermal Analysis, with Particular Reference to Rubberwood. Ph.D. Thesis, University of Canterbury, New Zealand.
3. EICKNER, H.W. (1962) Basic Research on the Pyrolysis and Combustion of Wood. *Forest Prod. J.*, **12**, 194.
4. NGUYEN, T., ZAVARIN, E. AND BARRALL II, E.M. (1981) Thermal Analysis of Cellulosic Materials. Part I. Unmodified Materials. *J. Macromol. Sci. — Rev. Macromol. Chem.*, **C20(1)**, 1.
5. SHAFIZADEH, F. (1982) Introduction to Pyrolysis of Biomass. *J. analyt. appl. Pyrolysis*, **3**, 283.
6. SHAFIZADEH, F. AND DEGROOT, W.F. (1977) Thermal Analysis of Forest Fuels. *Fuels and Energy from Renewable Resources (Tillman, D.A., Sarkanen, K.V. and Anderson, L.L., ed)*, p. 93. New York: Academic Press.
7. TANG, W.K. (1972) Forest Products. *Differential Thermal Analysis: (Mackenzie, R.C., ed)*, Vol. 2, p. 523. London and New York: Academic Press.

J. nat. Rubb. Res., **2(2)**, 94–98

The Significance of the Structure of Laticifer with Relation to the Exudation of Latex in Hevea brasiliensis

ZHAO XIU-QIAN*

Many investigations on the structure of the laticifer in Hevea brasiliensis *had been carried out about a century ago. The methods used in previous studies were by tissue sectioning. However, the present study is based on a new technique of isolating the complete network structure of the laticifer from the bark of the tree without sectioning. The procedures of the technique consist of mainly removing the bark from the tree, softening in boiling 10% KOH for about 15–20 min, dehydrating up to 80% alcohol and staining in Sudan III or Sudan IV dissolved in 75% alcohol. The stained tissue will show red indicating that it is ready for examination under the microscope and for taking photographs.*

Through this method of study, it has been revealed that there are two kinds of laticiferous tissues in the same plant. They are the non-articulated laticifer and the articulated laticifer. These tissues differ very much from each other by their origin, structure, ontogenetic development and distribution in the bark of the plant. The accompanying coloured photographs demonstrate the way of transportation and exudation of latex in the network structure of the laticifer.

The laticifer in *Hevea brasiliensis* is a cell type which produces latex. Since last century, investigations have been made through plant sectioning to study the structure of the laticifer and the physiological aspects. Not until recently has the author developed a technique by isolating the complete network structure of the laticifer from the bark of the tree without sectioning. This method of study reveals that the physiology of latex exudation is closely related with the morphological structure of the laticifer.

MATERIAL AND METHODS

The bark of the tree used in this study was obtained from young two- to three-year-old plants which were experimentally cultivated at Fujian Academy of Tropical Crops, Zhangzhou, Fujian, China. The technique used for the preparation of a complete network structure of the laticifer is simple. A description of the technique had been published by the author in Chinese[1]. The procedures are as follows:

1. Remove a piece of the bark from a tree about 10 – 20 cm in length or more.
2. Place the bark directly in boiling 10% KOH solution for about 15–20 minutes.
3. Wash the bark with several changes of water to remove dark brown contents.
4. Remove the peridermal layer of the stem with a pair of forceps to expose the laticiferous tissue which can easily be dissected out with dissecting needles under the dissecting microscope.
5. Dehydrate the tissuue through a series of gradations of methyl alcohol from 30%

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This paper is a part of a project supported by the Science Fund of the Chinese Academy of Science and had been presented at the IRRDB, Rubber Physiology and Exploitation Meeting, in South China Academy of Tropical Crops, Hainan, Guangdong, China in December, 1986.

up to 80%. Let the tissue remain in each grade of alcohol for about 10 – 15 minutes.

6. Stain the tissue in Sudan III or Sudan IV (1 g of the dye is added to 90 cc of 75% methyl alcohol) until it becomes red.

Special attention should be paid to the fact that it is better to transfer the tissue from the 80% alcohol to the Sudan III or Sudan IV solution for staining. Precipitations of the stain will usually occur if the tissue is transferred from a lower grade of alcohol to the staining solution in 75% alcohol. Hence this method of preparation is only adequate for quick and temporary observation under the microscope and for taking photographs.

TYPES OF LATICIFER TISSUE IN *HEVEA BRASILIENSIS* AND THEIR STRUCTURE

According to De Barry (as cited by Esau[2]) laticifers are divided into two main types, namely non-articulated and articulated. It has been reported[2-5] that there is only one kind of laticifer in rubber plant. However, in the present study, two types of laticifers were observed to exist simultaneously in the same plant body. Similar phenomenon occurs in other plants, as pointed out by Esau[2] in her book 'Plant Anatomy' that certain species of Asclepiadaceae had two kinds of laticifers in one plant body.

Structure of Non-articulated Laticifers

Figure 1 shows a portion of non-articulated laticiferous tissue isolated from primary phloem of a branch of a *Hevea* sapling, with the linear or thread-like structure of non-articulated laticifer stained in red in Sudan III. These laticifers are arranged alternately with cortical tissues which are not stained.

The non-articulated laticifer is aseptate and coenocytic; that is to say, there are no partitions inside the coenocytic cell. It is formed during embryonic development and then becomes dispersed in the primary structure of the seed (cotyledon), leaf, flower, fruit, root and stem. Hence, it is called a primary laticifer. This kind

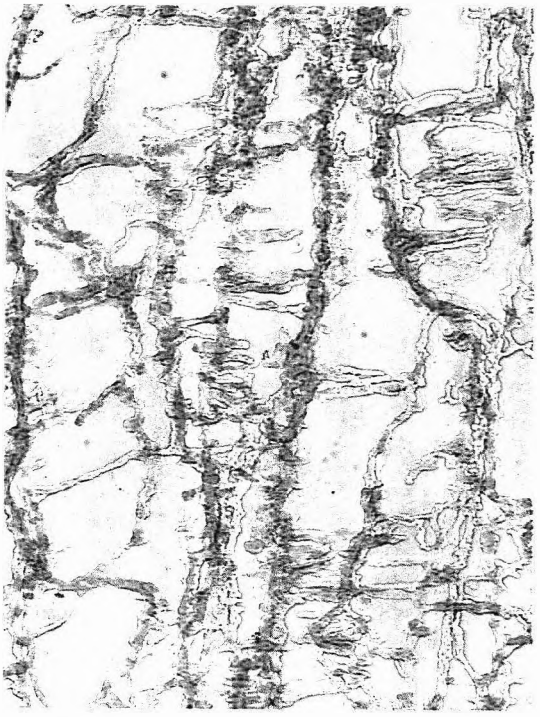


Figure 1. A portion of non-articulated laticiferous tissue from the primary phloem of young tree branch of 93-114 clone. Magnification: × 132.

of laticifer usually develops only in the growing season. When the growing season is over, it is shed with the cotyledons, leaves, flowers, fruits and the bark of the tree. Thus, it is also called an ineffective laticifer since it has no economic value.

Non-articulated laticifers spread from the terminal bud downwards to the branches and the stems of the tree. They penetrate into the intercellular spaces of the primary phloem and cortex forming a network structure. Their form and structure vary with the intercellular spaces. The laticifers are coarse and crowded when the intercellular spaces are large. Small laticifers are shown as very fine tubules. The length of the branches of laticifers varies greatly. Long branches may penetrate through several intercellular spaces, coming in contact with distant laticifers in a manner similar to that of a conjugation tube in the articulated laticifer,

whereas short branches look like small papillae or outgrowths.

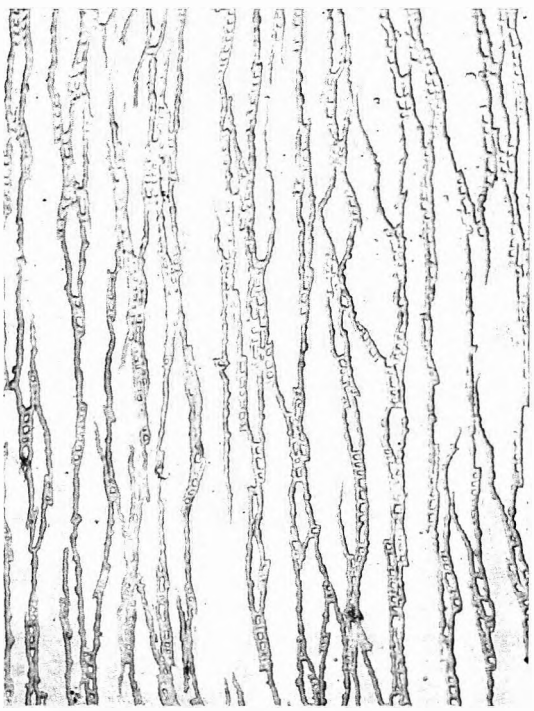
The development, morphological structure and distribution of non-articulated laticifers are different from those of articulated laticifers.

Structure of Articulated Laticifers

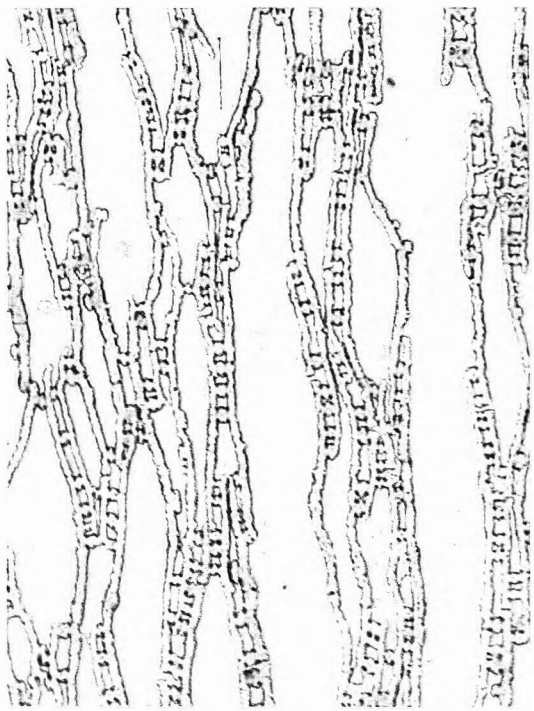
Figures 2a–2e show a portion of articulated laticifers isolated from secondary phloem in the stem of a *H. brasiliensis* sapling, with linear or thread-like laticifers. The interspaces are mostly shaped like convex lenses. They are spaces left after isolation of the phloem ray tissue of the secondary phloem. The articulated laticifers also exhibit a network structure.

Articulated laticifers originate from the cambium. In the beginning, they form a series of short columnar laticiferous initial cells. With the disintegration of the end walls of these initial cells, a long tube, the laticifer is formed. Since articulated laticifers originate from the cambium and constitute a portion of the secondary phloem, they are called secondary laticifers. They are also called effective laticifers because they provide the structure for producing latex.

In the developmental stage of the articulated laticifers, before the complete disintegration of the end walls of the laticiferous cells, conjugation tubes start to appear on the lateral walls of the cells. At first, short papillae or outgrowths emerge on the sides of the cells lying opposite one another in two adjacent laticifers. Soon after, the papillae lengthen and their ends meet. Finally, the end walls of the papillae dissolve and the cell cavities form an open tube, the conjugation tube, which connects the two laticifers. The latex in the laticifers can then pass freely through this tube. This process of the formation of the open tube in *H. brasiliensis*

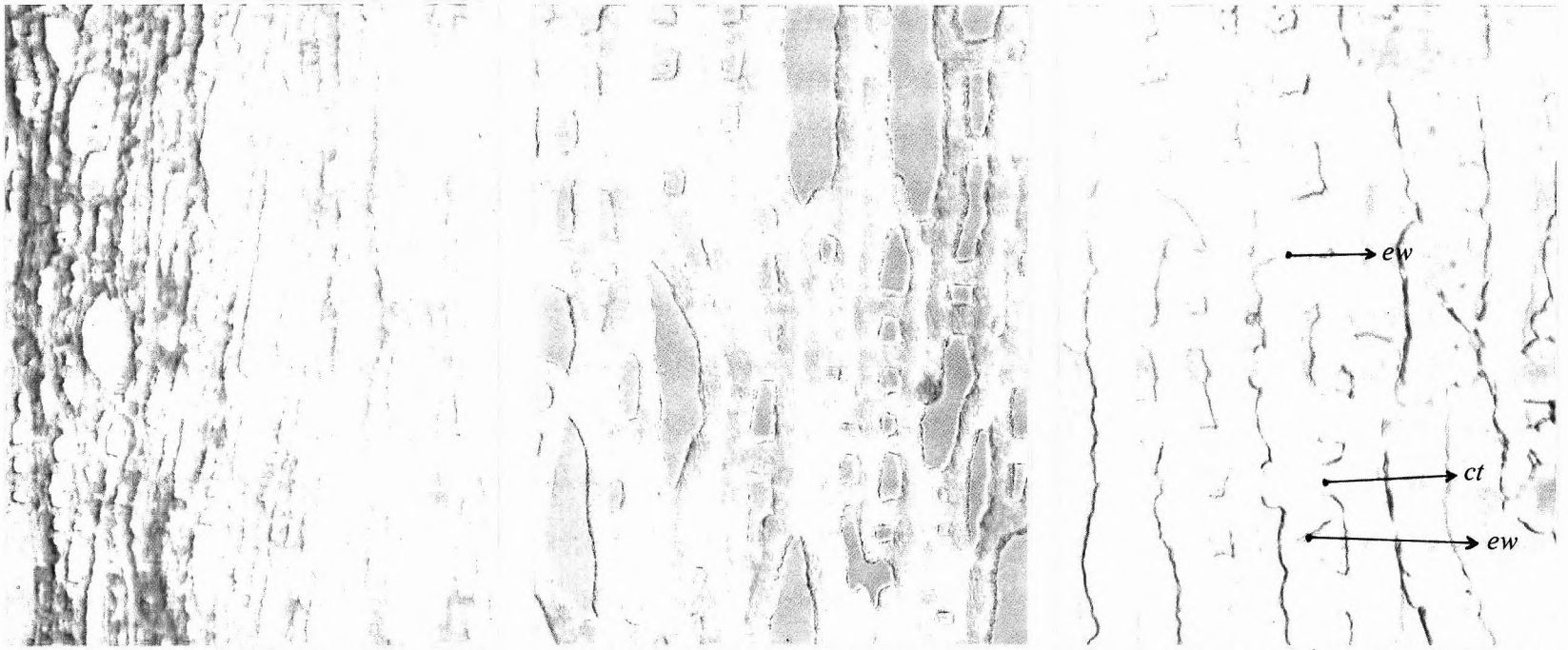


a. Wu-feng clone. Magnification: × 54.



b. Ming-ling clone. Magnification: × 132.

Figure 2. A portion of articulated laticiferous tissue from secondary phloem in the stem of a young tree.



c. RRIM 600 clone. Magnification: × 132.

d. RRIM 600 clone. Magnification × 264.

e. Tian-ren 31-45 clone. Magnification: × 528.
ew = End wall of articulated laticifer
ct = Conjugation tube

Figure 2. A portion of articulated laticiferous tissue from secondary phloem in the stem of a young tree (contd)

is similar to the scalariform sexual reproduction of *Spirogyra* in green algae. The open conjugation tube is thus considered a portion of the structure of articulated laticifer.

Observations show that there are many conjugation tubes in the articulated laticiferous tissue. Each laticiferous cell may have one to five such tubes arranged in a regular pattern, some growing on one side of the laticifer and others on both sides. This accounts for the ladder-like or fence-like appearance of the laticiferous tissue, as shown in *Figures 2b, 2d* and *2e.* The walls of the isolated laticifers as can be seen in the figures are always smooth, rarely having any protrusions. There are no conjugation tubes *(Figures 2a-2e)* on the side of the laticifer contiguous with the phloem ray. The length and the diameter of the conjugation tube as well as the distance between two neighbouring conjugation tubes are almost equal to the diameter of the laticifer. The number of laticifers are always greater in high productive clones than in low productive clones of the plant. These phenomena are noted in the accompanying figures. Since the laticiferous tissues form the structure that produces latex, it is evident that the morphological structure of the laticiferous tissue bears a close relation with the mode of transportation and exudation of the latex.

DISCUSSION AND SUMMARY

The significance of the morphological structure of the laticifers in latex exudation may be summarised as follows.

There are two kinds of latex vessels in *H. brasiliensis,* namely, non-articulated laticifers and articulated laticifers. The former have no bearing on latex production, while the latter originate from secondary meristematic tissues and produce new laticifers every year. All laticifers are inter-connected to form a network structure arranged in concentric layers, thereby providing a special system for the transportation of latex which may pass vertically through longitudinal laticifers or transversely through conjugation tubes. All latex of the plant is confined to the laticiferous system and the latex in certain parts of the bark tends to be transmitted to and exuded at the place of injury or tapping side of the tree *(Figures 2a–2e).*

REFERENCES

1. CHAO HSIU-CHIEN (1980) New Methods for Slide Preparation of the Laticifer of *Hevea brasiliensis* (in Chinese), *Acta Botanica Sinica,* **22(1)**, 101.

2. ESAU, K. (1965) *Plant Anatomy.* London and New York. John Wiley & Sons.

3. BOBILIOFF, W. (1923) Anatomy of *Hevea brasiliensis.* Zurich: Institut Orell Fussil.

4. GOMEZ, J.B. (1982) *Anatomy of* Hevea *and Its Influence on Latex Production.* Kuala Lumpur: Malaysian Rubber Research and Development Board.

5. FHAN, A. (1982) *Plant Anatomy,* 3rd ed. New York: Pergamon Press.

J. nat. Rubb. Res., 2(2), 99-110

Farming Systems Research for the Small Farm Rubber Sector

C.C. GOLDTHORPE*

Smallholding and large-scale plantation agriculture are two distinct farming systems for the production of tropical, perennial export crops. The farm management characteristics of smallholding rubber production are that it is a low input/low output system of agriculture. Rubber estate production is a farming system based on high levels of inputs and outputs. The research requirements of small farm and plantation production technology therefore differ. Rubber research programmes in previous years have concentrated on production techniques appropriate for the estate sector. This neglect of research into the specific needs of peasant farmers has led to generally poor acceptance and uptake of innovations by the smallholding sector. Research programmes that take a farming systems perspective are likely to identify new technologies for increasing the productivity of small rubber growers. New rubber cultivars and farm management practices selected for the specific agro-ecosystems and socio-economic circumstances of smallholding agriculture are more likely to be adopted by peasant farmers compared to production systems designed for plantations. Productivity hence farm incomes may be expected to improve with the implementation of production technologies identified for the small farm sector.

The large-scale plantation sector is an important component of the worldwide natural rubber (NR) industry. *Hevea* rubber is, however, predominantly cultivated by small farmers who derive all or part of their cash income from sales of the crop. The farmers growing rubber typically are recognised as belonging to low income, poverty groups within the national economies of the producing countries. There is widespread appreciation among rural development policy makers that one way of increasing the overall income of rubber farmers is through increases in productivity (output per tree, output per hectare, or output per farmer). One of the main methods by which *Hevea* productivity can be improved is by applied biological research on this economically important crop.

The objectives of the paper are two-fold; firstly to assist scientists to identify research programmes of immediate, direct benefit to the smallholder sector. The second, longer term objective is to contribute to the important debate within the NR industry on how to improve the standard of living of small growers. The paper argues that the main thrust of research in the past has generally been towards improving technologies appropriate to large plantations rather than small-scale producers. The emphasis on large-scale production techniques has led concomitantly to generally poor acceptance and uptake of the new technologies by the smallholding sector, though this differs between countries. An additional factor is that the concentration on estate production methods has led to a top-down approach to technology transfer through rubber smallholder extension services.

The development of the farming systems approach to research problems over the past decade has provided researchers with a valuable new tool for the identification of innovations for specific farm types. It is argued that by adopting a farming systems perspective more suitable technologies may be evolved for the small farm sector. The outcome of research

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programmes determined by farmers' needs (bottom-up approach) rather than the preconceptions of researchers is more likely to be put into practice by smallholders. Productivity hence farm incomes may be expected to improve with the widespread adoption of farmer-oriented rubber production techniques.

DEFINITION OF ESTATE AND SMALLHOLDING AGRICULTURE

Estate or plantation agriculture is defined as the production of rubber (and other perennial export crops) by a strictly supervised, wage-earning labour force in large-scale land units under central management. Smallholding producers of the same commodity crops, on the other hand, are independent decision makers who use family labour, which may work on its own or in conjunction with some hired workers, on small-scale land holdings typically 5 ha and below in size. Land owners of small plots who do not work their land but rent the usufruct to landless peasant farmers are not regarded as smallholders; they belong to a petty rentier class. In this case the tenant farmers or sharecroppers who work the land are the actual smallholders even though they do not possess legal title to the land holdings[1-6].

Small growers of perennial crops on large-scale, centrally managed, tightly controlled, Government financed development projects such as land settlement schemes in Malaysia, and nucleus estate projects in Indonesia, West Africa and other parts of the tropics are not regarded as belonging to the genuine smallholding sector. Projects of this type are generally referred to as organised smallholdings. The technology of production on these management and capital intensive development projects has many features of the plantation mode of production[1,3,4,7-10].

FARMING SYSTEMS RESEARCH

The classification of agricultural systems is complex[11] and a number of studies examine farming patterns on both a world wide and regional basis[12-21]. The standard work on tropical agricultural systems is that of Ruthenberg[5] who takes a multi-dimensional, systems theory perspective[22]. Ruthenberg defines an agricultural system as a distinct type of farm organisation based on cropping pattern and cultural practices, which has been developed in response to the ecological, economic and socio-institutional conditions of differing locations[5]. In taking a systems approach to agricultural production the farm (both small-scale and large-scale) is considered not as a fixed state means of production but as a dynamic institution constantly adapting to changes in its external environment[23].

The concept of an organisation as a system in which a number of interdependent variables interact with each other, and with the environment, is known as an open system[24]. In their analysis of organisations, Katz and Kahn[25] consider that nine characteristics define all open systems. For the purposes of this analysis three of the more important characteristics of open systems may be taken as:

INPUT → THROUGHPUT → OUTPUT.

Open systems use inputs from the external environment and transform them within the organisation into some form of product or output which is returned into the environment. Thus a rubber smallholding takes sunlight, air, water and mineral elements from the physical environment, and utilising manual labour and management skills together with the technology of rubber production (the input) transforms them within the organisation structure of the farm (the throughout) into raw rubber (the output) which is sold at the farmgate and returned to the external environment. The basic characteristics of a rubber smallholding when viewed as an open system are shown in diagrammatic form in *Figure 1*.

The World Bank[22] regards studying the farm as a system rather than addressing only its technical or economic dimensions as a substantial step forward in the overall area of agricultural research. There is however no general agreement on what constitutes farming systems research[22,26] although most workers in

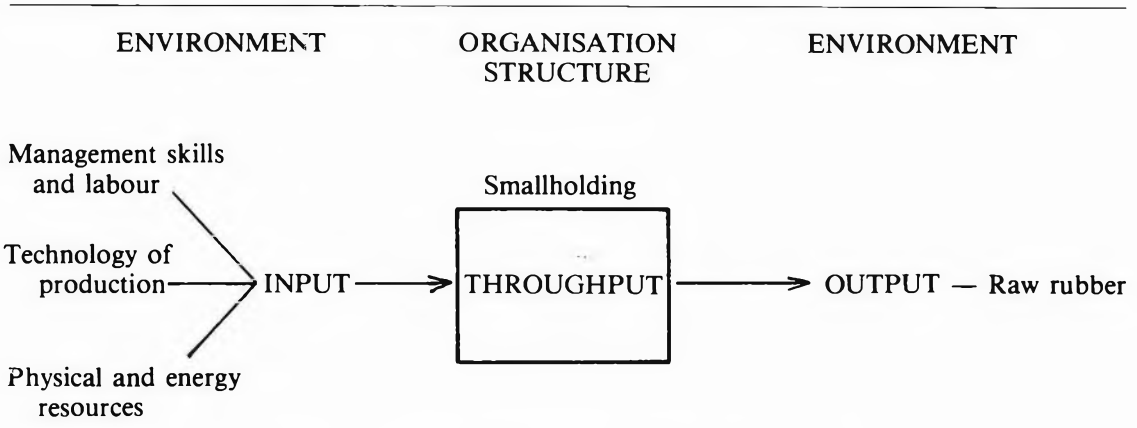

Figure 1. Rubber smallholding as an open system.

the field accept that the farming systems research approach:

- Regards the farm as a system
- Involves the farmers in the diagnosis of the farming system and verification of recommended new practices or inputs
- Is multi-disciplinary and holistic in perspective
- Accepts that the research is applied and aimed at generating near-term viable technologies.

The longer term objective of farming systems research is to design inputs and techniques tailored to the needs of specific agroecosystems and particular socio-economic niches[27].

CHARACTERISTICS OF SMALLHOLDING PRODUCTION

Socio-economic Factors

The general socio-economic characteristics of small farmers in the tropics have been summarised as follows:

- They are poor and have little ready cash.
- Loans to them are usually unavailable or expensive.
- They are conscious of an uncertain environment, of cash shortage, and of family responsibilities and therefore,
- They are risk-averse.
- They often suffer cyclical labour shortage and under-employment.
- They may have opportunities for competing off-farm employment.
- They are economically rational but not necessarily profit-maximising because;
- They have their own scales of utility.
- They live in countries in which the social infrastructure of markets, supplies, and communications is often weak and not to be relied upon.
- They live in societies which normally have clear codes as to what is socially acceptable and what is not[22].

Smallholder producers of perennial crops are inherently weak in husbandry by comparison with the performance of estates which leads to losses in yields[5]. Tree crop farmers rarely attain the high standards of farming found in plantations, the quality of their products is generally low and they are slow to adopt new methods[15,16]. Perennial peasant agriculture is typically characterised by small and often uneconomic holdings, lack of capital, poor

standards of crop husbandry, low levels of productivity, simple processing methods and poor quality produce which all-in-all result in low farm incomes. An important consequence of low levels of production technology especially low yields and poor quality produce, is the generation of low farm incomes despite the fact that unpaid family labour is employed[5,15,16,28].

The characteristics of the smallholding rubber sector are similar to those described for other small-scale tree crop farmers. The peasant system of rubber smallholdings suffers from several defects including poor yields and inferior quality of products compared to the estate mode of production. The typical small producer finds it difficult to take advantage of new technologies and economies of scale. Rubber farmers generally lack facilities for upgrading their processed rubber and face problems in the marketing of their produce. Labour performance in the smallholding sector is observed to be less productive than in plantations[8,29–35].

The crux of the matter, taking a socio-economic perspective, hinges on the low income of rubber smallholder families which is a resultant factor of low productivity and the small size of their holdings. Poor standards of production technology result in the smallholding sector being inferior to the estate sector so that independent small farmers are left behind in the overall development of the NR industry[6,34].

Poor Yields

Yield data from large estates are easily recorded and the figures have a high degree of accuracy. The collection of yield figures from a large number of independent smallholdings, on the other hand, is difficult and it is generally acknowledged that data from this sector are less reliable than from estate sources. Nevertheless, published figures from a number of producing countries show that yields from smallholdings are about 30% to 50% lower than estate yields *(Table 1)*. The same trend in productivity is discernible when yields from the plantation and small farm sectors are compared for other commodity crops that enter into the world export trade. *Table 2* illustrates the point.

Low Cost Production

The fact that peasant production can still compete with estates, despite obviously poor cultivation and husbandry techniques is considered by Ruthenberg to be explained by low costs of production. The recurrent cost

TABLE 1. RUBBER ESTATE AND SMALLHOLDING YIELDS

Country	Yield (kg/ha)	
	Estate	Smallholding
Malaysia[29] (peninsula)	1 428	1 050
Malaysia[35] (peninsula)	1 194	727
Indonesia[29]	1 284[a]	504
Indonesia[36]	850–950	350
Sri Lanka[29]	1 112[a]	750
Sri Lanka[37]	1 000	450
Papua New Guinea[38]	500–600	200–600
	1 400 best managed	
Liberia[39]	1 200	400–600
Liberia[40]	1 250–1 350	470
Nigeria[40]	900–1 000	500–800

[a]State-owned plantations

TABLE 2. ESTATE AND SMALLHOLDING YIELDS (OTHER CROPS)

Crop	Country	Yield (kg/ha)	
		Estate	Smallholding
Oil palm[41]	Papua New Guinea	2 286	748
Coconut[42]	Papua New Guinea	900	500
Cocoa[43]	Papua New Guinea	300–700	250
Cocoa[44]	Malaysia	1 080	850
Cocoa[45]	West Africa	785–841	183–221
Tea[46]	Kenya	2 230	851
Coffee[47]	Kenya	1 078	633
Coffee[48,49]	Papua New Guinea	2 000	700

schedules of estates and smallholdings are different since most of the labour employed by small farmers is either unpaid family labour or receives far less than the rates laid down by minimum wage legislation that apply on estates[5].

Ruthenberg argues that the whole situation is different as far as costs are concerned between plantations and small farms. He notes that a large-scale estate must bear an investment cost for land clearing and crop establishment; workers' houses, hospitals, roads and other infrastructure; and the factory. In family holdings there is no expenditure on expensive infrastructure nor is there investment in capital intensive processing equipment. Estates must bear the cost of clearance work, whilst on smallholdings the land is in any case cleared to grow subsistence crops. It costs little extra for farmers to set plants of a future perennial crop in the cleared land. Intercropping the perennial crop with arable food crops in the early years bridges the period when there is no harvest. These combined benefits give the smallholder a distinct cost advantage[5].

The competitive advantage of smallholders over estates for rubber production because of greater economy of resources, particularly labour, has been stressed by Bauer[50–55]. This viewpoint has been challenged by Benham and Silcock who question the argument that estates compare unfavourably with smallholdings that employ family labour[56,57]. Courtenay is also of the opinion that is extremely difficult to compare with any certainty the true productivities of smallholders and estates. Thus Courtenay writes that it is likely that the differences, even with a crop like rubber where real competition is feasible, are not invariably in the smallholder's favour[1]. Grigg similarly takes the viewpoint that it is difficult to make a convincing economic case for the superiority of the smallholding over the plantation and that the latter's efficient farming methods and power of earning foreign exchange seem to be powerful assets to a developing country[15].

Low Risk

Smallholders are more resilient to trade depression than estates since farmers tend to be highly price elastic with their inputs. In times when producer prices fall peasant farmers cease production and neglect their crop. The farmer has his cash earnings reduced in this period but he still has his livelihood from subsistence cultivation. When commodity prices increase the trees are harvested once again, and farm incomes rise accordingly[5,58].

In contrast, estates tend to maintain husbandry and production levels, irrespective of ups and downs in commodity prices. Ruthenberg considers these differences explicable by the relatively short time horizon of smallholders compared to the long-term view taken by plantations[5]. Another important reason why estates continue production is the large fixed

costs in overheads and labour wages that have to be paid irrespective of world market price fluctuations.

CHARACTERISTICS OF ESTATE PRODUCTION

The farm management advantages of growing perennial tree crops on a large scale do not lie in the labour economy. The use of labour-saving equipment is important only with perennial field crops like sugarcane, and where harvesting can be fully mechanised as on the tea plantations in the Western Highlands of Papua New Guinea. The competitiveness of plantations growing rubber and other tree crops in relation to smallholdings with their cheap production methods is based on:

- The rapid and consistent use of technical advances in crop production
- The more efficient organisation of delivery of the crop to the processing factory
- The more efficient processing of the product
- The better access to markets and capital[5].

Large plantation enterprises take advantage of modern technology and typically operate their own in-house experiment stations or contribute financially to the maintenance of a national research institute. Innovations and advances in agricultural methods and processing technology are rapidly applied on the estates. The skilled supervision of labour, and scientific management of land and the crop, by professional managers and agricultural specialists result in high standards of crop production and concomitant high yields. Compared to smallholdings, plantations harvest larger quantities per hectare. Furthermore, the high degree of control in handling a perishable commodity results in raw materials of considerably better quality being delivered to the estate factory.

Processing of the crop is carried out to high standards so that the quality of the end product can be sold at advantageous prices. Large size makes for economies of scale especially in the use of complex, expensive processing equipment and transport facilities. By-products and residues being sold off to local manufacturers, or used as fuel in the processing factory, or returned to the land as fertiliser, are used efficiently.

All told, a return per hectare or per worker is obtained that is typically greater than that from small farmers. Plantations, therefore, produce high net earnings of foreign exchange and a high taxable income which can be used for general economic development[1,4,5,15-19,31,35,58-72].

AGRICULTURE SYSTEMS PERSPECTIVE

It is argued that the estate sector is a high productivity/high quality/high income producer of export commodity crops while the opposite holds true for small farm producers. Peasant production of perennial crops tends to be characterised by low levels of yield and quality of produce which lead to poor returns per worker, per hectare and per tonne of output; consequently to low family incomes. Smallholders are low cost producers compared to plantations. Nevertheless, they are inefficient producers of export crops because low yields and poor quality caused by low input cultural techniques and reliance on low standards in processing technology result in low farm incomes to the family and loss in export earnings to the nation.

Although plantations have high fixed costs brought about by the employment of professional management and specialists such as processing engineers, agricultural scientists and accountants, and large numbers of hired workers, they are efficient producers. The efficiency of plantations is due to high yields, the processing of a good quality end-product attracting premium prices, and the spreading of fixed costs over a large land area. Plantations are the innovators in the introduction of new crop varieties, new cultural methods and improved agricultural produce. The relevant economic aspect of these innovations is that rising wages can be absorbed by higher yields per hectare and improved output per worker.

It is argued that although small growers are low cost producers they are not necessarily price efficient producers when efficiency is measured in monetary terms. This is because family cash incomes and export revenues generated from sales of the commodity crop are low. Another way of measuring efficiency, however, is in terms of labour energy inputs and calorific energy outputs. In the complex, mixed, multi-storey, small farm cropping systems characteristic of oil palm, coconut, cocoa and coffee farming in many parts of the tropics total production measured as calorific values is high. Rubber trees are grown in pure stands but rubber is, on many holdings, only part of a mixed cropping system. Rubber farmers may also grow cereals, other field crops and fruit trees as part of their total farm enterprise. In these mixed farms the energy value of home-grown foodstuffs consumed by the family is substantial while the monetary value of luxury crops (such as betel vine and areca nuts, kola nuts, or tobacco) may be great.

Hevea rubber is planted as a monocrop on both smallholdings and estates unlike, for example, peasant and plantation cultivation of oil palm in West Africa. However, the farm management systems followed by the two sectors of the NR industry are different. Smallholders typically plant their trees at high densities, neglect the immature plants, tap the trees intensively, and use minimal fertiliser and other agrichemical inputs. The estate sector, on the other hand, plants trees at lower densities, maintains each tree individually during immaturity, exploits the mature trees by using a number of sophisticated tapping systems, and has high agricultural standards based on the use of agricultural chemicals, fertilisers and skilled management inputs.

The viewpoint is put forward that plantation and smallholding crop production technologies may be regarded as two separate farming systems. It is argued that plantations are not very large-scale smallholdings; nor are smallholdings very small plantations. In a nutshell the plantation mode of production is characterised by a high input/high output system of agriculture. The main feature of smallholdings is a production system based on low inputs and low outputs for the cultivation and processing of the same export crops. Support for this proposition comes from the World Bank in a recent review of its role as a development institution. Experience of World Bank lending for tree crop agricultural projects has revealed that different production technologies are needed for estates and for small village plots[73].

RESEARCH BIAS

If plantation agriculture and peasant farming are distinct agricultural production systems it follows that the research requirements of plantation and smallholding agriculture differ. Barlow and Peries[74] have reviewed research programmes in the major rubber-producing countries and have concluded that until recently there has been an almost exclusive concentration on techniques and technologies appropriate for large-scale plantations. They argue that, because of the emphasis on capital intensive, labour-saving innovations, the new technologies are not generally suitable for small rubber farms. A similar situation is reported in the field of coconut research where efforts to increase production have been based on a high input technology inappropriate for smallholders[75]. Indeed a general feature of tropical perennial export crop research is that research and development programmes tend to be controlled by the industry[22] which typically is dominated by plantation company interests[76]. For example, research into oil palm husbandry and palm oil processing in South-East Asia, the main production area is carried out almost exclusively by in-house research stations belonging to large estate groups.

The bias against the small farmer in tropical agricultural research is not, however, restricted to rubber and other export commodity crops. Three factors that influence the rate and bias of technical change against the small grower have been identified by a study carried out by the Consultative Group in International Agricultural Research (CGIAR). The first is the difficulty of society (including political decision-makers) in perceiving the expected payoffs

from research which places the scientist in a position of having to create the demand for his future work. Secondly, there is the predisposition for scientists to seek peer recognition through scientific achievements instead of seeking maximum impact on civil society through technological advances. And the third, is the tendency for scientists to link up with the groups in society with the greatest financing capacity, typically the more aggressive producer associations[77].

The result of the bias to large-scale production methods has been that technologies adapted for rubber smallholders have tended to come as a spillover from research carried out on the estate sector[78]. The transmission of the results of research through specialised extension and advisory services has also tended to be a top/down process[22] typically by the introduction of scaled-down versions of plantation techniques. An alternative approach, (implemented with some degree of success in Malaysia) is the collectivisation of adjacent smallholder plots into tracts of land large enough for plantation-scale technology to be applied[32,33,35,79,80]. In the case of scattered, non-collectivised, independent smallholdings the acceptance of the new technologies has been poor[79,81] because the outcome of tree crop research programmes generally has not been tailored to the specific needs of small farmers[22,74,75]. Simmonds suggests that the reason why new production methods proposed by agricultural research have not been adopted is that generally the innovations are unsuitable for the socio-economic circumstances of the farmers[22].

It is argued by Simmonds that compared to traditional research methodology, a research programme taking a farming systems perspective is likely to be successful in identifying suitable technologies for small farmers in the tree crop sector[22]. There is a growing awareness that the smallholding has to be the focus of research programmes and that researchers should work specifically to solve the farmers' problems. The emphasis is on production techniques for small farmers to be able to maximise production with low cost inputs and management practices rather than aim for the highest yield potential[43,74,75,78,82]

DISCUSSION

The adaptation of temperate zone farm management economics to small farmers in the tropics indicates that the farmers are poor, economically rational (but not necessarily profit maximising), risk-averse and subject to high interest rates. They are ready enough, however, to adopt innovations that they themselves perceive to be economically attractive. Many innovations (new exploitation methods, for example) proposed by rubber research institutes have not been adopted readily by the majority of smallholders. Other new techniques, such as herbicide usage for weed control, have been taken up by many small growers. It is argued that the reason for the low uptake of new technologies is because research programmes have generally in the past been oriented towards the large-scale estate sector. This technology which is satisfactory for plantation agriculture has tended to be imposed by extension services on smallholders who may have neither the funds nor the skills to implement successfully the recommended programmes.

Farming systems research is an approach that focuses specifically on methods to solve farmers' problems and which regards the farm as a production organisation (including, importantly, the socio-economic aspects), which reacts with its external environment. Large-scale plantation and small-scale peasant production of perennial crops are regarded as two distinct agricultural systems when a systems perspective is employed. Small farm production technology is a low input/low output system; plantation agriculture, on the other hand, is a high input/high output production system. It follows that the technology suitable for plantations is unlikely to be appropriate for smallholdings. It should prove possible, however, to formulate production technology recommendations suitable for both small farms and large plantations if a systems approach is taken in devising research programmes.

The adoption of a farming systems research approach to *Hevea* production begins with the basic premise that estate and smallholding farm management practices have marked differences and that the technology suitable for one sector is unlikely to be appropriate for the other.

Research programmes for the rubber plantation sector need to be tailored to a high technology mode of production. The objective in breeding programmes for example will be yield maximisation given plantation standards of inputs and management. The goals of a smallholder oriented programme in contrast should be to achieve the highest possible yields in a farming system using only small amounts of capital and few purchased inputs. Breeding for resistance to a wide range of diseases at the expense of the highest yield potential, for example, is considered to be of greater priority in a research project designed for smallholders compared to one for estates.

The implication of this argument is that a two-pronged approach needs to be taken in the formulation of applied research programmes for the rubber industry. Research into the productivity of *Hevea* over the past six decades has been oriented almost totally towards the high input estate mode of production. This programme has been remarkably successful in raising the yields obtained on plantations from between 250–500 kg per hectare for unselected seedlings to 2000–2500 kg per hectare with the latest generation of commercially available high-yielding clones. A research and development programme based on a farming systems perspective could, it is suggested, raise productivity in the smallholder sector by a similar order of magnitude. It is argued, for example, that rubber-breeding programmes would have as their main objective the selection of new generation cultivars responsive to the management system of smallholders. The planting material selected for the small farm sector should, for example, give moderate yields (say 1000–1500 kg per hectare) under the following management regime:

- Close density planting
- Responsive to intensive tapping systems e.g. half-spiral/daily
- Standard fertiliser application while immature but no fertiliser during maturity
- Intercropping and/or a mixed grass interrow during immaturity.

Besides being vigorous in the immature phase and giving moderately high yields when in tapping the new material should also be strongly resistant to the major leaf, stem and tapping panel diseases.

The adoption of a farming systems perspective to research needs for the small farm sector is likely to identify suitable new technologies for increasing production and productivity in smallholdings. Low input methods of production are needed rather than models that emphasise the maximisation of production. There is a need to develop improved cultivars and farm management practices that produce moderate yields but which require only low management skills and few cost inputs. This adaptive approach to village farming systems allows farmers to test progressively various adjustments to their initial low-level input technology. The addition of improvements to the base of the existing technology in a sequential learning process where farmers acquire information and skills over time through a gradient concept is likely to be readily adopted[8,83]. Smallholders who move gradually to a higher technological plane of improved rubber planting material and new, low input farming practices will secure much better yields and in consequence earn higher gross incomes and enhance their standard of living.

ACKNOWLEDGEMENT

The permission of the Executive Director, International Natural Rubber Organisation to present this paper is gratefully acknowledged. The author thanks Dr Abdul Madjid, Director of the Indonesian National Rubber Research Centre for his helpful criticisms. Thanks are also due to colleagues in the International Natural Rubber Organisation for their comments. The views expressed are those of the author who accepts responsibility for the errors and omissions.

REFERENCES

1. COURTENAY, P.P. (1980) *Plantation Agriculture*, 2nd ed revised. London: Bell and Hyman.
2. EDWARDS, C. (1977) Rubber in the World Economy. *Pacif. Res.*, **8**, 1.

3. GOLDTHORPE, C.C. (1987) A Definition and Typology of Plantation Agriculture. *Singapore J. trop. Geogr.*, **8**, 1.

4. GRAHAM, E. AND FLOERING, I. (1984) *The Modern Plantation in the Third World.* London: Croom Helm.

5. RUTHENBERG, H. (1980) *Farming Systems in the Tropics,* 3rd ed. Oxford: Oxford University Press.

6. SUNDARAM, J.K. (1986) *A Question of Class: Capital, the State and Uneven Development in Malaya.* Singapore: Oxford University Press.

7. BARLOW, C. (1986) Oil Palm as a Smallholder Crop. *Proc. Semin. Palm Oil Res. Inst. Malaysia Kuala Lumpur 1986,* Preprint.

8. BARLOW, C. AND JAYASURIYA, S.K. (1984) Problems of Investment for Technological Advance: The Case of Indonesian Rubber Smallholders. *J. agric. Econ.*, **35**, 85.

9. LIM, S.C. (1983) Plantation Agriculture: Some Factors Impinging on the Future of the Industry. *Seventh Conv. Malaysian Econ. Ass. Kuala Lumpur.*

10. RUBBER RESEARCH INSTITUTE OF MALAYSIA (1985) Some Ways of Reducing Production Costs in Smallholdings. *Plrs' Bull. Rubb. Res. Inst. Malaysia No. 185,* 109.

11. SPENCER, J.E. AND STEWART, N.R. (1973) The Nature of Agriculture Systems. *Ann. Ass. Am. Geogr.*, **63**, 529.

12. ANDREAE, B. (1981) *Farming, Development and Space: A World Agricultural Geography* (Transl. from German by H.F. Gregor). Berlin: de Gruyter.

13. ANDREAE, B. (1982) *The Economics of Tropical Agriculture.* Slough, UK: Commonwealth Agricultural Bureaux.

14. DUCKHAM, A.N. AND MASEFIELD, G.B. (1970) *Farming Systems of the World.* London: Chatto and Windus.

15. GRIGG, D.B. (1970) *The Harsh Lands.* London: MacMillan.

16. GRIGG, D.B. (1974) *The Agricultural Systems of the World: An Evolutionary Approach.* London: Cambridge University Press.

17. LAUT, P. (1968) *Agricultural Geography.* Melbourne: Nelson.

18. MORGAN, W.B. (1978) *Agriculture in the Third World.* London: Bell.

19. MORGAN, W.B. AND MUNTON, R.J.C. (1971) *Agricultural Geography.* London: Methuen.

20. PELZER, K.J. (1958) Land Utilization in the Humid Tropics: Agriculture. *Proc. Ninth Pacif. Sci. Congr. Bangkok 1958.*

21. SYMONS, L. (1966) *Agricultural Geography.* London: Bell.

22. SIMMONDS, N.W. (1985) Farming Systems Research: A Review. *Wld Bank Tech. Pap. No. 43.* Washington D.C.: International Bank for Reconstruction and Development.

23. SPEDDING, C.R.W. (1975) *The Biology of Agricultural Systems.* London: Academic Press.

24. McGIVERING, I.C. (1971) Organizational Theory. *A Handbook of Management (Kempner, T. ed),* p. 258. London: Weidenfield and Nicolson.

25. KATZ, D. AND KAHN, R. (1966) *The Social Psychology of Organizations.* New York: Wiley.

26. REMENYI, J.V. (ed) (1985) Agricultural Systems Research for Developing Countries. *Proc. Wkshop Hawkesbury Agric. College, Richmond, N.S.W. Australia.* Canberra: Australian Centre for International Agricultural Research.

27. CONWAY, G.R. (1985) Agricultural Ecology and Farming Systems Research. *Agricultural Systems Research for Developing Countries (Remenyi, J.V. ed),* p. 43. Canberra: Australian Centre for International Agricultural Research.

28. WICKIZER, V.D. (1960) The Smallholder in Tropical Export Crop Production. *Fd Res. Inst. Stud.*, **1**, 49.

29. ASSOCIATION OF NATURAL RUBBER PRODUCING COUNTRIES (1986) Country Reports. *Proc. Sixth Semin. Prog. Dev. Rubb. Smallholders Indonesia 1986.* Preprint.

30. ANI, A. AND ARIFFIN, M.N. (1981) The Future of Natural Rubber in Peninsular Malaysia. *Planter, Kuala Lumpur,* **57**, 669.

31. BARLOW, C. (1978) *The Natural Rubber Industry: Its Development, Technology and Economy in Malaysia.* Kuala Lumpur: Oxford University Press.

32. MD. ISA, I.S. (1986) Mini-Estate: An Innovative Approach to Smallholder Development. *Planter, Kuala Lumpur,* **62**, 333.

33. MUSTAPHA, J. (1986) Increasing Productivity of Rubber Smallholding through Plantation Technology: Felcra's Experience. *Proc. Sixth Semin. Prog. Dev. Rubb. Smallholders Indonesia 1986.* Preprint.

34. RUBBER RESEARCH INSTITUTE OF MALAYSIA (1982) Some Consequences of Low Rubber Prices. *Plrs' Bull. Rubb. Res. Inst. Malaysia No. 173,* 111.

35. TASK FORCE OF EXPERTS (1983) *The Malaysian Natural Rubber Industry 1983-2000.* Kuala Lumpur: Malaysian Rubber Research and Development Board.

36. WORLD BANK (1984) Indonesia: The Major Tree Crops: A Sector Review. *Rep. No. 5318-IND.* Washington D.C.: International Bank for Reconstruction and Development (restricted circulation).

37. FAO/WORLD BANK (1979) Sri Lanka Smallholder Rubber Replanting Project. *Rep. No. 15/79 CEY 15.* Rome: Food and Agriculture Organization (restricted circulation).

38. CARRAD, B. (1981) *The Potential for Increased Rubber Production in Papua New Guinea.* Konedobu, National Capital District, PNG: Department of Primary Industry.

39. GRILLI, E.R., AGOSTINI, B.B. AND HOOFT-WELVAARS, M.J.t. (1981) *The World Rubber Economy.* Baltimore, Md: John Hopkins Press.

40. INTERNATIONAL RUBBER STUDY GROUP (1986) *The Future of Natural Rubber in Africa.* London: Secretariat IRSG.

41. NEW BRITAIN PALM OIL DEVELOPMENT LTD (1985) Private communication.

42. SACKETT, M.A. AND WILLIAMSON, P.G. (1973) The Copra Industry in Papua New Guinea cited by Wheeler *et al., Agriculture in the Economy, 1979 (Densley, D.R.J. ed).* Konedodu, National Capital District, PNG: Department of Primary Industry.

43. PRIOR, C. (1985) Approaches to the Control of Diseases of Cocoa in Papua New Guinea. *Planter, Kuala Lumpur,* **61,** 306.

44. AGRICULTURAL BANK MALAYSIA (1981) The Financing of Palm Oil and Cocoa Industries with Special Reference to the Agricultural Bank of Malaysia. *Planter, Kuala Lumpur,* **57,** 401.

45. INGHAM, B. (1981) *Tropical Exports and Economic Development.* London: MacMillan.

46. SWAINSON, N. (1986) Public Policy in the Development of Export Crops: Pineapples and Tea in Kenya. *Developmental States and African Agriculture (Mars, T. and White, G. ed). Inst. Dev. Stud. Bull.,* **17,** 39.

47. WHITAKER, M.J. (1984) *Some Characteristics of Agricultural Production on Coffee Smallholdings in the Main Coffee Producing Districts of Kenya.* Nairobi, Kenya: Coffee Research Foundation.

48. DEPARTMENT OF PRIMARY INDUSTRY (1985) *Trends in Plantation Data 1964-1982.* Konedobu, National Capital District, PNG: Department of Primary Industry.

49. CARRAD, B. (1985) Private communication. Ministry of Primary Industry, Papua New Guinea.

50. BAUER, P.T. (1948) Report on a Visit to the Rubber Growing Smallholdings of Malaya. *Colonial Res. Publ. No. 1.* London: His Majesty's Stationery Office.

51. BAUER, P.T. (1948) *The Rubber Industry: A Study in Competition and Monopoly.* London: Longmans Green.

52. BAUER, P.T. (1961) The Economics of Planting Density in Rubber Growing. *Readings in Malayan Economics (Silcock, T.H. ed).* Singapore: Eastern Universities Press.

53. BAUER, P.T. (1961) The Working of Rubber Regulation. *Readings in Malayan Economics (Silcock, T.H. ed).* Singapore: Eastern Universities Press.

54. BAUER, P.T. (1961) A Rejoinder. *Readings in Malayan Economics (Silcock, T.H. ed).* Singapore: Eastern Universities Press.

55. BAUER, P.T. (1961) Malayan Rubber Policy. *Readings in Malayan Economics (Silcock, T.H. ed).* Singapore: Eastern Universities Press.

56. BENHAM, F.C. (1961) The Rubber Industry. *Readings in Malayan Economics (Silcock, T.H. ed).* Singapore: Eastern Universities Press.

57. SILCOCK, T.H. (1961) A Note on the Working of Rubber Regulation. *Readings in Malayan Economics (Silcock, T.H. ed).* Singapore: Eastern Universities Press.

58. FISHER, C.A. (1968) Social and Economic Consequences of Western Commercial Agriculture in South East Asia. *Land Use and Resources: Studies in Applied Geography,* p. 147. London: Institute of Geographers.

59. ALLEN, P.W. (1972) *Natural Rubber and the Synthetics.* London: Crosby Lockwood.

60. ARTHUR, H.B., HOUCK, J.P. AND BECKFORD, G.L. (1968) *Tropical Agribusiness Structures and Adjustments: Bananas.* Boston Mass: Harvard University Press.

61. BINNS, B.O. (1955) Plantations and Other Centrally Operated Estates. *FAO Agric. Stud. No. 28.* Rome: Food and Agriculture Organization.

62. COUTENAY, P.P. (1965) *Plantation Agriculture.* London: Bell.

63. FARMER, B.H. (1963) Peasant and Plantation in Ceylon. *Pacif. Viewpoint,* **4,** 9.

64. GREAVES, I.C. (1959) Plantations in World Economy. *Plantation Systems of the New World.* Washington D.C.: Pan American Union.

65. GWYER, G.D. (1982) The Economic Role of Plantations in Development. *Trop. Agric. Ass. Newsl.,* 2.

66. HARRISON — CHURCH, R.J. (1969) The Firestone Rubber Plantations in Liberia. *Geography,* **54,** 430.

67. JOGARATNAM, T. (1964) Plantation Agriculture and Economic Development in Ceylon. *International Explorations of Agricultural Economics (Dixey, R.N. ed).* Ames USA: Iowa State University Press.

68. JONES, W.O. (1968) Plantations. *International Encyclopedia of the Social Science (Sills, D.L. ed),* vol. 12, p. 154. New York: MacMillan.

69. MYRDAL, G. (1968) *Asian Drama: an Inquiry into the Poverty of Nations.* Harmondsworth, UK: Penguin.

70. PHILLIPS, J.F.V. (1961) *The Development of Agriculture and Forestry in the Tropics.* London: Faber and Faber.

71. ROBERTSON, C.J. (1963) The Integration of Plantation Agriculture in Economic Planning with Special Reference to South East Asia. *Pacif. Viewpoint,* **4,** 1.

72. WICKIZER, V.D. (1958) The Plantation System in the Development of Tropical Economics. *J. Fm Econ.,* **11,** 63.

73. BAUM, W.C. AND TOLBERT, S.M. (1985) *Investing in Development: Lessons of World Bank Experience.* Washington D.C.: Oxford University Press.

74. BARLOW, C. AND PERIES, O.S. (1977) On Some Biases in the Generation of Technologies by Rubber Research Institutes. *J. Rubb. Res. Inst. Sri Lanka,* **54,** 445.

75. LIYANAGE, D.V. (1986) Increased Coconut Production and Coconut Land Productivity through Extension Services. *Monogr. on Organization, Programmes and Training of Officials and Small Coconut Farmers Leaders in Coconut Extension Work,* 1. Jakarta: Asian and Pacific Coconut Community.

76. BECKFORD, G.L. (1972) *Persistent Poverty: Underdevelopment in Plantation Economies of the Third World.* New York: Oxford University Press.

77. DE JANVY, A. AND DETHIER, J.J. (1985) Technological Innovation in Agriculture: The Political Economy of Its Rate and Bias. Consultative Group on International Agricultural Research. *Stud. Pap. No. 1.* Washington D.C.: The World Bank.

78. PLANTER (1985) Rubber Smallholdings of the Future. *Planter, Kuala Lumpur,* **61,** 344.

79. ANI, A. (1982) Increasing Agricultural Productivity through Technology Transfer. *Proc. Semin. State of Malaysian Agriculture: A Crucial Review,* 135. Kuala Lumpur: Agricultural Institute of Malaysia.

80. SEKHAR, B.C. (1982) Malaysian Agriculture in the Year 2000. *Proc. Semin. State of Malaysian Agriculture: A Crucial Review,* 225. Kuala Lumpur: Agricultural Institute of Malaysia.

81. MD. ZAIN, Y. (1986) Effective Transfer of Technology Towards Independent Smallholders. *Proc. Sixth Semin. Prog. Dev. Rubb. Smallholders Indonesia 1986,* Preprint.

82. ETHERINGTON, D.M. AND CARRAD, B. (1984) The Appropriate Scale for South Pacific Agriculture: Some Evidence from Papua New Guinea and the Solomon Islands: *Economic Policy Issues and Options in Papua New Guinea (Gupta, D. and Polume, S. ed),* p. 85. Canberra: Australian National University.

83. SHAW, B. (1985) *Agriculture in the Papua New Guinea Economy.* Port Moresby: Institute of National Affairs.

J. nat. Rubb. Res., **2(2)**, 111-117

Identification of Races and in vitro *Sporulation of* Microcyclus ulei

ISMAIL HASHIM* AND L.C.C. DE ALMEIDA**

Cultural characteristics viz. *colony appearance, growth habits and* in vitro *sporulation of pure isolates of* Microcyclus ulei *from different* Hevea *clones were studied. The physiologic races of these isolates were identified by inoculating leaf discs and differential plants grown in polyethylene bags. The isolates could be classified into two morphological groups and four physiologic races* i.e. Races 2, 4, 5 *and* 6. *Generally, results of leaf discs were similar to those of plants in polyethylene bags.*

Microcyclus ulei (P. Henn.) v. Arx, the fungus causing South American leaf blight (SALB) of *Hevea* rubber forms dark slow-growing stroma on artificial medium. Variations in cultural characteristics *viz.* growth rate, colony appearance and sporulation among isolates had been observed[1-4] but how these characteristics relate to the race structure of the fungus has not been studied.

The exact number of races of *M. ulei* are not known. Experimentally, Langdon[5] identified *Races 1* and *2*. In addition, *Races 3* and *4* were differentiated by Miller[6]. Chee *et al.*[7] indicated the existence of nine races, eight of which were present in the state of Bahia, Brazil. They identified the races by inoculating field conidia from different sources (clones) onto leaf discs of various *Hevea* clones from which the differentiating clones were selected. The objective of this paper is to identify the physiologic races using pure isolates of *M. ulei* obtained from different *Hevea* clones by inoculating leaf discs and also plants grown in polyethylene bags, and also to compare the cultural characteristics of these isolates.

MATERIALS AND METHODS

Isolation of *M. ulei*

M. ulei was isolated by touching sporulating leaf lesions with the tip of an inoculating needle and transferring them into test tubes containing potato sucrose agar (PSA) amended with Panvit[R] (a commercial mixture of amino acids and vitamins) and chloroamphenicol[4]. When the colony had established, usually after two to three weeks, the stroma was transferred onto PSA amended with Panvit and Bonzo[R] dog-food, referred to as sporulation medium[4]. Fungal isolation was done at different times during 1985 to 1986.

In vitro Sporulation of *M. ulei* and Preparation of Inoculum

A piece (2 mm) of stroma of *M. ulei* was inoculated onto slants of PSA sporulation medium (10 ml per tube) in test tubes (2 × 20 cm). The tubes were incubated at 24°C under subdued light. After ten days, 1 ml of sterilised distilled water was pipetted into the tubes and the stroma was crushed against the side of the tube and spread evenly onto the surface of the medium. The tubes were incubated in the dark at 24°C. Commencing from the fourteenth day after spreading the stroma, the tubes were exposed to fluorescent light (2600 lux) for 90 min per day for three consecutive days. Spores were harvested on the seventeenth day by adding 5 ml of sterile distilled water and brushing off the conidia with an artist paint brush. The concentration of the

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spores in the suspension was determined with a Nurbauer hemocytometer.

In the preparation of conidial inoculum, the same procedure was followed except that instead of test tubes, 125 ml Erlenmeyer flasks were used and the cultures were exposed to light by the tenth day after spreading.

Inoculation of Leaf Discs and Plants in Polyethylene Bags

Leaf discs of differential clones were prepared and inoculated as previously described[8]. In addition, about seven-day-old leaves of plants grown in polyethylene bags (polybag plants) in a glasshouse were inoculated by spraying the lower surface of the leaves with conidia ($1 \times 10^{4-5}$ per millilitre) using an Atomist atomiser. The sprayed shoots were then covered with clear polyethylene bags for 16 - 24 h to maintain high humidity.

Assessment of Infection

Infection of leaf discs was assessed by determining the presence of lesions on the eighth day after inoculation. Infection of polybag plants was assessed fourteen days after inoculation based on leaf symptom diagrams of percentage of leaf area necrotic[8]. In addition, disease reaction was also classified by using the following scale:

0 = No symptoms
1 = Chlorotic flecks, no necrosis and no sporulation
2 = Chlorosis and necrosis with very little sporulation
3 = Necrosis with little to medium sporulation
4 = Necrosis with medium to heavy sporulation
5 = Necrosis with very heavy sporulation.

Races Differentiation

The clones used to differentiate races of *M. ulei* were IAN 710, IAN 717, FX 2804, FX 3925, FX 2261, FX 985 and FX 25[7]. FX 4098 was included for confirmation of *Race 6.*

RESULTS

Morphological Forms

On PSA, similar to Chee[1], two distinct morphological forms of the fungus were observed. The first was greenish black with smooth velvety appearance. The stroma was more flattened forming a crust on the agar surface with extensive mycelial growth occurring in the medium. Isolates of this group were mostly from progenies of *H. benthamiana* clone F 4542 (FX 2804, FX 567, FX 3899, FX 3925, IAN 3272, IAN 717 and IAN 3703). These isolates were identified to belong to *Race 2.* The other morphological form had dark raised carbonaceous stroma with little growth in the medium. They belong to *Races 4, 5* or *6.*

The appearance of the conidia produced in culture differed from those obtained from the field in that the 'twist' which is prominent in field conidia, was lacking in conidia from cultures. Further, they were smaller and a greater proportion of them were of single cell.

Sporulation in Culture

Most of the cultures had fully covered the surface of the medium two weeks after the stroma was crushed and spread except for isolates 25-1, 25-2, 2261-1 and 3864-3. The number of conidia produced varied with isolates *(Table 1).* Isolate 567-1 from FX 567 produced the highest number of conidia followed by isolates 2804-1, 985-1, 600-2 and 3899-2. Few spores were obtained from 2261-4, 25-1, 4163-2 and 3844-2 despite their comparatively good growth. When the isolates were grouped according to their respective races, *Race 2* produced significantly more conidia than *Races 4, 5* and *6* which were not significant among themselves.

Reaction of Isolates on Leaf Discs of Differential Clones

Leaf discs of certain differential clones developed distinct lesions when they were inoculated by certain isolates of *M. ulei* and not by others indicating that the isolates belong to different races. However, discs of clones FX 25

TABLE 1. SPORULATION OF ISOLATES OF *M. ULEI* IN CULTURE

Race	Source clone	Isolate no.	No. ($\times 10^5$) of conidia	
			Mean of 3 tubes	Race mean
2	FX 2804	1	11.802	9.392
		2	3.290	
	FX 3899	1	3.926	
		2	8.056	
	FX 567	1	20.167	
	RRIM 600	2	9.111	
4	FX 2261	4	0.524	2.545
	IAN 873	1	5.975	
		2	1.136	
5	FX 2261	1	2.944	2.303
	FX 3844	1	1.358	
		2	0.950	
	FX 3846	1	5.556	
	FX 25	1	0.395	
		2	4.883	
	FX 3864	1	1.741	
		3	0.593	
6	FX 985	1	9.178	3.870
		2	3.963	
	RRIM 600	1	1.833	
		3	2.543	
	FX 4163	1	3.210	
		2	0.951	
		3	2.605	
	FX 3864	4	6.673	
				F = 4.776*

*Significant at p = 0.05

and IAN 710 developed lesions when inoculated by all isolates including those found by Chee *et al.*[7] to be resistant. Some isolates formed few or small lesions on these clones.

The infection reaction of sixty-eight isolates by *M. ulei* could be differentiated into four races *i.e. Races 2, 4, 5* and *6 (Table 2)*. Except for clones RRIM 600 and IAN 710, *Race 2* was isolated from clones which are progenies of *H. benthamiana* (F 4542, FX 516). *Race 4* was isolated from FX 2261 as reported by Chee *et al.*[7] and also from IAN 873. *Races 5* and *6* were isolated from clonal hybrids of many crosses of *H. brasiliensis*. Isolates from some clones e.g. IAN 710, RRIM 600, FX 985, FX 25, FX 2261, FX 3864 and FX 4163 were found to belong to more than one race.

Reaction of Isolates on Plants in Polyethylene Bags

The reaction of representative isolates of *Races 2, 4, 5* and *6* on differential clones grown in polyethylene bags was as indicated in *Table 3*. Generally, results obtained from these plants were similar to those of leaf discs *(Table 2)*. However, clone FX 2261 when inoculated with *Race 5* isolates which formed lesions on leaf discs, formed nil or only chlorotic flecks or small lesions with nil or very little sporulation on polybag differential plants. For confirmation, some of the leaves of polybag plants following inoculations with these isolates were detached and incubated as for leaf discs. Subsequently, prominent lesions developed on the detached leaves while none or very small lesions

TABLE 2. REACTION OF ISOLATES OF *M. ULEI* ON LEAF DISCS OF DIFFERENTIAL CLONES

Hosts (parents)	No. tested	Reaction of differential clones		Race
		Susceptible	Resistant	
FX 2804 (F 4542 × Tjir 1)	13			2
FX 3899 (F 4542 × AVROS 363)	4			
FX 3925 (F 4542 × AVROS 363)	1	IAN 717		
FX 567 (F 4542 × AVROS 368)	1	IAN 710	FX 2261	
IAN 717 (F 4542 × PB 86)	4	FX 3925	FX 985	
IAN 3272 (FX 516 × PB 86)	1	FX 2804		
IAN 3703 (FX 516 × PB 86)	1	FX 25		
IAN 710 (F 409 × PB 86)	3			
RRIM 600 (Tjir 1 × PB 86)	1			
FX 2261 (F 1619 × AVROS 183)	4	IAN 710, FX 25	IAN 717, FX 3925	4
IAN 873 (FA 1717 × PB 86)	5	FX 2261	FX 2804, FX 985	
FX 3844 (B 45 × AVROS 183)	2			5
FX 3846 (B 45 × AVROS 183)	2	IAN 710	IAN 717	
FX 2261 (F 1619 × AVROS 183)	3	FX 25	FX 3925	
FX 985 (F 315 × AVROS 183)	3	FX 2261	FX 2804	
FX 25 (F 351 × AVROS 49)	2	FX 985		
FX 4163 (F 170 × Tjir 1)	1			
FX 3864 (F 38 × PB 86)	2			
FX 985 (F 315 × AVROS 183)	8			6
FX 3864 (F 38 × PB 86)	3	IAN 710	IAN 717	
FX 4163 (F 170 × Tjir 1)	3	FX 25	FX 3925	
FX 25 (F 351 × AVROS 49)	1	FX 985	FX 2804	
			FX 2261	

developed on polybag plants.. Other isolates which formed distinct lesions on leaf discs, also formed lesions on intact plants and *vice versa*. Similar to leaf discs, polybag plants of clones IAN 710 and FX 25 developed lesions when they were inoculated with the four races tested, but lesion size and sporulation differed between race-clone combinations.

Disease reaction of *Races, 1, 2, 3* and *4* on differential clones had earlier been described[5,6]. From this study, reaction of *Races 2, 4, 5* and *6* on a different set of differential clones are summarised in *Table 4*. *Race 2* infected *H. benthamiana* clones IAN 717, FX 3925 and FX 2804 while no symptoms developed on FX 2261 and FX 985. On IAN 710, *Race 2* formed small lesions with low to medium sporulation. FX 25 was rated as highly resistant to *Race 2* because the lesions possessed very little sporulation. These observations agree with Langdon[5] who indicated high susceptibility of IAN 717 and FX 3925 while IAN 717 and FX 25 were rated highly resistant (few non-sporulating lesions).

Race 4 did not infect IAN 717, FX 3925, FX 2804 and FX 985. FX 2261 was very susceptible, while IAN 710 was also susceptible but with less sporulation. FX 25 was rated marginally resistant since sporulation was low. Similar description of *Race 4* was given by Miller[6]. *Race 5* caused no symptoms on IAN 717, FX 3925 and FX 2804, while on IAN 710 and FX 25 necrotic lesions with low sporulation occurred. On FX 985, *Race 5* formed lesions with heavy sporulation. In this trial, *Race 5* formed no lesions or only small lesions with nil or little sporulation on FX 2261. *Race 6* formed no lesion on IAN 717, FX 3925, FX 2804 and FX 2261. IAN 710 inoculated with *Race 6* developed lesions with medium sporulation. *Race 6* formed lesions with very little conidia on FX 25, while on FX 985 it produced large lesions with very heavy sporulation *(Table 4)*.

TABLE 3. REACTION OF ISOLATES OF *M. ULEI* ON DIFFERENTIAL CLONES IN POLYETHYLENE BAGS

Race	Isolate	Reaction on differential clone																											
		IAN 717				FX 3925				IAN 710				FX 2804				FX 2261				FX 985				FX 25			
		LS	DA	SP	DR	LS	DA	SP	DR	LS	DA	SP	DR	LS	DA	SP	DR	LS	DA	SP	DR	LS	DA	SP	DR	LS	DA	SP	DR
2	567-1	2-4	4	4	5	2-4	4	4	5	1-3	3	3	3	1-3	4	4	5	<1	1	0	1	<1	1	0	1	1-3	4	2	2
	2804-10	2-3	3	4	5	2-3	4	4	5	1-2	2	1	2	4-6	4	5	5	<1	1	0	1	<1	1	0	1	2-3	3	2	3
	3899-2	2-5	3	5	5	2-9	3	5	5	2-4	3	3	3	2-6	3	5	5	0	1	0	0	—	—	—	—	2-3	2	1	2
	600-2	1-2	3	3	4	—	—	—	—	1-3	3	3	3	3-6	3	5	5	<1	1	0	1	<1	1	0	1	1-2	2	1	2
	710-1	1-3	4	4	4	3-6	3	4	4	3-8	3	2	3	—	—	—	—	<1	1	0	1	1-2	3	0	1	1-3	4	1	2
	710-2	1-3	4	4	4	3-7	5	4	5	1-3	4	3	3	—	—	—	—	0	1	0	0	1-2	3	0	1	1-2	3	1	2
4	873-3	<1	2	0	1	1-3	2	0	1	3-4	2	4	4	0	1	0	0	3-7	4	3	3	0	1	0	0	1-3	4	1	2
	873-4	<1	2	0	1	0	1	0	0	1-2	4	4	4	—	—	—	—	2-6	4	3	3	1-2	2	0	1	2-5	4	1	2
	2261-3	0	1	0	0	0	1	0	0	1-3	4	3	4	—	—	—	—	2-3	4	4	5	1-2	1	0	1	1-2	4	0	1
	2261-6	0	1	0	0	0	1	0	0	1-2	2	3	3	0	1	0	0	2-3	4	4	5	1-2	2	0	1	1-2	2	1	2
5	985-7	1-2	2	0	1	1-2	2	0	1	1-3	3	2	2	0	1	0	0	<1	3	0	2	1-3	4	5	5	2-4	4	2	3
	3846-1	0	1	0	0	<1	1	0	1	2-3	4	3	3	0	1	0	0	<1	2	0	1	4-7	3	4	5	1-2	3	1	2
	3846-2	0	1	0	0	—	—	—	—	2-4	2	3	3	0	1	0	0	0	1	0	0	—	—	—	—	2-4	3	3	3
	3846-3	0	1	0	0	0	1	0	0	2-4	3	3	3	0	1	0	0	1	2	0	1	2-5	4	5	5	1-3	3	2	3
	3844-3	0	1	0	0	0	1	0	0	1-3	4	2	3	0	1	0	0	0	1	0	0	2-4	4	4	5	1-2	3	1	2
	25-2	0	1	0	0	0	1	0	0	2-4	4	3	3	0	1	0	0	0	1	0	0	2-4	4	4	5	1-3	4	3	3
6	3864-4	0	1	0	0	—	—	—	—	1-3	2	3	3	0	1	0	0	0	1	0	0	4-9	2	5	5	2-3	2	1	2
	985-1	0	1	0	0	<1	1	0	1	1-2	2	2	3	0	1	0	0	0	1	0	0	2-4	2	4	5	1-3	3	1	2
	985-12	<1	1	0	1	—	—	—	—	1-2	4	1	2	<1	1	0	1	0	1	0	0	2-6	4	5	5	2-4	4	1	2
	4163-4	0	1	0	0	—	—	—	—	2-4	4	3	3	<1	1	0	1	0	1	0	0	2-4	4	5	5	2-4	4	2	3

LS = lesion size (mm); DA = leaf area necrotic, 1 = <1%, 2 = 1%-5%, 3 = 6%-15%, 4 = 16%-30%, 5 = >30%; SP = sporulation, 0 = none, 5 = very heavy; DR = disease reaction type, 0 = no symptoms, 1 = chlorotic flecks with no sporulation, 2 = chlorosis and necrosis with little sporulation, 3 = necrosis with little to medium sporulation, 4 = necrosis with medium to heavy sporulation, 5 = necrosis with very heavy sporulation; — = no data.

TABLE 4. DIFFERENTIAL *HEVEA* CLONES AND THEIR REACTION TO *RACES 2, 4, 5* AND *6* OF *M. ULEI*

Race	Reaction type on differential clone						
	IAN 717	FX 3925	IAN 710	FX 2804	FX 2261	FX 985	FX 25
2	S,5[a]	S,5	MR;2-3	S,5	R,0	R,0	HR,1-2
4	R,0	R,0	S,3-4	R,0	S,5	R,0	MR,1-2
5	R,0	R,0	MR,2-3	R,0	HR,0	S,5	MR,2-3
6	R,0	R,0	MR,2-3	R,0	R,0	S,4-5	MR,1-2

R = Very resistant to immune, no symptoms or chlorotic flecks or small necrotic lesions with no sporulation
HR = High resistance, small necrotic lesions with little sporulation
MR = Marginal resistance, larger lesions with little to medium sporulation
S = Large lesions with heavy sporulation
[a]Sporulation rating from zero (no spores) to five (very heavy)

DISCUSSION

In 1966, an isolate of *M. ulei* from Belem, Brasil was identified as *Race 4*[6]. Junqueira *et al.*[9] observed three main groups of isolates from cultures of different regions in Brazil but no attempts were made to classify them into races. It is certain that Brazil has more than one race of *M. ulei,* but rather than describing new races, *Races 4a, 4b* and *4c* were designated by SUDHEVEA[10] which Chee *et al.*[7] renamed as *Races 4, 5* and *6* respectively. However, it is to be noted that *Race 6* (Chee *et al.*[7]) did not infect FX 2261 and FX 4098 while *Race 4c* infected FX 4098 and not FX 2261[10]. In fact *Race 4c* was closer to the description of *Race 7* of Chee *et al.*[7] In the present study, isolates from clones FX 985, FX 3864, FX 4163 and FX 25 were recognised as *Race 6* on the basis that it did not infect FX 2261, and later inoculations indicated that these isolates infected FX 4098 thus agreeing with the description of *Race 4c. Races 1* and *3* as described by Miller[6] and *Races 6, 8* and *9*[7] were not encountered among the sixty-eight pure isolates studied. Chee *et al.*[7] indicated that *Race 1* infected clone IAN 873 while Miller[6] reported that most of the time IAN 873 was highly resistant to *Race 1* but sometimes *Race 1* infected this clone. Langdon[5] showed that this clone was resistant to *Race 1.* In the present study, the five isolates from IAN 873 were *Race 4.* Miller[6] also indicated that this clone was susceptible to *Race 4.*

Race 2 was isolated mainly from progenies of F 4542. *Races 4, 5* and *6* were isolated from clones which derived their resistance from many sources as indicated in *Table 2. Race 4* was isolated from progenies of F 1619 and FA 1717. Progenies of F 351, F 315, F 170 and B 38 yielded *Races 5* and *6. Race 5* was also isolated from progenies of B 45 and F 1619. Thus these results indicated the races which could break down the sources of resistance derived from respective parents e.g. *Race 2* breaks down resistance from F 4542.

Laboratory inoculation of lead discs, interpreted carefully, could be used as a quick method to differentiate races of *M. ulei.* Excised leaf discs had earlier been used to investigate the disease expression by races of *Melampsora* spp. on poplar cultivars *in vitro*[11]. The present study indicated that in laboratory tests, the differentials IAN 710 and FX 25 were not too useful to differentiate *Races 2, 4, 5* and *6* as these races formed lesions on these clones. In fact the results presented here on infection of these two clones by *Races 2* and *4* differ with the findings of Chee *et al.*[7] The difference could be due to the fact that the present authors gave a 'susceptible' rating whenever lesions were observed irrespective of sporulation while Chee *et al.*[7] took sporulation into consideration. One problem with FX 25 is that sporulation was not free and few conidia were detected even fourteen days after inoculation. Clone FX 2261 indicated contrasting

results on leaf discs and in polybag plants. It is possible that environmental conditions could have caused the difference. During the period when FX 2261 was inoculated with *Race 5,* the weather was hot and the relative humidity was low which could have inhibited the development of the fungus. In addition, FX 2261 was rated as susceptible in the laboratory although it has more resistance in the field[12]. On other clones simultaneously inoculated with FX 2261, lesions and sporulation developed. It was also observed that in the summer months in Bahia, Brazil, some infection occurred on clone FX 985 but was hardly detected on FX 2261. In his trial, Miller[6] indicated the influence of weather on clones IAN 873 and MDF 180. He concluded that IAN 873 was more sensitive to weather. Similar argument could be true for FX 2261.

The results presented here confirm the observation of Chee *et al.*[7] that a clone could be infected by more than a race in the field simultaneously. In fact, two races were isolated from different lesions of the same leaf. The use of field conidia randomly harvested from different leaves in the field in the study of races will not guarantee that a pure race is being used and the same race or races are used in the repeated experiments. This might explain differences in results observed by Chee *et al.*[7] and the present authors.

The isolates so far studied could be classified into four races, and morphologically into two groups based on differences in their growth form and sporulation in medium. This paper describes the reaction type of *Races 2, 4, 5* and *6* and confirms their existence in the state of Bahia, Brazil. The occurrence of races could account for the different morphology of the fungus observed earlier between isolates[1-4]. Further work is necessary to isolate and determine the reaction type of other races and only then could their existence be confirmed. If they do exist, it could be assumed that they are less predominant than *Races 2, 4, 5* and *6.*

ACKNOWLEDGEMENT

This project was supported by the Rubber Technical Cooperation Programme between SUDHEVEA/EMBRAPA/CEPLAC/RRIM.

REFERENCES

1. CHEE, K.H. (1978) South American Leaf Blight of *Hevea brasiliensis:* Culture of *Microcyclus ulei. Trans. Br. mycol. Soc.,* **70,** 341.
2. HOLLIDAY, P. (1970) South American Leaf Blight *(Microcyclus ulei)* of *Hevea brasiliensis. Phytopath. Pap. No. 12.*
3. JUNQUEIRA, N.T.V., CHAVES, C.M., ZAMBOLIM, L. AND GASPOROTTO, L. (1984) Variabilidade Fisiologica de *Microcyclus ulei,* Agente Etiologico do Mal Das Folhas de Seringueira. *Fitopatologia Brasiliera,* **9,** 385.
4. JUNQUEIRA, N.T.V., CHAVES, G.M., ZAMBOLIM, L., ROMEIRO, R.S. AND GASPOROTTO, L. (1984) Isolamento, Cultivo e Esporulacao de *Microcyclus ulei,* Agente Etiologico do Mal Das Folhas da Seringueira. *Revta Ceres,* **31,** 322.
5. LANGDON, K.R. (1965) Relative Resistance or Susceptibility of Several Clones of *Hevea brasiliensis* × *H. benthamiana* to Two Races of *Dothidella ulei. Pl. Dis. Rep.,* **49,** 12.
6. MILLER, J.W. (1966) Differential Clones of *Hevea* for Identifying Races of *Dothidella ulei. Pl. Dis. Rep.,* **50,** 187.
7. CHEE, K.H., ZHANG, K.M. AND DARMONO, T.W. (1986) The Occurrence of Eight Races of *Microcyclus ulei* on *Hevea* Rubber in Bahia, Brazil. *Trans. Br. mycol. Soc.,* **87,** 15.
8. CHEE, K.H. (1976) Assessing Susceptibility of *Hevea* Clones to *Microcyclus ulei. Ann. appl. Biol.,* **84,** 135.
9. JUNQUEIRA, N.T.V., ZAMBOLIM, L. AND CHAVES, G.M. (1985) Resistencia de Clones de Seringueira ao Mal Das Folhas. *Infme Agropecuario, Belo Horizonte,* **11,** 42.
10. SUDHEVEA (1970) Plana Nacional da Borracha. *Anexo XI-Pesquisas e Experimentacao com Seringueira.*
11. CHANDRASHEKAR, M. AND HEATHER, W.A. (1980) Reaction of Poplar Clones to Physiologic Races of *Melampsora larici-populina. Euphytica,* **29,** 401.
12. DARMONO, T.W. AND CHEE, K.H. (1985) Reactions of *Hevea* Clones to Races of *Microcyclus ulei* in Brazil. *J. Rubb. Res. Inst. Malaysia,* **33(1),** 1.

J. nat. Rubb. Res., **2(2)**, 118-128

Ultrastructure of Rubber Particles in PA 80 Latex. I. Changes during Sulphur Vulcanisation Reaction

SAMSIDAR HAMZAH*, J.B. GOMEZ* AND P.S. RAMA RAO*

Latex withdrawn during the various processing stages of sulphur vulcanisation was examined using two types of fixatives: standard osmium tetroxide (OsO_4) and potassium permanganate ($KMnO_4$). Observations indicated that samples fixed in 5% $KMnO_4$ for 15 min showed ultrastructural changes in the rubber particles during vulcanisation, especially when sections were post-stained with OsO_4 vapour.

As the process of vulcanisation proceeds, the internal stainable material (grain-like appearance), indicating the unoxidised portion of the particle, is increased. In a mixture of 80:20 of the vulcanised to unvulcanised latex i.e. *PA 80, these two types of particles could be readily differentiated by the trained eye. The distinction appears in the degree of oxidation of the interior of the unvulcanised particles being greater than in vulcanised latex particles.*

PA 80 (processing aid 80) is a concentrated masterbatch form of superior processing rubber (SPR) which was developed as early as 1958[1] so that the consumer can obtain any desired degree of superior processing character in any type of rubber, natural or synthetic. It is essentially an intimate mixture of 80% sulphur vulcanised rubber to 20% unvulcanised ammoniated rubber in a latex form[2,3], after which it is coagulated and made into sheet or crepe according to the grade selected. The properties that it possesses are advantageous to application where extrusion and calendering of rubber compounds are involved.

In most cases, the vulcanised state is achieved by a chemical reaction between the linear chains of rubber and the vulcanising agent (in this case sulphur) which produces covalent crosslinks between the rubber chains. Sulphur is combined in the vulcanisation network in a number of ways as enumerated by Porter[4]. The possibility of existence of non-network material in electron-lucent spaces around the rubber network may explain the presence of some residual materials[5]. Naturally abnormal properties are bound to arise in PA 80 due to certain production practices and/or structural differences. Methods have been described[6] to overcome these problems attributed to a 'second vulcanisation' reaction.

This study examines the ultrastructural changes of the rubber particles at the various predetermined sampling stages of vulcanisation, using two types of fixatives for comparison. It was initiated with the hope that practical problems arising at the consumers' end could be related to the ultrastructure of the rubber particles themselves.

MATERIALS AND METHODS

Latex samples were collected at the various stages of vulcanisation which were carried out at the Processing Instructors' School in the RRIM Experiment Station, Sungai Buloh. These stages were 0.7% ammoniated latex, 0, 1, 2, 3 and 5 h after steam was injected in *i.e.* at temperatures 25°C, 46°C, 77°C, 81°C and 83°C respectively and also at the completion of vulcanisation which was sampled the following day. A mixture of 80:20 vulcanised and the ammoniated latex were also sampled. The formula of the vulcanising dispersion used per 100 p.h.r. is as tabulated in the *Planters' Bulletin*[3].

Each sample was fixed in 5% aqueous solution of potassium permanganate for 15 min or

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in 2% aqueous osmium tetroxide for 24 h, both under chilled conditions. This was followed by thorough washing in several changes of water with the aid of a low-speed centrifuge to facilitate sedimentation of particles.

Dehydration was in graded series of ethanol before the samples were embedded in styrene-methacrylate (BDH Chemicals Ltd). Ultra-thin sections were examined, stained or unstained using Philips EM 300. Sections fixed in $KMnO_4$ were post-stained with osmium vapour overnight. Osmium-fixed samples were either post-stained with 2.5% lead citrate for 30 min or with 5% hydrogen peroxide for 30 minutes. Where experimental batches of PA 80 were made for examination by electron microscopy, samples taken from two other factories producing PA 80 were also taken at other times for comparison.

OBSERVATIONS AND DISCUSSION

Ammoniated Latex

The field latex to be vulcanised was initially ammoniated and in this case with 0.7% NH_3 before the vulcanising dispersion was added. (The normal practice is to add 0.5% NH_3.) *Figure 1* shows the appearance of ammoniated rubber particles in the various fixatives and staining conditions. Unammoniated latex fixed in OsO_4 under stringent conditions *(Figure 1A)* showed rubber particles with rigid boundaries unlike in the ammoniated latex *(Figure 1B)* where the boundaries were crenulated. This was presumably due to the addition of ammonia which had affected the elasticity and perhaps the surface characteristics of the membrane. Otherwise the electron density of the rubber particles was similar except when the sections were stained with lead citrate *(Figure 1C)* where the electron density of the rubber and of the membrane was further enhanced. In some particles differentiation into a lighter ring (380 Å - 1500 Å thick) and a darker core was also observed. When these osmium-fixed sections were treated with hydrogen peroxide, a bleached outer ring of variable thickness (750 Å - 2000 Å) was seen in all particles *(Figure 1D),* as has been shown elsewhere[7]. These bleached rings represented areas where free osmium was reduced and there is a possibility that part of these bleached zones were somewhat similar to the areas that were differentiated when the sections were stained with lead citrate.

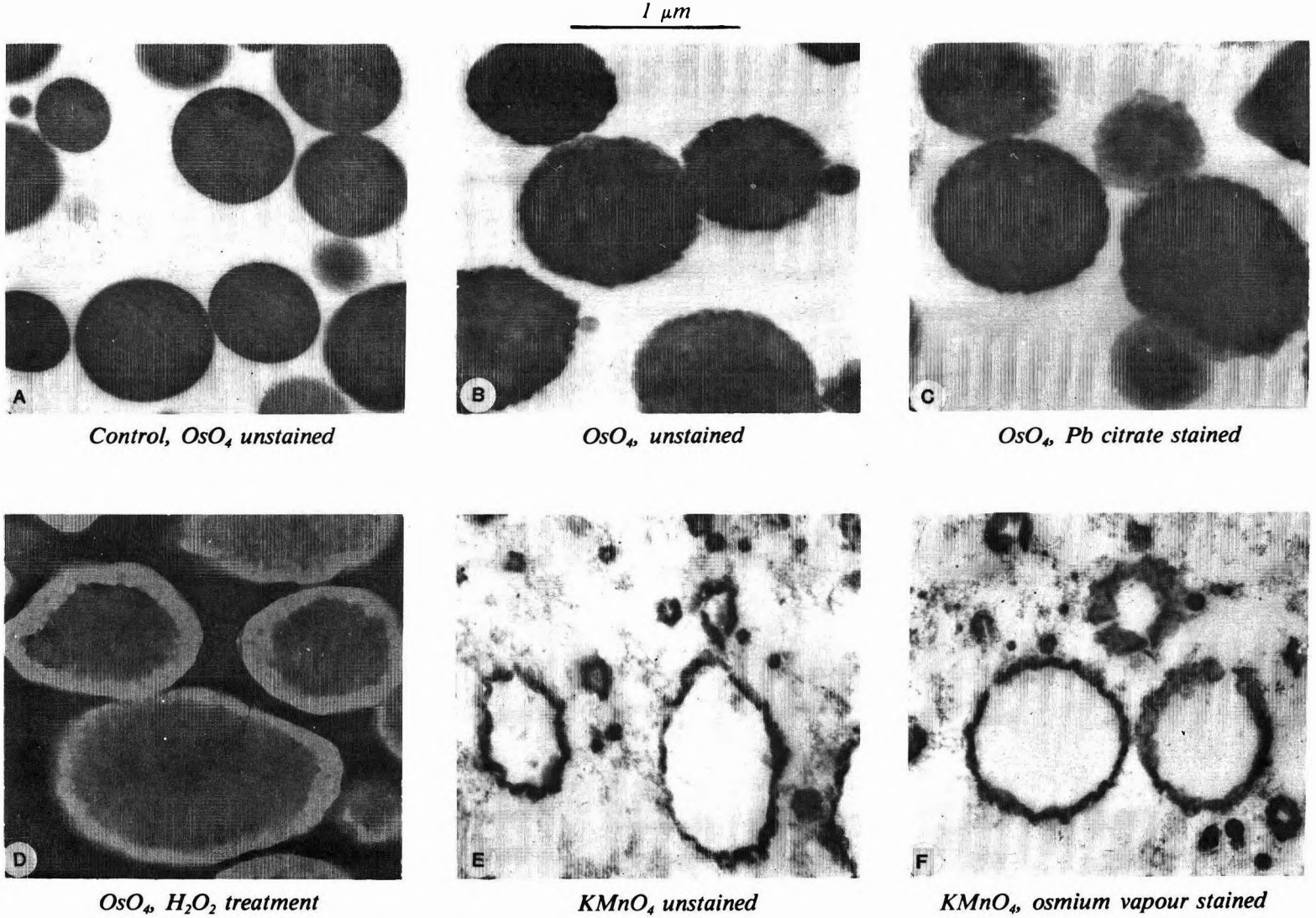
Fixation with permanganate, however, showed particles with rather thick shells ranging from 400 Å - 1500 Å *(Figures 1E* and *1F)* as compared to about 100 Å in unammoniated latex[7]. Smaller particles (1500 Å in diameter and below) showed completely dark particles. Staining did not show any differences to the interior which indicates that the material had been oxidised by the permanganate.

Changes during Vulcanisation

The vulcanising dispersion is stirred thoroughly to ensure homogeneity before it is weighed and added to the ammoniated latex. This mixture is gently stirred and heated by live-steam injection so that the temperature is raised to 80°C over a period of time depending on the factory concerned. In the Processing Instructors' School, the duration it took to reach 80°C was about 3 h and this temperature was maintained for another 2 h (approximately 83°C) in order to produce a well-vulcanised latex.

Figures 2-6 show the latices at various duration of vulcanisation under the different fixatives and staining. Fixing in osmium and examining unstained did not reveal any significant changes to the rubber particles during the process of vulcanisation *(Figure 2).* However, a change in the boundary of the particles was observed on completion of vulcanisation *(Figure 2F):* the boundary was then rigid as compared to the slightly crenulated condition at the beginning of vulcanisation.

Staining of the sections with lead citrate accentuated the membraneous region of the particles *(Figure 3)* in which some were thicker than others[8]. The presence of extraneous dark precipitates was also characteristic of stained sections. These were either found on the periphery or within the particles itself, in the amorphous or irregular crystal-like forms. The number of particles with the amorphous form,



Control, OsO_4 unstained | *OsO_4, unstained* | *OsO_4, Pb citrate stained*

OsO_4, H_2O_2 treatment | *$KMnO_4$ unstained* | *$KMnO_4$, osmium vapour stained*

Figure 1. Control (A) and 0.7% ammoniated latices (B-F) in the various fixatives.

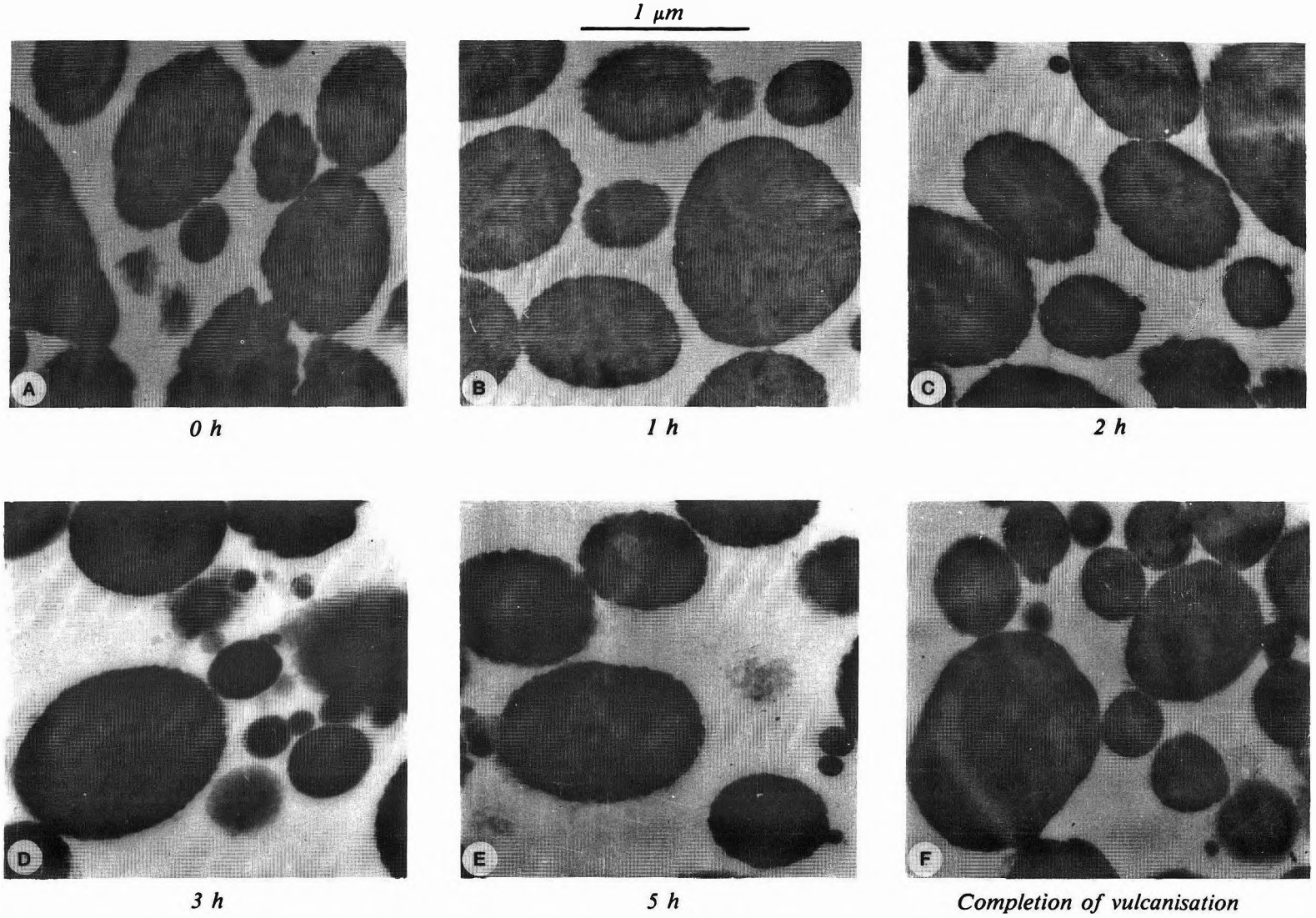


Figure 2. Rubber particles from various stages of vulcanisation, fixed in OsO_4 and unstained.

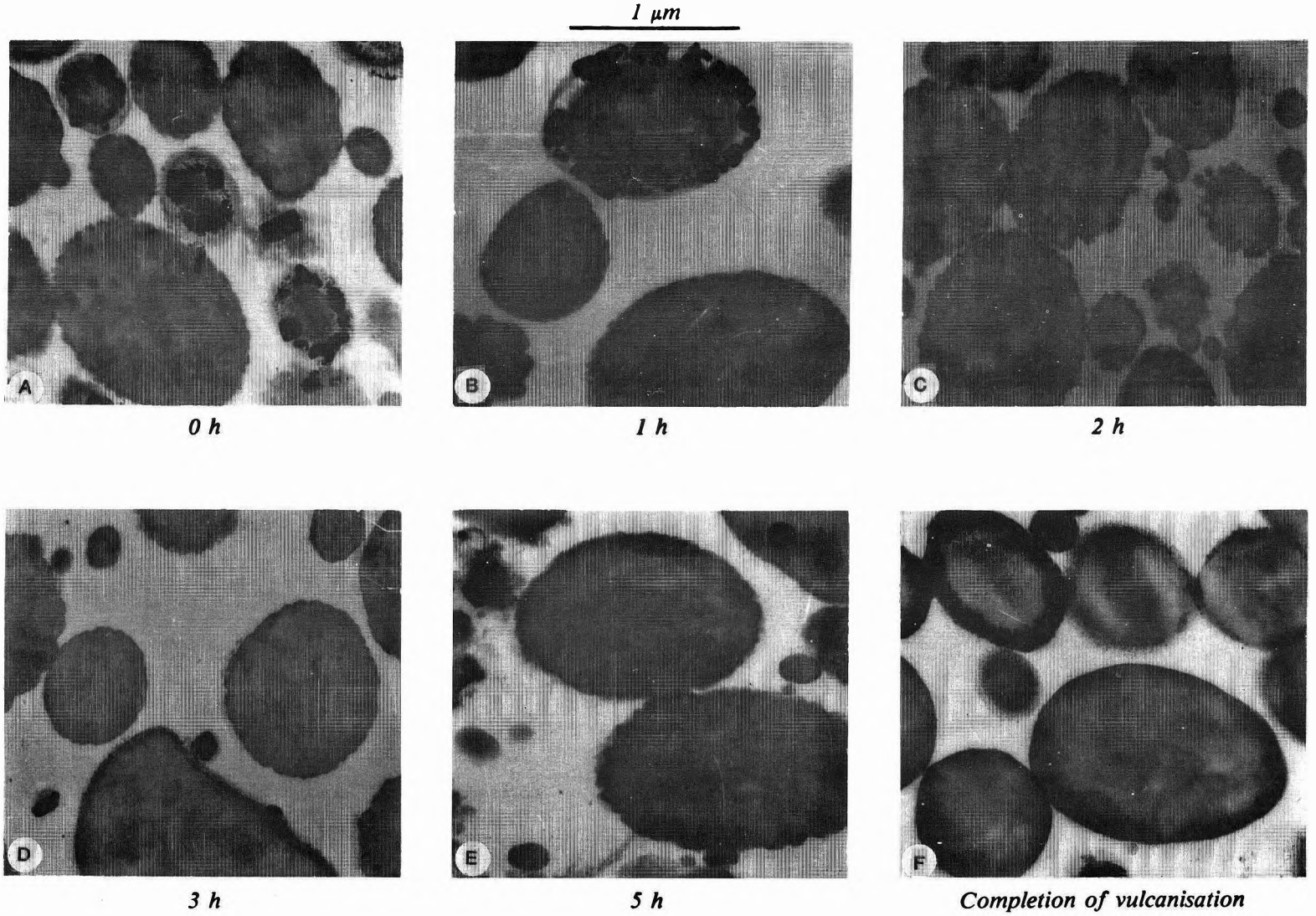


Figure 3. Rubber particles from various stages of vulcanisation, fixed in OsO_4 and stained in lead citrate.

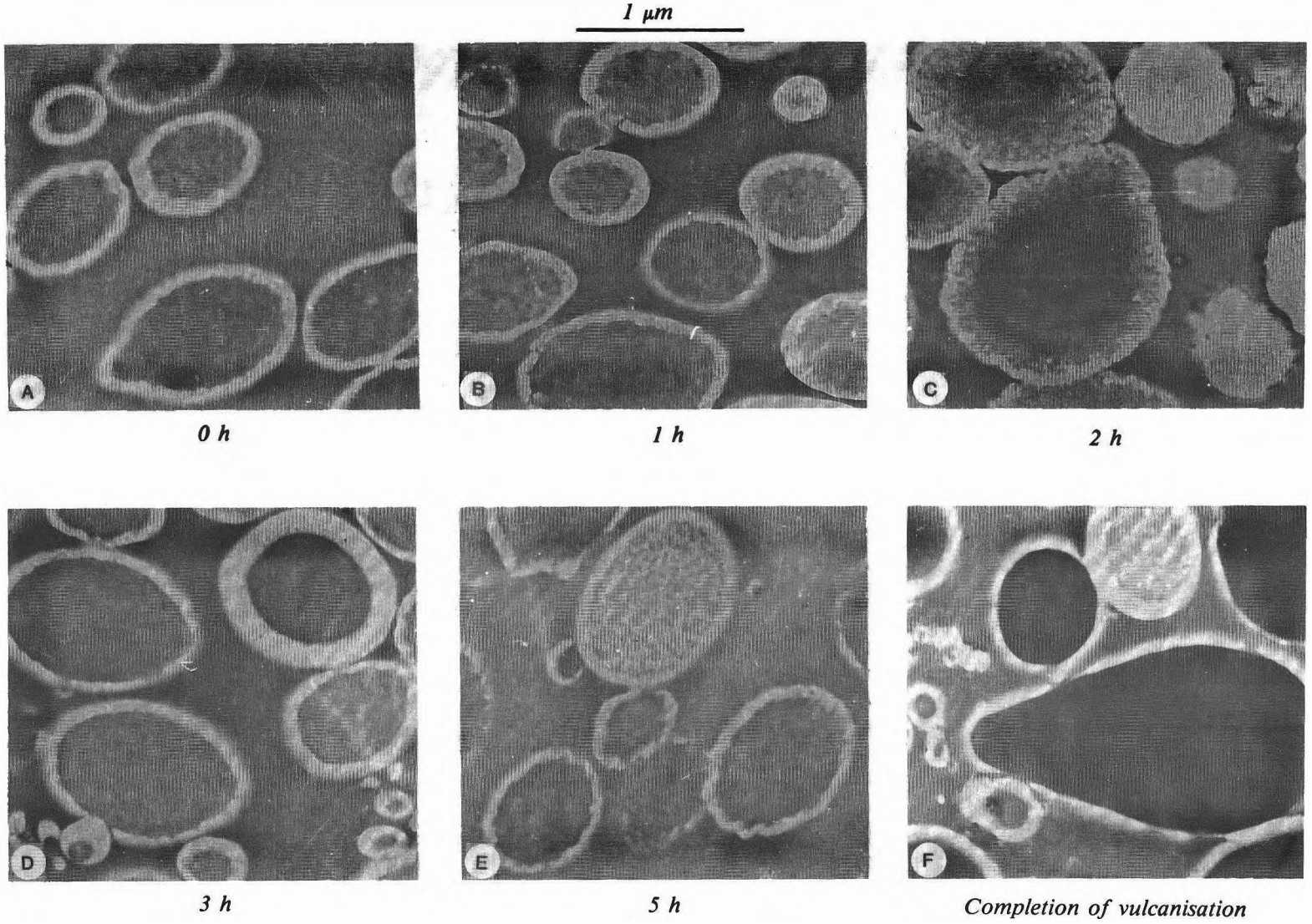


Figure 4. Rubber particles from various stages of vulcanisation, fixed in OsO_4 and stained with H_2O_2.

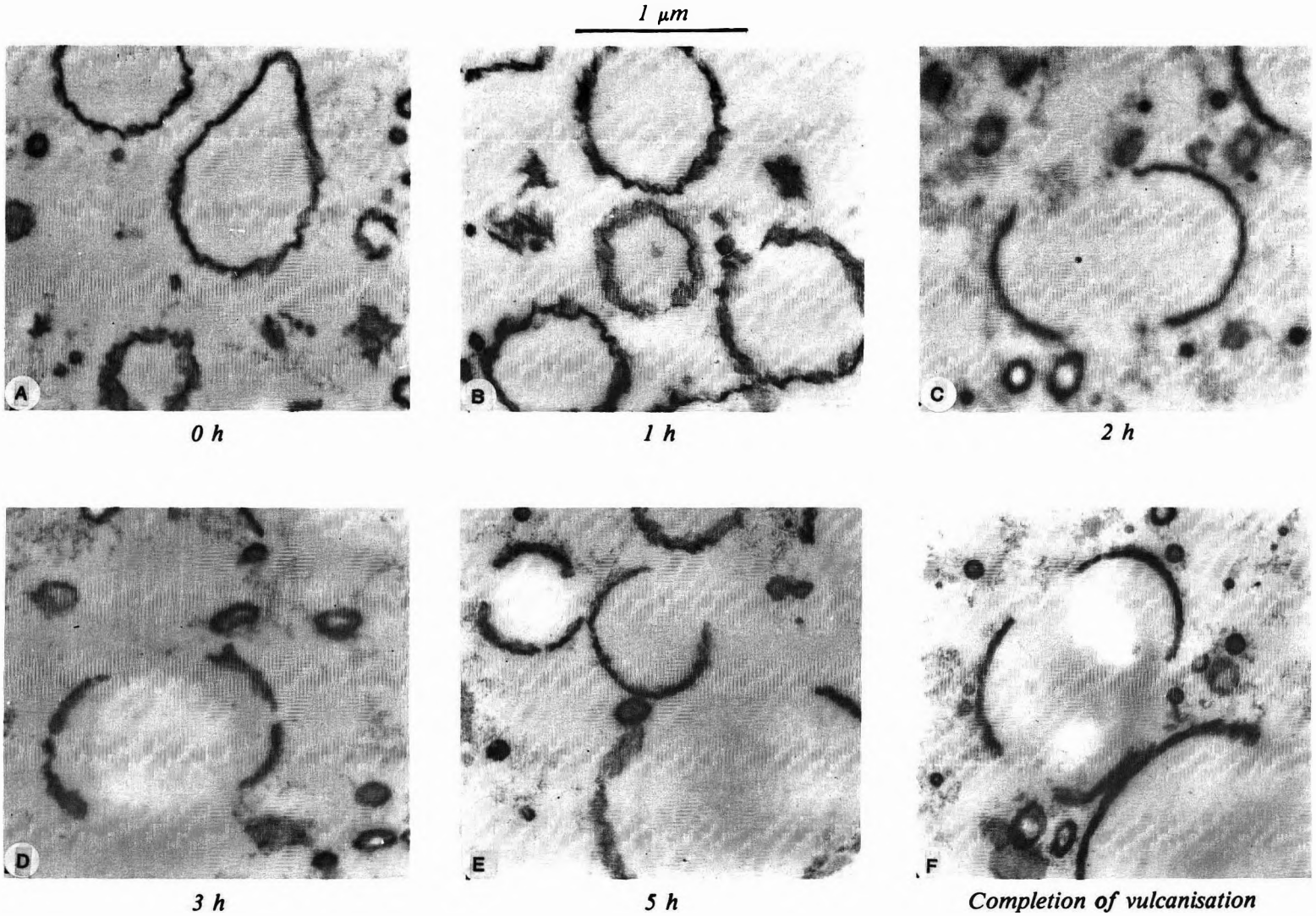


Figure 5. Rubber particles from various stages of vulcanisation, fixed in $KMnO_4$ and unstained.

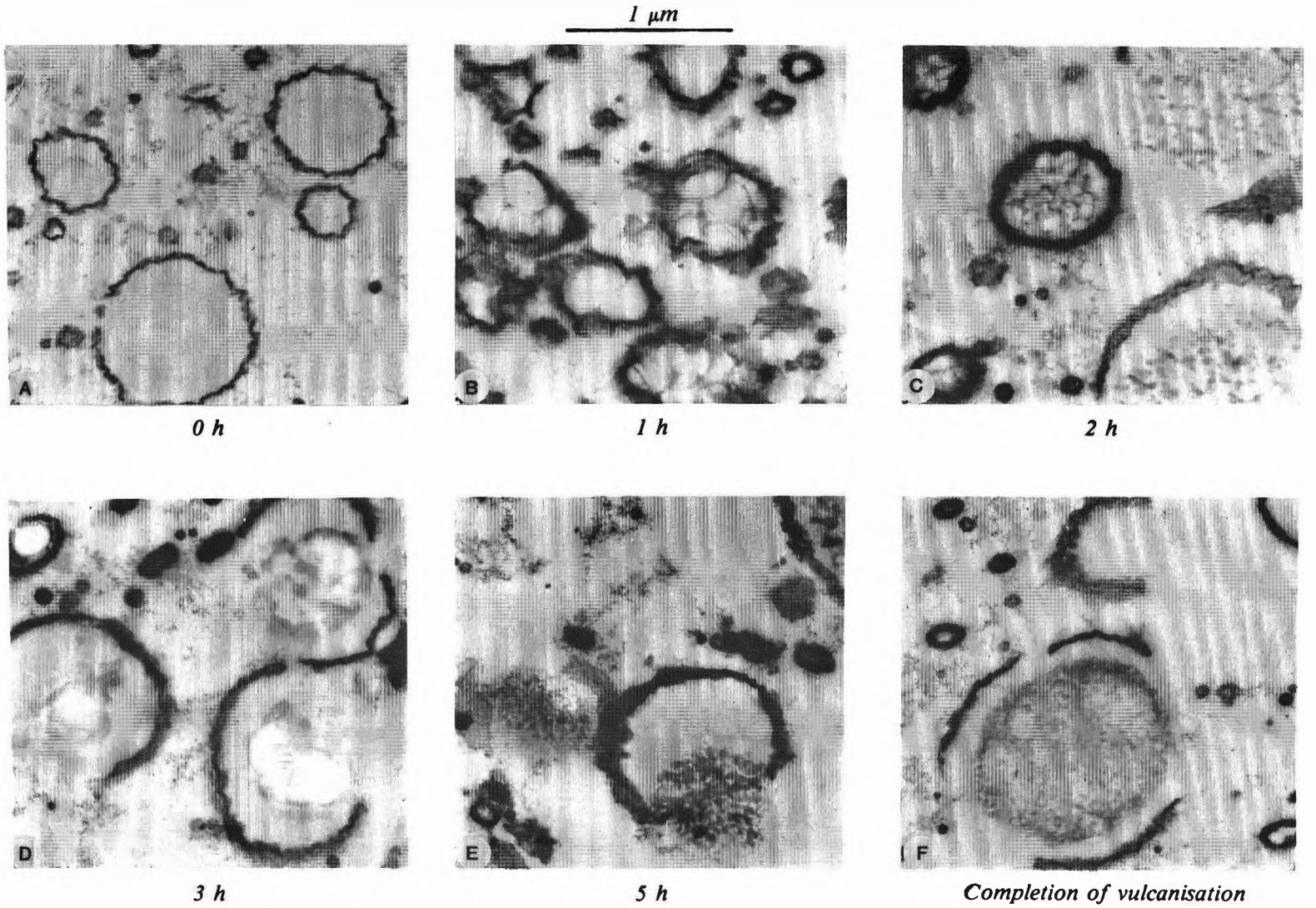


Figure 6. Rubber particles from various stages of vulcanisation, fixed in $KMnO_4$ and post-stained with osmium vapour.

usually located on the periphery of particles, appeared to be increased on the completion of vulcanisation *(Figure 3F)*. It was also noticed that where there were precipitates, the rubber particles had rigid boundaries.

In sections reacted with hydrogen peroxide, no significant trends were shown by the rubber particles during vulcanisation *(Figure 4)*. The thickness of the external bleached layer was variable throughout the process and so was the interior core which ranged from grey to speckled to very electron dense. The rigidity of the boundaries of rubber particles on the completion of vulcanisation was also shown by this peroxide reaction *(Figure 4F)*. This white external ring was also shown in the smaller particles.

With permanganate-fixed samples, however, micro-changes in the particles were observed during vulcanisation *(Figure 5)*, more clearly so in sections which were post-stained with osmium vapour *(Figure 6)*. The change was so marked that differentiation could be made between vulcanised and unvulcanised rubber particles.

Changes to the rubber particles were observed immediately on commencement of steam injection into the latex *(Figure 6A)*. The ordinary empty particles with thick shells could then be seen containing a faint circular ring inside them; the shells mostly remaining intact. After 1 h of steam (46°C), the single ring structure changed to a few smaller-ringed structures closely packed together within the particles *(Figure 6B)*; most of the shells were intact. But after 2 h of steam (77°C), some of the shells began to disrupt, especially the larger ones. *Figure 6C* shows particles with two types of interior after 2 h of steam: bunch-of-grapes type and a grainy type. By the third (81°C) and fifth hour (83°C) of steaming, about 50% and 80% of the particles respectively showed disrupted shells and grainy interiors *(Figures 6D* and *6E)*, together with another type of interior *(Figure 6E)* amidst the usual particles. On the completion of vulcanisation, which was sampled the following day, most particles had grainy interiors and broken shells indicating swelling.

The nature of the interior of the particles would not have been observed if the sections were unstained (compare *Figures 5* and *6)*. The smaller particles might have undergone similar changes during vulcanisation but as their lumen was rather small (750° in diameter and below), these changes could not be detected readily except for some which showed slightly greyish interiors.

Mixture of 80:20 (Vulcanised:Unvulcanised)

Blending of the two types of particles (latices) was carried out the following day after proper cooling of the prepared vulcanised latex. Mixture of this latex blend in the various fixatives and staining is shown in *Figure 7*. Fixation by osmium, irrespective of post-staining or peroxide treatment could not differentiate clearly the vulcanised from the unvulcanised particles *(Figures 7A–7C)*. With the permanganate-fixed samples, however, especially those post-stained with osmium vapour *(Figure 7E)*, these two types of particles could be singled out clearly. The unvulcanised rubber particles had thick shells and were mostly intact while the vulcanised particles had disrupted shells and grain-like interiors. The latter appeared detached from the shell indicating some swelling *(Figure 7F)*. The particles from vulcanised latex that has been fixed in osmium have been shown to be fairly stable to solvent effects [8].

The methods discussed in this paper offer a convenient technique to gain insight into the ultrastructure of rubber particles undergoing vulcanisation and on completion of the vulcanisation reaction as normally practised in factories producing PA 80. It is evident that with progress in vulcanisation, there is an increase in the resistance to permanganate oxidation, a phenomenon obviously connected with the network structure of the rubber in the particles. We are not sure whether the graininess observed corresponds to the occurrence of clusters of crosslinks as elucidated by chemical techniques[9] but such a possibility has to be borne in mind. Unfortunately, the resolution used in the present study does not permit us to offer such an explanation. However, we

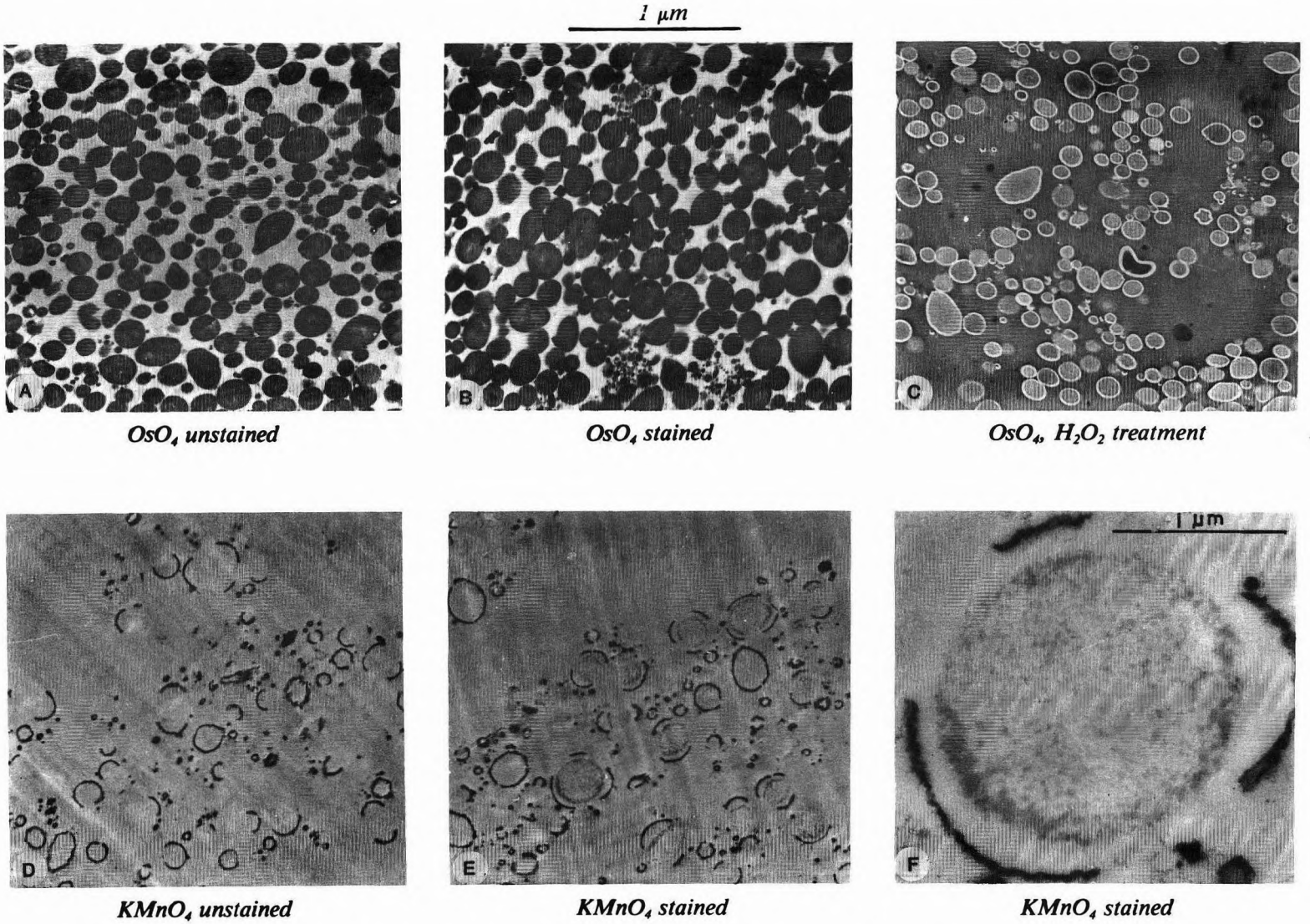


Figure 7. Blend of 80:20 vulcanised and unvulcanised latices.

note that this topic is open for further investigations on micro-structure.

CONCLUSION

From this study, potassium permanganate was found to be the most suitable fixative for detecting micro-changes in the rubber particles during sulphur vulcanisation. Hence, the behaviour of the rubber particles during vulcanisation could be followed through and distinction between vulcanised and unvulcanised particles could be made by this fixation, especially when sections were post-stained with osmium vapour. This fact can be utilised to detect changes in PA 80, if there are any, when it undergoes storage during shipment. This topic is currently under further investigation.

The various domains found in the grainy particles *(Figure 6F)* of permanganate-oxidised particles suggest that these might indicate clusters of crosslinks if the whole picture is envisaged as an exposition of the micro-network inside the particle.

ACKNOWLEDGEMENT

The authors are grateful to Encik-Encik Yee Shin Meng, Ariffin Haji Hassan, Abdul Karim, Tsan Fan Kui and Puan Chan Jee Leene and Cik Ho Lai Har for their valuable technical assistance and to Puan Sooryakumari for typing the manuscript.

REFERENCES

1. SEKHAR, B.C. AND DRAKE, G.W. (1958) Superior Processing Rubber Master-batch. *J. Rubb. Res. Inst. Malaya,* **15(4),** 216.
2. RUBBER RESEARCH INSTITUTE OF MALAYA (1960) Production of PA 80 (Processing Aid 80). *Plrs' Bull. Rubb. Res. Inst. Malaya No. 47,* 26.
3. RUBBER RESEARCH INSTITUTE OF MALAYA (1967) Improvements in the Production of PA 80. *Plrs' Bull. Rubb. Res. Inst. Malaya No. 91,* 127.
4. PORTER, M. (1969) Effect of Re-inforcing Fillers on the Chemical Structure of Sulphur Vulcanisation of Natural Rubber. *Kaut. Gumm. Kunst.,* **2(8),** 419.
5. LEWIS, P.M. (1986) Vulcanisate Structure and Its Effects on Properties. *NR Technol.,* **17(4),** 57.
6. GORTON, A.D.T. (1968) Effect of Production Procedures on Properties of PA 80 Rubber. *J. Rubb. Res. Inst. Malaya,* **20(5),** 257.
7. GOMEZ, J.B. AND SUBRAMANIAM, A. (1985) Some Recent Electron Microscopic Studies of *Hevea* Latex Particles. *Proc. Int. Rubb. Conf. 1985 Kuala Lumpur,* **2,** 510.
8. SAMSIDAR HAMZAH, GOMEZ, J.B. AND RAMA RAO, P.S. (1986) Recent Observations on the Microstructure of PA 80 Latex. *Proc. XIth Int. Congr. Electron Microscopy Kyoto 1986,* **2,** 1755.
9. BRYDSON, J.A. (1978) *Rubber Chemistry,* p. 198. London: Applied Science Publishers Ltd.

J. nat. Rubb. Res., **2(2)**, 129–133

SHORT COMMUNICATION

Proteolipids of Natural Rubber Particles

H. HASMA*

About 40% of the membrane proteins of the rubber particles were found to be proteolipids. They were hydrophobic proteins containing 70% non-polar amino acids and were closely associated with phospholipids and glycolipids. SDS-gel electrophoresis revealed one main protein band at a molecular weight of 14 300 with two minor ones in between 14 300 and 24 000.

Proteolipids, first discovered by Folch and Lees[1], are a type of protein-lipid complex which differ from lipoproteins in being soluble in chloroform/methanol mixture but insoluble in water. They have been found characteristically as membrane components of many plant, animal and bacterial cells[2] especially in brain white matter (BWM) where they constitute about 50% of the central nervous system myelin proteins[3]. Generally, they are hydrophobic and non-covalently associated with lipids[1,4,5]. However, proteolipids containing covalently bound long-chain fatty acids linked to specific amino acids have also been found[6,7].

Proteolipids have not previously been reported to be present in *Hevea brasiliensis* latex. This communication describes the isolation and characterisation of proteolipids associated with natural rubber (NR) particles.

EXPERIMENTAL

Isolation of Proteolipids

Fresh latex from clone RRIM 600 was centrifuged on a Beckman L8–70 ultracentrifuge at 19 500 r.p.m. using rotor 21 for about 1 hour. The rubber phase and bottom fraction were collected.

The rubber phase was redispersed in the minimum of water, filtered and added dropwise to five volumes of a continuously stirred chloroform/methanol (2:1, volume/volume) mixture. The extracts separated from the rubber coagulum were washed with salt solutions [0.6% aqueous NaCl and 0.1*M* NaCl in 0.023*M* *tris*-HCl (pH 8.8)[4]]. A lower chloroform fraction and a thin whitish interfacial layer were isolated. The chloroform layer was concentrated on a rotatory evaporator. Further addition of a chloroform/methanol (2:1, volume/volume) mixture left an insoluble portion. This insoluble portion containing proteolipids was collected. The interfacial layer was precipitated with acetone, centrifuged and dried.

The bottom fraction was freeze-thawed three times and then centrifuged. The sedimented membrane components, washed several times with water to remove the remaining B-serum, were extracted with a chloroform/methanol (2:1, volume/volume) mixture and the proteolipids isolated as described in the rubber phase.

Determination of Proteins, Phospholipids, Glycolipids and Amino Acids

Protein content was determined by multiplying the amount of nitrogen[8] with its conversion factor 6.25. Similarly, multiplying phosphorous content[9] by 25.9[10] and the sugar content[11] by 4.7[10] gave the amount of phospholipids and glycolipids, respectively.

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Amino acid analyses were done on a Technicon automatic amino acid analyser. The amino acids were obtained by hydrolysing the proteolipids in 6*N* HCl at 110°C for 24 hours.

SDS-urea Polyacrylamide Gel Electrophoresis

Electrophoresis on SDS-urea polyacrylamide gel was carried out according to the method of Chans and Lees[12] at a constant current of 99mA for 2½ hours.

RESULTS AND DISCUSSIONS

By extracting the rubber particles with chloroform/methanol, there was a loss of about 40% nitrogen content in the rubber phase. As this nitrogen is attributed mainly to the proteins associated with the rubber particles it implied that the chloroform/methanol mixture extracted some proteins. Concentrating the water washed chloroform/methanol extracts to almost dryness left a portion of the extracts insoluble in the same solvent mixture. Nitrogen determination showed the residue to contain about 7%–10% nitrogen which was considered too high for any nitrogen-containing lipids. Acid hydrolysis of the residue showed a high concentration of neutral amino acids. All these observations seem to fit well with the description of proteolipids by Folch and Lees[1]. Further analysis described below confirmed the presence of proteolipids in the membrane components of rubber particles.

As observed by other workers[1,4,5], the proteolipids from the rubber particles also showed a variation in lipid components, depending on the method of isolation. Lipid contents decreased from 30% in water washed chloroform/methanol extracts to 7%–13% in proteolipids obtained from the interfacial layer on washing the extracts with sodium chloride solutions of different pH *(Table 1)*. The decrease in lipid content could be due to the 'splitting' of the protein-lipid linkages by the salt[4] or the proteolipid apoprotein (the protein moiety of the proteolipid) itself undergoing changes at alkaline pH leading to its precipitation with parallel release of associated lipids[5]. The level of lipids reflected the purity of proteolipid apoprotein as *Table 1* shows that the protein content increased from 62% to 81% with the level of lipids decreasing from 30% to 7%. A crystalline proteolipid containing 86% proteins and 2% lipids was obtained from the interfacial layer of the chilled unwashed chloroform/methanol extract of the rubber phase which had been redispersed in excess water.

Characteristic of membrane proteolipid, the lipids highly bound to the apoprotein were mainly phospholipids and glycolipids. Some neutral lipids were also detected. Preliminary investigations showed that the phospholipid, glycolipid and neutral lipid composition of the rubber particle proteolipid was similar to that present in the total lipid extracts of the whole *H. brasiliensis* latex[13].

Complying with the hydrophobic nature of the proteins, the amino acids of *H. brasiliensis* proteolipids comprised about 70% neutral amino acids *(Table 2)*. Out of the eighteen common protein amino acids only fourteen were detectable. Tryptophan is usually destroyed by 6*N* HCl hydrolysis of proteins[14]. The presence of carbohydrates in the glycolipid

TABLE 1. PROTEIN AND LIPID CONTENT OF THE PROTEOLIPIDS ASSOCIATED WITH THE RUBBER PARTICLES

Washing reagent	Composition (%)		
	Proteins	Phospholipids	Glycolipids
Water	62.5	23.0	6.9
Sodium chloride	75.0	10.2	2.6
Tris-HCl (NaCl)	81.3	6.0	1.5

TABLE 2. AMINO ACID COMPOSITION OF PROTEOLIPIDS ASSOCIATED WITH THE RUBBER PARTICLES

Amino acid	Proteolipids at the interphase	Proteolipids from the $CHCl_3$ faction
Lysine	8.0	6.9
Histidine	—	—
Arginine	2.5	2.6
Aspartic acid	10.1	10.0
Threonine	3.7	4.2
Serine	7.0	6.8
Glutamic acid	9.6	9.5
Proline	3.9	6.4
Glycine	7.5	7.6
Alanine	12.9	11.8
Cysteine	—	—
Valine	12.4	11.5
Methionine	—	—
Isoleucine	5.5	6.1
Leucine	8.2	8.0
Tyrosine	3.8	3.9
Phenylalanine	4.5	4.4
Polar (%)	30.2	29.0
Neutral (%)	69.8	71.0

component of the rubber proteolipids enhanced the destruction of methionine and cysteine during acid hydrolysis[15]. Histidine was not present in the proteolipids of NR particles as simultaneous hydrolysis of cow BWM proteolipids showed that the amino acid was not destroyed by the acid hydrolysis process. *Table 2* shows that there was no major difference in the amino acid composition of the proteolipids from the chloroform layer and from the interphase. Both proteolipids had high content of alanine, valine, glutamic acid and aspartic acid, each constituting about 9%–13% of the total amino acids. The relatively high concentration of the latter two polar amino acids in rubber proteolipids was not unusual as it has been reported that such exceptions to the general abundance of non-polar amino acids do occur in the proteolipids of sarcoplasmic reticulum Ca^{2+}-ATPase[2] and defatted soybean meals[16].

SDS-urea polyacrylamide gel electrophoresis of the rubber particle proteolipids showed a prominent band at a molecular weight of 14 300 with a second and a faint third one between 14 300 and 24 000 *(Figure 1)*. A similar pattern was observed in proteolipids obtained at the interphase, regardless of their state of delipidation. The proteolipids from the chloroform layer, however, contained mainly the lower molecular weight proteolipids. The other plant proteolipids which showed a similar protein pattern to the NR particle proteolipids were the soybean proteolipids[16]. They too gave two protein bands on SDS-urea polyacrylamide gel electrophoresis; a denser band at the

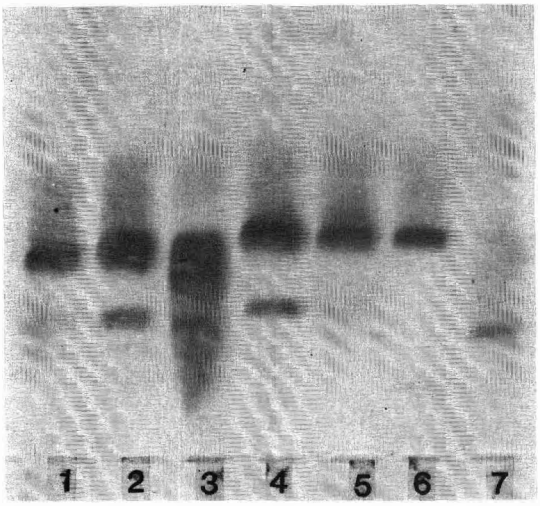

Figure 1. SDS-urea polyacrylamide gel patterns of proteolipids from NR particles. 1 = proteolipids obtained after washing the chloroform-methanol extracts with water, (2) with sodium chloride, (3) with tris-*HCl (NaCl), 4 = proteolipids at the interphase, (5) in the chloroform fraction, 6 = lysozyme, and 7 = trypsinogen.*

molecular weight of 15 000 and a less dense one at 13 000.

The triple proteolipid apoprotein bands on SDS-urea polyacrylamide gel electrophoresis might not indicate three different kinds of proteolipids but merely represent specific states of aggregation of a single protein as shown in the case of BWM proteolipids[12]. Differences in molecular shape would also result in differences in SDS binding capacity.

Although proteolipid is a membrane protein, it is not characteristic to all membranes[1]. In *H. brasiliensis* latex the concentration of the proteolipids in the membrane surrounding the non-rubber particles was low, being only 3%–6% of the total proteins, compared to 40%–50% in the rubber particles. Moreover, a certain portion of the proteolipids of the non-rubber particles must be derived from the rubber particles which were sedimented together with the former in the bottom fraction of the ultracentrifuged latex. Thus, the hydrophobic proteolipids of *H. brasiliensis* seem to show preference for the membrane surrounding the equally hydrophobic rubber molecules rather than the plasma membrane of the non-rubber particles. This perhaps suggests a specific function of the proteins in the membrane of the rubber particles; for example in the stability of NR latex.

ACKNOWLEDGEMENT

The author thanks Dr A. Subramaniam for reading the manuscript and some suggestions, Encik Mohd Yusof Rais, Encik Anthonysamy Michael and Puan Low Chooi Lan for experimental assistance, and Encik Chan Tin Wan and Head of the Department of Food Science, Universiti Pertanian Malaysia, for amino acid analysis.

REFERENCES

1. FOLCH, J. AND LEES, M. (1951) Proteolipids, a New Type of Tissue Lipoproteins, Their Isolation from Brain. *J. Biol. Chem.*, **191**, 807.

2. LEES, M.B., SAKURA, J.D., SAPIRSTEIN, V.S. AND CURATOLO, W. (1979) Structure and Function of Proteolipids in Myelin and Non-Myelin Membranes. *Biochim. Biophys. Acta,* **559**, 209.

3. ENG, L.F., CHAO, F.C., PRATT, B.G. AND TAVASTSTJERNA, M.G. (1968) The Maturation of Human White Matter Myelin. Fractionation of the Myelin Membrane Proteins. *Biochemistry,* **7**, 4455.

4. WEBSTER, G.R. AND FOLCH, J. (1961) Some Studies in the Properties of Proteolipids. *Biochim. Biophys. Acta,* **49**, 399.

5. TENENBAUM, D. AND FOLCH-PI, J. (1966) The Preparation and Characterisation of Water-Soluble Proteolipid Protein from Bovine Brain White Matter. *Biochim. Biophys. Acta,* **115**, 141.

6. SCHLESINGER, M.J. (1981) Proteolipids. *Ann. Rev. Biochem.*, **50**, 193.

7. BIZZOZERO, O.A. AND LEES, M.B. (1986) Spectroscopic Analysis of Acylated and Deacylated Myelin Proteolipid Protein. *Biochem.*, **25**, 6752.

8. RUBBER RESEARCH INSTITUTE OF MALAYSIA (1973) R.R.I.M. Test Methods for Standard Malaysian Rubbers. *SMR Bull. No. 9.*

9. A.O.C.S. OFFICIAL METHOD (1964) *Official and Tentative Methods of the American Oil Chemists' Society,* Vol. 1, Ca 12-55.

10. HASMA, H. (1984) Lipids in Latex and Rubber of *Hevea brasiliensis Muell. Arg.* and Their Effects on Some Properties of Natural Rubber. Doctor in Agricultural Sciences Thesis, Rijksuniversiteit Gent.

11. DUBOIS, M., GILLES, K.A., HAMILTON, J.K., REBERS, P.A. AND SMITH, F. (1956) Colorimetric Method for the Determination of Sugars and Related Substances. *Analyt. Chem.*, **28**, 350.

12. CHANS, D.S. AND LEES, M.B. (1974) Gel Electrophoresis Studies of Bovine Brain White Matter Proteolipid and Myelin Proteins. *Biochem.*, **13**, 2704.

13. HASMA, H. AND SUBRAMANIAM, A. (1986) Composition of Lipids in Latex of *Hevea brasiliensis* Clone RRIM 501. *J. nat. Rubb. Res.*, **1(1)**, 30.

14. SCOFFONS, E. AND FUNTANA, A. (1975) *Identification of Specific Amino Acid Residues, Protein Sequence Determination (Needleman, S.B. ed)*, p. 162. New York: Springer-Verlag.

15. ANDREWS, R.P. AND BALDAR, N.A. (1985) *The Amino Acid Analysis of Feed Constituents.* England: LKB Incentive Group, LKB Biochrom Ltd.

16. MIURA, M., YAMAUCHI, F., OGAWA, Y. AND SHIBASAKI, K. (1982) Isolation and Characterization of a Proteolipid in Defatted Soybean Meals. *Agric. Biol. Chem.*, **46**, 1631.

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