.08 ml of normal human serum amounts to 13.5 γ, so that a serum solution of .25 mg% results. Studies of carcinogenesis in rats are under way.

The last two lines in Table 1 allow a comparison of the amount of fat-soluble azo compound bound to the serum proteins by electrophoretic elution and the amount of lipophil Sudan red eluted from animal skin membranes (*2*). In both techniques the greatest solubilization takes place in the β-globulins, with albumin second. It has recently been shown that the β-globulins associate with cholesterol (*3*) and also with phosphatides (*4*).

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Differential Migration of Rubber by Reversed-Phase Partition Chromatography[1]

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Differential migration of rubber has been accomplished by using silicone-treated paper as the stationary phase and certain viscous oxygenated solvents for the more polar, mobile phase. This is a reversal of the conventional procedure of paper partition chromatography in which the paper holds the polar solvent, ordinarily water, as a stationary phase. The reversed-phase technique has already been applied to other compounds of limited water solubility such as steroids (*1*), where the partition coefficents are greatly in favor of the mobile, nonpolar phase.

Levi and Cajelli (*2*) have shown that ethanolic benzene solutions of Hevea rubber, prepared from fresh latex, can be fractionated by passage through animal charcoal to give a series of eluates of progressively decreasing relative viscosities.

The present report discloses a procedure whereby natural and synthetic rubbers, and possibly other high polymers, can be chromatographed on treated filter paper to give patterns characteristic of their migration tendencies. These patterns are reproducible and, in some instances, can be related to various inherent physical properties of the polymer—e.g., molecular weight distribution, viscosity and polymer breakdown (Fig. 1).

Ordinary filter paper (Whatman No. 1) is cut into sheets 5 in. square and drawn through a 5% (by vol)

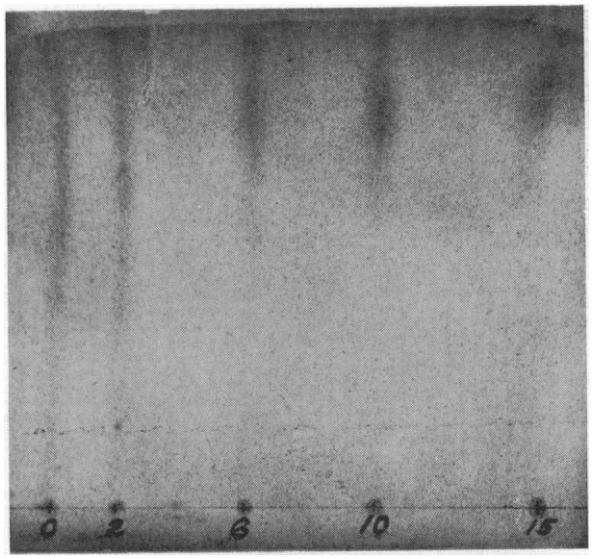

FIG. 1. The effect of physical breakdown by milling on the differential migration of rubber (deresinated guayule) on silicone-treated paper with cyclohexanone for solvent; numbers refer to minutes of milling.

solution of methyltrichlorosilane in benzene. The sheets are pressed to remove excess solution and are dried in an explosionproof circulating air oven at 65° C for 2 hr. This treatment produces a water-repellent paper which must be handled with care on account of its extreme brittleness.

Most of the experiments were carried out according to the microtechnique of Rockland and his co-workers (*3, 4*). The rubber is applied as microdrops of 1% solution in benzene to give a line of spots 0.5 in. from one edge of the paper sheets. Microwire loops (0.7 mm ID) provide a convenient means for applying one or more drops at each spot. Ascending chromatograms are obtained by suspending the paper sheets in covered museum jars containing sufficient solvent to wet the lower edge of the sheets.

The most satisfactory moving solvents have been cyclohexanone and mixtures of butyl diethyleneglycol acetate with cyclohexanone or xylene. The rate of solvent ascent with cyclohexanone is about 3 in./hr. When the moving solvent has traveled the desired distance (4 in.), the sheet is removed and dried in the circulating air oven at 65° C for 1 hr.

The spots or streaks of partitioned rubber are rendered visible by immersion of the paper for 15 min at room temperature in a .25% solution of oil blue NA (Calco) in 50% (by vol) aqueous ethanol.[2] A brief rinse in 50% aqueous ethanol destains the paper but leaves the rubber, and any other lipophilic substances, stained a bright blue or purple on a white background.

The use of oil blue NA as a stain for rubber in plant tissues has been recommended by Whittenberger (*5*) and Addicott (*6*). The intensity and permanency of

[1] The advice and interest of W. de Ráfols, Instituto Nacional de Investigationes Agronomicas, Madrid, Spain, and L. B. Rockland of this bureau are gratefully acknowledged.

[2] The oil blue NA should be first dissolved in a minimum of butanol before addition of the aqueous ethanol and then the resulting solution filtered.

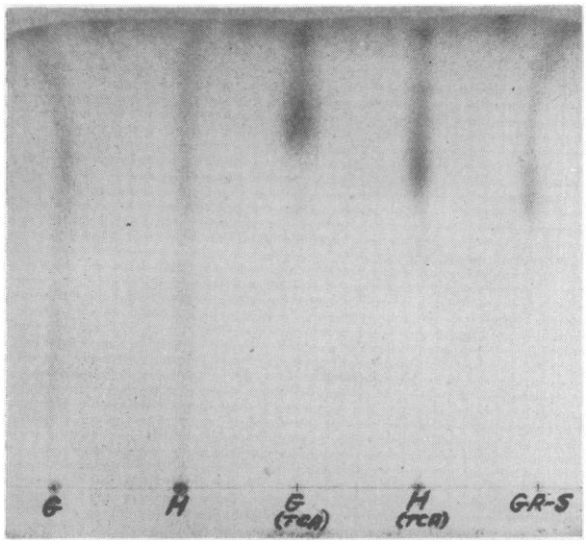

FIG. 2. Chromatograms of several types of elastomers on silicone-treated paper with cyclohexanone for solvent; guayule, Hevea, guayule (cont. 1% trichloracetic acid), Hevea (cont. 1% trichloracetic acid), GR-S.

color conferred to the rubber with this stain remain unsurpassed, to date, by any of the other means found for detecting the partitioned rubber. These other means include the use of Sudan III and Sudan IV (*7*), fluorescein-bromine spray (*8*), modified by the use of isopropanol as a wetting agent, and a spray or dip with .5% neutral aqueous potassium permanganate solution. Although all these reagents are of some value for the detection of rubber on untreated paper,[3] only oil blue NA has given consistently good results with silicone-treated papers. The hydrophobic character of these treated papers probably accounts for their decided "inertness" and lack of response to many of the familiar chromogenic sprays.

By means of the technique outlined above, differential migration patterns have been obtained for several natural and synthetic rubbers. In Fig. 2, where cyclohexanone was used for the mobile phase, comparisons are shown between Hevea (No. 1 smoked sheet), guayule (Salinas), and GR-S (standard). A definite resemblance may be seen in the migration behavior of Hevea and guayule. The somewhat greater mobility of guayule probably attests to a lower mean molecular weight, and a much smaller proportion of the poorly soluble gel fraction. The well-known effect of trichloracetic acid in dispersing the gel and otherwise decreasing solution viscosity is clearly demonstrated in this same chromatogram. The relatively lower mean molecular weight of GR-S is also reflected in its faster migration.

A chromatographic demonstration of the physical breakdown which results from the milling of crude rubber is shown in Fig. 1. A sample of high-quality acetone-deresinated guayule rubber (*9*) was systematically broken down by milling on closely set rolls. Samples were taken at the time intervals indicated, while the viscosity of the rubber was being reduced from an initial Mooney value of 95 to a final value of about 15. Taylor and Veith (*10*) have shown that for rubber broken down in a Brabender plastograph the Mooney viscosity is a semilogarithmic function of the masticating time. The differential migration patterns of Fig. 1 suggest that a similar relationship exists between polymer mobility and milling time.

The results presented here are intended mainly to demonstrate several ways in which partition chromatography can be a useful tool for the study of elastomers. The work is being continued and will be reported in greater detail at a later date.

[3] With untreated paper, much of the rubber either moves with the solvent front or remains at the origin. Migration in this instance is probably governed more by rate of dissolution than by partition.

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Splash-Cup Dispersal Mechanism in *Chrysosplenium* and *Mitella*[1]

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Upon reading the stimulating account by Brodie (*1*) of the splash-cup dispersal mechanism in the Nidulariaceae and other groups of plants, the writer recalled looking down upon fruiting plants of the arctic golden saxifrage, *Chrysosplenium tetrandrum* (Lund) Th. Fries, at Chesterfield Inlet, Keewatin, and thinking how closely the erect, open capsules resembled the gemma cups of *Marchantia.* Examination of specimens collected at that time confirmed that the open capsule formed a deep cup with a flaring lip. Several plants were sent to Brodie, who agreed that the form of the cup appeared to be suitable for splash dispersal of the seeds.

Chrysosplenium americanum Schwein. was found in fruit some miles from Ottawa on June 4, 1952, a few hours after a heavy thunderstorm. The fully expanded

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