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Theoretical Aspects of Synthetic/Polycross Populations of Rubber Seedlings

N.W. SIMMONDS*

Rubber seedlings, the products of seed gardens, have been grown successfully for decades. Such populations are polycrosses or first generation synthetic varieties (SYN_1). The genetics of SYN_1 in rubber have not been investigated but some (rather speculative) calculations are possible on fragmentary data. It is likely: that parents are unequally represented among progeny; that crossing is very imperfect but that the deleterious effects of inbreeding are partly mitigated by seedling culling; that, nevertheless, SYN_1 suffers from some inbreeding depression; that second generation seedlings (SYN_2) offer interesting practical potential worthy of investigation; and that seed-garden design deserves far more attention that it has had. Suggestions for relevant experiments are made and it is pointed out that isozyme genes would offer a powerful method of analysing the genetical dynamics of seed gardens and their products.

Synthetic (SYN) populations reproduced by seed are used in many crops in which an outbreeding habit forbids the use of inbred lines but clones are technically infeasible[1]. They are genetically somewhat variable and are formed by crossing selected parental clones or populations. Sometimes the original synthetic population (SYN_1) is used agriculturally, as in coconuts, oil palms and cacao; sometimes, one or two generations of bulk propagation are interjected so that the agricultural population is SYN_2 or SYN_3, as in many grasses[2] and legume forages[3]. The number of parents used depends largely upon the degree of pollination control that can be achieved. If perfect crossing is possible, then two parents may suffice and the SYN_1 product approaches an F_1 hybrid variety except that it is variable; the palms (formed from population crosses by hand pollination) and the cacaos (formed from bi-clonal crosses using the incompatibility mechanism) are examples. If self-pollination cannot be prevented, then it is usual to minimise inbreeding depression by interplanting several-many parents in a polycross seed-garden with the object of producing a more or less random array of outbred hybrids between good parents; the presence of some weak inbred progeny has to be accepted, along with recognition of the fact that differential fertilities will usually imply that parents will be unequally represented in the products.

A nomenclatural point deserves mention. Any SYN population based on several parents is a polycross. However the word 'synthetic' is also used of an open-pollinated population compounded from inbred lines of an outbreeder such as maize; genetically it would be equivalent to a later-generation SYN (as used here). The word 'polycross' originally applied to a testing procedure used to assess clones or lines or populations for which hand pollination was too laborious for use in a test-crossing procedure[1,2,3]. The performance of polycross progeny estimates general combining ability (GCA) (but not SCA, of course). Nowadays, the word is widely used to mean multiple uncontrolled crossing of several-many parents, for whatever purpose.

In rubber, though clones predominate, SYN_1 seedling populations produced from polycrosses of clonal parents have long been planted on a substantial scale. That they grow and yield well is due to the predominantly additive genetic control of vigour and yield;

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GCA for these characters is high (Tan[4], Simmonds[5,6]) so the choice of vigorous and high yielding clonal parents ensures good, though not outstanding, progeny. But rubber suffers severely from inbreeding depression, so the fact that the flowers are self-compatible and not very efficiently crossed incurs the presence of some selfed progeny in the products of even an excellent seed garden.

The extent of inbreeding in rubber seed gardens and its consequences for SYN_1 seedling performance have not been investigated; nor have the potential performance of SYN_2 populations and the question of optimal structure of the seed garden itself. These are quite difficult questions and relevant experimental data are few; but some theory is possible and my object in this paper is to present an outline of it, drawing attention to the kinds of experiments and observations needed to attain better understanding.

RESULTS

Inheritance of Seed Fertility

It has long been known, in a general sort of way, that rubber clones vary rather widely in fruitfulness[7,8] but there is no systematic study of which I am aware bearing on the genetic control of fertility, male or female. The data of Tan and Subramaniam[9] on a five-parent diallel cross, however, can be usefully interpreted in this context as follows. They give data on per cent success of large numbers of pollinations for all ten crosses (crosses in both directions aggregated) and for the five parents selfed. The cross data are treated as a half-diallel in *Figure 1* and the results of selfing are plotted in the same diagram as observed fertilities against expectations based on GCA. With few degrees of freedom and no replicates, the variance due to parents is non-significant tested against residuals but of the predominantly additive nature of average (male and female) fertility there can be little doubt. Residuals will, of course, include male-female differences as well as any specific interactions.

Samples were large (pollinations ran into thousands) so errors estimated from a binomial assumption are small *(Figure 1)*. This assumption is probably not justifiable (due to heterogeneity of probability of success of a pollination); these rough errors therefore serve merely to support the idea of a predominant GCA component of fertility, with a fair probability that SCA effects would be detected by the appropriate experiment (given proper estimates of errors).

One of the most striking features of the data is the relative ineffectiveness of selfing. Wycherley[8] also noted the point but his data did not reveal so extreme a difference. There is no evidence of self-incompatibility in rubber so the effect must be attributed to zygotic inviability due to inbreeding; the failed seeds might be regarded, therefore, as very early-expressed runts[6]. However, the unit of setting in rubber is the fruit rather than the seed and genetic interpretation of the difference is deferred to the next section.

The results summarised in *Figure 1* are striking and a test of the conclusion regarding a high GCA component of control of fertility would be worthwhile. Wycherley's data[8] showed very large environmental effects of time and place so any experiment should provide for blocking and hence valid estimation of error.

Seed and Fruit Set

Rubber fruits are usually tricarpellary and the evidence is clear that all three seeds must (nearly always) set for the fruit to survive[10]. Suppose p_s and p_c are the probabilities of *seed*-setting for selfs and crosses respectively. Ignoring mixed pollinations, the probabilities of *fruit*-setting are then $u_s = p_s^3$ and $u_c = p_c^3$; hence $p_s = \sqrt[3]{u_s}$ and $p_c = \sqrt[3]{u_c}$. Estimates of u are available from the data of Wycherley[8] and Tan and Subramaniam[9] as follows: $u_s = 0.027$, $u_c = 0.048$ and $u_s = 0.005$, $u_c = 0.037$ when $p_s = 0.30$, $p_c = 0.35$ and $p_s = 0.17$, $p_c = 0.33$ respectively. Thus large differences (two- to seven-fold) in u reflect much smaller differences in the underlying p. At the embryo, as against the fruit level, selfing is not so defective as it would seem.

The matter should be accessible to experimental investigation. It should not be difficult to determine rates of fertilisation success and

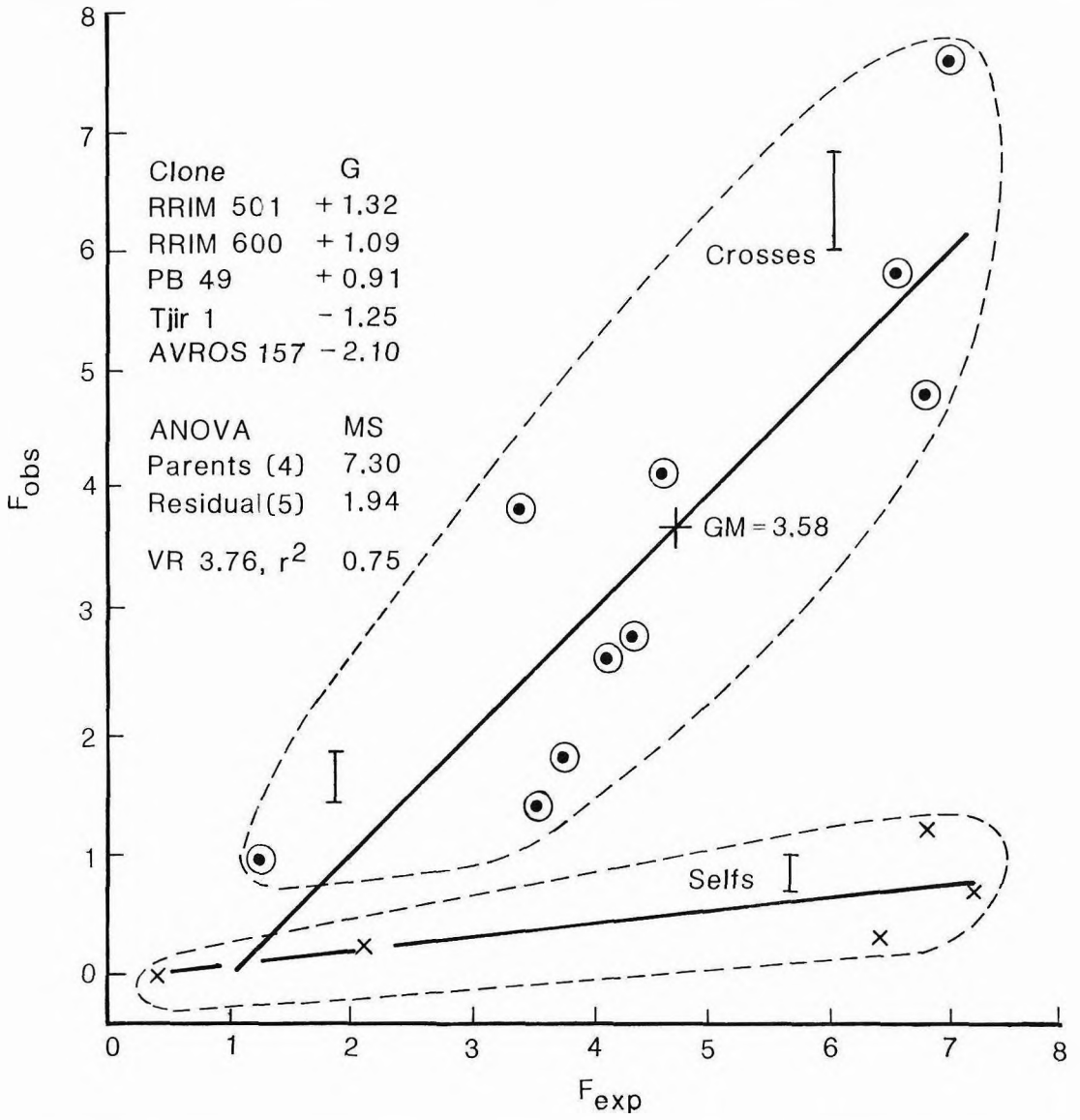

Figure 1. Heritability of seed fertility in rubber. Based on data of Tan and Subramaniam[9]. Observed per cent flowers setting fruit in crosses (F_{obs}) plotted against expectations based on GCA. Selfs (bottom) are much less fertile than expectations based on GCA. The vertical bars are confidence limits of a point based (probably illegitimately) upon binomial assumptions.

embryo survival by sampling flowers at intervals after controlled self and cross pollination.

Crossing in a Seed Garden

Consider one clonal parent and its progeny. Let its female flowers be q self-pollinated and $(1-q)$ crossed. We know that selfed fruits are less likely to survive than hybrid ones, say at rates u_s and u_c respectively (as in the preceding section). For simplicity we ignore mixed pollinations. Then young seedlings will have frequencies:

Selfed	Crossed
$\frac{qu_s}{qu_s + (1-q)u_c}$	$\frac{(1-q)u_c}{qu_s + (1-q)u_c}$

We have estimates of u from the same sources cited above, thus (in per cent):

$u_s = 2.7$, $u_c = 4.8$ and $u_c/u_s = 1.8$ . . . 1

$u_s = 0.5$, $u_c = 3.7$ and $u_c/u_s = 7.4$ . . . 2

Writing s for the overall effective selfing rate at the young seedling stage [crossing $(1-s)$], we have:

$$s = \frac{q}{1.8-0.8q} \quad \text{and} \quad s = \frac{q}{7.4-6.4q}$$

respectively, for the two pairs of estimates of u. Curves of s against q are plotted in *Figure 2.*

We need direct estimates of s. The only one I know of is from Prang Besar data[6] on the recessive yellow mutant in PB 5/51. Estimates of s ranged from 16% to 28% with a mean of 22%. The uncertainties attaching to u show that q may be taken to lie over a wide range of 26% to 74% selfing at pollination *(Figure 2).* Clearly, due to severe zygotic elimination, $q \gg s$.

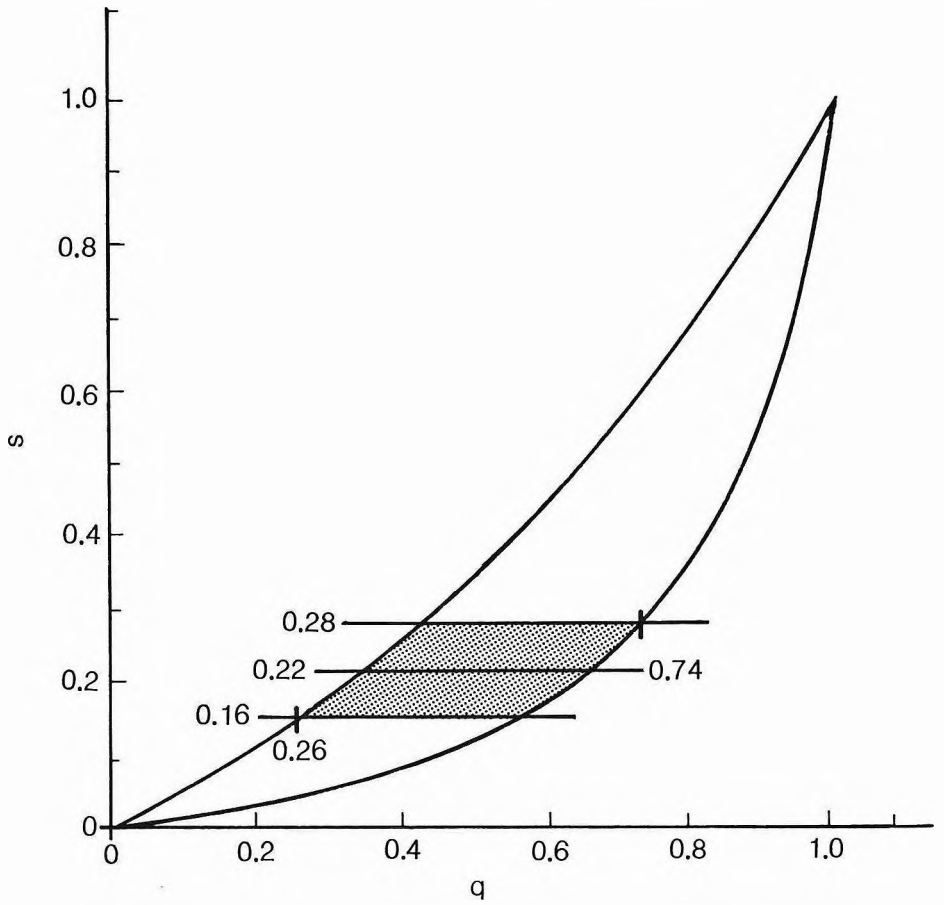

Figure 2. The relation between selfing rate at the young seedling stage (s) *and at pollination* (q). *For sources of data on relative fruit survival parameters* (u_c/u_s) *see text; the critical area defined by the intersection of the curves with estimates of* s *is shaded. In general* q ≫ s.

Attention is drawn below to the possible use of isozyme genes in getting better estimates of *s*. One should recall that the one estimate available may be far from an average value.

The structure of the seed-garden from which the PB 5/51 data were derived is not known. There is no means of telling, therefore, whether the selfs were the products of pollination within the tree or between neighbouring trees of one clone (homo-neighbours, see below), or both. The point is of some importance because it has bearing upon the extent to which wise choice of field plan for a seed garden can minimise the undesirable selfing; it might be that there is an irreducible minimum of selfing that no choice of field plan could mitigate.

Clearly, the fact that the fruit sets or fails as a tricarpellary structure (when $u = p^3$ — see above) implies that selfing is somewhat discriminated against at the embryo level. It can be roughly calculated in fact that *s* might be of the order of twice as large as it is (say 30%–50% as against about 20%) if embryos behaved independently rather than as triplets.

Effect of Selfing on Yield of a Polycross

Any selfs that survive in a polycross population of rubber must tend to depress yield. The relevant questions are: how many selfs survive and what effect will they have? Some calculations are possible. *Figure 3* exhibits a specific case using several necessary parameters which are defined in the following paragraphs.

Tan and Subramaniam[9] found that the vigour (estimated as girth) of nursery seedlings, *after* culling out runts, was much the same for

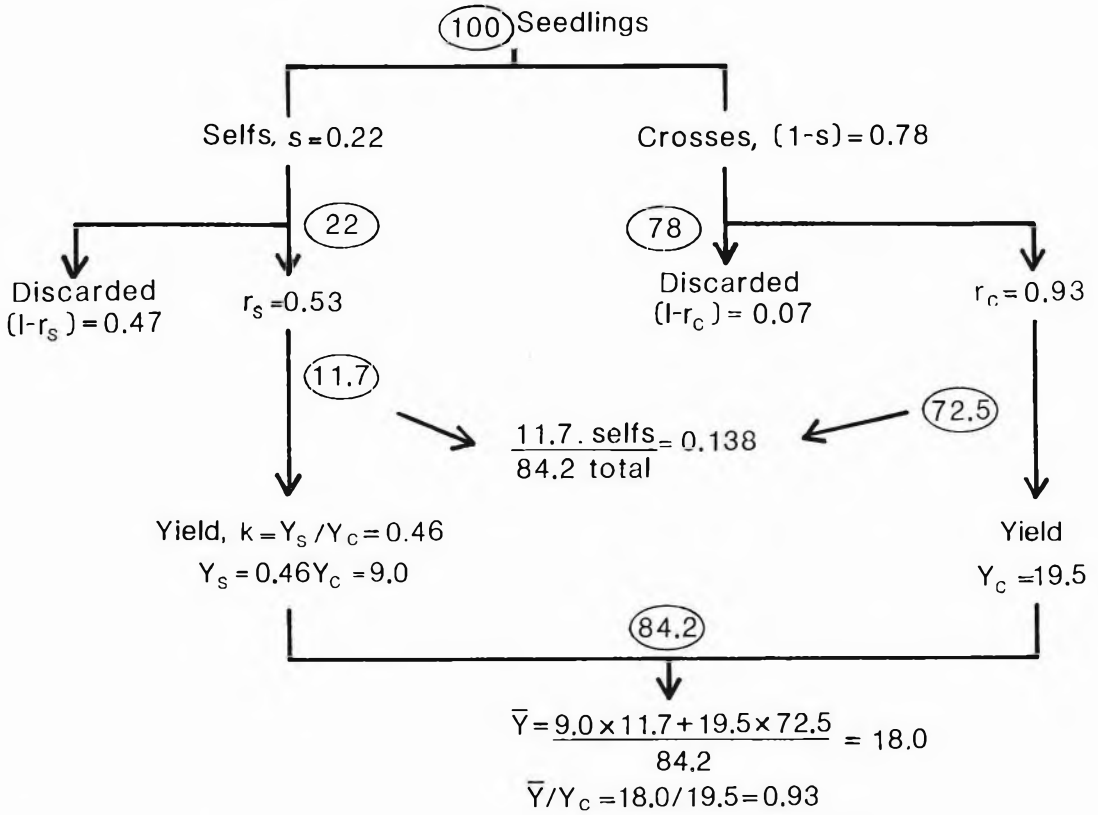

Figure 3. Selfing, crossing, survival and yield in a rubber SYN$_1$ population. Numbers of plants encircled. For parameters and assumptions see text.

selfs and crosses, thus: selfs (four families) 5.35 ± 0.38; crosses (nine families) 5.83 ± 0.22. There were differences between families but the set of data was of diallel form and genetically well balanced. The impression that inbreeding had little or no effect on average vigour, however, is false because culling rates of runts were quite different, thus: survival in crossed families, $r_c = 0.93$; in selfed families $r_s = 0.53$. When a (necessarily rough) correction is made for culling, the selfs would have had only a little over half the girth of the crosses[6]. Even though culled survivors differed little or not at all in girth, they differed greatly in yield. Tan and Subramaniam[9] found: selfs (four families) 3.10 ± 0.86; crosses (nine families) 6.67 ± 0.43. Thus, as survivors, selfs only yielded about 46% of crosses and we may write *(cf Figure 3)* $Y_s/Y_c = 0.46 = k$. Assuming that this figure is roughly predictive of mature yield, the effect of leaving some selfs in a polycross population can be estimated.

I note that, in the above calculations, only nine crossed families were considered, rather than the ten actually studied. One cross was excluded because it was, in fact, a backcross (RRIM 600 × Tjir 1) which displayed (as could be expected) an intermediate runt frequency [$(1-r) = 0.25$, $r = 0.75$]; to have included it would have unreasonably biassed the estimate of r_c.

The Ross and Brookson[11] data analysed by Simmonds[6] suggested that a 'perfect' polycross of the four best clones having well estimated GCA for yield would give (pounds per tree, 15 years):

$$14.2 + \tfrac{1}{2}(3.1+4.9+0.2+2.4) = 19.5$$

This, of course, assumes what has to be assumed (but can hardly be correct), that all parents contribute equally to the progeny. *Figure 1,* in fact, suggests that contributions could be very different.

Using the various estimates derived above and used in *Figure 3* (namely $s = 0.22$, $r_s = 0.53$, $r_c = 0.93$, $Y_s/Y_c = k = 0.46$) we see that another, derived, parameter is useful: t_s is the selfed fraction of the population as planted, t_c the crossed fraction and $t_s + t_c = 1$. Evidently:

$$t_s = \frac{sr_s}{sr_s + (1-s)r_c}, \quad t_c = \frac{(1-s)r_c}{sr_s + (1-s)r_c}$$

The t_s is estimated in *Figure 3* as 13.8% and the average yield *($\bar{Y}$),* as an imperfect polycross, as 18.0% or 93% of Y_c. Clearly:

$$\bar{Y} = Y_c [kt_s + t_c]$$
$$= Y_c [(k-1)t_s + 1]$$

There would therefore probably be appreciable loss of yield from selfing; better estimation of it must await better estimates of the parameters.

An interesting question arises here to which no answer, on the data available, can be given. What would be the effect on inbred survival frequency of more intense culling for vigour at the young seedling stage? The answer must depend upon the shapes of distributions (which are unlikely to be normal). On the face of it, more intense selection would be expected to reduce the inbred component. The point is also relevant to SYN_2 (see below).

Another point about these calculations can be made here because it will be useful later. If crosses are between unrelated parents, they will have heterozygosity $H = 1$. Selfs will have $H = 0.5$ and one notes that, on the Tan and Subramaniam[9] data, they have culling survival rates *(r_s)* and yields *after* culling of close to one half those of crosses. As a rough approximation, therefore, we may take H to predict r and yield in considering the SYN_2 population (see below). Conversely, r could be taken as a rough measure of H in open-pollinated seedling populations, if tested against the appropriate inbred and outbred controls.

The preceding results suggest an interesting experiment that could throw much light upon SYN_1 population structure. Consider an m-parent seed garden. The contributions of m clones to seed-fall could be estimated by sampling or (easier and cheaper, if possible) by classifying bulk seed as to female parent on seed characters. Grow samples (say 200 each) of the seed of m clones and record culling rates and then nursery yields of random samples of survivors. Correlation of yields of individuals with mature yields are low but means of substan-

tial samples should provide much better estimates (and only family means are of interest here). As controls it would be good to have samples of the $m(m\text{-}1)/2$ crosses and m selfs from hand-pollinations. These could be laborious to get but open-pollinated seed from monoclone blocks of m clones would be cheap and a reasonable compromise. A refinement of the experiment would be to grow buddings (rather than seedlings) up to test-tapping and include m parent clones themselves as controls. The prediction of mature yield would be improved because correlations are higher[12] and the experiment would immediately provide comparisons of the parents' GCAs in polycross with their known performance as clones *per se*.

From such an experiment one could derive estimates of: proportional representation of parents as females among progeny; selfing rates, clone by clone; yield decrements due to inbreeding; epistatic effects contributing to differences between clones as clones and as parents. This would be valuable information for understanding polycrosses and it would not be very expensive: about 100 m plants taken to test-tapping should suffice.

The SYN_2 Population

I remarked above that the commercial SYN populations of grasses and forage legumes are mostly SYN_2 or SYN_3, one or two bulk multiplications intervening between SYN_1 and sale of seed. It is generally accepted that such populations must tend to be at least slightly inferior to the SYN_1 if SYN_1 performance were to depend much upon SCA and if the parental population size (m) were small enough to cause some inbreeding (even if SYN_1 were perfectly outbred). However, grass and forage breeders have no real choice in the matter.

In rubber, SYN_1 alone is used and the properties of SYN_2 have never, so far as I know, been investigated, either theoretically or practically. The matter is worth considering because two possible uses for SYN_2 can be identified; both rest upon the need for abundant supplies of cheap seeds of high-class materials, not so good as a good SYN_1 maybe, but superior to any conceivable mono-clonal seeds and very probably also to mono-clonal sources even from field edges. These needs are as follows. First, there is the possibility of wanting to plant large areas of dual purpose timber-rubber trees, following the suggestion of the Task Force of Experts[13]. For this purpose cheap seeds of large numbers of vigorous and reasonably high yielding trees would be required and the use of SYN_3 from good existing SYN_1 stands could be an interesting (and economical) possibility. Second, the work of Ng *et al.*[14] strongly suggests (what has not yet been tested) that the best seedling rootstocks would be SYN_1 plants; if so, SYN_2 might be hardly inferior and would surely be cheaper. They should surely be tested.

Results of some calculations are given in *Tables 1* and *2*. The assumptions are heavy: that t (frequency of surviving selfs — *cf Figure 3*) is constant over generations; that matings are proportional to frequencies of parents; and that H predicts probability of survival. However, it is at least clear from *Table 1* (right hand column) that, the larger m is, the less the inbreeding in SYN_2; also that, even if t were zero but m were small, there would still be appreciable inbreeding. The estimates of H in *Table 2* show a range to cover some variation in assumptions. The indications are that SYN_2 would be somewhat inferior to SYN_1 (due to inbreeding) at low m but that the depression might be quite small at $m = 10$. One notes that the frequency of fully outbred plants ($H = 1$) would be more affected than mean outbreeding level ($\bar{H}$).

These calculations, it must be emphasised, are very rough but would be open to great improvement with better knowledge of the genetical dynamics of seed gardens (see below). They could also easily be subjected to limited experimental test, as follows. Samples of SYN_1 seedlings could be compared within SYN_2 from the same source as to runt frequencies and culling rates as a rough direct measure of inbreeding. Random samples of survivors taken into a budded nursery trial with the parent clones as controls would then provide a reasonably good forecast of mature yield potential. The principles are the same

TABLE 1. INBREEDING AND HETEROZYGOSITY (H) IN SYN_1 AND SYN_2 FROM AN m-PARENT SEED GARDEN

Source	Type	H	Frequency
SYN_1			
Self	(AA)	0.5	t
Cross	(AB)	1.0	(1-t)
SYN_2			
Self-self	(AA . AA) double self	0.25	t^2
Self-cross	(AA)(AB) backcross	0.375	$t(1-t) \times [2/(m+1)]$
	(AA)(BC) outcross	1.0	$t(1-t) \times [(m-1)/(m+1)]$
Cross-self	(AB . AB) self	0.5	(1-t)t
Cross-cross	(AB)(AB) sib	0.75	$(1-t)^2 \times [2/m(m-1)]$
	(AB)(BC) half-sib	0.875	$(1-t)^2 \times [2(m-2)/m(m-1)]$
	(AB)(CD) outcross	1.0	$(1-t)^2 \times [(m-2)/m]$

Putting t = 0 (no selfing), the last three lines show that $\bar{H} = (m-1.25)/(m-1)$, which rises to $\bar{H} = 0.97$ at m = 10.

TABLE 2. HETEROZYGOSITY IN SYN_2 IN RELATION TO PARENT CLONES IN A SEED GARDEN OF m SIZE

t in SYN_1	H (%)	SYN_1	H in SYN_2			
			m = 5	m = 10	m = 15	m = 20
0.10	$\bar{H}$	95	88 – 92	92 – 95	93 – 96	93 – 96
	H_1	90	55 – 62	72 – 79	78 – 84	81 – 87
0.20	$\bar{H}$	90	82 – 89	88 – 92	87 – 93	87 – 93
	H_1	80	49 – 60	64 – 75	70 – 80	72 – 83

t is taken as 0.1, 0.2 (cf estimate in text for SYN_1 of 0.14); $\bar{H}$ is mean heterozygosity, H_1 is frequency of fully outbred progeny.

as for the experiment on SYN_1 materials described above. Thus a preliminary judgement as to the utility of SYN_2 could be got quite quickly and cheaply.

If SYN_2 were thought to have practical potential, the same question would have to be asked about seedling culling rates as was posed above in relation to SYN_1. Given that there must be some inbreds in the population, to what extent could their frequency be reduced by intensifying seedling selection before planting? Data by Tan and Subramaniam[9] and the calculations on SYN_1 make it clear that some selection is, in fact, exercised; whether more intense selection would be beneficial (and economic) is not clear.

Finally, there is an interesting question about the effect of inbreeding which has bearing upon the possible use of SYN_2 and could be relatively easily investigated. A monoclone seedling planting (call it MON_1) could be regarded as a SYN_1 with $m = 1$. The progeny must have $H = 0.5$ but the effect of inbreeding will be partially offset by seedling selection (recall that $r_s \simeq 0.5$). The question then is: how would MON_2 perform, with H in the range 0.25 to 0.5? A fair idea could be got from study of r and of yield in the budded nursery of parent clones, MON_1 and MON_2 in the same experiment. Suitable MON_1 stands cannot be numerous but several could presumably be found and Tjir 1 monoclone, at least, must be available. Such an experiment,

especially if it could be based on several parents, would be a valuable complement to the SYN_2 experiment discussed above.

Use of Genetic Markers

The only estimate we have of *s* (the proportion of selfs in the young seedling stage) comes from the use of the yellow recessive mutant for which PB 5/51 is fortuitously heterozygous (see above). Any more precise knowledge of crossing/selfing parameters in rubber must rest upon the use of genetic markers. I know of no other available morphological mutants but isozyme genes are an obvious and very attractive possibility. Chevallier[15] has recently used three polymorphic isozyme loci to study the geographical distribution of variability in new collections from Brazil. He identified two, four and five alleles at three loci (GOT, AP and ESTI respectively) and remarked that several of them were already known in Wickham material. These genes (and others that would surely be revealed by systematic search) would offer an excellent basis for studies of crossing patterns. In comparison with morphological mutants they have, of course, the great technical attraction of heterozygous expression, so that all three phases are determinable.

Examples of how they might be used follow. First, a clone homozygous for one allele (say A_1A_1) could be embedded in other clones carrying other alleles (A_2, A_3 *etc.* but not A_1) in spatially varied layouts. Seed samples from specific trees would then yield seedlings which would immediately provide estimates of selfing and crossing rates, range and direction of pollination, the relative efficiency of two-dimensional and one-dimensional layouts (see below), the effectiveness of diagonal and orthogonal hetero-neighbours (see below) and so on. Second, the power of such experiments would be greatly increased if each clone in them could be uniquely marked. Third, if, in existing seed gardens, the constituent clones could be at least partly characterised as to their isozyme loci, the way would be open to at least some investigation of spatial aspects of pollination (by seedling sampling). Further, from sampling the mature SYN_1 from that garden, at least some estimates of effective selfing rates after culling and overall contributions of clones as parents would be possible; at the limit, if all clones were distinctively homozygous-marked (improbable!), a rather complete picture could be achieved.

The uses enumerated above all bear upon the dynamics of seed-garden polycrosses. There are at least two other uses for isozyme genes that could be attractive for the rubber breeder. First, in selecting clones from polycross progenies (e.g. in 'ortet selection'), characterisation of isozymes could sometimes enable the breeder to identify at least one parent or, alternatively, to assert that such-and-such a parentage was impossible. This would be equivalent to partial pedigree information and it could be distinctly useful in deciding on further crosses. Second, it has been found in maize[16] and tomatoes[17] that isozyme alleles are sometimes linked with economically important genes (or chromosome segments). If this were true in rubber (and sometimes, by chance, it must be true) then there would be the interesting possibility of assisting selection (e.g. in SYN_1 populations) by preliminary isozyme testing. The virtually complete marking of the genome which is in prospect in tomato[17] would, of course, be impossible in rubber but occasional useful linkages are conceivable.

Yet another use for isozyme markers would lie in checking clonal identities in doubtful cases. The technique is widely used in testing the authenticity and purity of inbred lines of barley, brassicas and maize.

The Choice of Parents

The ideal method of choosing a set of *m* clonal parents for a seed garden may be stated as follows. The $m(m-1)/2$ crossed families should all be known, from experimental test, to be high yielding and acceptable in other respects; the *m* clones should be known to be at least fairly seed and pollen fertile; and *m* should be fairly large (arbitrarily 5, but 10 would be better if there were any prospect of using SYN_2 — see above).

The practice is, of course, quite different. Historically, seed gardens have been based on m = 3 to 7 and clones have been chosen on performance *per se*. The latter has been retrospectively justified by the finding that economic characters are dominated by GCA. However, it must be probable that there are minor epistatic components of clonal performance, implying that even the best families would fall short of their parent clones. We cannot yet know this to be true, of course, because the performance of SYN_1 populations is confounded by the presence of some inbreds, as discussed above. However, abundant practical experience goes a long way to justifying the phenotypic choice of parental clones and consideration of time-scales supports the procedure; good clones for parents are not so numerous that their use as parents can long be delayed while they are being subjected to an elaborate test-crossing procedure.

As to numbers (m), one must accept that, if there are few candidate clones available, then m must perforce be small. If most selfing occurs within the tree (which we do not know) then the size of m might not have much effect on inbreeding in the SYN_1, though, as we saw above, it must be influential at SYN_2. Thus small m may not have been too harmful in practice, but only better genetical understanding of seed garden dynamics could clarify the point. My own feeling is that one should aim at m = 5 as a minimum but this is a 'magic number' not a genetical calculation.

One possibility referred to in the literature[18] is to plant an m-parent seed garden but be prepared to cut out one or more parents on later evidence. This suggests the interesting possibility of, so to speak, 'validating' a new seed garden, not by bulk testing of SYN_1 progeny, but by testing substantial samples of progeny of the m-parents separately at, say, the nursery test-tapping stage. Poor parents could probably thus be identified with fair confidence. If this sounds laborious, one might reflect that mistakes might be in the ground for thirty years.

Should the decision have to be taken to discard a parent, some, but probably not very serious, impairment of the balance of the designs discussed below would have to be accepted.

Field Layouts for Polycrosses

The objects are to maximise outcrossing, minimise inbreeding and to equalise crossing between all possible pairs of clones in the array. Thus identical clones must, if possible, not be neighbours (homo-neighbours) and the frequencies of the various hetero-neighbours with respect to a given clone are to be made identical, or at least as nearly equal as possible.

Two broad categories of design are apparent: two-dimensional and one-dimensional. In the former, we consider neighbours, both within and between adjacent rows of trees, by the criteria stated above. In the latter, the rows are widely separated in an 'avenue' planting and the trees crowded within the row; the assumption (untested) is then made that between-row pollination can be disregarded so that only spatial relations within the row need be considered.

Consider, first, two-dimensional designs. It would be good if hetero-neighbours were all equally distant from a given point but this is not always possible. I assume, as a starting point, that, in square layouts, diagonal are equivalent to orthogonal neighbours so that any tree has eight neighbours, but qualify this assumption later. Results for an m-parent seed garden are summarised in *Table 3* and *Figure 4*. A value for m as small as three is too small to be genetically satisfactory and no good design is possible. For the rest, there are four 'perfect' designs (hetero-neighbours exactly equal) (m = 4, 5, 7, 9), two of them (m = 4, 7) based on non-square patterns. For m = 6, 8, 10, >10, randomisation will ensure near-equality of hetero-neighbours and would be open to arbitrary adjustment anyway if it were thought desirable. The cases m = 7, 9 could equally well be treated as for m = 6, 8, 10 if a triangular layout (m = 7) were unacceptable or if the orthogonal-diagonal imbalance for m = 9 were thought to be unsatisfactory. Lacking experimental evidence as to the effectiveness of crossing between orthogonal and diagonal neigh-

TABLE 3. TWO-DIMENSIONAL DESIGNS FOR RUBBER POLYCROSSES[a]

Number of parents, m	Design
3	No good design possible. Systematic one-step Latin Square best because it confines homo-neighbours to diagonals. An avenue layout would be advantageous.
4	Honeycomb-hexagon perfect and the form shown maximises distance of homoclones. For unit area per tree, side has length 0.620, hexagon has width 1.074. No simple square is possible, but three blocks of repeating subsquares are imperfect only at the boundaries.
5	Systematic, repeating two-step ('knight's move') Latin Square is perfect.
6,8,10,>10	No perfect designs are possible. Arbitrary first line of m entries, followed by lines randomised under the constraint of no homo-neighbours are good. See also m = 7,9.
7	Equilateral triangle-hexagon is perfect. For unit area per tree, distance in row = 1.075, between rows 0.931. For a square lay-out, the same procedure as for m = 6,8,10 would be acceptable.
9	Systematic, repeating three-step rectangle is perfect in that all eight hetero-neighbours are represented. However, neighbours are unbalanced as to orthogonal/diagonal placement and, if this were thought important, the same procedure as for m = 6,8,10 would be acceptable.

[a]See *Figure 4*.

bours, it might be well to assume provisionally that the former are more effective: thus one would aim to balance the two as to hetero-neighbours while strictly avoiding all homo-neighbours, whether orthogonal or diagonal. This the plan for $m = 6, 8, 10$ does. All such seed gardens should have an outside discard row, defined as the row that would have been planted there to conform to the design.

Turning now to one-dimensional designs, we seek linear sequences such that each of the m clones is flanked equally by all the other $(m\text{-}1)$ clones, homo-neighbours again being forbidden, of course. Williams[19] has described methods of generating such sequences and tabulates examples for $m = 3$ to 10. An example for $m = 6$ is given in *Figure 5*. Designs are readily, if laboriously, generated by considering paths through a network generated by lines joining all points in a polygon of size m *(Figure 5A)*. If the number of concurrences (specific pairs of neighbours), c, is to be unity, designs exist only for m odd. If $c = 2$, designs are possible for any m (as in *Figure 5*). Williams' designs are blocked, with r replicates, and it turns out that $r = (m\text{-}1)$ for $c = 2$. Thus any sequence has total length $N = m(m\text{-}1)$ if thought of as a closed line. However, in the field, rows will generally be straight, so must be terminated by the obvious discard tree at each end to complete the sequence; therefore, in practice, $N = m(m\text{-}1) + 2$ *(Figure 5B)*. If, for convenience in fitting the field, a sequence must be broken, this can be done at any point provided the necessary extra discards are inserted to preserve balance *(Figure 5C)*. In building up the field, there are several possibilities. Many different sequences could be generated; or one sequence used, reversed in alternate rows *(Figure 5D)*. Some homo-neighbours as between rows are unavoidable but these are, by assumption, acceptable.

A general point should be made. The object of all the layouts discussed here is to maximise and equalise crossing between clones. It is not to provide designs susceptible of valid statistical analysis. In practice, some analyses (e.g. of seed yields) might be done with reasonable propriety in some instances but professional statistical advice should be sought before doing so.

It is not part of my purpose to discuss the agronomy of seed gardens but it is worth noting here that Wycherley[8] found that fairly low planting densities (*ca* 240 trees per hectare) were probably best for seed yield, with indications in favour of an avenue layout. But the choice between one- and two-dimensional designs ought to be based on knowledge of spatial patterns of pollination, of which we are still totally ignorant. Such knowledge would best be based, as I remarked above, on experiments with isozyme markers.

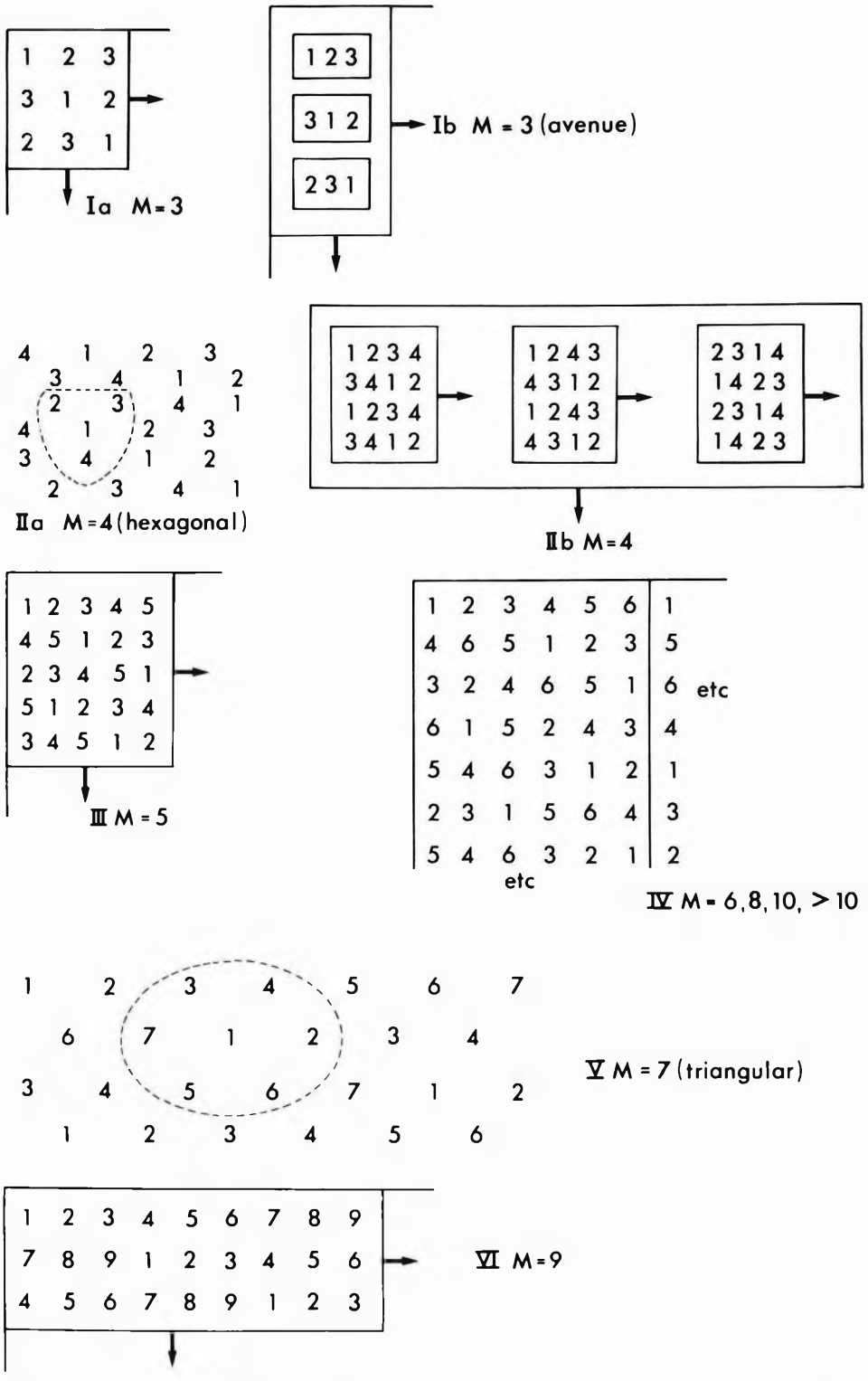

Figure 4. Two-dimensional rubber polycross designs. See Table 3 *and text. Arrows indicate repetition of a boxed pattern;* 'etc' *indicates continuation of a randomised pattern.*

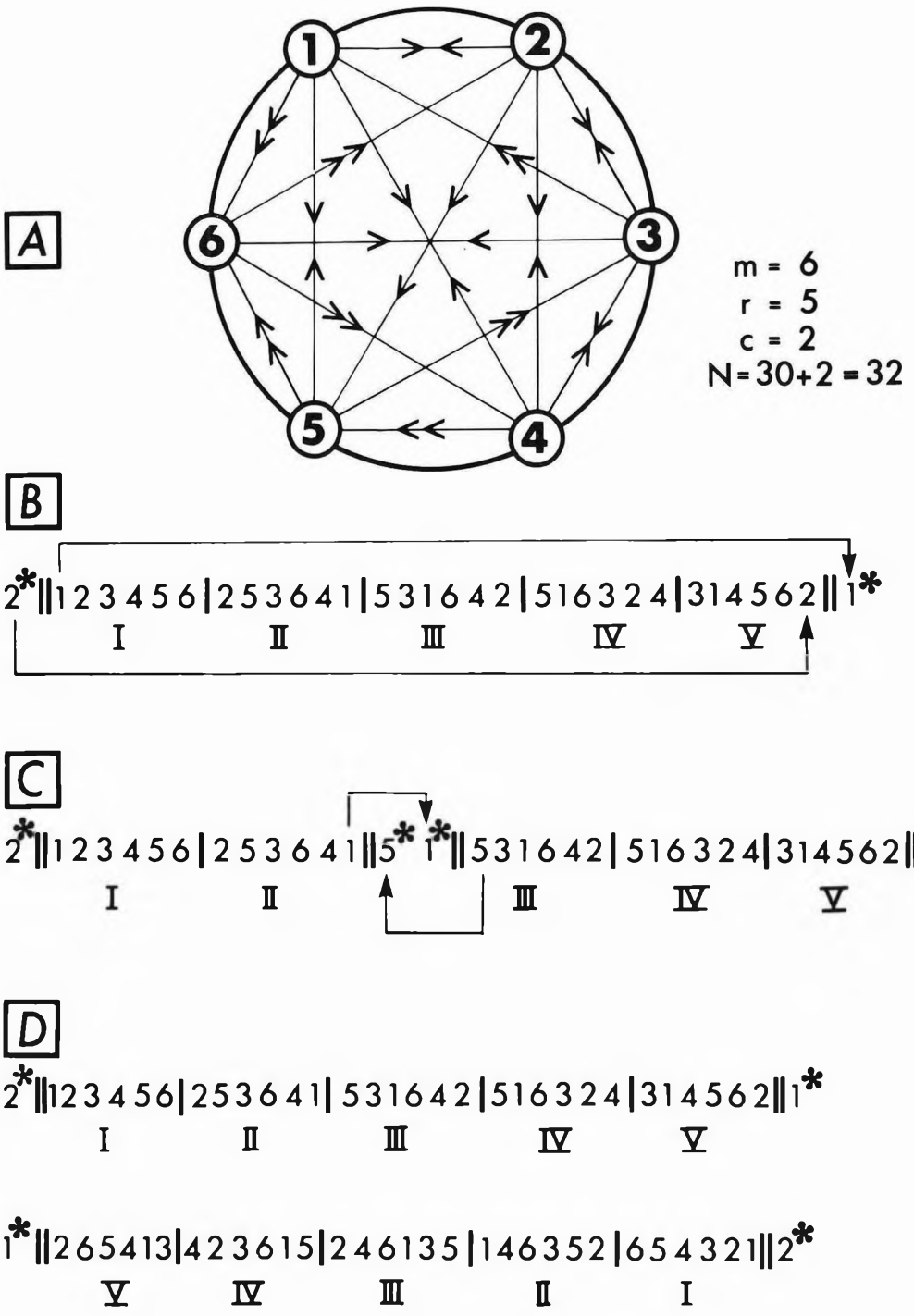

Figure 5. One-dimensional rubber polycross design for m = 6 clones. A, the hexagon that generates the design; B, a design, including discard trees at the ends of the sequence (asterisked); C, splitting the sequence, with insertion of compensating discards; D, possible field layout with single basic sequence reversed in alternate rows. In B, C, D, blocks are indicated by I–V.

Turning, finally, to the wider literature, there is very little on rubber polycrosses. Some published designs[18, 20] are unsatisfactory but reference has been made[21] to use at the RRIM of the $m = 7$ triangular design *(Figure 4V)*. The more general literature of polycrosses[22-25] is mostly based on Latin Squares, ignoring the presence of homo-diagonals, which I believe it would be prudent to avoid in a self-compatible outbreeder. Freeman[25] demonstrates the perfect square for $m = 5$ *(Figure 4)* and elsewhere[26] suggests the use of cyclic balanced incomplete blocks for large m. Fasoulas[27] draws attention to the balanced triangles for $m = 7$ *(Figure 4)* (in the context of plant selection rather than polycrossing) and other authors[28] have advocated similar ('beehive') layouts for competition experiments.

CONCLUSIONS

Multiparent, first-generation synthetic varieties (SYN_1 = polycrosses) of rubber have been economically successful for many decades and are still, in Malaysia, subject to Class 1 RRIM Recommendation. They have, in practice, been founded empirically upon planting mixtures of superior clones in seed gardens in the hope/expectation of random interpollination; and, a high GCA component of performance. The latter assumption has largely been justified by recent biometrical studies; the former is yet uncertain because we are still essentially ignorant of crossing and selfing patterns in the seed garden and of the relative contributions of parents to progeny. The calculations set out above suggest that: parental contributions are likely to be unequal; there is probably a great deal of self-pollination, partly mitigated by selection against inbred weakness; nevertheless, it is likely that selfed progeny detract appreciably from SYN_1 performance; and inbreeding could very probably (almost certainly) be reduced by paying more attention to seed-garden layout.

Several suggestions are made above as to experiments that would throw light on the genetical dynamics of seed gardens and the performance of SYN_1 seedling populations. Undoubtedly, the best information would come from the use of isozyme marker genes but such experiments would be of a fairly long-term nature. In the shorter term, it is suggested that quite simple experiments using vigour as a measure of inbreeding could be informative.

The characteristics of the SYN_2 population in rubber are unknown but some calculations are possible. Such a population might be not much inferior to its parental SYN_1 but might suffer from some extra inbreeding depression due to small numbers of original parental clones. SYN_2 offers interesting practical possibilities for cheap production of dual-purpose timber-rubber trees and/or seedling rootstocks. But these possibilities have yet to be investigated. It would be well worthwhile to know more about SYN_2.

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Invertase Activity in Hevea *Latex Serum: Interaction between pH and Serum Concentration*

H.Y. YEANG*, J.L. JACOB**, J.C. PRÉVÔT** AND A. VIDAL**

When C-serum was diluted from its natural (undiluted) concentration, there was a shift of pH optimum in its invertase activity from basic (about 8.8) to near neutral (about 7.4). This effect was especially obvious when the dilution was 50% or greater. There was, at the same time, a marked apparent increase in enzyme activity at pH below 8.4 when the serum was diluted. The shift in the pH optimum in undiluted serum towards neutral could be partially effected by Trisacryl GF05 filtration of the serum. Addition of boiled C-serum to dilute unboiled serum tended to shift the optimum towards the basic pH values. Besides the known effect of the pH of latex on its invertase activity, the factors concerned with the phenomena observed might also serve as a means of fine adjustment of this activity.

Invertase is a key enzyme in *Hevea* latex that activates the initial step of the glycolytic pathway. This itself initiates a chain of biochemical reactions both in respect of respiration and, essentially, rubber biosynthesis. Invertase activity in C-serum has been shown by Tupy[1,2] to be highly sensitive to pH. Activity of this enzyme was found to be negligible at pH lower than about 6.5. At pH above this, there was a rapid rise in invertase activity[1,2,3] reaching an optimum at pH 7.2 - 7.5. It was this abrupt increase in enzyme activity that prompted the ascription of serum pH as a major factor regulating the activity of this enzyme in the tree. As the distinct invertase peak in the region of pH 7.4 had always been readily perceived, activity of the enzyme was rarely carried out beyond pH 8 (where, moreover, the normally used buffers such as phosphate were inadequate in buffering capacity and a second buffer would have been obligatory). Using a glycine-NaOH buffer to adjust for the pH range 8 - 10, Chong[4] noted a pH optimum of 8.9 for invertase in the latex serum from unstimulated trees. In view of the discrepancy in the reported pH optima, a study was initiated to re-examine the pH dependency of *Hevea* C-serum invertase with special emphasis on its activity above pH 8. The interaction between pH and serum concentration was also investigated.

MATERIALS AND METHODS

The first half-hour flow of latex from RRIM 501, RRIM 600, RRIM 701, GT 1, PR 107 and Tjir 1 trees (all tapped on *Panel BI-1)* grown in the RRIM Experiment Station, Sungei Buluh, was collected into chilled containers. C-serum was obtained from the latex by centrifugation for 1 h in a Sorvall RC-2B centrifuge at 19 000 r.p.m. (44 000 g max.) at 3°C - 4°C. Sera from all the clones were used fresh in the first experiment (represented by the results from RRIM 701 in *Figure 1*). In all subsequent experiments, sera from RRIM 600 and GT 1 were freeze-dried and then re-constituted before use by the addition of water and/or buffer-substrate to the appropriate concentration. The natural concentration of C-serum was taken as 50 mg freeze-dried solids per millilitre.

The invertase assay for fresh sera was carried out as previously described[5] by incubating the serum with sucrose. The products of the enzymatic reaction (glucose and fructose) were assayed as reducing sugar by the Nelson-Somogyi reaction[6,7]. With the freeze-dried sera, a generally similar incubation procedure[8] was employed but an enzymatic method[9] was adopted for the quantification of glucose and fructose separately. Sodium fluoride (60 mM) was added to the incubation mixtures containing

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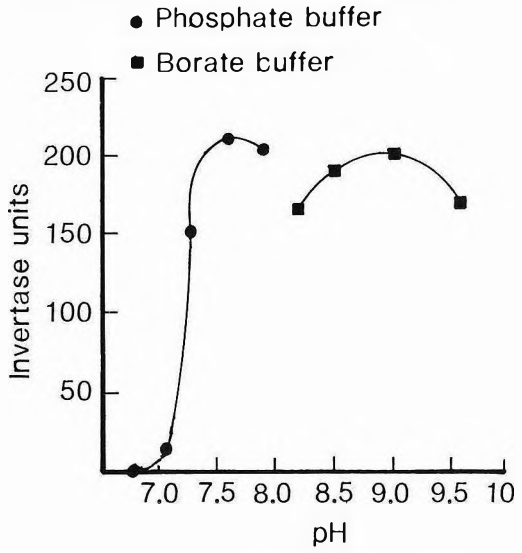

Figure 1. Invertase activity in fresh RRIM 701 C-serum at various pH adjusted with phosphate and borate buffers. The proportion of serum in the incubation mixture was 50%.

fresh serum and to some of the incubation mixtures containing freeze-dried serum (in experiments where high serum concentrations were used). This inhibited regeneration of uridine diphosphate glucose and so obviated interference in the sucrose levels by the action of sucrose synthetase present in the serum[10]. Three buffer systems were used in the invertase assays. Phosphate buffer (0.1*M*) was used in assays carried out at pH 6.0 - 7.8 while borate-KCl-NaOH buffer (0.1*M*) was used at pH 8.1 - 9.9. A third buffer, Prideaux's universal buffer[11] (consisting of a 50 mM solution with respect to boric, acetic and phosphoric acids adjusted with NaOH), was used in assays over the entire pH range from pH 6.0 to 9.9. Invertase activity was expressed in invertase units where 1 unit = 1 μmole sucrose hydrolysed per 10 ml undiluted C-serum in 30 min.

To remove low molecular weight molecules from the serum, concentrated serum (517 mg per millilitre) was filtered through a 30 cm Trisacryl GF05 gel column equilibrated with 50 mM phosphate buffer at pH 7. The pooled filtrate containing all the serum proteins (as determined by adsorption at 280 nm) was concentrated using an Amicon filter with a cut-off at molecular weight 10 000. The resultant filtrate was essentially free of salts and other small molecules.

Inactivated C-serum was prepared by boiling freeze-dried GT 1 serum that had been reconstituted to the required concentrations. The precipitate formed on boiling was removed by centrifugation.

RESULTS

Invertase activities in fresh C-serum from RRIM 501, RRIM 600, RRIM 701, GT 1, PR 107 and Tjir 1 trees were assayed over a range of pH using two buffer systems: phosphate buffer for pH 6.8 to 7.9 and borate buffer for pH 8.2 to 9.6. The proportion of serum in the incubation mixtures was 50%. The results for RRIM 701 are given in *Figure 1.* Two pH optima were observed — pH 7.5 and pH 8.9 — one for each buffer system. Essentially similar trends were observed with the other clones (data not presented). It was not clear from these experiments, however, if the two pH optima observed had resulted from interactions of the enzyme with the two dissimilar buffer systems used. To resolve this ambiguity, it was therefore desirable to have a single buffer covering the whole pH range being investigated. Prideaux's universal buffer was adopted for this purpose.

Invertase assays were carried out with freeze-dried RRIM 600 C-serum with the proportion of serum in the incubation mixtures being adjusted to be equivalent to 4.4%, 50% and 100% that of fresh serum. For the purpose of comparison with the universal buffer, phosphate and borate buffers were also used in parallel assays within their effective ranges. When the serum concentration in the incubation mixture was dilute (4.4%) the change in invertase activity with pH change took the form of a single smooth curve peaking at pH 7.5 *(Figure 2).* With increased serum concentration (50% and 100%), two curves — somewhat discontinuous with one another — were seen in

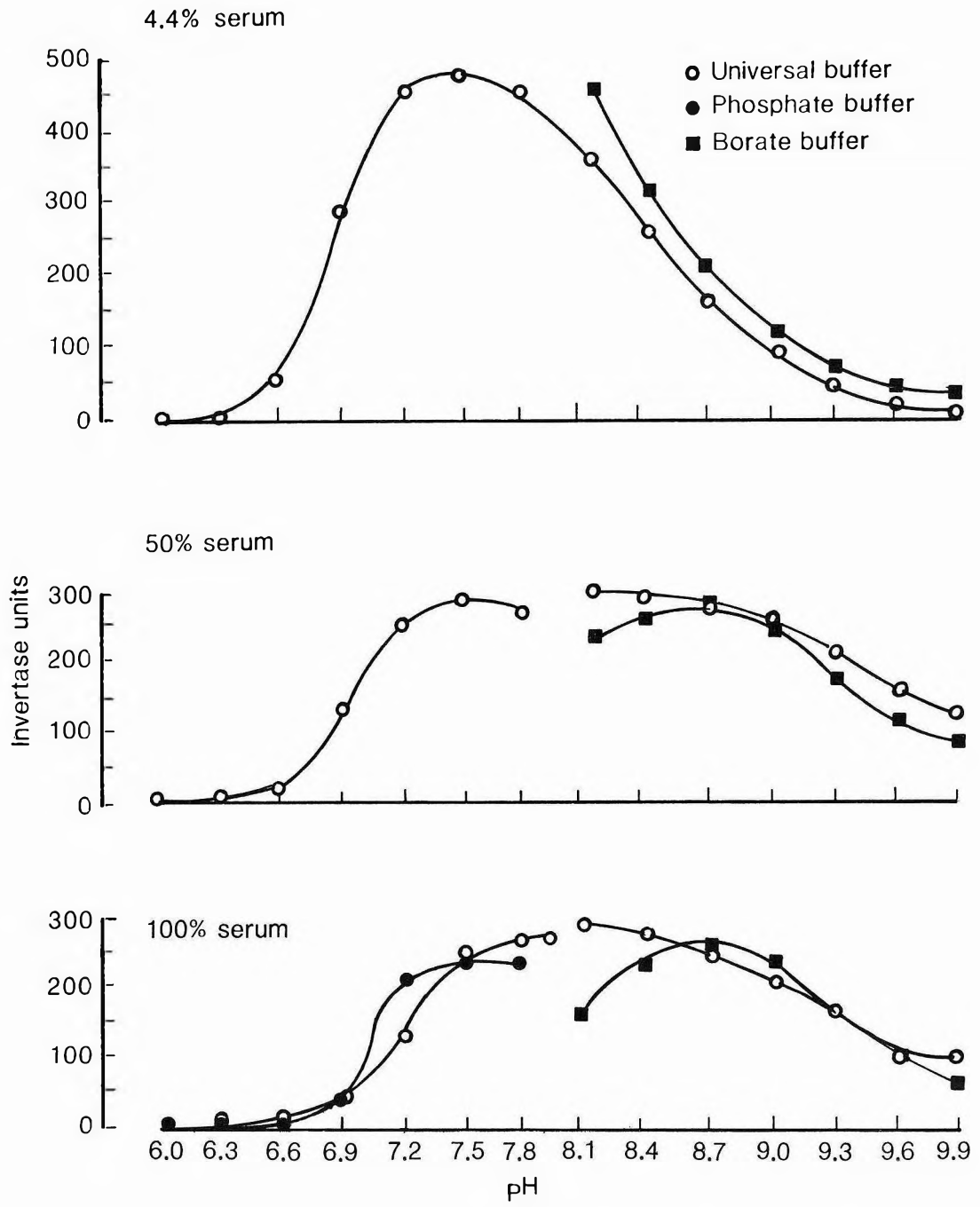

Figure 2. Invertase activity in freeze-dried RRIM 600 C-serum at various serum concentrations and at various pH adjusted with universal, phosphate and borate buffers.

assays using the universal buffer *(Figure 2)*. At 50% serum concentration, the pH optimum at 7.5 was still evident while at 100% concentration, invertase activity continued to rise beyond pH 7.5. The net effect was that the pH optimum in universal buffer tended to shift to the right (*i.e.* towards basic pH) with increasing serum concentration. A second distinctive feature of the results of this experiment was the very significant apparent decrease in activity as the proportion of serum in the incubation mixture rose from 4.4% to its undiluted state, when the pH was below 8.4 *(Figure 2)*. Beyond this pH, there was an increase in enzyme activity as concentration of the serum rose. When phosphate and borate buffers were used in the assays, the curves obtained were generally similar to those for the universal buffer. With borate buffer especially, a distinct peak in the region of pH 8.8 was observed when serum concentration in the incubation mixture was 50% or 100% *(Figure 2)*.

To verify further the results obtained with RRIM 600 serum, a similar series of assays were carried out in universal buffer using freeze-dried GT 1 serum. A total of five dilutions of serum in the incubation mixture were tested: 5%, 25%, 50%, 75% and 100%. The results *(Figure 3)* were generally similar to those for RRIM 600 serum. More so than in RRIM 600, GT 1 serum invertase showed a gradual increase in activity from pH 6.9 to its peak at pH 8.7 when the serum was in its undiluted state. This contrasted with the behavior of the enzyme in highly dilute (5%) serum when its activity rose abruptly from pH 6.9 to pH 7.2 and declined rapidly thereafter. There was a marked increase in invertase activity as the serum was diluted when assays were carried out at pH below 9. This increase was not seen between undiluted serum and serum diluted to 75% its natural concentration. Some increase occurred at 50% serum while at the higher dilutions (25% and 5% serum) a sharp increase in activity was observed. This increase was especially marked at pH 7.2, the optimum pH for invertase activity in dilute serum. At pH greater than 9.0, dilution did not result to an increase in invertase activity *(Figure 3)*. The effects of serum dilution on the pH optima of invertase and its level of activity were therefore quite similar in the two clones, RRIM 600 and GT 1.

Reconstituted freeze-dried GT 1 C-serum was filtered through a Trisacryl GF05 gel column to remove salts and other low molecular weight molecules. The eluted fractions containing proteins were subsequently concentrated using an Amicon filter. Invertase assays in universal buffer were then carried out with filtered and unfiltered sera, their concentrations in the incubation mixtures having been adjusted to 100% (resembling natural, undiluted serum) or 9.5%. The results, presented in *Figure 4,* showed the typical curves (resembling those in *Figure 3*) of invertase activity for dilute and undiluted *unfiltered* sera over a range of pH. With *filtered* sera, it was found that filtration had the effect of lowering the invertase activity in the dilute serum. The shape of the pH-activity curve was unaltered as compared to the unfiltered serum. On the other hand, there was considerable change in the response of invertase activity to pH when the filtered serum was used in its undiluted form. The peak around pH 8.8 was no longer evident while there was a significant increase in activity at the lower pH values (pH 6.9 - 8.1) as compared with concentrated serum that was unfiltered *(Figure 4)*. The net effect was that, with filtration, the pH optimum of invertase shifted towards the left (neutral pH).

Boiled C-serum was added to GT 1 freeze-dried C-serum that had been reconstituted to 10% of its natural concentration. The final content of boiled serum in the invertase incubation mixture was equivalent to 28 mg freeze-dried serum solids per millilitre, this being equatable to 56% of natural serum (assumed to be 50 mg per millilitre). The results revealed a loss of activity at the lower pH range (pH 6.9 - 7.8), but an increase in activity at pH greater than 7.8 *(Figure 5)*. In effect, there was an apparent shift of the pH optimum to the right from near neutral towards basic pH. When a higher concentration of boiled C-serum (150 mg per millilitre, equivalent to 300% of serum) was added to the dilute serum, the results were generally similar to those when less boiled serum was used, other than that, activities at the basic pH values were higher.

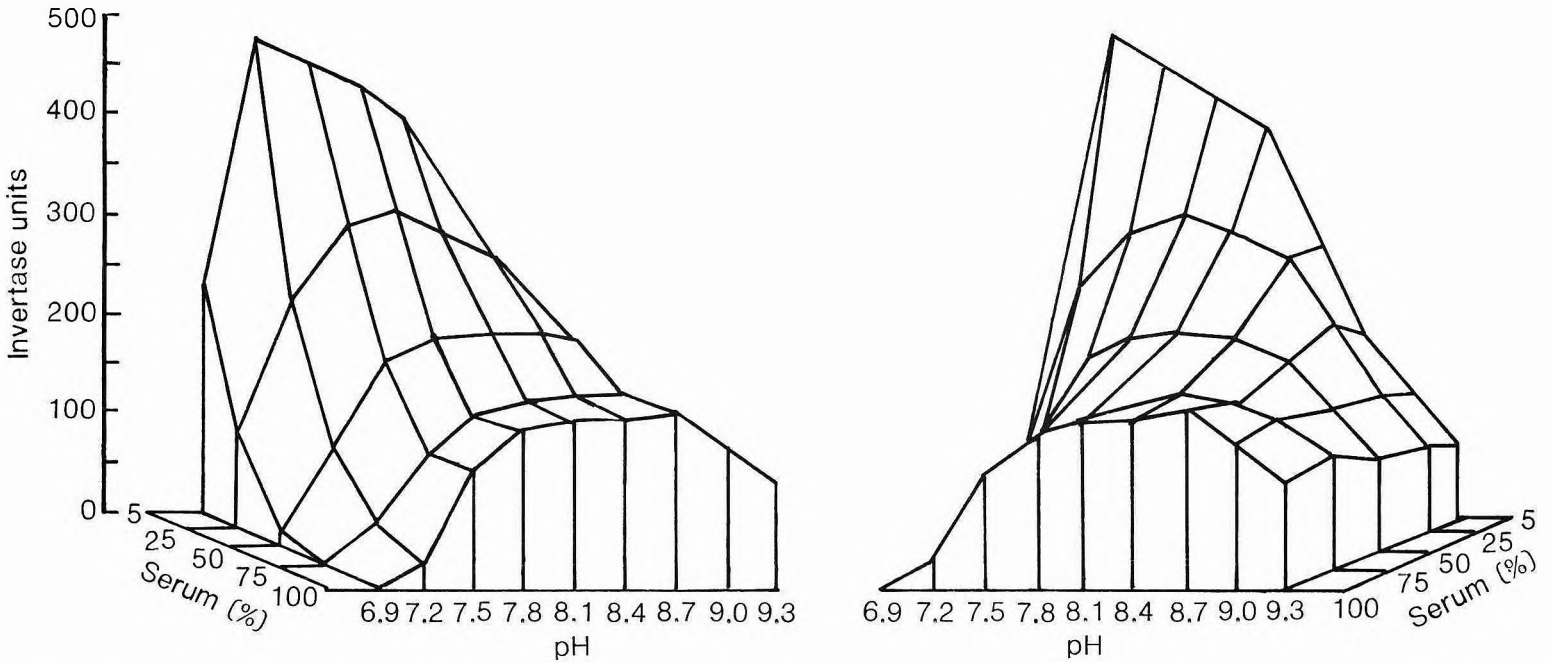

Figure 3. Invertase activity in freeze-dried GT 1 C-serum at various serum concentrations and at various pH adjusted with universal buffer (two perspectives).

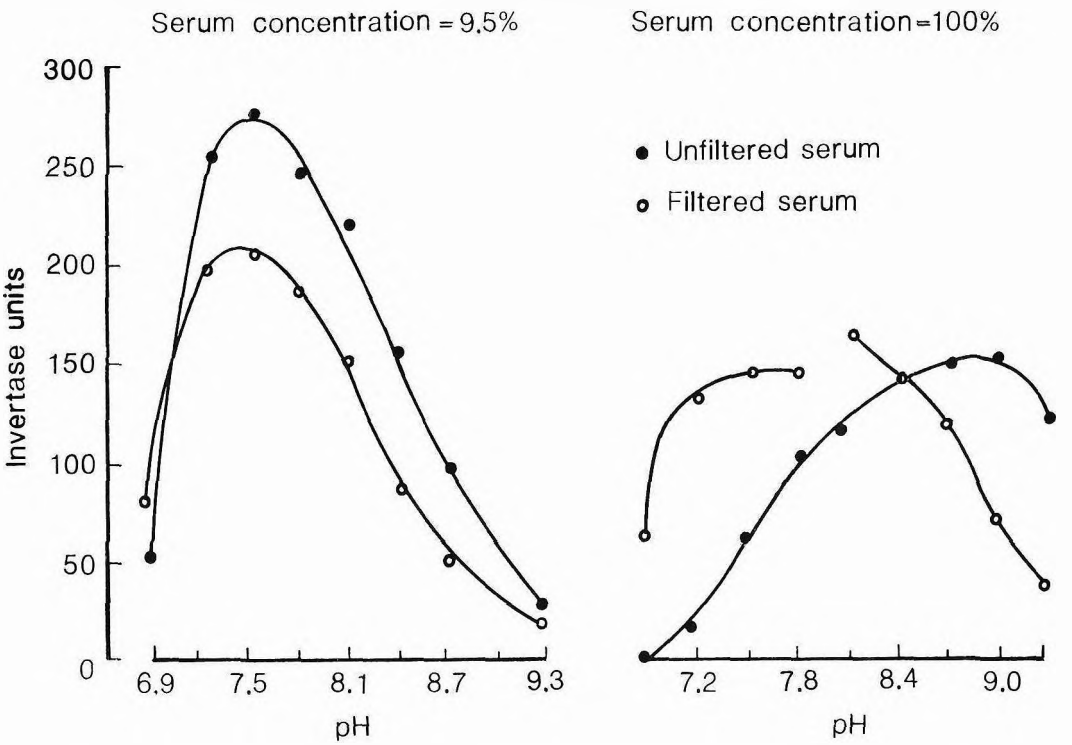

Figure 4. Effect of Trisacryl GF05 filtration of freeze-dried GT 1 C-serum on its invertase activity. Universal buffer was used to adjust the pH.

DISCUSSION

Two important characteristics of invertase have been repeatedly observed when serum in the enzyme incubation mixture was diluted from its natural concentration in serum.

Firstly, there was generally an apparent increase in invertase activity with serum dilution, a property already noted by Tupy[2]. This increment did not extend over the entire pH range investigated but was apparent only at pH below 8.4 (RRIM 600) and 9.0 (GT 1). Beyond these pH values, dilution of the serum decreased activity of the enzyme. Nevertheless, at the physiological pH of the latex (about 7), the tendency would be towards the increment of invertase activity with dilution.

Secondly, there was a shift in pH optimum from about 8.8 to about 7.4 with dilution. That two pH optima were also found in experiments using fresh serum indicated that the process of freeze-drying did not affect this enzyme characteristic. The use of a single buffer over the entire pH range investigated showed that these observations were not an effect of interactions with dissimilar buffers.

Two hypothetical mechanisms which can explain most of the observations from the experiments are proposed.

One possible mechanism is as follows. Invertase is subject to both the inhibiting and activating actions of one or more pH sensitive effectors which are small molecules. The putative effectors activate invertase at basic pH but inhibit it at pH closer to neutral. At normal (*i.e.* high) serum concentrations, there is a high concentration of the effectors; thus, activity in the vicinity of neutral pH is suppressed while that towards the basic end is enhanced. On diluting the serum (to 10% and below), there

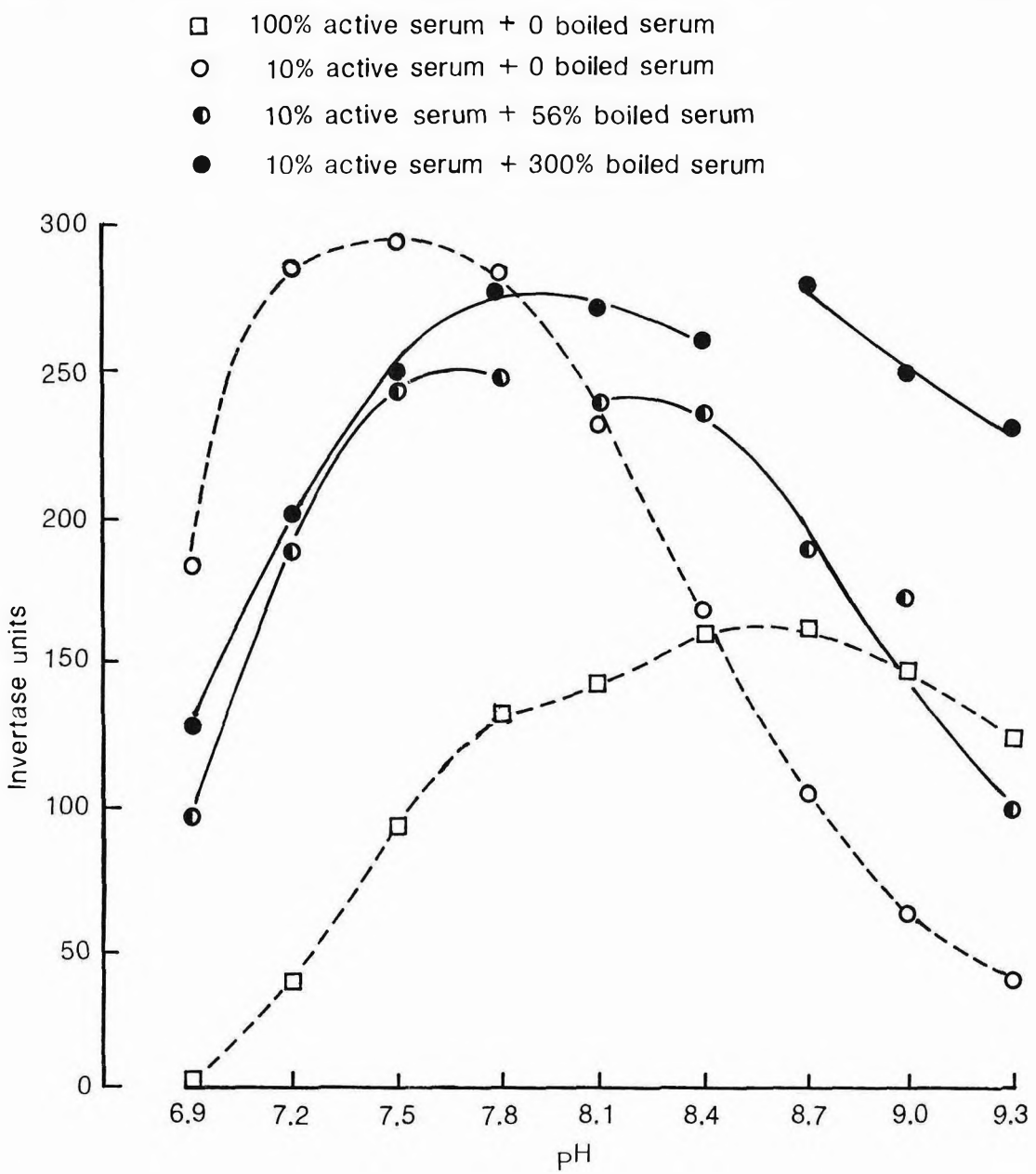

Figure 5. Effect of adding boiled C-serum to freeze-dried GT 1 C-serum on its invertase activity. Universal buffer was used to adjust the pH.

is a rise in activity around and just above neutral pH as the inhibition by the effectors is removed. At the same time, activation by the effectors at the high pH range is diminished resulting in a decrease in activity at this end of the pH scale.

On removing small molecules by gel filtration, the concentrated serum experiences a loss in activity at basic pH. This may be attributed to the loss of the small molecule effectors from the serum. For the same reason, there is a concomitant increase in activity towards the neutral pH values. To some extent, therefore, filtration has the same general effect as the dilution of the serum in shifting the pH optimum from basic towards the neutral values.

On addition of boiled serum to the incubation mixture, invertase activity at basic pH is elevated because of the action of additional effectors present in the boiled serum. The effectors' inhibitory property, on the other hand, suppresses activity when pH tends towards neutral. Hence, the addition of boiled serum partially simulates the effect of increasing the C-serum concentration.

An alternative explanation is as follows. There are two interchangeable forms of invertase having pH optima around 7.4 *(Type A)* and 8.8 *(Type B)*. Of the two, *Type B* is the less active. A small molecule acts as a transformer to change the invertase molecules from *Type A* to *Type B* and to maintain it in the latter state. Thus, at the natural (*i.e.* high) concentration of the serum, the transformer molecule is in abundance, and accordingly, the basic type *(Type B)* of invertase predominates. On dilution, the transformer molecule is scarce and consequently, *Type B* invertase is transformed into *Type A* invertase, this being accompanied by the shift of pH optimum from 8.8 to 7.4.

With filtration, some of the transformer molecules in the concentrated serum is lost; hence, a partial shift of the pH optimum towards the neutral values. Why there should be a reduction in activity in the dilute serum after filtration is not clear but various promoters of invertase present in the serum would have been lost in the course of filtration.

On addition of boiled serum to dilute serum in the enzyme incubation mixture, the transformer contained in the boiled serum converts *Type A* invertase to *Type B*, shifting the pH optimum towards the basic in the process.

In summarising the proposed regulatory mechanisms, therefore, there could exist in C-serum small molecule effectors that inhibit invertase activity in the vicinity of neutral pH but activate it at higher pH. Alternatively, a small molecule transformer might transform the enzyme molecule from one form (with pH optimum about 7.4) to another (with pH optimum about 8.8). A polymeric form of invertase which can dissociate into and reassociate from active sub-units has been reported in *Neurospora*[12].

A critical role of serum pH in the regulation of invertase activity has repeatedly been emphasised. This line of thought stemmed from the previous observation in sera, diluted 50% or more, that a very steep increase in activity accompanied the increase of pH from about 7 to 7.5. The findings in the present investigation, especially in GT 1 serum *(Figures 3 - 5)*, showed that at its natural concentration the increase in activity with pH over this range was considerably more gradual than had been thought; the enzyme did not have as narrow a pH optimal as supposed. As such, the regulation of invertase activity by latex pH in the tree, while substantiated, is probably of lesser importance than has been generally believed.

Although serum dilution can lead to a drastic change in pH optimum and relative activity, it is doubtful if this mechanism gives rise to extreme fluxes in invertase activity in the tree. The effects of dilution was marked only when it reached 50% or greater *(Figure 3)*, this extent of dilution not being normally encountered *in vivo*. On the other hand, changes in serum concentration within the physiological range might serve as a means of fine adjustment to latex invertase activity.

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Differential Sensitivities of Physiologic Races of Microcyclus ulei *to Fungicides*

ZHANG KAI-MING* AND K.H. CHEE**

Benomyl, thiophanate methyl, chlorothalonil, and mancozeb were tested against Races 4, 6, 7, *and* 8 *of* Microcyclus ulei *for inhibition of conidial germination, leaf disc infection, and field disease control.* Races 6 *and* 8 *were less sensitive to benomyl and thiophanate methyl than* Races 4 *and* 7. *Spraying benomyl (25 mg and 50 mg per litre) in the nursery gave satisfactory control of South American leaf blight on clones FX 2261 and FX 985 infected by* Races 4 *and* 7 *respectively, but not FX 3864 and FX 2804 infected by* Races 6 *and* 8 *respectively.*

South American leaf blight (SALB) of *Hevea* rubber caused by *Microcyclus ulei* (P. Henn.) Arx is indigenous to South America where it is a serious impediment to the development of a natural rubber industry. In Bahia, the annual expense of controlling SALB is equivalent to some 20% of the value of the crop. In one estate in Belem, Para chemical control of SALB represents 40% of the operational cost. Work on methods of chemical control represents some three-quarters of total research and development effort on SALB in Brazil. From 1970 onwards large-scale applications of fungicides have been carried out annually over several thousand hectares of rubber in Bahia, under the sponsorship of a Government Agency — PROMASE/SUDHEVEA. As a result, many areas of debilitated rubber have been rehabilitated. Chemicals used are the systemic compounds benomyl and thiophanate methyl, and the contact fungicides chlorothalonil and mancozeb. They are applied either from a helicopter or from the ground using a thermal fogger when the trees are refoliating. Applications are usually made on six to eight occasions at intervals of four to seven days but twelve or even more applications may be needed if climatic conditions and the phenology of the trees warrant it. Even then, control is not always completely effective and research effort is still being devoted to improving it.

This paper describes the effect of fungicides on the germination of conidia of different races of *M. ulei*, as well as the pathogenicity of these races on leaves in the laboratory and their control in the field.

MATERIALS AND METHODS

Fungicide and Inoculum

The fungicides tested were benomyl (Benlate), thiophanate methyl (Cercobin), chlorothalonil (Daconil), and mancozeb (Dithane M45). They were diluted with tap water to give concentrations of 200 mg, 50 mg and 4 mg per litre, and mixed in equal volumes with a suspension (obtained by brushing conidia off lesions) containing 2×10^5 spores per millilitre, so that

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final concentrations of both spores and fungicide were half those stated, *i.e.* 100 mg, 25 mg and 2 mg per litre. Conidia of *Race 4* were obtained from clone FX 2261, *Race 6* from FX 3864, *Race 7* from FX 985 and *Race 3* from FX 2804[1].

Conidial Germination

Drops of the spore/fungicide mixtures, and of spore suspension diluted with distilled water, were placed on microscope slides and incubated in a humid chamber at 24°C for 6 h before germination of 200 spores was counted. Conidia were considered to have germinated when the germtubes were more than half the length of the spores. Each experiment was carried out three to six times.

Prophylactic Treatment of Leaf Discs

Leaf discs 15 mm diameter were cut with a cork borer from healthy seven-day-old leaves of clones FX 2261, FX 3864, FX 985 and FX 2804 and floated with the abaxial surface uppermost[2] on distilled water in petri dishes. The fungicide/spore suspension mixtures were sprayed on the discs using an atomiser, and the dishes were incubated at 24°C under light (2600 lux) for seven days. The inoculation was carried out twice, each time using ten leaf discs. Assessment of infection was based on the percentage area of each disc which was covered with lesions, using a scale of 0%, 1% - 5%, 6% - 15%, 16% - 30%, and > 30%. The effectiveness of each fungicide was measured by the reduction of infection compared with the fungicide-free treatment. The experiments were carried out three to six times. For the method of calculating percentage control, see *Formula 2* of the later section.

Effect of Fungicide Concentration and Inoculum Density

Benomyl and thiophanate methyl were mixed with conidia of *Races 6, 7* and *8* to give mixtures containing 12.5 mg or 50 mg per litre of fungicide and conidial concentrations of 2×10^5 (high) and 4×10^4 (low) spores per millilitre. The mixtures were used as described above.

Field Experiments

Young flushes of leaves of clones FX 2261, FX 3864, FX 985 and FX 2804 planted in the EMBRAPA Station in Una, Bahia exposed to natural infection were sprayed to run-off using a 5-l pressurised hand sprayer, with benomyl, at concentration of 25 mg or 50 mg per litre. The flushes were sprayed four times at weekly intervals until the leaves were mature. Unsprayed flushes served as controls. Flushes were cut one week after the last application and brought back to the laboratory where 300 leaves were assessed for severity of infection based on a 0 to 4 scale:

0 : No infection

1 : Lesions small and sparsely scattered, affected leaf area ≤ 5%

2 : Lesions mostly small and scattered, affected leaf area 6% - 15%

3 : Lesions large and dense, sporulation and leaf distortion, affected leaf area 16% - 30%

4 : Lesions rampant, confluent, leaf torn, affected leaf area > 30%.

The severity of infection (%) was calculated by *Formula 1,* and the percentage control by *Formula 2*:

$$\frac{\text{No. of leaves in different disease classes} \times \text{Score of the class}}{\text{No. of leaves in different disease classes} \times 4} \times 100 \quad \ldots 1$$

$$\frac{\text{Severity of infection with no treatment} - \text{Severity of infection with treatment}}{\text{Severity of infection with no treatment}} \times 100 \quad \ldots 2$$

RESULTS

Germination of Conidia

Table 1 shows that chlorothalonil (1 mg per litre) was strongly inhibitory to germination of conidia of all races of *M. ulei,* and mancozeb (12.5 mg per litre) slightly less inhibitory. However, the inhibitory effect of mancozeb suspension diminished somewhat after 24 h storage. Conidia of the four races were affected to different degrees by benomyl and thiophanate methyl. *Races 6* and *8* were much less susceptible to thiophanate methyl than *Races 4* and *7. Race 6,* and to a lesser extent *Races 4* and *8* were also less affected by benomyl than *Race 7.*

Prophylactic Treatment of Leaf Discs

Table 2 shows that the effect of fungicides on leaf disc infection differed among four races, especially with benomyl. Benomyl was much less effective in suppressing infection by *Races 6* and *8* than *4* and *7* when tested at 12.5 mg per litre. Even at 50 mg per litre, benomyl was about half as effective on infec-

TABLE 1. PERCENTAGE GERMINATION OF CONIDIA OF FOUR RACES OF *M. ULEI* IN FOUR FUNGICIDES

Fungicide	Concentration (mg/litre)	Percentage germination[a]			
		Race 4	Race 6	Race 7	Race 8
1. Chlorothalonil	1.0	0	0	0	0
2. Mancozeb	12.5	0	4.1	0	28.4
3. Benomyl	12.5	42.6	81.7	24.6	48.7
4. Thiophanate methyl	12.5	28.4	53.5	17.2	46.6
L.S.D. (Between 3 and 4)		9.23	7.68	4.26	6.21
L.S.D. (Between 2 and 3)					6.81

[a]As compared to control converted to 100%

TABLE 2. PERCENTAGE CONTROL OF LEAF DISC INFECTION BY CONIDIA OF FOUR RACES OF *M. ULEI* IN THE PRESENCE OF THREE FUNGICIDES AT TWO CONCENTRATIONS

Fungicide	Concentration (mg/litre)	Percentage control			
		Race 4	Race 6	Race 7	Race 8
Mancozeb	12.5	64.4	67.0	67.7	39.6
	50.0	84.3	83.3	94.1	51.7
Benomyl	12.5	54.2	26.8	61.4	30.7
	50.0	71.0	41.7	79.2	52.1
Thiophanate methyl	12.5	49.6	47.8	60.7	45.9
	50.0	83.9	62.2	76.9	57.5
L.S.D. (Chemical)		12.87	4.81	5.22	6.49
L.S.D. (Concentration)		10.51	3.92	4.26	5.30

tion caused by *Race 6* than on infection caused by *Races 4* and *7*. Mancozeb was less effective in controlling infection caused by *Race 8* than by *Races 4, 6* and *7,* whereas thiophanate methyl was less effective against *Races 6, 8* and *4* at low fungicide concentration.

Effect of Fungicide Concentrations and Inoculum Density

As expected, the results in *Table 3* show that high fungicide concentration (50 mg per litre) combined with low inoculum density (4×10^4 spores per mililitre) reduced infection more than low fungicide concentration (12.5 mg per litre) combined with high inoculum density (2×10^5 spores per millilitre). Percentage control of leaf disc infection by benomyl in the former combination with *Races 6, 7* and *8* were 65.0, 86.6 and 68.6 respectively, whereas in the latter they were 27.2, 53.4, and 35.5 respectively. However, the results differed little when the comparisons were between high fungicide concentration with high inoculum density, and low fungicide concentration with low inoculum density *(Table 3)*. Irrespective of fungicide concentrations and inoculum densities, *Races 6* and *8* were consistently less sensitive to benomyl and thiophanate methyl than *Race 7*.

Field Control

The results in *Table 4* show that under field conditions, benomyl at 25 mg and 50 mg per litre were most effective against SALB on FX 2261 (infection by *Race 4*), slightly less effective on FX 985 (infection by *Race 7*), and least effective on FX 3864 (infection by *Race 6*) and FX 2804 (infection by *Race 8*). Field results were consistent with laboratory finding in that benomyl was effective against certain races of *M. ulei* and not others at the concentrations tested.

DISCUSSION

Fungicides had hitherto been tested against *M. ulei* without regard to possible differences in the sensitivity of races to the chemicals. The present study demonstrated that benomyl,

TABLE 3. PERCENTAGE CONTROL OF LEAF DISC INFECTION BY *M. ULEI* IN DIFFERENT COMBINATIONS OF CONIDIA DENSITIES AND FUNGICIDE CONCENTRATIONS

Fungicide	Concentration (mg/litre)	Conidia density	Percentage control		
			Race 6	Race 7	Race 8
Benomyl	12.5	High	27.2	53.4	35.5
		Low	40.0	67.3	47.1
	50.0	High	42.3	76.4	53.5
		Low	65.0	86.6	68.6
Thiophanate methyl	12.5	High	36.4	60.2	36.1
		Low	40.0	67.3	45.3
	50.0	High	45.5	80.3	50.0
		Low	65.0	86.6	65.9
Transformed data					
L.S.D. (Chemical)			5.50	5.91	2.67
L.S.D. (Concentration)			3.89	4.18	1.89
L.S.D. (Chemical × Concentration)			7.78		

TABLE 4. EFFECT OF BENOMYL ON THE CONTROL OF SALB CAUSED BY FOUR RACES OF *M. ULEI*

Clone	Race	Severity of infection				Percentage control as compared to no treatment	
		Treatment		No treatment			
		Expt. 1 25 mg/litre	Expt. 2 50 mg/litre	Expt. 1 25 mg/litre	Expt. 2 50 mg/litre	Expt. 1 25 mg/litre	Expt. 2 50 mg/litre
FX 2261	4	19.1	12.3	51.5	57.9	62.9	78.8
FX 3864	6	39.9	45.5	38.0	51.4	0	11.9
FX 985	7	44.0	25.9	77.3	61.8	43.4	58.1
FX 2804	8	57.5	39.2	62.9	50.0	8.6	21.6

which is commonly used against SALB, was more effective against some races of *M. ulei* than others. Thus *Races 6* and *8* were tolerant to benomyl while *Races 4* and *7* were sensitive. Hence benomyl gave satisfactory control of SALB on FX 2261 (infection by *Race 4*) and FX 985 (infection by *Race 7*), and poor or no control on FX 3864 (infection by *Race 6*) and FX 2804 (infection by *Race 8*). This finding helped to explain some of the inconsistent results obtained in the treatment of SALB in Bahia with benomyl.

It is important therefore, that along with a study of the race structure of *M. ulei* in Brazil, research should also be conducted on the effect of fungicides, especially the systemic compounds, on controlling infection caused by separate races of *M. ulei.* There are many different clones of *Hevea* in commercial plantings and it is likely that they are differently susceptible to each of the races present[1] and therefore the degree of control given by benomyl can vary from clone to clone. Benomyl has been used for SALB control in Bahia for over ten years, and it is not known if certain races of *M. ulei* have developed resistance to it. Rao *et al.*[3] in Bahia tested benomyl at 10 mg per litre and found that conidial germination was merely 4%. In the present study however, germination averaged 49.9% when tested against benomyl at 12.5 mg per litre. Chee[4] in Trinidad found no germination with conidia of *Race 6* (25 mg per litre), whereas in the present study, germination of the same race was 28.6% (50 mg per litre). If indeed some races do become tolerant to benomyl through repeated usage there is a need to consider alternating or combined use of conventional fungicides, such as chlorothalonil, for the control of SALB.

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Composition of Lipids in Latex of Hevea brasiliensis *Clone RRIM 501*

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Lipids were extracted from fresh latex of Hevea brasiliensis *clone RRIM 501 and analysed qualitatively and quantitatively by a combination of chromatographic, spectroscopic and colorimetric techniques. The total lipids constituted about 1.6% of the latex, out of which 54% was due to neutral lipids, 33% glycolipids and 14% phospholipids. The neutral lipids comprised of carotenoid pigments, free and esterified sterols, free and esterified tocotrienols, free fatty alcohols and their acetates, tri-, di- and monoglycerides and free fatty acids. Triglycerides alone constituted about 63% of the neutral lipids or 0.6% of the latex, making them the major components of* H. brasiliensis *lipids. The glycolipids consisted of free and esterified steryl glucosides, di- and monogalactosyl diglycerides while the phospholipids contained mainly phosphatidyl ethanolamine, phosphatidyl choline and phosphatidyl inositol.*

Natural rubber derived from *H. brasiliensis* latex contains 4%–5% non-rubber substances comprising of amines, amino acids, carbohydrates, inorganic constituents, proteins, nucleic acids, nucleotides and lipids. Some of these non-rubber substances, especially the lipids and the precipitated proteins, which are retained in the dry rubber after coagulation and drying have been found to affect the rubber properties. Thus there have been several investigations on the nature and role of these substances in *H. brasiliensis* latex and rubber[1-7].

Most of the studies on non-rubber substances were made twenty to fifty years ago using classical methods of analysis without utilising modern techniques such as thin layer chromatography (TLC), gas liquid chromatography (GLC) and spectroscopic techniques. Consequently no complete analysis of all the lipids present in *H. brasiliensis* latex has been reported. Furthermore no special attention was given to the source (clonal or bulked) and nature (fresh, preserved or concentrated) of the latex for analysis. These factors may give rise to variable lipid compositions.

Ho *et al.*[8] were the first to clearly fractionate the total neutral lipids and phospholipids from fresh latex of a *H. brasiliensis* clone on TLC. They were however unable to confirm the identities of some of the lipids and no attempts were made to isolate and further analyse the composition of these lipids. This paper reports the characterisation and composition of lipids in fresh latex of a *H. brasiliensis* clone, RRIM 501.

MATERIALS AND METHODS

Materials

Fresh latex was obtained from mature unstimulated trees of RRIM 501. The lipid standards were purchased from Sigma Chemical Company and Applied Science Laboratories. The glycolipid standards were obtained from Supelco Incorporated.

Extraction and Fractionation of Lipids

Lipids were extracted from fresh latex by drop-wise addition of the latex to at least five volumes of a continuously stirred chloroform/methanol (2:1, v/v) mixture. The extracts separated from the rubber coagulum were washed with sodium chloride solution according to the procedure of Folch *et al*[9].

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Total extracts were separated by silicic acid column chromatography (CC) into neutral lipids, glycolipids and phospholipids[10].

Thin Layer Chromatography

Each lipid class was analysed by TLC on plates coated with silica gel G. The neutral lipids were separated with hexane/benzene (85:15, v/v) followed by hexane/diethyl ether/acetic acid (69:29:2, v/v/v), the glycolipids with chloroform/methanol/water (95:20:2.5, v/v/v) and the phospholipids with chloroform/acetone/methanol/acetic acid/water (50:20:10:10:5, v/v/v/v/v). The sterol acetates were fractionated on 20% $AgNO_3$ - TLC plates and developed in hexane-benzene (1:1, v/v). The neutral lipids were detected by 3% solution of cupric acetate in 8% ortho phosphoric acid[11], the glycolipids by α-naphthol followed by concentrated sulphuric acid[12] and the phospholipids by the modified molybdenum reagent[13]. The lipid layers including the sterol acetates on the preparative TLC plates were located by 2′,7′-dichlorofluoresceine.

Quantitative Determination of Lipids

The various lipids were quantified following the scheme outlined in *Figure 1*. The total lipids were fractionated on silica gel CC and eluted with hexane-ether mixtures followed by methanol to give various fractions of neutral lipids and polar lipids, respectively. The hexane-diethyl ether (95:5, v/v) eluent contained some rubber. The eluent was thus concentrated and acetone added to separate the acetone insoluble rubber from the acetone soluble lipid esters. The combined weights of all the hexane-diethyl ether eluents (minus rubber) give the amount of neutral lipids. The amounts of glycolipids and phospholipids were determined by multiplying the amount of sugar[14] and phosphorus[15] in the methanol eluent with their conversion factors (CF), 4.7 and 25.9 respectively[16]. Similarly the triglycerides were quantified by multiplying the amount of glycerol[16] in the methanolysate hexane-diethyl ether (75:25, v/v) eluent with its CF, 10.4.

The tocotrienols and sterols were determined by GLC after further purification on TLC.

Gas Liquid Chromatography

Gas liquid chromatography was performed on a Pye Unicam GCD Liquid Chromatograph and a Hewlett-Packard 5750 Research Chromatograph, equipped with flame ionisation detectors. The fatty acid methyl esters were analysed on a polyethyleneglycol adipate column; sterols, tocotrienols and long-chain alcohol acetates on a 3% SE-30 silicone gum column; and the trimethylsilyl (TMS) ether derivative of methyl glycosides on a UCC-W-982 (vinyl methyl) column.

Spectrometry

The mass spectra were obtained on a GC/AEI MS3074 instrument attached to a DS-50S mass spectroscopy (MS) data system. The nuclear magnetic resonance (^{1}H NMR) spectra were recorded from carbon tetrachloride or deuterated chloroform solution on a Hitachi-Perkin Elmer R-20B instrument at 60 MHz using tetramethysilane as internal reference. The infra-red (IR) spectra were obtained from carbon tetrachloride solution on a Beckmann IR 4250 spectrophotometer.

The results reported are the average of three samplings.

RESULTS

Characterisation of Lipids

Neutral lipids. Thin layer chromatography of the neutral lipids *(Figure 2)* gave thirteen spots. The identities of the spots *A, B, E, I, J, K, L* and *M* were obtained by comparing with their standards. Lipids *C, D, F* and *G* were isolated from preparative TLC along with the other major neutral lipids and analysed for their structural compositions by a combination of chromatographic and spectroscopic techniques. *Table 1* gives the composition of the major neutral lipids of RRIM 501 latex.

The free sterols were analysed chromatographically and by MS. Fractionation on

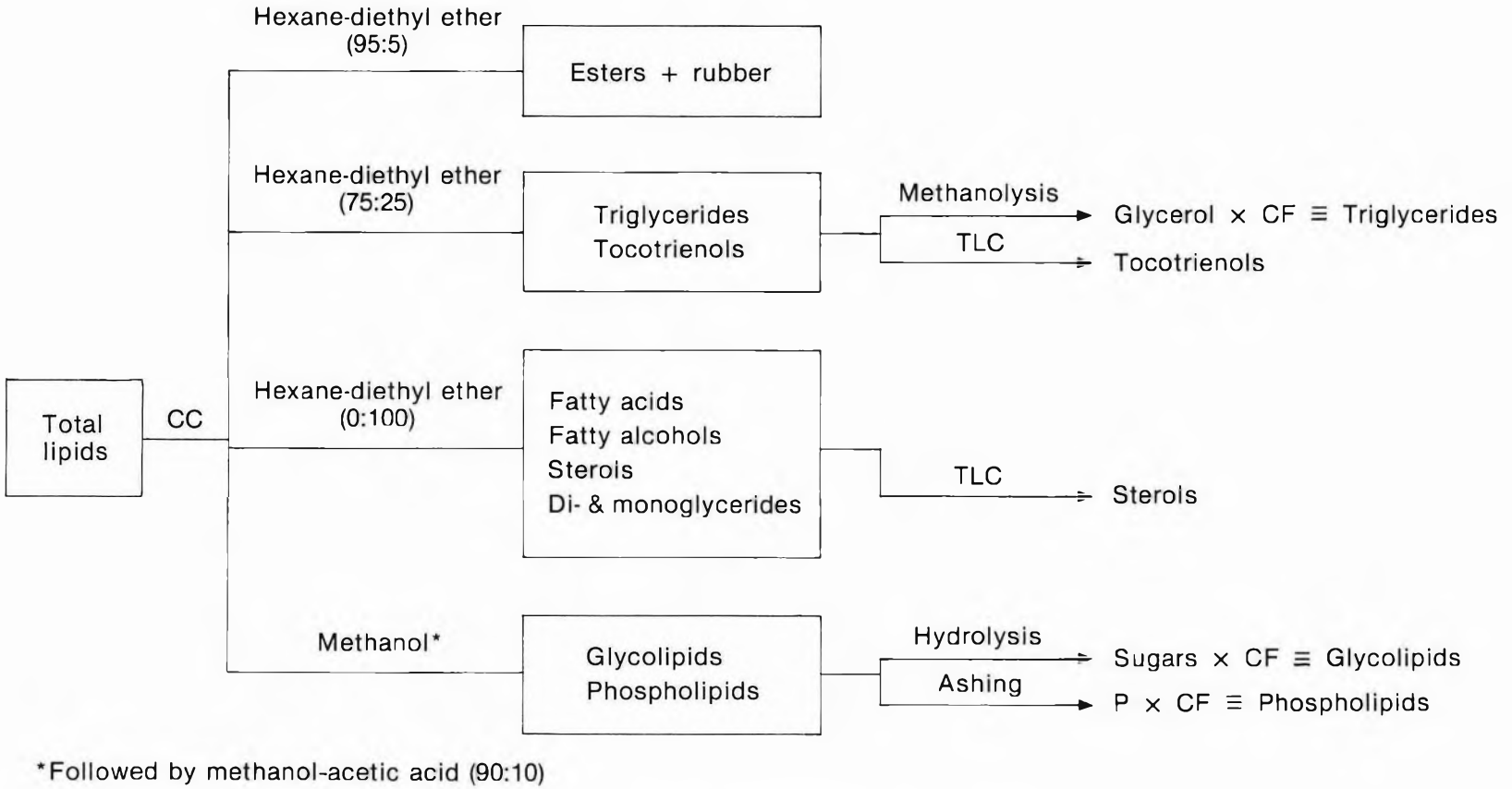

Figure 1. Quantitative determination of lipids from Hevea brasiliensis *latex.*

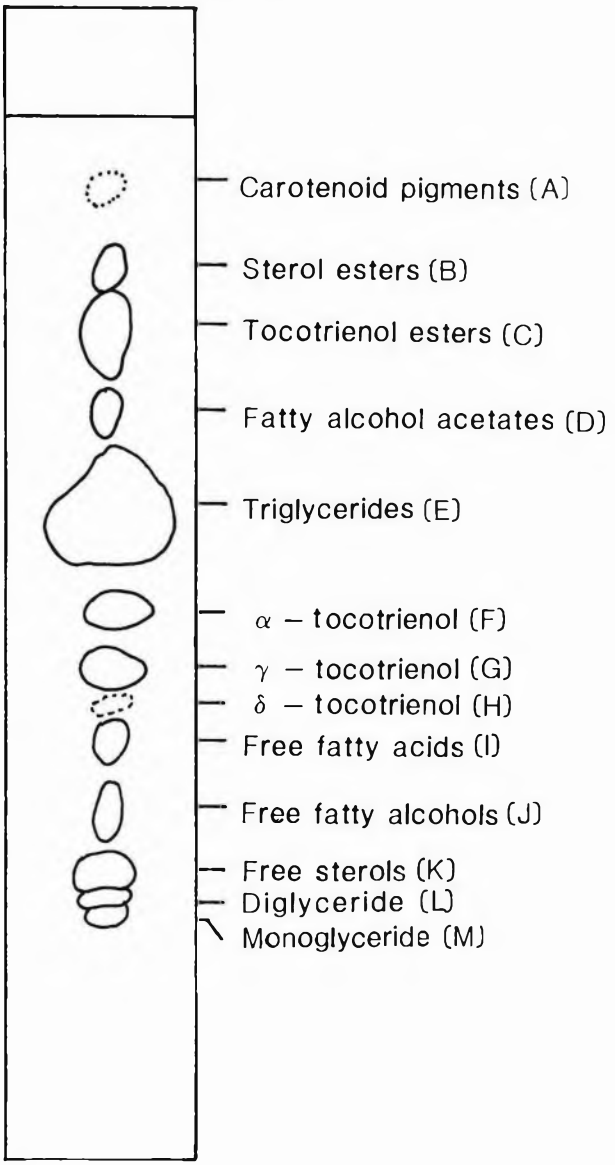

Figure 2. Thin layer chromatography of neutral lipids from fresh H. brasiliensis *latex of RRIM 501 developed in hexane-benzene (85:15, v/v) followed by hexane-diethyl ether-acetic acid (69:92:2, v/v/v) and detected by cupric acetate reagent.*

$AgNO_3$-TLC plates resolved the acetate derivative of the sterol mixture into two bands. Gas chromatography of the upper band gave two peaks with retention time corresponding to standard β-sitosterol acetate (2.24) and stigmasterol acetate (1.88) relative to cholesterol taken as 1. Gas chromatography of the lower band however revealed only one peak with a retention time of 2.26. Further analysis of the sterol by MS showed it to be fucosterol:

TABLE 1. COMPOSITION OF THE MAJOR NEUTRAL LIPIDS FROM FRESH *H. BRASILIENSIS* LATEX OF RRIM 501

Neutral lipids	Unsaponifiables	Fatty acids							
		16:0	16:1	18:0	18:1	18:2	18:3	20:0	F_2
Sterol esters	β-sitosterol (64%), fucosterol (19%) and stigmasterol (17%)	6.9	3.8	26.0	13.0	33.0	7.0	10.0	-
Tocotrienol esters	γ- and δ-tocotrienols	11.0	0.8	49.0	19.6	11.0	0.5	8.9	-
Fatty alcohol acetates	Cetyl, stearyl and arachidyl alcohols								
Triglycerides		-	-	0.4	1.1	0.6	-	-	98.0
Free tocotrienols	α- and γ-tocotrienols (40%) (60%)								
Free sterols	β-sitosterol (53%), fucosterol (33%) and stigmasterol (14%)								

F_2 = Furanoid fatty acid

mass/charge (m/e) ratios (relative intensity of fragments) of 412 (M^+, 17), 314 (100) and 271 (19).

The free tocotrienol spots *F, G* and *H* were initially identified by their specific reaction with the Emmerie-Engel[17] reagent and through comparison with the free tocotrienols from palm oil extracts. The larger bands *F* and *G* were isolated separately by preparative TLC and the presence of α- and γ-tocotrienols in *F* and *G*, respectively were confirmed by GC-MS *(Figure 3)* and ^{1}H NMR *(Figure 4)* spectroscopy.

Like the mass spectra of their tocopherols[18] the mass spectra of α- and γ-tocotrienols are also characterised by the absence of numerous fragmentation processes. The DS-50 atomic composition report showed that the parent ion of γ-tocotrienol at m/e 424 has a general formula of $C_{29}H_{44}O_2$ which splits to a very stable dihydroxy tropylium ion at m/e 165 with a formula of $C_{10}H_{44}O_2$. Similarly the parent ion of γ-tocotrienol was shown to be at m/e 410 with a formula of $C_{28}H_{42}O_2$ and a base peak at m/e 151 with the formula $C_9H_{11}O_2$.

The ^{1}H NMR spectra of the two tocotrienols show similar proton shifts as α-tocopherol[19] except in the proton shift of the side chain due to the unsaturation in the former. The only difference between the spectra of the two tocotrienols is in the aromatic proton shift at δ 6.12 p.p.m. which is absent in α-tocotrienol. The γ-tocotrienol was distinguished from β-tocotrienol by two-dimensional TLC. The fatty alcohol acetates were deduced by the presence of acetyl function at δ 1.95 p.p.m. on ^{1}H NMR. Infra-red spectroscopy showed the carbonyl band at 1746 cm^{-1} which corresponds to that of the standard fatty alcohol acetates. Gas chromatography analysis showed three peaks corresponding to standard cetyl, stearyl and arachidyl acetates. These were further confirmed by GC-MS *(Table 2)*. No molecular ion was detected in any of the three spectra, characteristic of the spectra of the acetate derivatives due to the loss of acetic acid as evidenced by the peak M^+-60. The fragment m/e 61 strengthens the presence of the acetate compounds.

All the acyl lipids were methanolysed and the fatty acid methyl esters were analysed by GLC. It can be seen *(Table 1)* that stearic, oleic and linoleic acids formed the major acyl components of the sterol and tocotrienol esters. The triglycerides however contained 98% of a furanoid fatty acid as reported earlier[20].

The unsaponifiable fraction of sterol esters contained stigmasterol, β-sitosterol and fucosterol while the tocotrienol ester contained mainly γ- and δ-tocotrienol in a mixture with

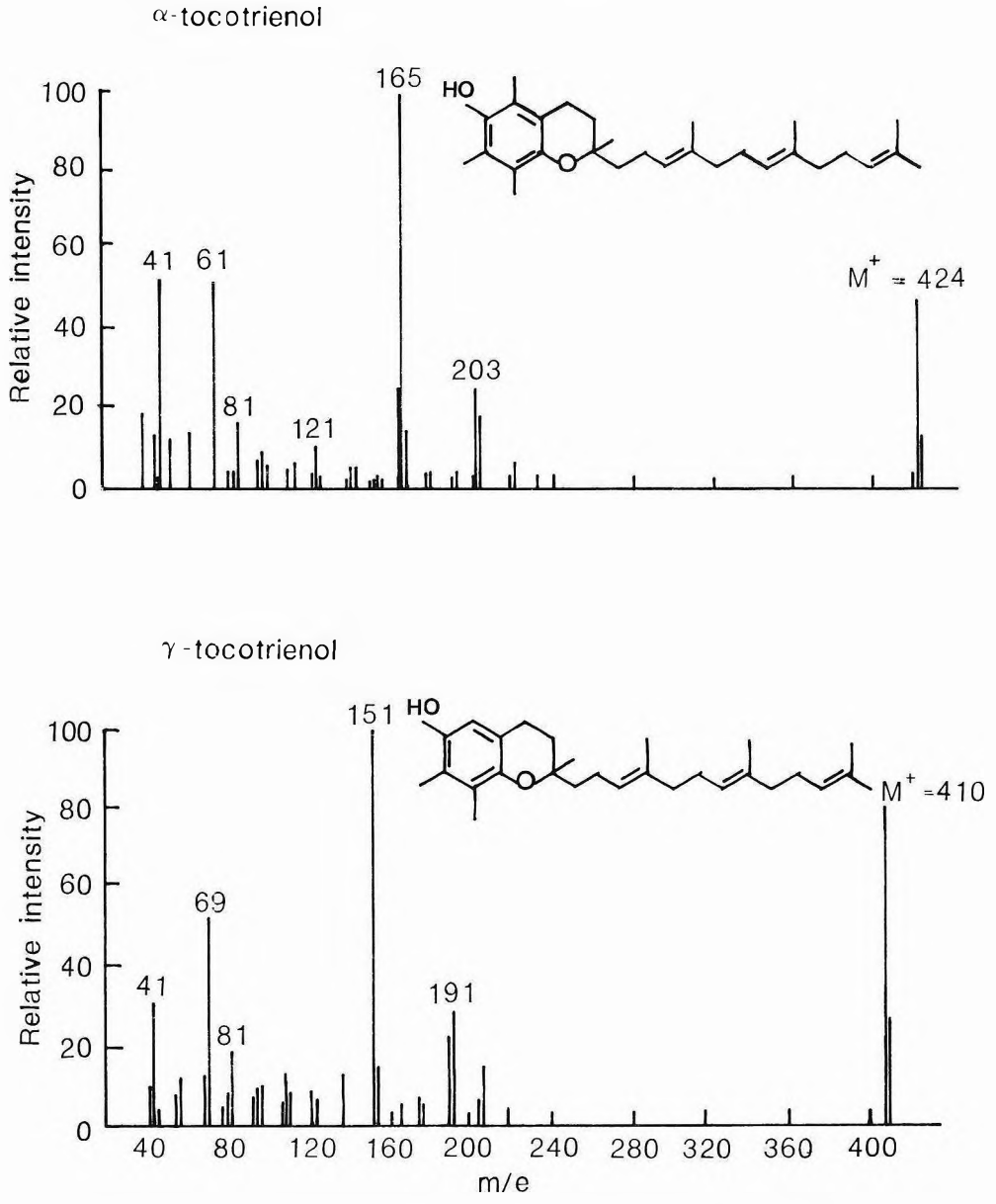

Figure 3. Mass spectra of α- and γ-tocotrienols from H. brasiliensis *latex.*

other phenolic compounds, probably the oxidised products of the tocotrienols.

Glycolipids. Thin layer chromatography of the glycolipids sprayed with α-naphthol reagent showed four main spots which corresponded to the glycolipid standards *viz.* esterified steryl glycoside (ESG), monogalactosyl diglyceride (MGDG), steryl glucoside (SG) and digalactosyl diglyceride (DGDG).

The presence of sugar was confirmed by analysing the TMS ether derivative of the methyl glycosides by GLC. The chromatogram

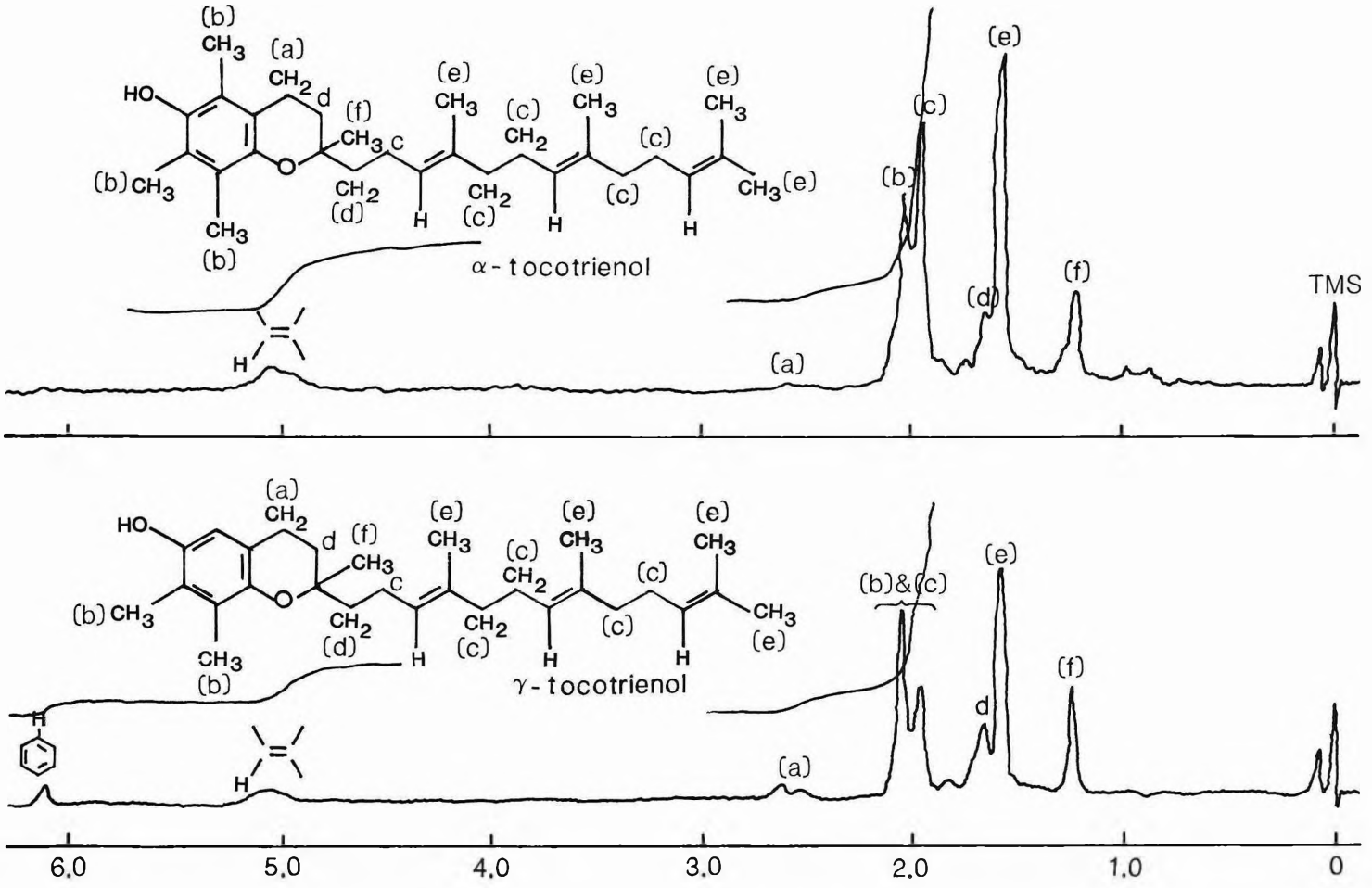

Figure 4. ^1H NMR spectra of α- and γ-tocotrienols of Hevea brasiliensis *latex.*

TABLE 2. MASS SPECTRAL DATA OF THE LONG-CHAIN ALCOHOL ACETATES FROM FRESH LATEX OF RRIM 501

Acetate	Characteristic fragments m/e (relative abundance)
Cetyl	224 (M^+ - CH_3COOH, 3), 99(4), 85(21), 71(45), 61(5), 57(80), 43(100)
Stearyl	252 (M^+ - CH_3COOH, 5), 99(3), 55(16), 71(33), 61(64), 57(73), 43(100)
Arachidyl	280 (M^+ - CH_3COOH, 12), 99(7), 85(30), 71(51), 61(69), 57(100), 43(82)

showed two peaks for the α and β anomers of methyl glucosides and three peaks for the α, β and γ anomers of methyl galactosides as reported by Sweeley and Walker[21]. Each glycolipid was isolated by preparative TLC, extracted and analysed for its composition. The sterol component of ESG and SG consisted of β-sitosterol, fucosterol and stigmasterol with β-sitosterol occurring as the major component (about 89%) in both cases. The fatty acid methyl esters of ESG, MGDG and DGDG *(Table 3)* consisted mainly of stearic, oleic and linoleic acids. Furanoid fatty acid was also present but the amount was less than one-fifth of that present in the triglyceride fraction. It is interesting to note that ESG had a higher proportion, about 64%, of saturated fatty acids compared to MGDG and DGDG which had less than 40% of the saturated fatty acids.

Phospholipids. Thin layer chromatography of the phospholipids showed three main spots corresponding to phosphatidyl ethanolamine (PE), phosphatidyl choline (PC) and phosphatidyl inositol (PI). There were also two smaller spots of phosphatidic acid and diphosphadityl glycerol. The latter two could be the artifacts of the hydrolytic action of phospholipase *D* which is known to occur in *H. brasiliensis* latex[5].

The identities of PE, PC and PI were further confirmed by their positive reaction with specific detecting reagents and thorough comparison with their standards. PC and PE were stained orange and pink by Dragendorff and ninhydrin reagents, respectively. The colour changed to blue on respraying the TLC plate with the modified molybdenum reagent. Infra-red spectra of PI showed a broad absorption band at 3400 cm^{-1} (OH) which was not prominent in the spectrum of PC and absent in the spectrum of PE.

Analysis of the fatty acid methyl esters of the three main phospholipids from fresh latex of RRIM 501 *(Table 3)* showed that the acyl component of PE, PC and PI consisted of only aliphatic fatty acids: mostly of palmitic, stearic, oleic and linoleic acids. Furanoid fatty acid was not detected in the phospholipid fraction of *H. brasiliensis* latex.

Quantitative Composition of the Lipids

The total lipid content of RRIM 501 was about 1.64% of the latex *(Table 4)*. The neutral lipids formed the major class of *H. brasiliensis* lipids, constituting about 53.6% of the total lipids. The glycolipids and phospholipids constituted about 32.9% and 14.0%, respectively. Among the neutral lipids the triglyceride fraction alone contributed about 63.3% of the total or 0.56% of latex, making it the major component of *H. brasiliensis* lipids. The tocotrienols which are the natural antioxidants of rubber comprised of only 0.03% which was comparable to the amount of free sterols.

In the glycolipids the amount of galactosyl diglycerides exceeded that of the steryl glucosides. DGDG formed the second major component of *H. brasiliensis* latex constituting about 0.34%. Phosphatidyl choline which was the major component of phospholipids constituted about 0.13% of the latex.

DISCUSSION

Despite considerable work done by previous workers in the characterisation of lipids in

TABLE 3. FATTY ACID COMPOSITION OF ACYL POLAR LIPIDS FROM FRESH LATEX OF RRIM 501

Lipid	Fatty acid composition (%)								
	14:0	16:0	16:1	18:0	18:1	18:2	18:3	20:0	F_2
ESG	1.9	25.1	2.8	35.0	11.2	11.3	0.9	2.5	9.3
MGDG	-	13.5	4.1	22.9	22.2	17.9	4.1	-	15.1
DGDG	1.4	5.2	2.0	22.9	21.2	26.2	3.5	-	17.6
PE	2.0	20.8	3.7	18.3	14.1	37.5	3.6	-	-
PC	0.9	9.6	10.5	21.9	20.6	34.3	2.3	-	-
PI	0.5	30.2	3.4	16.8	10.3	35.4	3.4	-	-

ESG = Esterified steryl glycoside
MGDG = Monogalactosyl diglyceride
DGDG = Digalactosyl diglyceride
PE = Phosphatidyl ethanolamine
PC = Phosphatidyl choline
PI = Phosphatidyl inositol
F_2 = Furanoid fatty acid

TABLE 4. LIPID COMPOSITION OF *H. BRASILIENSIS* LATEX OF RRIM 501

Lipid	Composition		
	% of total lipids	% of latex	% of respective lipid classes
Neutral lipids	53.6	0.88	
Esters		0.14	16.1
TG		0.56	63.3
T_3		0.03	3.4
Sterols		0.04	4.5
FA, ROH, DG, MG		0.10	11.9
Glycolipids	32.9	0.54	
ESG		0.05	9.5
MGDG		0.04	6.5
SG		0.11	21.1
DGDG		0.34	62.8
Phospholipids	14.0	0.23	
PE		0.05	21.0
PC		0.13	58.4
PI		0.05	20.6
Total lipids		1.64	

TG = Triglycerides
T_3 = Tocotrienols
FA = Fatty acid
ROH = Alcohols
DG = Diglycerides
MG = Monoglycerides
Other abbreviations as in *Table 3*.

H. brasiliensis latex some important components were still not detected. The existence of the glycolipids which form an important fraction of total lipids has never been clearly demonstrated before, although many workers[3,4,6] have detected sugars in their polar lipid fractions. Whitby *et al.*[1] and Altman and Kraay[2] however, identified a sterol glucoside in their lipid fraction and Sentheshanmuganathan *et al.*[22] suggested the possibility of the presence of glycolipids but the composition of the glycolipids has not been clearly demonstrated as in the present study. Here the glycolipids are shown to contain four main components, namely, ESG, MGDG, SG and DGDG.

Besides the glycolipids, the occurrence of a high amount of furanoid fatty acid (98%) in the triglyceride fraction together with the high concentration of the fraction in the lipids of *H. brasiliensis* is another interesting finding. The acid although found in triglycerides and glycolipids has not been detected in other *Hevea* lipids including rubber seed oil. Further investigations have shown that the occurrence of the furanoid fatty acid in the triglyceride fraction of the *H. brasiliensis* lipids is a clonal characteristic and is dependent to some extent on the period of the year[23]. Similar seasonal and individual variations have been observed in the distribution of furanoid fatty acids in the Northern Pike *(Esox lucius)*[24]. It is possible that the acid has a physiological role in *Hevea* but this has not yet been ascertained.

The fatty alcohols, unlike the other non-saponifiables, *i.e.* the sterols and tocotrienols, were surprisingly found esterified to acetic acid and not to fatty acids. This is rather uncommon among plant lipids and like the furanoid fatty acids the function of these fatty alcohol acetates is also uncertain.

Apart from the above findings, the other results of the qualitative composition are generally in accordance with previous studies. The content of phospholipids is lower than that reported by previous workers. It is likely that the 'phospholipids' in previous investigations were actually polar lipids and would have included glycolipids.

In this study a systematic method of analysis of lipids in *Hevea* latex is used. It consists in first separating the lipids into different classes, then characterising their identities by chromatography and spectroscopy and finally quantifying their composition. The study thus gives a better picture of the composition of lipids in the fresh latex of a *Hevea brasiliensis* clone. This method can be used for a general study of clonal variations in the lipid content of *Hevea*.

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Experiments on the Lubrication of Raw Natural Rubber

E.L. ONG* AND A.D. ROBERTS**

The surface lubrication of a few grades of raw natural rubber (NR) was investigated with simple friction apparatus. Various liquids at room temperature were used. In particular the effect of aqueous solutions of inorganic salts, lipids and rubber protein was examined.

The 'tackiness' of raw rubber can be reduced by liquid or solid lubricants and also by surface chemical attack such as chlorination. This is important in rubber manufacture. The way liquids lubricate raw rubber is of wider interest in unvulcanised rubber products, such as shoe sole crepe, adhesives and plastics modifiers, and has a bearing upon the mechanical stability of rubber particles in latex. While much has been published on the lubrication of vulcanised rubber, there appears to be little published information on raw rubber. We report experiments on the lubrication of standard grades of raw Malaysian rubber by hydrocarbon liquids and thin films, inorganic solids, soap and aqueous solutions of inorganic salts and rubber protein.

EXPERIMENTAL

Rubber samples were designated grades of Standard Malaysian Rubber (SMR) and were compression moulded into smooth-surfaced hemispheres[1]. Friction measurements, used to indicate the efficacity of a lubricant, were made with simple apparatus[1]. A raw rubber hemisphere was drawn over a flat track of 'Perspex' (polymethylmethacrylate) in the presence of the lubricant under test. The 'Perspex' track was selected as the contact interface could be viewed directly through the 'Perspex' plate[1] but there was no provision for measuring lubricant film thickness between surfaces. In some tests, for symmetry, the track surface was the same rubber (thin sheet) supported by the 'Perspex' track. Under a normal load, W, the sliding friction force, F, was measured at a given speed and the friction coefficient, $\mu = F/W$, used as a comparision for different lubricants. Unless stated otherwise, the 'Perspex' track surface was solvent cleaned by wiping with tissue paper moistened with isopropanol, and allowed to dry. All rubber surfaces were tested as moulded without solvent treatment.

RESULTS

Contaminant Films

In an earlier communication[1], it was noted that the presence of trace amounts of surface contaminant, such as silicone release agent, could reduce the sliding friction of raw rubber by up to 50%. A raw rubber hemisphere 'as moulded' generally has a dry friction coefficient greater than 3. Surface cleaning of it with solvents (impregnated cotton wool wipe and allow to dry) can cause an increase in the friction, and if the hemisphere is extracted in cold acetone (frequent changes of acetone over a period of a month) it is found that the friction increases further *(Table 1)*. This suggests that solvent wipe cleaning of rubber surfaces removed some low molecular weight materials that act as a boundary lubricant, and longer term soaking in solvent removed more of them. This interpretation may, however, be complicated by residual solvent left after drying that

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TABLE 1. EFFECT OF SURFACE CONTAMINANTS ON FRICTION

Surface condition	Friction coefficient	Observations
Rubber 'as moulded'	3.1	Ridge[1] formed during sliding
Rubber surface cleaned with acetone	3.3	No colouration on wipe tissue
Rubber extracted in cold acetone	5.7	Acetone acquires brownish colour
Cast film of NR extract — prolonged running	0.9 0.1	No ridge or surface damage
Rubber, covered with NR extract, re-run on clean track	0.7 2.0	Initial friction later rises with damage to surface

SMR CV hemisphere (R = 21 mm) sliding at 0.2 mms^{-1} on smooth 'Perspex' track under 1.57 N load, temp. 23°C-24°C, RH = 55% - 65%. Friction coefficients quoted are averaged values; the scatter was about ±20%

softens the rubber leading to more intimate contact and a higher friction.

The lubricating effect of materials extracted by acetone from raw rubber (RSS 1) was examined. The acetone extract was evaporated down and solids were re-dissolved in ethanol (necessary because acetone attacks 'Perspex') then cast from the solution as a dry, thin surface film on a smooth 'Perspex' track. A clean raw rubber hemisphere was drawn over the extract film. The friction was initially high, but fell with prolonged running to a low order *(Table 1);* a sliding mark was left in the surface film, but there was no damage to the rubber which had acquired a waxy surface appearance after this test. Materials from the cast film became transferred onto the rubber, eventually in sufficient quantity to greatly reduce the friction. Whenever the waxy surfaced hemisphere was re-run (without solvent cleaning) on a new area of cast film, the friction remained low, but when it was re-run on a clean area of 'Perspex' track (no cast film present) the friction rose with increasing sliding distance to a high value *(Table 1)*. With increased friction, there was some damage to the rubber surface. The waxy material on the rubber hemisphere appeared to be wiped away by sliding against the clean track, and this may always happen to trace contaminants on a raw rubber surface during sliding.

Solid Lubricants

In manufacturing operations the dusting of rubber with talc is extensively employed to reduce tack. The effect on the friction of raw rubber was examined. An unvulcanised rubber hemisphere was lightly dusted with talcum powder and then run dry against a clean 'Perspex' track. The friction was about half that of an untreated 'as moulded' hemisphere *(Tables 1* and *2)*. If water was added to the contact region the friction fell considerably, or alternatively if a liberal amount of talc was added *(Table 2)*. It was clear that a range of friction values could be obtained depending upon the amount of talc present, and this can be exploited in practice, for example, to obtain just the right amount of interply tack in latex thread.

Magnesium oxide powder (coarse particles) was dusted liberally onto a raw rubber hemisphere and the sliding friction measured against a 'Perspex' plate. There was surface damage of the rubber. After repeat testing it appeared that the friction coefficient was very considerably higher *(Table 2)* than for a liberal dusting with talc. This presumably reflects the difference in shear properties of the two powders.

In practice, the tack of rubber articles is often reduced by surface chlorination. The

TABLE 2. SOLID LUBRICANTS OF RAW RUBBER

Treatment	Friction coefficient	Observations
Slight dusting with talc	1.7 - 2.4	Rubber slightly scuffed
— water added	0.01	No surface damage to rubber
Liberal amount of talc	0.01	No damage
Magnesium oxide powder	1.7 - 2.3	Rubber surface damaged
Surface hypochlorination	0.3 - 0.5	Surface whitening
Sulphuric acid attack	0.4 - 0.5	but no gross changes

SMR CV hemisphere (R = 21 mm) sliding at 0.25 mms^{-1} against smooth 'Perspex' under 1.57 N load, temp. 23°C-28°C, RH = 55%-80%

surface of a raw rubber hemisphere was treated with 0.3% aqueous chlorine solution (3 min immersion; followed by neutralisation with 10% ammonia, washed and dried). After chlorination the surface was slightly whitish. The dry sliding friction against smooth 'Perspex' was now *(Table 2)* nearly an order of magnitude less than an 'as moulded' hemisphere, and there was no surface damage done to it. Another hemisphere was treated with a few drops of concentrated sulphuric acid (3 min; then ammonia neutralised, washed and dried). Again a whitish surface resulted and the sliding friction was much reduced *(Table 2)*. Sulphuric acid is used in industry to reduce the tack of unvulcanised rubber. The reduction in friction by both chemical agents is probably mainly brought about due to physical roughening of the rubber surface.

A comparison of the changes in surface texture due to chlorination for different immersion times *(Figure 1)* was made by Scanning Electron Microscopy (SEM). At 2 min immersion a 'domain' pattern becomes etched into the surface on a scale of 5-10 μm. After 8 min the domains are lost and a 1 μm particulate structure prevails. These textural changes will lead to a loss of real contact against a hard substrate (track). They explain why the friction falls, without having to invoke uncertain arguments about the changed chemical state of a treated surface. A further feature of chemical treatment is a hardened skin. The higher surface elastic modulus means less material comes into real contact with the substrate for a given applied load. Thus two factors, surface roughness and hardness, conspire to reduce tack and sliding friction as a consequence of surface chlorination.

Sulphuric acid treatment also renders the rubber surface more rough and hard. SEM analysis, however, shows in this case no domain structure. After 3 min contact with sulphuric acid a fine particulate texture appears, on a scale 0.1 - 0.2 μm. Again, it is clear why the friction falls with this treatment.

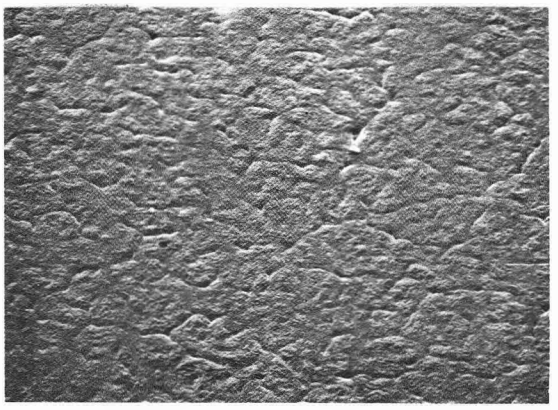
Electron probe analysis of chlorinated SMR CV surfaces was carried out, using chlorobutyl rubber as a standard. After 2 min immersion in chlorinating solution it was found that approximately 2.2% Cl was retained in the SMR surfaces; at 4 min and 8 min the levels were 3.3% and 5.2%. It was discovered that by fracturing rubber samples cooled in liquid nitrogen, a sufficiently clean fracture could be obtained to allow a cross-sectional chlorine profile to be made. The chlorine penetration depth for a few minutes immersion in chlorination solution was typically 25 μm.

A similar electron probe procedure using a sulphur standard indicated that after 3 min contact with sulphuric acid about 2% S was retained in an SMR CV surface.

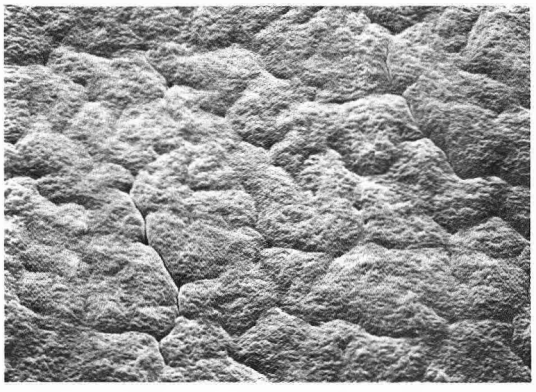
Lubrication by Soap

Different amounts of the detergent 'Teepol' (mixture of soap, the main one being sodium dodecyl sulphate) were deposited in different

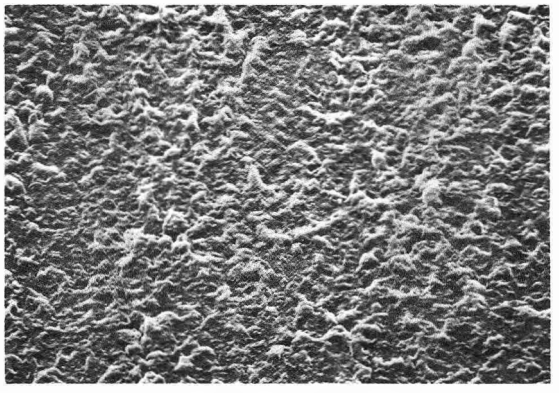
Untreated



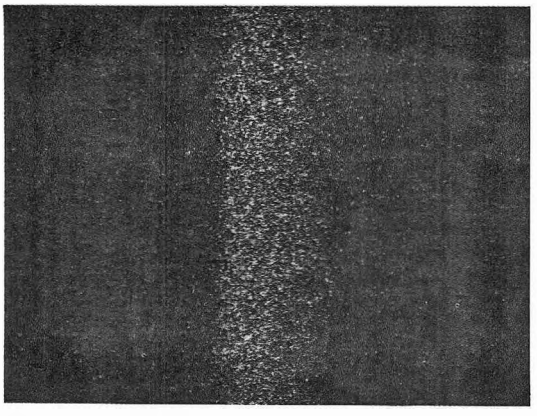
2 min immersion



4 min immersion

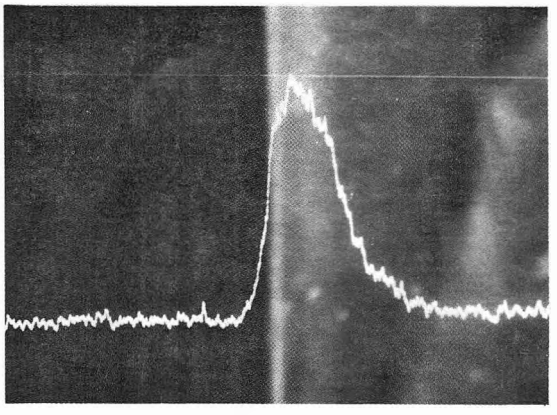


8 min immersion



Cl x-ray map

2 min immersion



Cl level

2 min immersion

Figure 1. Surface of SMR CV after chlorination. Magnification: 800 ×.

ways on the 'Perspex' track and the lubricating effect observed *(Table 3)*. The results demonstrate that Teepol can be a most effective lubricant. In the presence of Teepol, sliding damage to the rubber surface was usually reduced to a negligible amount, although for dilute aqueous solutions a certain conditioning time appeared to be necessary to achieve the lowest friction with least damage.

In rubber processing, stearic acid and stearates function mechanically as lubricants to reduce die drag, aid milling, act as mould release agents and to improve knitting. In the present tests, they were deposited as thick films from alcohol onto the 'Perspex' track and the friction of a raw rubber hemisphere measured in their presence. As dry boundary lubricants at room temperature, they just about halve the friction compared to clean surfaces, but when water was added to the stearates they lubricated more effectively, presumably due to the action of an aqueous soap film despite their sparing solubility in water. The ammonium salt is more soluble and gave the lowest friction when water was added.

In view of the good lubricating action of the detergent 'Teepol' the action of a non-ionic surfactant was also examined. A drop of 'Triton X-100' (iso-octylphenoxy polyethoxyethanol) was added to 20 ml of water and the solution spread on the 'Perspex' track. A rubber hemisphere (SMR CV) was drawn over the track at 0.25 mms^{-1} under 1.57 N load. There was no damage to the rubber surface and the friction coefficient was 0.01 or less, no conditioning time being required to reach this low friction. After the test, the sliding surfaces were thoroughly rinsed with water, the rubber cleaned with acetone and then the friction was re-measured with distilled water as lubricant. The friction coefficient was 0.7 - 1.6 (stickslip), suggesting that most of the Triton X-100 had been removed. Clearly, the Triton is a good lubricant of raw natural rubber and it appears to act immediately.

Action of Various Liquids

Raw rubber hemispheres were pulled across the 'Perspex' track in the presence of liberal amounts of various liquids at room temperature. Friction values varied widely *(Table 4)*. Both water and glycerol appeared to be poor boundary lubricants, as judged by the high 'stick' value of the sliding stickslip motion. During the slip, the friction fell towards zero. The impression gained was that the rubber moved rapidly (~ 1 ms^{-1}) when slipping and at this moment both liquids were good hydrodynamic lubricants. The slightly lower stiction

TABLE 3. LUBRICATION OF SMR CV BY SOAP

Track treatment	Friction coefficient	Observations
16% aqueous Teepol	0.01 (av)	2 min conditioning time
Thin smear	0.1 (av)	Friction falls with Teepol pickup
Thin 'invisible' film	0.4 - 1.8	Surprisingly effective
Ammonium stearate film	1.7 - 2.0	Some damage to rubber
— water added	0.06	No damage
Zinc stearate film	1.3 - 2.0	Rubber surface damage
— water added	0.6	Only slight scuffing
Stearic acid film	1.5 - 2.2	Rubber surface damage
— water added	0.8 - 1.1	Stickslip and scuffing
Clean, dry surfaces	> 3	Gross damage to rubber (ridge deformed)

Rubber hemisphere (R = 21 mm) against 'Perspex' track; normal load 1.57 N, temp. = 24°C, sliding speed 0.25 mms^{-1}

TABLE 4. LUBRICATION BY VARIOUS LIQUIDS

Liquid	Friction coefficient	Observations
Distilled water	2.7 (max)	Severe stickslip, rubber scuffed
Glycerol	2.1 (max)	Stickslip
Ethanol	1.2	Steady sliding, no marked damage to rubber surface
Isopropanol	1.5	
Dutrex oil	2.1	
Viscous silicone	0.03 - 0.06	Micro-stickslip
Silicone emulsion	1.9 - 2.4 (max)	Severe stickslip

SMR CV hemisphere (R = 21 mm) sliding at 0.25 mm^{-1} on smooth 'Perspex' track under 1.57 N load, temp. 23°C-24°C, RH = 55%-65%

value for the glycerol may reflect its lower surface tension (63 mJm^{-1} at 25°C).

Ethanol and propanol were better lubricants and this may in part be due to their low liquid surface tensions (21 mJm^{-1} - 22 mJm^{-1}). With both, the sliding motion was steady. The slightly higher friction with isopropanol may be due to its viscosity being twice that of ethanol. Dutrex oil is more viscous and an even higher friction was observed.

Two types of mould release agent were examined, one consisting of a viscous silicone fluid dissolved in a volatile solvent (Bomb-Lube, Addison Chemical) and the other a silicone fluid-in-water emulsion (Shell Chemicals). With the first type the volatile solvent was allowed to evaporate and then the 'Perspex' track was lubricated with the residue; with the second type the track was directly lubricated with the emulsion. Both were tested at room temperature and the results were very different. With the first, the silicone residue was an effective lubricant, but the second showed the same behaviour as lubrication by distilled water, though stiction was less severe. If the second type was used in practice on a hot mould, water would evaporate off and its lubrication behaviour would approach that of the first type.

Aqueous Electrolytes

Various investigations[2-4] have shown that salt solutions can lubricate vulcanised rubber better than distilled water, but the observations do not support any single concept as to why this is so. The effect on *raw* rubber friction was tested by sliding hemispheres of unvulcanised rubber (SMR CV) over the 'Perspex' track flooded in turn with different salt solutions in water. The investigation also encompassed their effectiveness for rubber-rubber contact. The results *(Table 5)* show an effect of both the actual salt employed and the nature of the contact surfaces. Further data for rubber-'Perspex' contact indicates an effect of concentration *(Figure 2)*.

Lipids and Proteins

There is ample evidence[5] of the presence of lipids and proteins in the surface layer around rubber particles in fresh field latex. The following experiments were performed to examine their possible role in lubrication mechanisms.

The polar lipid lecithin (phosphatidyl choline) is believed to be present around particles of rubber in latex. For our experiments lecithin from egg yoke was used (BDH, 33%). Chemical analysis of the sample showed it to be partially hydrolysed, a variety of phospholipids being present e.g. phosphatidyl choline, phosphatidyl serine, phosphatidyl inositol, and phosphotidic acid. A dilute ethanol solution (>0.5%) was prepared from which a thin lipid film was deposited onto the 'Perspex' track. This cast

TABLE 5. LUBRICATION BY AQUEOUS SALT SOLUTIONS

Lubricant	Friction coefficients	
	'Perspex'/rubber	Rubber/rubber
Dry surfaces	4.1	10
Distilled water	2.6	7.4
Sodium nitrite	2.6	7.3
Sodium sulphite	2.1	4.9
Sodium chloride	1.5	4.6
Sodium carbonate	1.1	2.1

Normal load 3.43 N, sliding speed 0.2 mms^{-2}, temp. = 22°C–24°C, RH = 55%–65%. SMR CV hemisphere (R = 21 mm) slid on smooth 'Perspex' track or on smooth sheet of SMR CV (5 mm thick) supported on the 'Perspex' track. All salt solutions were 0.1*M* concentration. Stickslip motion tended to occur and maximum friction coefficients are quoted.

film consisted of both neutral and polar lipids on the approximate ratic 10:1 respectively. Neutral lipids present were monoglyceride, diglyceride, triglyceride, free fatty acids and sterols. A raw rubber hemisphere (SMR CV) was drawn over the cast film when dry. A friction coefficient value of 1.8 was typical; no ridge formed on the rubber but there was some slight surface damage. A plough mark was left in the cast lipid film and some of the lipid became transferred onto the rubber surface. When the track was flooded with distilled water

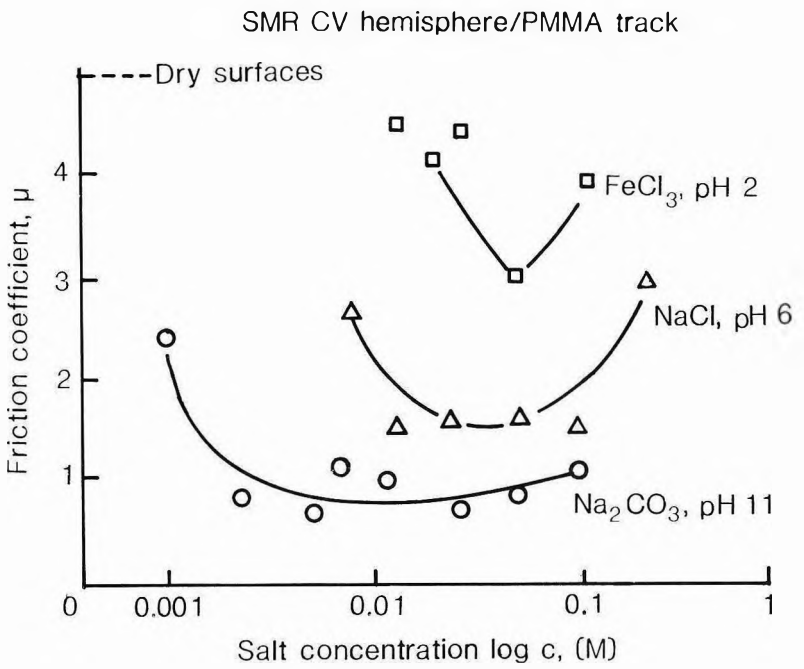


Figure 2. Effect of salt solutions on friction. (Sample was acetone-extracted SMR CV whose dry friction coefficient was 5.1.)

the friction fell considerably, so suggesting enhanced lubricating action in the presence of water *(Table 6)*.

The neutral lipid, tristearin (99% pure sample) was friction tested in a similar manner. A film of it was cast from hexane solution onto the 'Perspex' track, the film obtained being powdery. In dry sliding contact with a raw rubber hemisphere the friction was rather high, there was transfer of the tristearin onto the rubber but no apparent damage to its surface. When the track was flooded with water the friction fell to a low value, although the water did not wet (water beads) the tristearin coated track *(Table 6)*.

A sample of freeze-dried C serum* was made up to a 6% by weight aqueous solution and this was spread on the 'Perspex' track. A rubber hemisphere (SMR CV) was drawn over the track. The friction coefficient varied around 0.9 and the rubber surface was slightly damaged. For comparison the dry friction coefficient for clean dry surfaces was greater than 3, and so the serum appeared to provide a degree of modest lubrication.

Surface protein can be extracted off rubber particles. A solid sample was made up into aqueous solution by dissolving 10 mg in 2 cc of borate buffer solution (pH = 8.9). The 'Perspex' track was lubricated with the aqueous solution and a rubber hemisphere (SMR CV) drawn over it. On the first pass, the friction coefficient was 0.9, but on the second pass made a few minutes later the coefficient was 0.006. This suggests a conditioning effect; presumably the protein requires time to absorb to the sliding surfaces. The friction coefficient with borate buffer alone was 1.7. Thus it would appear to be the protein (or any contaminants it may contain) that provides for good lubrication.

For comparison, another protein solution was used as lubricant. The 'Perspex' track was flooded with a 1% aqueous solution of casein and the rubber hemisphere drawn over it as before. On the first run the friction coefficient was 0.31 - 0.28 (falling with increasing displacement), and by the third run it had steadied to 0.18. There was no surface damage to the rubber. It appears that this protein can also act as a lubricant in aqueous solution, though it is not as effective as the rubber particle protein solution.

DISCUSSION

The foregoing experiments represent an attempt to explore broadly how the friction of raw natural rubber is altered, if at all, by the presence of different liquids and solids that

TABLE 6. LUBRICATION BY LIPIDS AND PROTEINS

Material	Dry friction coefficient	Wet friction coefficient
Mixed lipids Neutral lipid/Polar lipid 10:1	1.6 - 2.5	0.3 - 0.4
Tristearin	1.1 - 1.7	0.2
NR latex C serum		0.7 - 1.1
NR surface protein		0.006
Casein		0.18

SMR CV hemisphere (R = 21 mm) sliding at 0.2 mms^{-1} on smooth 'Perspex' track under 1.57 N load, temp. 24°C-26°C, RH = 60%-70%

*The ambient serum around the rubber particles in latex that contains 15% or more of protein plus smaller amounts of carbohydrates and organic acids.

might be expected to act as lubricants. 'Soapy' liquids and 'flakey' solids gave the most dramatic results. Detergent solutions, such as sodium dodecyl sulphate, were able to bring about a hundred-fold drop in the level of friction, as was talcum powder; the former probably because of boundary action[2] and the latter due to its solid lamella structure.

Less spectacular changes were brought about by contaminant films of organic substances. For example, at room temperature a film of stearic acid could barely halve the value of the dry friction. Stearates were a little more effective, particularly in the presence of water if they were sparingly soluble. Presumably, this is due to boundary action akin to aqueous detergent films, such as electric double-layer repulsion and, or, steric hindrance. This may be compared with distilled water that is a poor lubricant at low sliding speeds, though improved by dissolving certain salts in the water. Any trapped water film in the contact zone probably collapses rapidly, so leading to high interface adhesion and friction. Some salts and soap can help to resist the collapse. The lubricating ability of hydrocarbon liquids was not much better than that of water, any improvement probably reflecting a lower surface tension or higher bulk viscosity.

An intriguing aspect of lubrication by aqueous salt solutions was the change in efficacity with the actual salt employed, and with its concentration. It has been observed in studies of the lubrication of fully vulcanised rubber that some salt solutions can reduce the friction[2-4], and this may be due to the creation of an electrical double-layer on both contact surfaces. The mutual electrostatic repulsion is enough to reduce the friction. Detailed interpretation is complicated by the surface characteristics of the contacting bodies. Any mutual repulsion (or attraction) can in part arise from chemical changes at the surfaces, in addition to any adsorbed layer of charge.

The most effective salt lubricants of raw natural rubber were found to be solutions of sodium carbonate and bicarbonate. These are alkaline and one may speculate that if residues in the contacting surfaces are involved in the lubrication mechanism, then conditions that favour enhanced charge repulsion due to them are more likely to reduce the surface friction. At high pH the protein residues in the rubber are in the charged form $H_2N.Pr.COO^-$. Furthermore, hydroxyl ions may act on the 'Perspex' surface to give the charged species $CH_2\ CH_3\ C\ COO^-$.

Support for these charge effects comes from experiments *(Figure 2)* indicating that the friction falls as the pH of a salt solution increases.

The various friction tests carried out on lipids and proteins suggest that they act, in varying degrees, as boundary lubricants of raw rubber. In particular, the protein surrounding rubber particles appears most effective and this may relate to the mechanical stability of latex.

CONCLUSION

Lubrication studies of raw rubber indicate similar trends as for vulcanised rubber. Aqueous soap solutions and certain proteins are excellent boundary lubricants, probably on account of electrical double-layer repulsion and protective adsorbed layers. Liquids containing few or no free ions for example, water, are less effective. Alcohol is a little better and this may be due to its lower surface tension. Oils appear to depend upon viscosity and low surface tension for their lubricating effect. Solid lubricants, such as zinc stearate or talc, act to reduce the adhesion between raw rubber and track so that the friction can be as low as with soap solution. Poor lubricants result in rather more surface damage to raw rubber than would occur to a vulcanisate.

ACKNOWLEDGEMENTS

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Molecular Mobility in Rubbers

GEOFFREY ALLEN*

Neutron beams can be obtained in pulsed beams of virtually monochromatic character at wavelengths of about 5 Å. The momentum associated with the beam is high and the energy low. Thus neutrons are ideal for studying the diffusional motions of liquids in scattering experiments. In the past ten years, neutron scattering has been used to determine the average dimensions of individual chains in bulk rubbers and plastics, to study the dynamics of polymer chains in raw rubbers and melts and cross-linked networks and to measure the rotation of side groups. Results show that the Rouse model of molecular chain dynamics is more appropriate than the Zimm model for rubber. In networks, the sections of chain between cross-links form essentially the same chain motion as in the raw rubber, but the junction points move more slowly. The energy barrier to internal rotation of the methyl side groups in polypropylene oxide is found to be approximately 15 kJ mol^{-1} in line with the expected value.

The essential properties of rubbers of high extensibility and rapid recovery have their origins in the long-chain structure of the polymer molecules and the fact that each molecule is undergoing rapid changes in shape by virtue of internal rotations about the σ-bonds in the long chain. The chemical process of cross-linking enhances the retention of the strained and unstrained shapes of the rubber without seriously impairing its ability to undergo rapid, large deformations. This is because the chains are chemically linked at infrequent intervals to form a three-dimensional network within which the long segments of chains between cross-links are still able to perform rapid changes in conformation. If as in natural rubber, the rubber molecule has side groups ($-CH_3$ in this case), these groups undergo internal rotation about the C-C bond relative to the main chain in addition to the main-chain wriggling. When a rubber is cooled to a glass or crystalline material, the main-chain wriggling is frozen out in the glass or crystalline domains, however the internal rotation of the side groups usually continues down to much lower temperatures.

A variety of spectroscopic and scattering techniques have been used to investigate molecular motion in rubbers and to support the rotational isomeric model of the polymer chain which is central to molecular theories of polymeric materials. X-ray scattering has contributed to our understanding of the molecular structure in crystalline rubbers. Light scattering has been used to measure the diffusion of the centre of mass of individual polymer molecules in dilute solutions. In the past ten years, using neutron scattering, it has been possible to determine the average dimensions of individual chains in bulk rubbers and plastics, to study the dynamics of polymer chains in raw rubbers and melts and cross-linked materials and to measure the rotation of side groups. All these measurements are possible because of the unique nature of neutron scattering from molecules.

Neutron Scattering

Intense neutron beams can be obtained from specially designed nuclear reactors. Using crystal filters and mechanical selectors it is possible to isolate pulsed beams of virtually monochromatic character at wavelengths of about 5 Å. The beam is composed of neutrons which have atomic mass 1, zero charge and nuclear spin ½, and velocities of the order of 1000 m per second. Compared with beams of electromagnetic radiation, the momentum

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associated with the beam is high and the energy low. The energy associated with Avogadro's number of neutrons is only 280 joules.

Thus, neutrons are ideal for studying the diffusional motions of liquids in scattering experiments in which very small amounts of energy are exchanged between these quasi-classical modes of motion and the incident neutron. The scattered neutron may gain or lose energy in the scattering event or may simply be scattered elastically. After scattering its velocity may be increased, reduced or remain unaltered.

Thus, if the energy profile of the neutron beam is measured before and after scattering it will be broader after scattering. The broadening gives a spectrum of the energies of diffusional motion in the sample. This broadening is termed 'quasi-elastic' or Doppler broadening. It is a function of the momentum Q transferred in the scattering event. When the energy exchanged is very small, Q is defined by the angle of scatter:

$$Q = \frac{4\ \pi\ \mathrm{Sin}\ (\theta/2)}{\lambda}$$

where λ is the wavelength.

The experiment is shown schematically in *Figure 1*. The incident and scattered velocities of the neutrons are obtained from the times of flight of the neutrons over fixed distances measured at fixed angles before and after the scattering event. A typical plot of the variation in the breadth of the profile of scattered neutrons is shown in *Figure 2* as a function of the momentum transfer ($\cong$ angle of scatter) Q. The fact that broadening is observed shows that molecular motions are occurring in this polypropylene oxide rubber.

Molecular Motion in Liquids and Rubbers

The Langevin equation can be used to predict the shape of the quasi-elastic broadening. For simple liquids such as water and benzene where the molecules diffuse as single units, the shape

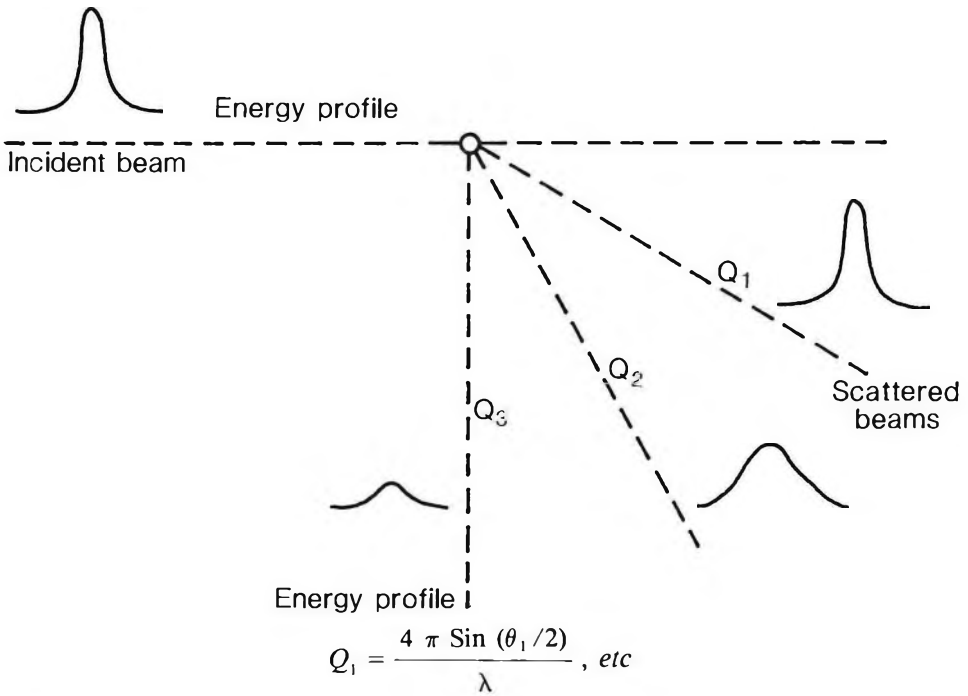

Figure 1. Quasi-elastic neutron scattering experiment showing Doppler broadening of the energy profile.

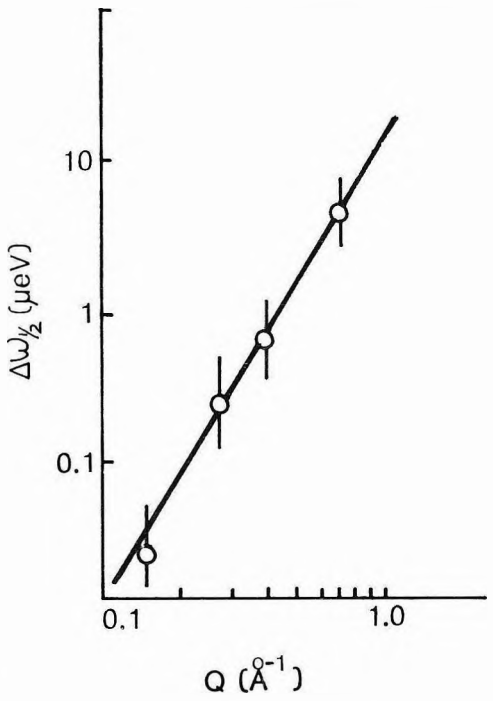

Figure 2. Quasi-elastic (Doppler) broadening plotted as a function of Q *for polypropylene oxide* $-CH_2-CH(CH_3)-O-$.

of the quasi-elastic (Doppler) broadening is predicted to be Lorenzian with:

$$\text{width of broadening } \alpha Q^2.$$

The broadening is measured as the increase in width at half the height of the peak, in units of micro-electron volts μeV.

For a polymer chain with no hydrodynamic interactions (*i.e.* the Rouse model), for example in a polymer melt of rubber where the chains are undergoing self-diffusion, (the wriggling motion is coupled with the centre of mass motion) the broadening is similar but now:

$$\text{width of broadening } \alpha Q^4.$$

For a polymer chain subjected to hydrodynamic interaction as in a polymer solution, where the polymer chain motion is subject to interaction in the solvent molecules:

$$\text{width of broadening } \alpha Q^3.$$

Experimental comparison of the three laws is given in *Figure 3* for three different samples — a liquid, a rubber and a polymer solution. In each experiment the scattering intensity is normalised to the same number of scattering units. The results largely confirm theoretical prediction of the variation of broadening with Q for the three different systems. Of course, in a polymer the diffusion process is observed only in the melt or rubber phase or in solution. In the glass the motion is frozen out, so no broadening is observed.

Having established that the broadening is approximately proportional to Q^4 for polymer melts, we can now compare the scattering from different rubbers or polymer melts at the same temperature.

At a given temperature, the higher the frequency of diffusional motion the larger the energy exchange, and hence the larger is the broadening of the quasi-elastic peak. Thus flexible chains, because they will have higher frequencies of motion, will be expected to give larger broadening at a given temperature and angle of scatter than will stiff chains. High molecular weight polymers are used in these experiments and the chain motions are being observed over distances of less than 30 Å at these wavelengths and Q values. *Figure 4* shows logarithmic plots of broadening against momentum transfer Q for a group of four polymers in the melt.

From measurements of the kind summarised in *Figures 2* and *3* we learn two facts. Firstly, the polymers are all closer to the Rouse model in the melt than to the Zimm model. Secondly, the order of chain flexibility as judged from a comparison of the Doppler broadening at the same temperature and Q value is:

Polydimethylsiloxane > polytetrahydrofuran ≃ polypropylene oxide > polyisobutene

i.e. $-O-Si(CH_3)_2 > -CH_2-CH_2\ -CH_2\ -CH_2-O- \simeq -CH_2\ -CH_2\ -O- > -CH_2(CH_3)_2$

Thus, we have shown that the Rouse model of molecular chain dynamics (*i.e.* chain flexibility) is more appropriate than the Zimm model for

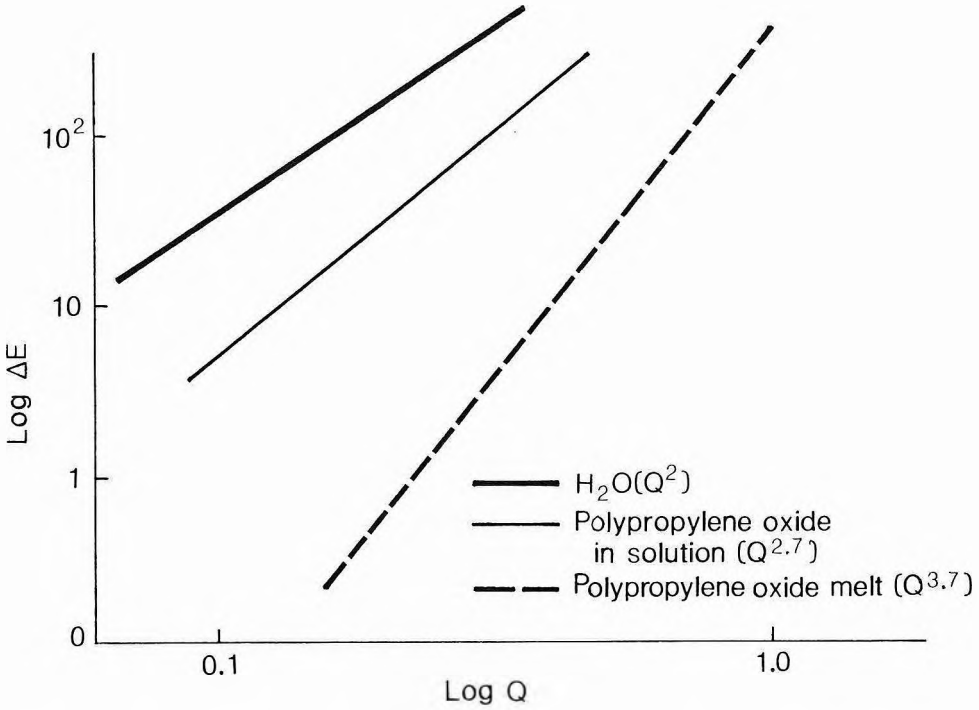

Figure 3. Comparison of the scattering laws for $H_2O(Q^2)$, polypropylene oxide in solution ($Q^{2.7}$) and polypropylene oxide melt ($Q^{3.7}$).

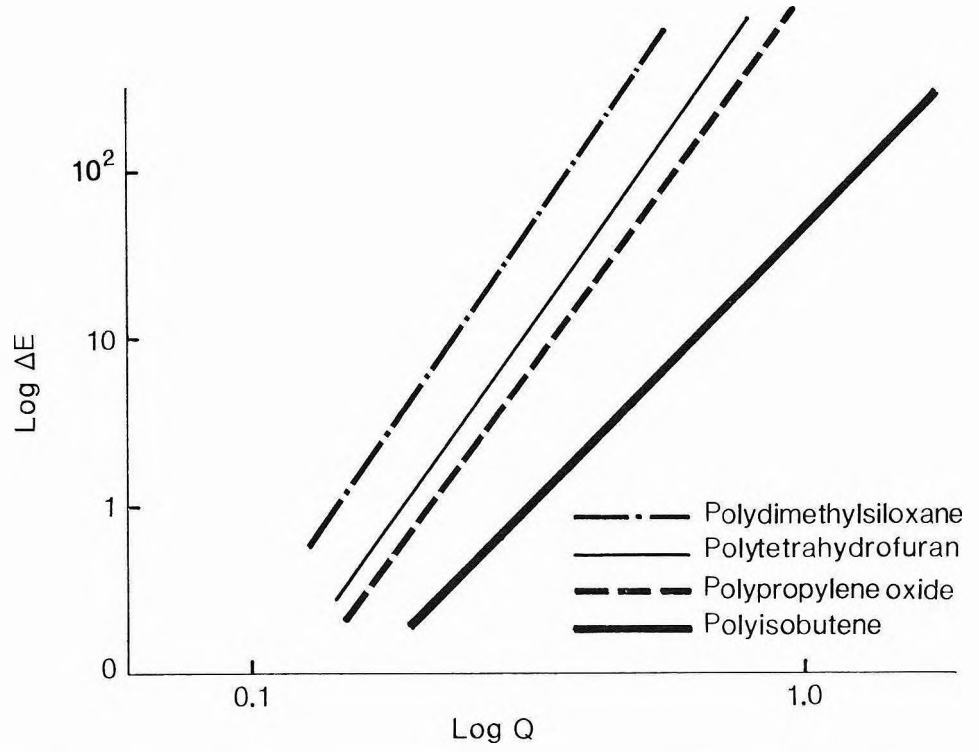

Figure 4. Quasi-elastic (Doppler) broadening plots for four polymer melts: polydimethylsiloxane, polytetrahydrofuran, polypropylene oxide and polyisobutene.

rubber and the four polymers whose chain flexibilities have been compared by quasi-elastic neutron scattering are placed in order of decreasing chain flexibility.

Molecular Motion in Networks

Recently one of my colleagues[2] studied quasi-elastic broadening of neutron scattering from polypropylene oxide networks and compared the results with scattering measurements from partially deuterated networks in which hydrogen was located only around the network function points as in *Figure 5*.

The networks were made by reacting polypropylene oxide diol of molecular weight 2000 with:

$$C_2H_5C(CH_2\text{-}O\text{-}CH_2CH_2\text{-}COCl)_3$$

For the partially deuterated network perdeuteropolypropylene oxide diol was used.

In these samples, the observed neutron scattering is dominated by scattering from the hydrogenated parts of the network. In the partly deuterated sample scattering from the deuterated chains is virtually invisible and the broadening is determined by the motion of the cross-links of the networks since they are hydrogenated. The results are shown in *Figure 6*. The lower line represents the broadening observed in the partially deuterated sample. Thus, it can be deduced that the cross-links are moving more slowly than the chains between cross-links because the partly hydrogenated sample shows greater broadening under the same conditions of Q and temperature. If the two sets of scattering data are subtracted from each other, the residues give the broadening as a function of Q arising from the chain segments *between* cross-links.

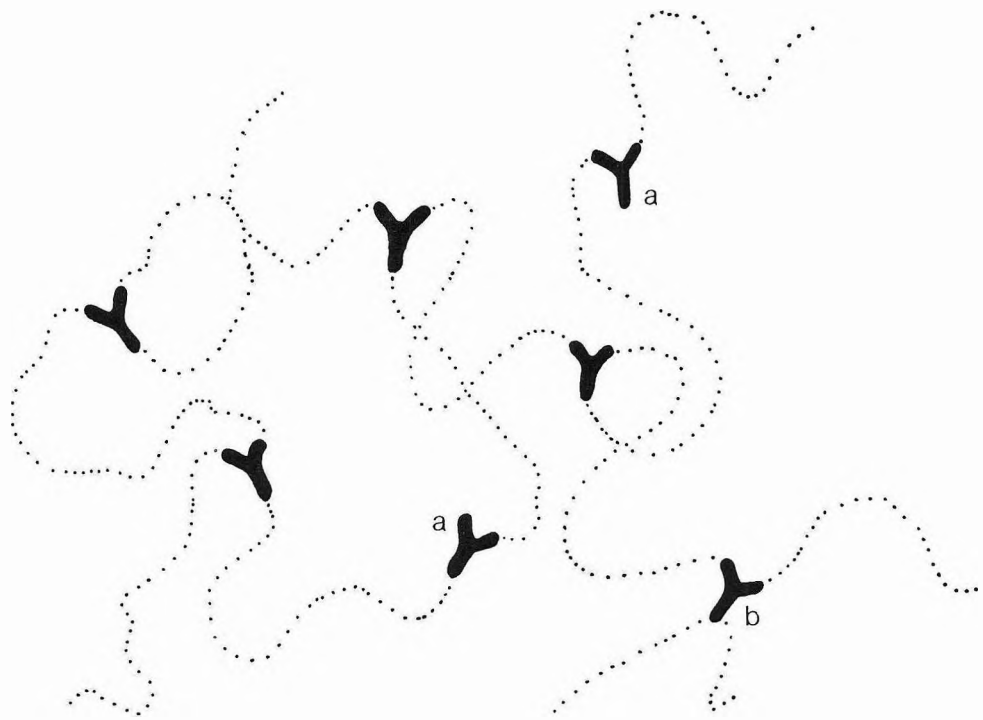

Figure 5. Diagrammatic representation of network showing some incomplete junctions.

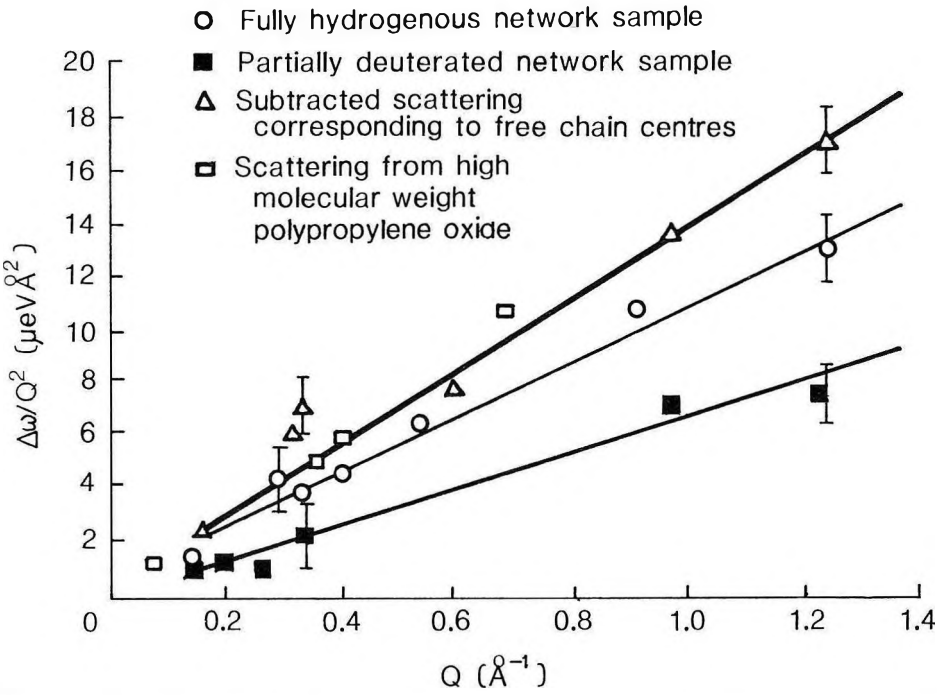

Figure 6. $\Delta\omega/Q^2$ against Q for scattering from a fully hydrogenous network sample, a partially deuterated network sample, subtracted scattering corresponding to free chain centres and scattering from high molecular-weight polypropylene oxide. The lines are guides to the eye.

These are the points on the upper line in *Figure 6*. Furthermore, these points lie on the same line representing the broadening from the uncross-linked rubber. Thus, we arrive at the conclusion that in a network the sections of chain between cross-links form essentially the *same* chain motion as in the raw rubber, but the junction points move more slowly. The timescales differ by about a factor of 2. However, we can begin to build up a physical picture of the dynamics of this network.

Side Chain Motions

The same method of quasi-elastic neutron scattering can be used[3] to study the motion of side groups. This has also been done for polypropylene oxide rubber. However, because the side groups rotate so rapidly and because their broadening contribution is convoluted into the main chain broadening, the rubber has to be cooled below *Tg*. This freezes out the main chain broadening because the main chain motion is frozen out. Ultimately, a very small broadening component can be seen *(Figure 7)* in the wings of the elastic peak. It is just within the experimental limits of the present instruments. The fact that this broadening originates from $-CH_3$ motion can be seen from the comparison with $-CD_3$ shown in *Figure 7*.

Whereas the main chain broadening is a function of Q ($\sim Q^4$) the side chain quasi-elastic broadening is *independent* of Q. This is because it arises from rotational diffusion of the CH_3 group as it rotates around a -C-C- bond fixed in space. The main chain motion is self-diffusional and the scattering centres move randomly through a relatively large volume of space. The temperature dependence of the rotational broadening gives an order of magnitude of the energy barrier linking rotation. For this CH_3 group in polypropylene

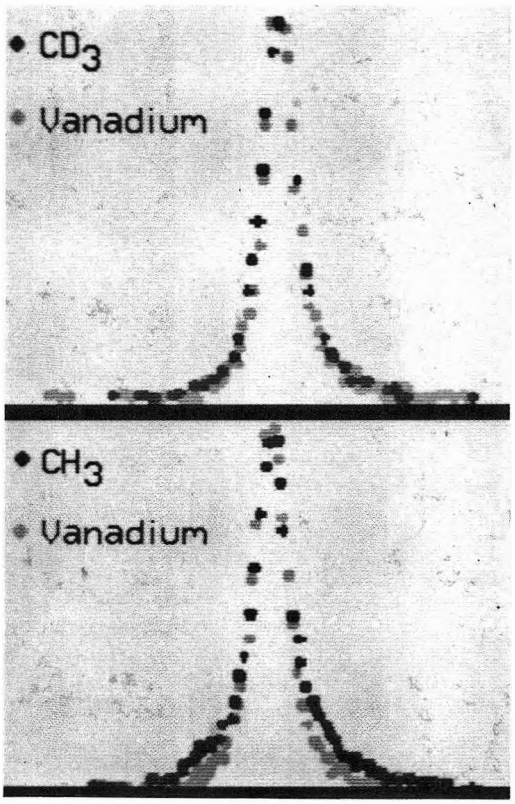

Figure 7. Comparison of the quasi-elastic scattering from polypropylene oxide (CD_2–$C(CH_3)$ D–O–) and (CH_2–$C(CD_3)$H–O)$_n$ at 173 K and Q = 1.62. Also shown is the instrumental resolution represented as the scattering from vanadium.

oxide it is approximately 15 kJ mol^{-1} in line with what would be expected from the spectroscopic value of the barrier to internal rotation.

SUMMARY

Using neutron scattering we can support the current models for molecular motion in raw and cross-linked rubbers. As the technique improves more quantitative values will be ascribed to the various forms of motion.

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Storage Hardening of Natural Rubber I. Effect of Epoxide Groups

A. SUBRAMANIAM* AND WONG WAI SUIN**

A small number of epoxide groups were introduced onto purified natural rubber and synthetic cis-*1,4-polyisoprene by reacting the rubbers with* m-*chloroperbenzoic acid. The rubbers were then subjected to the conditions of the accelerated storage hardening test in the presence of an amino acid (glycine). Results showed that epoxide groups made no contribution to storage hardening and are therefore not responsible for storage hardening of natural rubber.*

When natural rubber is stored at room temperature, it tends to harden, *i.e.* its viscosity increases. This phenomenon of storage hardening can be inhibited almost completely by reacting the rubber with hydroxylamine or its salts during production, as in the case of constant viscosity or CV rubber. While the technological problem has been solved, the actual reason for storage hardening is still not fully understood.

Three hypotheses have been put forward to explain storage hardening. In the first hypothesis[1-4], it is suggested that a small number of carbonyl groups — probably aldehydic — are present on the rubber molecule and that these crosslink with the amino acids and proteins present in the non-rubber fraction. In the second hypothesis[5], it is suggested that storage hardening is due to epoxide groups in natural rubber while the third hypothesis[6] considers the functional groups to be ester groups.

One method of resolving the problem is to actually introduce a small number of these functional groups onto the *cis*-1,4-polyisoprene molecule and study whether the product will show the same behaviour as natural rubber under conditions favourable for storage hardening. The introduction of epoxide groups onto *cis*-1,4-polyisoprene is relatively easy.

The work reported here involves the introduction of a small number of epoxide groups onto synthetic *cis*-1,4-polyisoprene and purified natural rubber and the determination of their degree of storage hardening.

EXPERIMENTAL

Preparation of Epoxidised Synthetic *cis*-1,4-polyisoprene

A sample of synthetic *cis*-1,4-polyisoprene (150 g) with a high *cis* content [Ameripol (Goodrich) or Natsyn 2200 (Goodyear)] was dissolved with constant shaking and stirring in toluene (3 litres) to give a 5% (weight/volume) solution. To this was added *m*-chloroperbenzoic acid (1.5 g to 13.5 g, depending on the level of epoxidation required) dissolved in a minimum volume of toluene. The reaction mixture was stirred with a magnetic stirrer for 4 h and the epoxidised rubber was precipitated from solution by pouring into an equal volume of methanol. The rubber thus collected was washed well with methanol and dried under reduced pressure.

Preparation of Purified Natural Rubber

Fresh latex was ultracentrifuged at 50 000 g for 1 h. The cream fraction was collected and dispersed in a solution containing sodium dodecyl sulphate (1% by weight on dry rubber content). The dispersion was filtered through muslin cloth and diluted to the original volume of fresh latex used before being ultra-

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centrifuged again for half an hour. The cream was then dispersed in water and recentrifuged and this procedure was repeated once more.

The final purified rubber latex dispersion was precipitated in ethanol and the rubber was extracted with hot ethanol for 16 h under nitrogen. After drying in vacuum, the rubber was left to stand in an excess of *n*-hexane without agitation. After two days, the solution was carefully poured out and dried by filming. More hexane was added to the rubber and the procedure was repeated several times until most of the rubber had gone into solution.

By this procedure, a highly purified gel-free natural rubber sample was obtained.

Preparation of Epoxidised Purified Natural Rubber

The purified natural rubber collected above was epoxidised as described above for synthetic *cis*-1,4-polyisoprene, except for the following changes:

- A lower quantity (10–20 g) of rubber was used for each preparation, with the concentration of *m*-chloroperbenzoic acid adjusted accordingly.
- The concentration of the rubber solution was 2.5% (weight/volume).
- The solution was left to epoxidise overnight.

Chemical Determination of Epoxide Content

The level of epoxidation in the rubber was determined by titration using both the method described in the British Standards[7] as well as the method of Durbetaki[8] which was used by Burfield[9] and Lee[10].

Determination of Storage Hardening

A weighed amount of glycine was dissolved in the minimum amount of water (a few drops) and the solution was dispersed in 20 ml of toluene containing 2 mg of antioxidant (Antioxidant 2246). Epoxidised *cis*-1,4-polyisoprene or purified natural rubber (10 g) was cut into small pieces and added to the toluene with vigorous shaking. The rubber quickly absorbed the solvent. The swollen rubber was stirred thoroughly to disperse the glycine. The swollen rubber was then pressed into a thin film, dried in air and finally in vacuum to remove the solvent. The dry rubber was passed six times through the narrow gap of a two-roll mill. Traces of glycine left in the container were added back to the rubber at this stage. Several Wallace pallets cut from the rubber sheet were heated for 24 h at 60°C over phosphorous pentoxide in vacuum. The test was similar to the accelerated storage hardening test for natural rubber (ASHT) except that it was done in vacuum. The Wallace plasticity values were measured before (P_O) and after (P_H) heating. The difference ($\Delta P = P_H - P_O$) was taken as the extent of hardening.

RESULTS AND DISCUSSION

The epoxidation of olefinic compounds by *m*-chloroperbenzoic acid is known to take place readily at or below room temperature with efficiencies greater than 90%. The theoretical values for the level of epoxidation calculated from the amount of *m*-chloroperbenzoic acid added are shown in *Table 1*. As mentioned in an earlier paper[11], the determination of epoxide groups by titration (both the British Standard and Durbetaki methods) gave values much lower than the theoretical values. These values measured by the British Standard method are shown in *Table 1* for both *cis*-1, 4-polyisoprene and purified natural rubber.

Based on the number average molecular weight, $\overline{M}n$, of 300 000 for the rubbers the theoretical levels of epoxidation are equivalent to 17, 52, 105 and 157 epoxide groups per molecule at concentrations of *m*-chloroperbenzoic acid of 10, 30, 60 and 90 mg per gramme, respectively. For *cis*-1,4-polyisoprene, the measured values are equivalent to 13, 40, 75 and 110 groups per molecule and for purified natural rubber, 8, 12, 57 and 101 groups per molecule, respectively.

Burfield and Gan[12] have reported that the level of epoxide groups naturally present in different clonal rubbers range from 46 to 111. Thus the number of epoxide groups introduced

TABLE 1. CALCULATED AND MEASURED VALUES OF EPOXIDATION LEVEL

Concentration of m-chloroperbenzoic acid (mg/g rubber)	Calculated value of epoxide content (mole per cent)	Measured value (mole per cent)	
		Polyisoprene	Purified natural rubber
10	0.39	0.3	0.18
30	1.18	0.9	0.27
60	2.37	1.7	1.3
90	3.55	2.5	2.3

into *cis*-1,4-polyisoprene in the present experiments is not dissimilar to the number of epoxide groups reported in NR. Therefore the discrepancy between the measured and calculated values is not important in the discussion of the storage hardening results.

The present experiments are based on the fact that highly purified natural rubber does not undergo storage hardening on its own. However, when an amino acid like glycine is incorporated into the purified rubber, the rubber hardens in the accelerated storage hardening test, just like unpurified natural rubber.

Epoxidised synthetic *cis*-1,4-polyisoprene when reacted with glycine in increasing concentrations and subjected to the accelerated storage hardening test, showed no hardening *(Table 2)*. The epoxidised synthetic *cis*-1,4-polyisoprene behaved exactly as the unepoxidised sample, thus indicating that the epoxide groups did not respond to the accelerated storage hardening test even in the presence of an amino acid.

The P_O and P_H values given in the tables are the averages of three readings. Assuming an error of ± 1 unit per reading, ΔP is expected to have a maximum error of 2 units. Many of the readings in *Table 2* show a negative value for ΔP. This could be expected if traces of *m*-chlorobenzoic acid (product of the epoxidation reaction) left in the rubber caused some oxidation of the rubber, despite the presence of antioxidant.

The results for unepoxidised and epoxidised purified natural rubber are given in *Table 3*. The unepoxidised or epoxidised purified natural rubber showed negligible hardening in the absence of glycine. The small value of ΔP shown could be due to other side reactions but this is small compared to the increase shown in the presence of glycine. In the presence of increasing concentrations of glycine, both unepoxidised and epoxidised purified natural rubber showed considerable hardening (P_H/P_O values were 2 or higher). The epoxidised purified natural rubber actually showed a lower hardening than the unepoxidised purified natural rubber. These results clearly show that the epoxide groups introduced did not contribute to storage hardening.

The lower degree of hardening seen in the epoxidised purified natural rubber could arise, for example, if some of the glycine molecules reacted with the epoxide groups and were therefore not available for storage hardening. Another possibility is that small amounts of epoxide groups could cause storage hardening but large amounts could disturb this reaction. However, the fact that the number of epoxide groups introduced into *cis*-1,4-polyisoprene was similar to the number of epoxide groups found in NR[12], would tend to lessen this possibility.

It is also possible that epoxide groups alone are not sufficient for storage hardening but that some other functional groups in NR are also required for the reaction. However this seems unlikely in view of the fact that epoxidised

TABLE 2. STORAGE HARDENING OF EPOXIDISED SYNTHETIC CIS-1,4-POLYISOPRENE

Concentration of m-chloroperbenzoic acid used (mg/g rubber)	Concentration of glycine (mg/g rubber)	Wallace plasticity, P_O	Wallace plasticity after hardening, P_H	Extent of hardening, ΔP
0[a]	0	44	44	0
	1	42	43	1
	3	40	41	1
	5	41	41	0
	10	41	42	1
10	0	46	37	-9
	1	44	41	-3
	2	45	41	-4
	5	40	37	-3
30	0	43	41	-2
	1	45	44	-1
	2	44	43	-1
	3	42	42	0
	5	44	44	0
	10	44	43	-1
60	0	45	37	-8
	3	43	40	-3
	5	41	38	-3
	10	42	40	-2
90	0	45	37	-8
	3	42	39	-3
	5	40	38	-2

[a]Control — treated like the epoxidised samples except that no m-chloroperbenzoic acid added.

TABLE 3. STORAGE HARDENING OF EPOXIDISED PURIFIED NATURAL RUBBER

Concentration of m-chloroperbenzoic acid used (mg/g rubber)	Concentration of glycine (mg/g rubber)	Wallace plasticity, P_O	Wallace plasticity after hardening, P_H	Extent of hardening, ΔP
0[a]	0	39	42	3
	1	41	74	33
	3	43	87	44
	5	41	86	45
	10	42	86	44
10	0	40	39	- 1
	1	40	62	22
	2	40	70	30
	5	41	85	44
30	0	40	43	3
	1	41	58	17
	2	41	70	29
	5	40	76	36
	10	42	89	47
60	0	41	43	2
	3	42	62	20
	5	46	79	33
	10	45	87	42
90	0	42	47	5
	3	39	52	13
	5	45	84	39
	10	45	87	42
	20	42	88	46

[a]Control

purified natural rubber did not show a higher degree of storage hardening than the unepoxidised sample in the present experiments.

The maximum hardening of the unepoxidised purified natural rubber was observed at a concentration of glycine higher than 27×10^{-3} mole per kilogramme rubber. The concentration of glycine required to give maximum hardening was earlier reported[4] to be 2×10^{-3} to 6×10^{-3} mole per kilogramme rubber. This discrepancy could be due to two reasons. The purified rubber in the earlier experiments was not as pure as in the present work. The glycine was added to the dry rubber in the present work and the homogeneity of mixing would not be as good as the addition in the latex stage as in the earlier work.

The results given in *Tables 2* and *3* were generally true whether the antioxidant was present or not. In the absence of antioxidant, the samples sometimes showed a sticky appearance and reduced P_H values, probably due to oxidation. This was also the reason for carrying out the test in vacuum. Samples hardened in air over phosphorus pentoxide showed lower P_H values.

Since the work was completed, natural rubber samples with much higher epoxidation levels *viz.* 10, 25 and 50 mole per cent have become available. Experiments with these rubbers showed that in the presence of glycine, these rubbers actually hardened less than the control unepoxidised rubber under the conditions used in the accelerated storage hardening test.

From all the above results it is clear that epoxide groups in synthetic polyisoprene or natural rubber do not contribute to storage hardening. It can thus be concluded that, while epoxide groups may be present in natural rubber, they are not responsible for storage hardening.

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Some Considerations in the Design of Natural Rubber Bearings for the Penang Bridge

CHIN FUNG KEE*

Natural rubber bearings have gained widespread application in reinforced concrete and in prestressed concrete bridges[1]. They were installed in the Penang Bridge Project. Certain important findings in regard to the durability and fatigue life of NR for bridge bearings are reviewed in relation to the requirements of the site of the Penang Bridge.

In good design, rubber is normally not used in tension. To ensure that no point in the rubber bearing is subjected to an upward movement and results in hydrostatic tension, rotations are limited by BE 1/76[2] in accordance to which the NR bearings for the Penang Bridge Project were designed.

The most likely stage in which the rubber may be subjected to tensile stresses is in the early period of the construction when the precast prestressed concrete beam is first installed on the top of the supporting bearings. Compressive load-deflection test results of some of the installed bearings are presented. On the basis of these test results and together with the actual deflections of a precast prestressed concrete beam measured at the site, a computation is presented to verify that at no stage in its service life the rubber bearing concerned will be subjected to tensile stress.

Rubber bearings for reinforced and prestressed concrete bridges have gained increasingly widespread use since they were first introduced some three-and-a-half decades ago. They have no mechanical moving parts and do not present corrosion and wearing problems as the traditional steel roller and plate bearings; they are virtually maintenance-free and cost much less than mechanical bearings. Laminated rubber bearings allow not only movement in any horizontal direction but also rotation about any horizontal axis. This flexibility is important for modern bridges where thermal expansion of a wide orthotropic bridge deck does not always result in equal horizontal movements or equal rotations at the ends of every one of the many beams. In regard to noise absorption and vibration isolation, rubber bearings are far superior.

It is not surprising that rubber bearings are now replacing steel bearings. The suspension chain saddles of the Hammersmith Bridge across the River Thames were originally mounted on large diameter steel rollers. About fourteen years ago the bridge was upgraded and the seized steel bearings were replaced with 1.5 m long smaller diameter units. In February 1984, a combination of forces on the bridge made the steel rollers move further than anticipated on their bearing plates and eventually right off their ends. As a result, the deck slumped 60 mm. A computer simulation of the lateral and longitudinal loads indicated that rubber bearings would be most suitable and Hammersmith Bridge was put back into service with the saddles now mounted on permanent rubber bearings[3].

The construction industry in Malaysia is in a special position to take advantage of rubber bearing technology as Malaysia is the world's largest producer of natural rubber and has the technological back-up services of the laboratories and the Technology Centre of the Rubber Research Institute of Malaysia.

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Except at the navigation channel, all the spans of the Penang Bridge Project consist of precast prestressed concrete beams resting on cross-beams using natural rubber bearings. The prestressed concrete beams for the 40 m spans over the sea were designed as simply supported for dead load and continuous over five spans for live load.

Altogether 8846 NR bearings were installed in this project which has 13.5 km of bridging of which 8.4 km are over the sea. The largest bearings were designed for a vertical compressive dead load of 800 kN and for a live load of 400 kN.

The NR bearings were designed in accordance with BE 1/76 the fore-runner of which is UK Ministry of Transport Memorandum No. 802/1962[4] entitled 'Provisional Rules for the Use of Rubber Bearings in Highway Bridges,' both of which are based on the developmental work at the Malaysian Rubber Producers' Research Association. The Rubber Research Institute of Malaysia assisted in the design of NR bearings for the Penang Bridge in accordance with BE 1/76.

DURABILITY OF NATURAL RUBBER BEARINGS

Extensive research on the durability and fatigue life of natural rubber for bridge bearings has been carried out by the Malaysian Rubber Producers' Research Association. Their findings are reviewed here in relation to the requirements of bearings for the Penang Bridge.

Deterioration Due to Environmental Factors

Low temperatures. Most rubbers stiffen at low temperatures. Natural rubber does so less than most materials. It is therefore the preferred material under conditions of low temperatures. In any case temperatures low enough for such crystallisation do not occur at the site of the Penang Bridge.

Oxidation. There is evidence that rubber can survive very long periods in service at ambient temperatures of at least 30°C with no serious deterioration at all. Rubber bearings installed under prestressed concrete beams are sheltered from the direct sun and being in the shade, temperatures high enough for significant age hardening are not likely to occur.

Oxidation and attack by ozone as a result of long exposure to the atmosphere will only affect a relatively thin layer of rubber and this oxidation will thus be no more than a surface effect for bridge rubber bearings. The reasons for the oxidation to be so limited are: the diffusion of oxygen through oxidised rubber is much slower than through new rubber[5,6]; the diffusion of oxygen into rubber is a very slow process; and the extremely low rate of diffusion is further retarded by virtue of the rubber block being under compression. As in the oxidation of aluminium, the oxidised material forms a thin protective layer on the surface. However, rubber can be protected from oxidation and ozone attack by the incorporation of anti-oxidants and antiozonants.

The degradation of the natural rubber pads installed in an Australian railway viaduct completed in 1891 and recently examined, was found to be only superficial. Most of the cracks were less than 0.7 mm deep and below a depth of about 1.5 mm there was no visible evidence of deterioration[7].

Sea water. Rubber does not appear to be adversely affected by water. The absorption of water by rubber is very small[8,9]. The diffusion coefficient and solubility of water in rubber[10] are very small being 1.4×10^{-6} cm^2 s^{-1} and 3.3×10^{-4} g cm^{-3} respectively. The concern of the effect of water is not so much on the rubber itself but on the rubber-to-metal bonds. The effect of water on rubber-to-metal bonds has been shown to be not important even under conditions where the water has direct access to the bonds *i.e.* the edges of the metal is not covered with rubber[11]. However, the bearings for the Penang Bridge are located at least 6 m above the sea and are therefore not in direct contact with sea water.

Time-dependent Crack Growth

Under static as well as cyclic load conditions, crack growth can develop in some rubbers.

Natural rubber is not normally prone to crack growth under static loads because of its ability to strain crystallise[12]. The formation of crystallites at the tip of a crack, where the high concentration of strain occurs, prevents further growth of cracks.

Crack Growth

The tearing energy[13], which is the energy required to cause unit area of new crack growth, is a function of crack length in tension. In shear and in compression, the growth of a crack will not accelerate except at very small crack length[14]. This is however not the case in direct tension. Consequently in good design, rubber is not normally used in tension but only in shear and in compression under service conditions.

A prestressed concrete beam hoggs up on transfer of the prestressing force. As a result, the previously horizontal plane of the underside of the beam assumes a parabolic profile. The ends of the beam are no longer horizontal but are inclined at an angle to the horizontal plane. The situation in which rubber in a laminated bridge bearing is most likely to be in tension is when the bearing has to rotate to accommodate the inclination of the ends of a prestressed concrete beam. Tension of laminated bearings can result in internal cracks due to hydrostatic tensions.

Compression Stiffness of Rubber Bearing

The direct compression of a laminated rubber bearing will depend on:

- the physical properties of the rubber after the bearing is manufactured
- the physical properties of the mild steel used for plate reinforcement
- the number and dimensions of the rubber laminates and mild steel plates.

At the design stage it is difficult to estimate the magnitude of the direct compression or deformation of a rubber bearing under a given compressive force. At best a minimum compressive stiffness based on past experience is

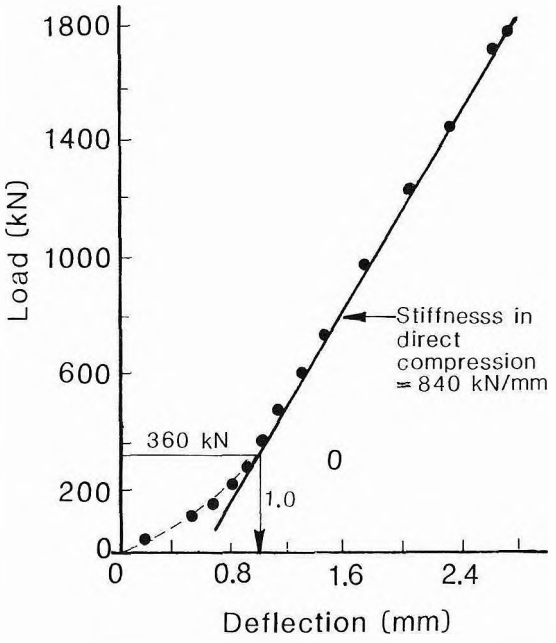


Figure 1. Type 1 bearings: the relation between load and deflection.

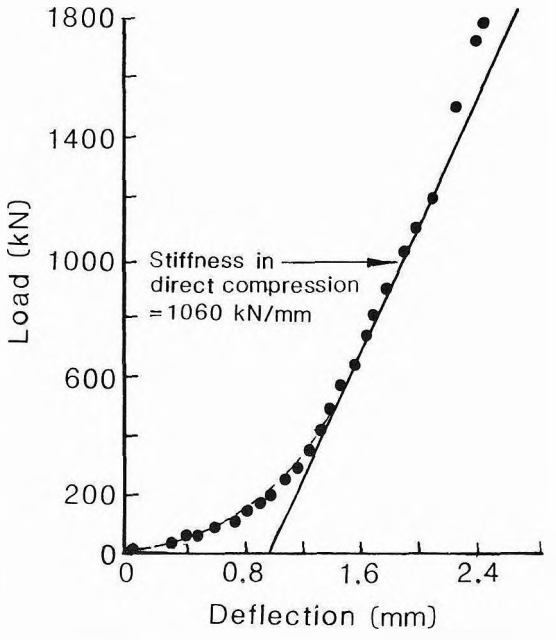


Figure 2. Type 2 bearings: the relation between load and deflection.

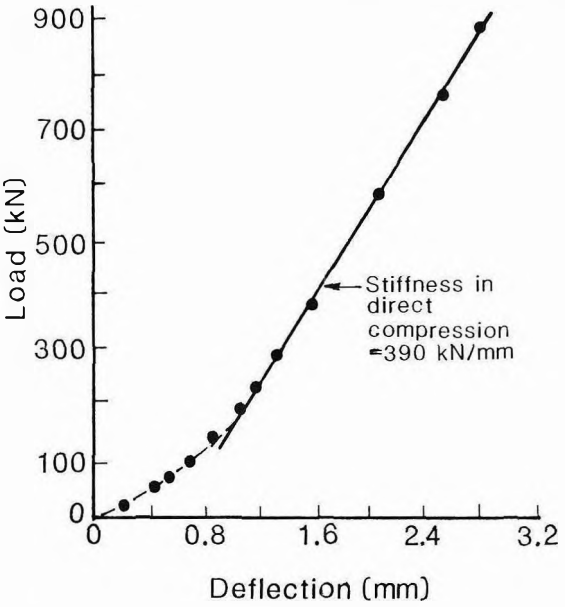


Figure 3. Type 3 bearings: the relation between load and deflection.

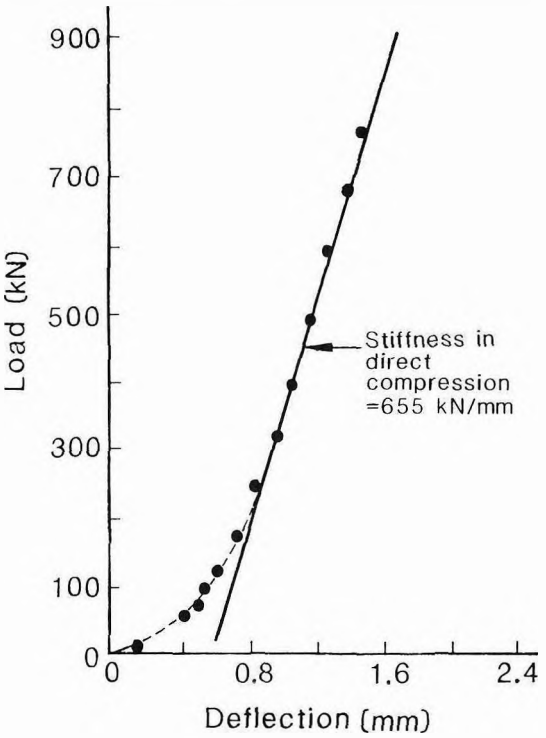


Figure 4. Type 4 bearing: the relation between load and deflection.

specified and the structural analysis of the orthotropic bridge deck is then carried out on the basis of this value.

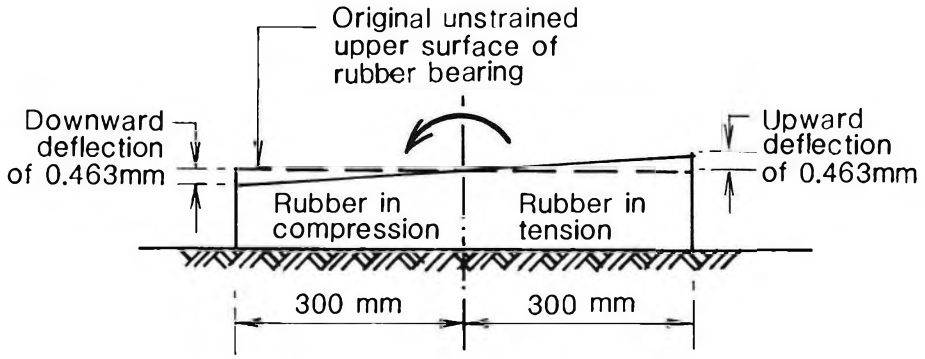
For all the types of bearings for the Penang Bridge, a stiffness in direct compression of not less than 210 kN per millimetre and a stiffness in shear of not greater than 2.6 kN per millimetre were specified.

Figures 1, 2, 3 and *4* give the load-deflection behaviour of some of the rubber bearings tested. In general, the load-deflection relationship begins to be linear only after a certain initial load. At loads less than this initial value the load-deflection relation is non-linear. At load values lower than this initial value the load per unit deflection is much lower. Consequently it is advantageous to design a bearing such that at no stage in the loading history the compressive force will fall below the linear part of the load-deflection curve.

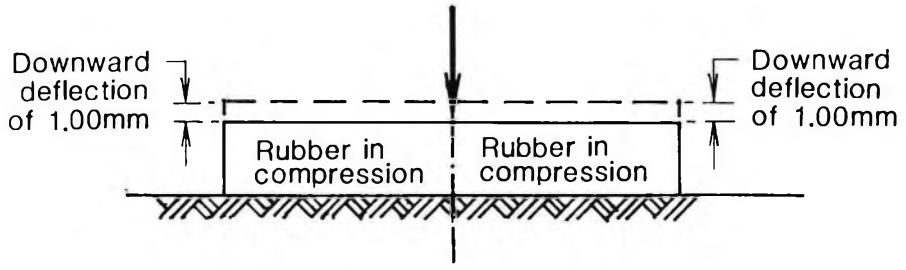
All the four tests show that when the relation between compressive load and deflection becomes linear, the stiffness of the bearings in direct compression is greater than the specified value of 210 kN per millimetre.

No Tension Assessment

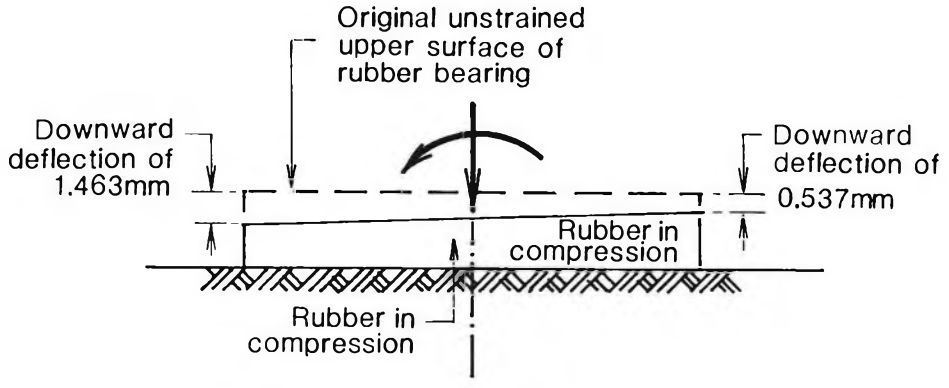
At the early stage of the construction when the prestressed concrete beam is placed on the top of a rubber bearing at each end of the beam, the direct compressive stress on the bearing is only that due to the weight of the beam itself. The inclination of the ends of the beam to the horizontal on the other hand, is the greatest. Because of the low direct compression and the highest angle of inclination of the ends of the beam, this is the stage at which the rubber is most likely if ever to be in tension. Under a normal programme of construction this situation prevails for only a short period. With the placement of the diaphragms and deck, their weights not only increase the direct compressive stress on the



Effect of rotation



Effect of compression



Combined effect of rotation and compression

Figure 5. Type 1 bearing: the combined effect of highest angle of rotation and lowest compressive load.

bearings but also reduce the inclination of the ends of the beam to the horizontal. The subsequent live load, *i.e.* the traffic load further increases the magnitude of the direct compressive stress on the rubber bearings and further decreases the inclination of the ends of the beam to the horizontal. Consequently the stage at which rubber in the bearings is most likely to be in tension is at the early stage of the bridge construction when the rubber bearings support only the weight of the prestressed concrete beam. At this stage the hogging or upward deflection of the prestressed concrete beam is the net result of the upward deflection due to the prestressing force in the prestressing cables and the downward deflection of the beam due to its own weight.

At the design stage, it is difficult to determine the actual magnitude of the hogging because the magnitude of the elastic modulus of the concrete is not known. This modulus is mainly dependent on the crushing strength of the concrete but is however, influenced by the elastic properties of the aggregates, the conditions of curing, the age of the concrete, the mix proportions and by the type of cement used. Approximate values are given in CP 110 (British Standard Code of Practice for the structural use of concrete) but if an accurate figure is required, the best method is still to measure the deflections on the actual beams under loads. However, this can only be done during the construction stage.

During the manufacture of the prestressed concrete beams in the Penang Bridge Project, the hogging at the centre of each beam at transfer of the prestressing force was also measured. Analysis of the measured results of sixty-five beams of length 39.820 m each subjected to the same total prestress force of 8004 kN showed that the average net upward deflection at the centre of the span due to the combined effect of the prestress and of the beam's weight, was 30.75 mm. At the ends of the beam the average slope of the underside was therefore 1 vertical to 647.5 horizontal. The length of the rubber bearing *(Type 1)* is 600 mm in the direction of the longitudinal axis of the prestressed concrete beam. Consequently the rotation of the bearing to accommodate this angle resulted in an upward rise of 0.463 mm on the tension edge of the bearing and an equal downward deformation on the compressive edge.

The weight of the prestressed concrete beam exerts a direct compressive force of 360 kN on the bearing. This results in a downward deformation of 1.00 mm over the whole bearing. The combined effect of the rotation and direct compression is that every part of the bearing is under compression *(Figure 5)*.

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