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The Incorporation of [1-^{14}C]Acetate into Fatty Acids, Non-Saponifiable Lipids, and Rubber by the Latex of *Hevea brasiliensis*

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In addition to the incorporation of [1-^{14}C]acetate into rubber by the latex of *Hevea brasiliensis*, reported by Harris & Kekwick (1961), incorporation has also been observed into the lipids of the non-saponifiable fat fraction, and into the fatty acids of latex. The total incorporation into the non-saponifiable fat fraction was about 10% of that into the rubber and the label appeared only in those components which co-chromatographed with authentic farnesol and squalene on thin-layer and column chromatography; no incorporation into the sterol component of this fraction was observed. The total incorporation into fatty acids varied but was usually of the order of 10% of that into rubber. The principal fatty acids labelled had the characteristics of palmitic, palmitoleic, oleic, and linoleic acids on gas–liquid chromatography.

The incorporation of acetate into each of the three fractions, rubber, non-saponifiable lipid and fatty acids, was inhibited to some extent by avidin. At low concentrations (1·5 units avidin/ml.) the incorporation into fatty acids was inhibited by 50% and the incorporation into the other two fractions was unaffected, but at high concentrations, (10 units/ml.) the incorporation into all three fractions was inhibited, that into fatty acids by 70% and that into both rubber and non-saponifiable lipid by 40%. The inhibitory effect of avidin was always reversed by prior incubation with biotin.

The effect of adding ATP on the incorporation of acetate into the rubber and into the non-saponifiable lipids was markedly different from that on the incorporation into fatty acids. Whereas maximal incorporation of acetate into rubber and non-saponifiable lipid occurred at 0·1mM added ATP, higher concentrations being inhibitory, incorporation into fatty acids increased with concentrations of added ATP up to 4mM. Incorporation of [3-^{14}C]pyruvate into rubber was also inhibited by concentrations of added ATP in excess of 0·3mM, but that of mevalonate was stimulated by concentrations up to 10mM.

These results suggest that biotin may play some part in the biosynthesis of isoprenoids in latex, possibly in the formation of malonyl-CoA as has been demonstrated by Brodie, Wasson & Porter, (1963). There appears to be an ATP-sensitive step in the formation of mevalonate from acetyl-CoA.

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The Formation of Sodium Complexes by Certain Pyrophosphates

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During the determination of dissociation constants for pyrophosphoric acid by a conductivity method, Monk (1949) found deviations from theoretical behaviour which were consistent with the association of pyrophosphate and sodium ions. We have investigated the association of sodium and pyrophosphate ions by a more direct method, employing a sodium-responsive glass electrode (E.I.L.) to measure the activity of sodium ions, both for inorganic pyrophosphate and the two organic pyrophosphates ATP and ADP.

Association was found to exist, and values for equilibrium formation constants were determined for the sodium complexes. Measurements were carried out in an aqueous buffer solution of tris-hydroxymethylaminomethane chloride of ionic strength 0·2. Total sodium concentrations were determined by flame photometer (E.E.L.).

It was found that at relatively low concentrations ($< 0{\cdot}15$ equiv./l.) of sodium ion, pyrophosphate and ATP could bind two sodium ions per molecule and ADP one only. Orthophosphate and AMP were also investigated and found to exhibit no appreciable association. At concentrations of sodium ion greater than 0·15 equiv./l. there was evidence for the association of more than two sodium ions per molecule of pyrophosphate and of ATP, and of more than one sodium ion per molecule of ADP.

Mean equilibrium formation constants have been calculated for the mixture of ionic forms present at pH 7·0 and are here given as log$K$ values. For the first and second sodium ions associated with pyrophosphate: 1·88 and 0·68 respectively. For the first and second sodium ions associated with ATP 2·18 and 0·98 respectively. For the first sodium ion associated with ADP: 1·64. Under our conditions lack of appreciable association of sodium ions with AMP and orthophosphate implies log$K$ values of less than 0·3 approx.

below 0·02. On starch-gel electrophoresis (Smithies, 1955) of whole sarcoplasm in borate buffer, pH 9·1, at least 12–14 different bands could clearly be recognized and some of these have been identified with known proteins. When whole sarcoplasm was fractionated on a column of diethylaminoethylcellulose there was a good direct correlation between the mobility of the components in each fraction and the ionic strength required for their elution from the diethylaminoethylcellulose. Whereas the majority of identifiable bands moved towards the anode, a striking feature of the electrophoretic pattern obtained was the reproducible, densely staining series of 4–5 bands which moved in the opposite direction.

In sarcoplasm isolated from the back and hind leg muscle of rabbit foetuses (26–28 days old) and from the newly born animal the components which moved towards the cathode were much reduced. The lowered content of these more basic proteins in foetal muscle was reflected by the fact that only about 15–30 % of the total protein was eluted from diethylaminoethylcellulose at ionic strength 0·02. Although the backward-moving components were very conspicuous in extracts made from the mixed back and leg muscles and from the psoas, they were less apparent in red skeletal muscle, and particularly in cardiac muscle of adult rabbits. It is of interest to speculate whether the marked increase in the backward-moving components, which is apparent on electrophoresis of white skeletal muscle after birth, is a consequence of the more highly developed anaerobic activity of this tissue compared to red skeletal and cardiac muscle.

Smithies, O. (1955). *Biochem. J.* **61**, 629.

(11) The Incorporation of Sodium [2-^{14}C]-Acetate into Rubber by the Latex of *Hevea brasiliensis*

By R. V. Harris and R. G. O. Kekwick. (*Department of Biochemistry, The University of Birmingham*)

Bandurski & Teas (1957) found that sodium [1-^{14}C]acetate was incorporated into rubber by the latex tapped from seedling trees of *Hevea brasiliensis*. The incubation mixture contained latex diluted threefold in 0·2 M-sucrose containing added cofactors. Park & Bonner (1958), and Gascoigne & Jones (1959) were unable to obtain incorporation of labelled sodium acetate under the conditions used by Bandurski & Teas (1957). Furthermore, neither Gascoigne & Jones (1959) nor Kekwick *et al.* (1959) were able to obtain incorporation of [2-^{14}C]mevalonic acid into rubber by latex from *Hevea brasiliensis* using these conditions. However, since Park & Bonner (1958) and Kekwick *et al.* (1959) did obtain incorporation of [2-^{14}C]mevalonic acid into rubber by undiluted latex, the ability of latex to incorporate labelled acetate into rubber was rechecked.

Samples of latex were obtained from eight-year-old seedling *Hevea brasiliensis* trees grown in a hot house at Birmingham. Incubations with sodium [2-^{14}C]acetate were carried out at 28° in the absence of added cofactors, using fresh undiluted latex.

The rubber was purified and analysed by the methods previously described (Kekwick *et al.* 1959). The percentage incorporation of the radioactive carbon into the rubber obtained varied from 1·4 % with latex obtained in midsummer (August) to 0·1 % with latex obtained in midwinter (January). Duplicate incubations and analyses carried out simultaneously agreed to within 2 %. While standing in air at room temperature, the latex rapidly lost its ability to incorporate labelled acetate in the absence of added cofactors and had completely lost this ability after 2 hr.

Tapping and incubation in an atmosphere of nitrogen increased the incorporation by nearly 100 %. Tapping in air and incubation in the presence of the reducing agents cyteine (25 mM) and sodium ascorbate (22 mM) resulted in a decrease in the incorporation of labelled acetate.

Sodium [3-^{14}C]pyruvate was a less efficient precursor than sodium [2-^{14}C]acetate. The incorporation obtained was only about 50 % of that of [2-^{14}C]acetate.

Examination of the incubation mixtures for acyl-coenzyme A derivatives by chromatography of the derived hydroxamic acids revealed labelling in acetohydroxamic acid. Patrick (1957) and the present authors have found evidence for an acetate-activating enzyme in *Hevea brasiliensis* latex.

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(12) The Catabolic Rate of Albumin Doubly Labelled with ^{14}C and ^{131}I in the Isolated Perfused Rat Liver

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[^{14}C]Glycine or [^{14}C]leucine was incorporated into rat plasma proteins either *in vivo* or by means of the perfused liver (Gordon & Humphrey, 1960).