(Smith & Larson, 1946; van Reen & Pearson, 1953), and in lower ones, e.g. *Corcyra* larvae (Sivarama Sastry, Radhakrishna Murthy & Sarma, 1958*a*; Sivarama Sastry & Sarma, 1958), is that growth inhibition can be reversed independently of other parameters. This also would seem to be of general occurrence.

Though metal antagonisms can ultimately be explained on the basis of a control of one metal uptake by another, the present results emphasize that this control could be either extracellular or intracellular. Such control processes also differ with the nature of the cells in question. For instance, in contrast with the results with *N. crassa*, iron does suppress total uptake of ^{65}Zn in erythrocytes (Sivarama Sastry, Viswanathan, Ramaiah & Sarma, 1960*b*). Thus a metal may control the uptake of another in one type of cell but not in another.

SUMMARY

1. The toxicity of cobalt, nickel and zinc to the growth of *Neurospora crassa* was studied as a function of magnesium supply. The extent of toxicity of all the metals was considerably enhanced by decreasing the normally high magnesium concentration in the medium to a minimal value for optimum growth.

2. Reversal of metal toxicities was possible with iron as well as magnesium. The antagonism between iron and the toxic metal was competitive, at high magnesium concentrations, for cobalt and nickel toxicities only. With a minimal magnesium supply a much higher ratio of iron to toxic metal was necessary in all cases to achieve normal growth rates.

3. The correlation between mycelial accumulation of the toxic metal and the growth of the mould was investigated under various conditions. In the absence of a counteracting metal there was a direct relationship between these two parameters.

4. The reversal of metal toxicities by magnesium is due to a suppression of the uptake of toxic metal. Further, such a control of metal uptake does not occur when iron is the counteracting metal.

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Particulate Ribonucleoprotein Components of *Hevea brasiliensis* Latex

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During the isolation of a soluble nucleoprotein component of natural-rubber latex from freeze-dried latex 'serum' it was noted that a very small amount of additional polynucleotide material was present in the sediment obtained from this reconstituted serum by centrifuging at high *g* (McMullen, 1959).

The present paper shows that most of this particulate nucleic acid is located in the 'bottom fraction' freeze-dried material as obtained from

Malaya but also that some is found to be closely associated with the 'rubber' phase in normal fresh (liquid) latex. A physical and chemical description of these polynucleotide components is reported.

EXPERIMENTAL

Materials. 'Bottom fraction' and 'C-serum' solids were prepared in Malaya from fresh centrifuged latex in a freeze-dried condition by the staff of the Rubber Research Institute of Malaya as described by McMullen (1959). In addition, a few samples of fresh latex preserved with ethylene oxide were flown from Malaya and centrifuged within 60 hr. of collection, as a check against the possible distribution of the particulate nucleic acid as an artifact by the freeze-drying procedures.

Chemicals. Sodium deoxycholate solutions were freed from particulate material by centrifuging at 100 000*g* for 2 hr. and from most of the soluble contaminants (absorbing at about 250 mμ) by repeated extraction with acid-washed (Norit SX30 special) charcoal. Sodium dodecyl sulphate was purified by foaming (Cockbain & McMullen, 1951) and cetyltrimethylammonium bromide by several recrystallizations from acetone.

Sedimentation fractionation. Preparative centrifugal separations were performed on approximately 10% (w/v) suspensions in 0·2M-tris buffer in a Spinco L-type ultracentrifuge at 0–5° and quantitative measurements of the sedimentation coefficients of ribosome particles were made in the Spinco (Special Instruments Co., Belmont, Calif., U.S.A.) model E equipment in the Biochemistry Department, Cambridge.

Detection and estimation of ribonucleic acid. This was carried out as described by McMullen (1959). In addition minor nucleotide components were investigated by the techniques of Dunn (1959).

Detection of polyisoprene. Micro-quantities of rubber were identified by comparison of their infrared-absorption characteristics in the region 2100–650 cm.$^{-1}$ with those of an authentic sample. Films were cast from benzene or chloroform solutions on NaCl plates. The approximate stereochemical nature was determined from detailed examination of the region 1200–1050 cm.$^{-1}$ (Cunneen, Higgins & Watson, 1959). A Hilger H. 800 double-beam spectrophotometer, fitted with a NaCl prism, was used.

Hyperchromicity. The variation in u.v. absorption with temperature of RNA-particle suspensions was observed in a Hilger Uvispek absorptiometer fitted with a thermostatically heated cell.

Isolation of particles containing ribonucleic acid. The freeze-dried latex solids were homogenized by an Ato-Mix apparatus (Measuring and Scientific Equipment Ltd., London) in buffer solutions with and without sodium deoxycholate. These solutions were previously saturated with coal gas, which is effective in inhibiting the enzymic oxidation of polyphenols present. Generally the extracts were made at the 10% (w/v) solids level, the usual buffer being 0·02M-tris–0·01M-magnesium acetate. Fig. 1 shows the general scheme of fractionation of 'bottom fraction' material; all sediments were thoroughly resuspended in a Potter-type homogenizer. A similar fractionation procedure was carried out on the fresh liquid-latex samples and in addition the upper redispersible rubber-layer particles were fractionated as illustrated in Fig. 2.

RESULTS

Reconstituted 'C-serum' solids were found to contain less than 0·003% by weight of nucleic acid sedimentable at 100 000*g* for 2 hr. This confirms previous work which demonstrated the almost complete absence of particulate RNA in this material (McMullen, 1959). 'Bottom fraction' solids, on the other hand, contained approximately 0·1% of RNA sedimentable after rehomogenization in deoxycholate under these conditions and is therefore the fraction mainly used as a source of insoluble RNA in the present work.

In the absence of deoxycholate or sodium dodecyl sulphate in the extraction medium, over 80% of the RNA is sedimented in the low-speed fraction (Fig. 1) and several extractions with deoxycholate are required to obtain most of the nucleic acid in the supernatant at this stage. The ease of extraction, however, varies somewhat with the particular sample of 'bottom fraction' solids being investigated, and may reflect differences in the pretreatment of the original latex.

Phosphate buffer alone is also more efficient in the extraction of RNA than, for example, tris buffer alone, and the extraction in these cases is greatly facilitated by incubation of the homogenate overnight.

The 'ribosomal' particles obtained in the high-speed sediment (Fig. 1) have a polynucleotide content of 25% but this value can be raised to about 60% (w/w) by solubilizing the nucleic acid in boiling 10% (w/v) sodium chloride, followed by precipitation first with 70% (v/v) ethanol and then by acid at pH 3·0. Further protein may be removed from this product by forming a complex between the RNA and cetyltrimethylammonium bromide as described by McMullen (1959), whence the RNA content can be raised to over 90% (w/w).

On dispersal in sodium dodecyl sulphate the ribosomal sediment is partially solubilized, the sediment obtained by prolonged centrifuging contains a rather higher proportion (30%) of nucleic acid and a low-density particulate layer is found at the upper surface of the supernatant. This low-density substance is very soluble in chloroform, partly soluble in benzene, and infrared spectroscopy shows it to consist mainly of *cis*-polyisoprene, possibly containing some polar material. Electron-microscope studies show also the presence of 'ultramicro rubber' particles of average diameter approximately 300 Å. When boiled in 10% (w/v) sodium chloride the particles coagulate and a solution containing ultraviolet-absorbing substances is obtained from which, on the addition of ethanol to give a final content of 70% (v/v), is precipitated material with the typical ultraviolet-

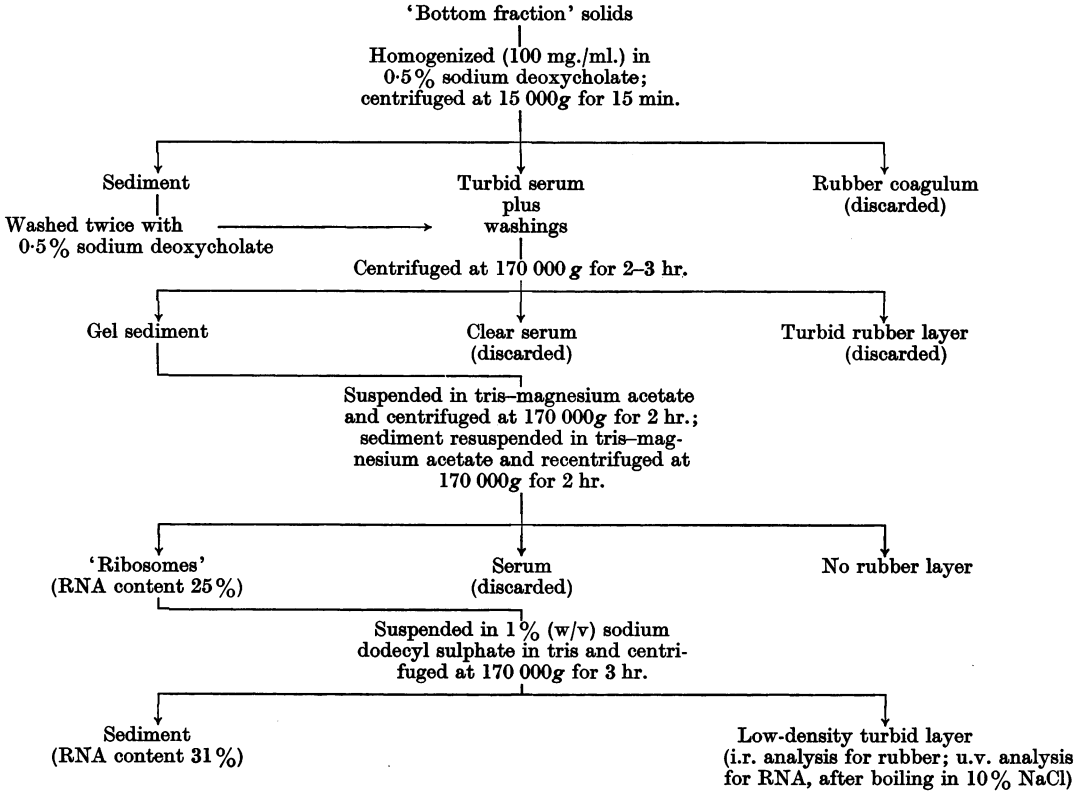

Fig. 1. Fractionation procedure with 'bottom fraction solids'.

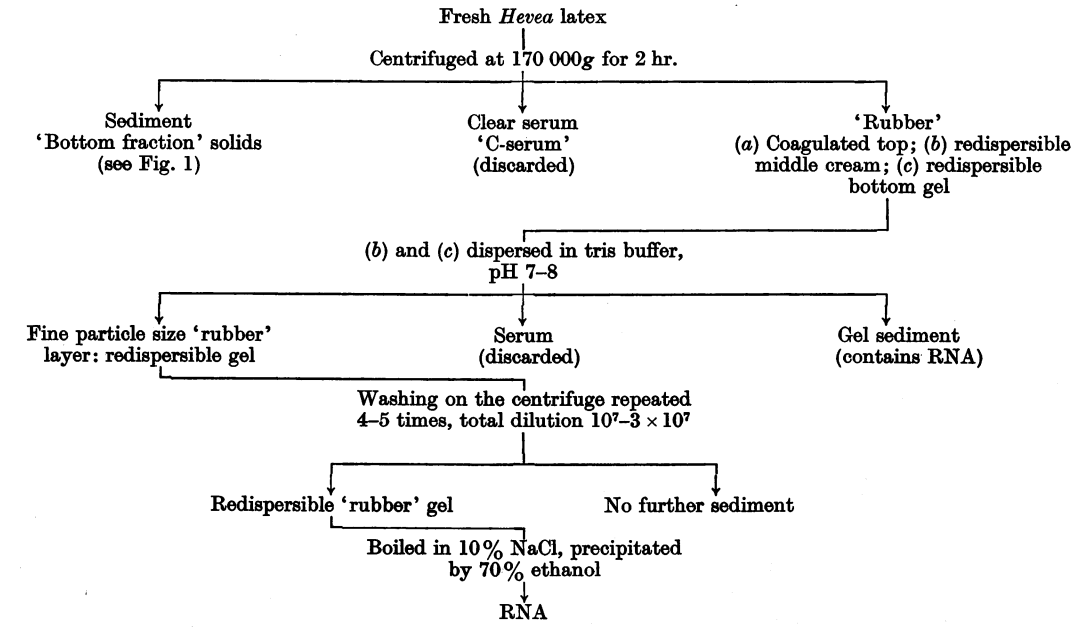

Fig. 2. Fractionation procedure with fresh liquid latex.

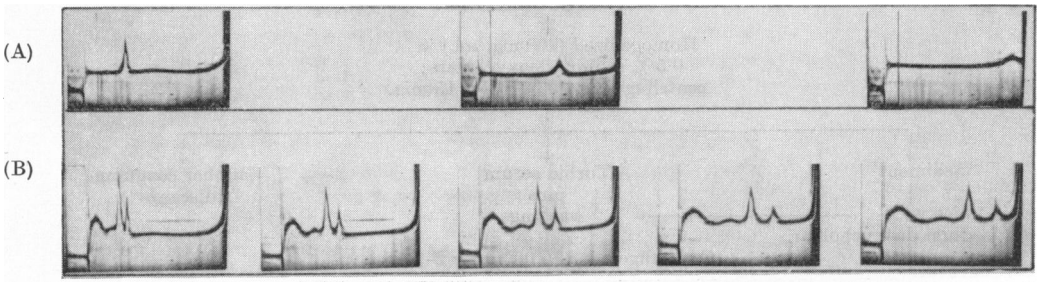

Fig. 3. Sedimentation pattern of RNA particles. (A) In 0·01 M-magnesium acetate: 94s component. (B) In the absence of Mg^{2+} ions: 9s, 44s, 64s and 77s components.

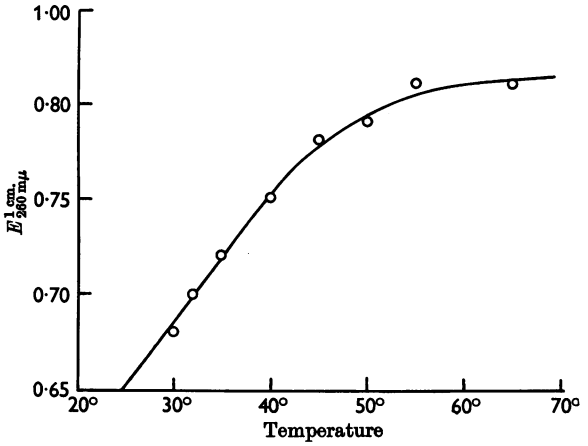

Fig. 4. Hyperchromicity curve of '25% RNA' particles in tris–magnesium acetate buffer, pH 7·2.

absorbing characteristics of RNA: $\lambda_{max.}$, 260 mμ; $\lambda_{min.}$, 237 mμ; $E_{max.}/E_{min.}$, 1·8.

Analytical sedimentation patterns of a sample of '25% RNA' ribosomal particles resuspended in (*a*) tris buffer plus magnesium acetate and (*b*) tris buffer alone are shown in Fig. 3. These indicate that a large particle with a $S_{20,w}$ of 94s is the stable entity in the presence of Mg^{2+} ions and that this dissociates into particles with $S_{20,w}$ values of 9, 44, 64 and 77s when Mg^{2+} ions are absent.

When fixed in formalin and stained by the procedure suggested by Huxley & Zubay (1960), particles with a fenestrated appearance are observed. These appear to consist of negatively stained bundles of helical elements and are positively stained in 5% (w/v) uranyl acetate. Suspensions of these particles exhibit ultraviolet hyperchromicity over the range 20–70° wherein the absorption at 260 mμ increases by over 25%. This is shown in Fig. 4. The average relative nucleotide composition of the 25% RNA particles is: guanylic acid, 1·44; adenylic acid, 1·00; cytidylic acid, 1·16; uridylic acid, 0·95. No 'minor components' were detected.

Centrifugal washing (Fig. 2) of the upper 'rubber layer' (at a dilution of 1 in 50) of the fresh liquid-latex samples results in the sedimentation, even after three such washings, of RNA-containing particles. Insufficient fresh latex was available for these experiments to determine satisfactorily whether the base-ratio composition was similar to that obtained from the freeze-dried ribosomes, though definite but faint RNA patterns were obtained on chromatography of hydrolysates of these sediments. When all the sedimentable RNA had been washed out from the rubber layer (after a total dilution of about 1 in 10^7), at which stage electron-microscope observations show that coalescence is commencing, it was possible to extract further nucleic acid constituents by boiling the layer in 10% (w/v) sodium chloride. When the serum from this was adjusted to 70% (v/v) ethanol content a precipitate was obtained whose solution in water exhibited a definite absorption maximum in the 260 mμ region. The amount of RNA detected in this way varied from 0·04 to 1·25% (w/w) (based on the polyisoprene content), depending on the average particle size of the suspension, the higher RNA content being present in the latex with smaller average particle diameter. The total amount of 'nucleoprotein' determined by comparison of the absorption at 260 mμ and at 280 mμ is approximateoy 10–20 times the RNA content and is about sufficient to represent a 'monolayer' coverage of the particle surface area.

DISCUSSION

In fresh liquid latex a significant proportion of the particulate RNA is found in the rubber layer during centrifuging. Whether this is entirely due to a mechanical trapping action or partly to a more specific interaction is the subject of further investigations. Other 'insoluble' RNA found in this

fraction appears to be strongly associated with the polyisoprenoid particles.

'Bottom fraction' solids contain RNA particles which are mainly associated with readily sedimentable material and which are liberated therefrom by treatment with deoxycholate. The free particles have the characteristics of ribosomal bodies in that they contain 25–30 % of nucleic acid, dissociate into sub-units in the absence of bivalent cations and have the typical negatively-stained fenestrated appearance in phosphotungstic acid. The probable helical substructure of these RNA particles receives confirmation from the observation that on heating their aqueous preparations the extinction maximum in the ultraviolet region increases by an amount which is attributable to a breakdown of a 40 % helical organization of such molecules (Hall & Doty, 1959). This value is similar to that found by these authors and by Schlessinger (1960) for ribosomal particles in *Escherichia coli*. The presence of 60s and 80s particles has been detected in *E. coli* by Bolton, Hayer & Ritter (1958) and the presence of 40s, 60s and 74s particles in pea seedlings has been shown (Ts'O, Bonner & Vinograd, 1956). The present results on *Hevea* are in conformity with such work.

SUMMARY

1. The isolation from *Hevea* latex of ribosomal particles containing 25 % of RNA and their nucleotide analysis is described.

2. Such particles are reversibly aggregated in the presence of Mg^{2+} ions to a large aggregate with a sedimentation coefficient of 94s.

3. These ribosomes, on treatment with detergent, liberate *cis*-polyisoprene ultramicro-particles into the supernatant on centrifuging.

4. These polyisoprenoid particles contain polynucleotide material.

5. 'Normal' polyisoprene particles, obtained in the low-density 'cream' layer on centrifuging fresh latex, also contain polynucleotide to a similar degree, after exhaustive washing procedures.

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Some Properties of a Kidney Adenosine Triphosphatase Relevant to Active Cation Transport

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It is well known that the active transport of ions across cell membranes depends on a supply of energy from cell metabolism, which may be aerobic or anaerobic. Since the only features common to both respiration and glycolysis are the synthesis of ATP from ADP and the oxidation and reduction of NAD, it has been widely inferred that the linkage of active transport to metabolism is through ATP, which is hydrolysed as ions are moved against concentration gradients. The possibility still exists, however, that a reversible oxido-reduction reaction, perhaps coupled to a reaction involving the fission of ATP, might be concerned in active transport. Evidence suggesting that the active-transport mechanism might have the effect of an enzyme which hydrolyses ATP was provided by the discovery in crab nerve (Skou, 1957), in various preparations from brain (Hess & Pope, 1957; Järnefelt, 1961; Deul & McIlwain, 1961; Aldridge, 1962) and in fragmented human-erythrocyte membranes (Post, Merritt, Kinsolving & Albright, 1960; Dunham & Glynn, 1961) of adenosine triphosphatases which are activated by Na^+ and K^+ ions. This concept was supported by the demonstration of the inhibition of adenosine-triphosphatase activity by cardiac glycosides, such as