1). Such maps are used to record the observations and steps of computation. Each 15% increase of activity is arbitrarily taken as one unit and is represented as a line beginning at the point of measurement and drawn at the same angle as that of the collimated tube positioned at this point. (Increase of over 15% is considered as symptomatic of tumor.)

In the case of an increased radiation zone (characteristic of tumor), these lines, which are an index of the magnitude and the location of increased activity, intersect over the critical area. Taking into consideration the angle of collimation (30°), we can then fairly well delineate the location, shape, and size of the area of increased activity.

We have the impression, based on a few observations, that by taking into account not only the increased but also the decreased uptake values, other pathological brain processes could eventually be detected and evaluated by the method described (e.g., necrotic, ischemic areas, nontumoral lesions, etc.—processes where, probably because of circulatory or other deficiencies, the distribution of the tagged material is impeded, thus demonstrating in the involved area measurably less than normal radiation).

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The Solubilization of 4-Dimethylaminoazobenzene (Butter Yellow) in Serum

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Many of the carcinogenic agents, including the azo dye 4-dimethylaminoazobenzene, are fat-soluble. As a vehicle for such substances emulsions of corn oil are used with polyglycerolester as emulsifier and stabilizer. In other experiments (*1*) rats were fed with a diet containing .058–.064% of 4-dimethylaminoazobenzene. It is a well-known fact that the mode of administration of the carcinogenic agent can have a decisive influence on the result; we were therefore looking for a technique to allow solubilization of such agents in homologous serum without any denaturation of the serum proteins.

To this end thick filter paper (Munktell No. 20/150, Grycksbo, Sweden) is thoroughly soaked in a solution of 12.5 mg% 4 dimethylaminoazobenzene in a 1:1 mixture of ethanol and ether. After drying the paper contains about 4 γ of the azo compound per cm². It is cut in strips of 40 × 7 cm, and .08 ml of normal human serum is placed on the middle of each strip. We arrange the apparatus for paper electrophoresis in a deep-cooling tank, keeping a constant temperature of 2° C and giving the effect of a closed atmosphere. Within 9 hr, 5–6 mg of serum protein can be separated, when 5 v/cm and 5–6 mamp are applied. The paper is wet with veronal/veronal sodium buffer of .06 ionic strength and pH 8.9. Of the two paper strips used simultaneously, one is afterwards stained with bromphenol blue; this strip serves as a guide for cutting off the fractions from the still wet unstained strip. The protein/azocompound is eluted from the cuttings with saline (.85%), the volume of each of the solutions being brought to about 3 ml. Then the optical density of the perfectly clear solutions is read in the Beckman spectrophotometer at 420 mμ and thereupon the content of the azo compound is calculated. The eluate from a protein-free paper cutting serves as a blank.

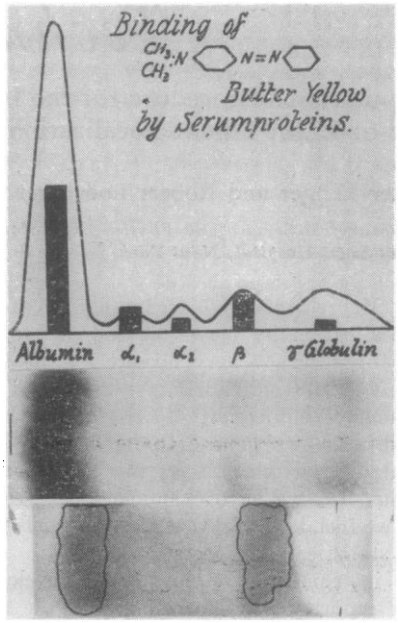

FIG. 1. Above: Serum protein fractions colored with bromophenol blue. Below: Same paper strip without coloring, showing the accumulation of butter yellow in the regions of albumin and β-globulin; blank space corresponds to site of γ-globulins.

The black columns in Fig. 1 show graphically the γ of azo compound bound to each serum protein fraction. The total of azo compound transported by this

TABLE 1

	Albumin	α_1	α_2	β	γ-Globulin
Serum fractions in rel %	63.2	4.0	7.1	9.6	16.1
Bound azo compound in γ	9.01	1.50	0.46	2.20	0.32
Bound azo compound in % of the total of transported azo compound	66	11	3	16	4
γ Azo compound/mg protein	6.7	4.2	3.0	11.0	0.9
Elution of Sudan red by isolated fractions of normal human serum protein[2] γ/mg protein	9.5		10.1	11.3	5.4

.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) 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.

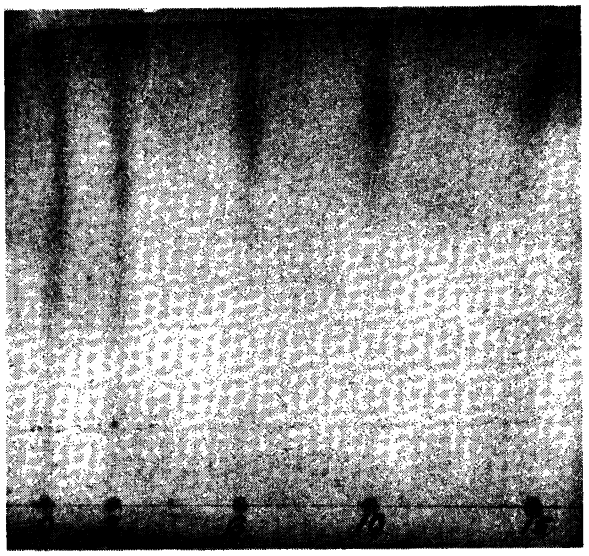

FIG. 1. The effect of physical breakdown by milling on the differential migration of rubber (deresinated guayule) on silicone-treated paper with cyclohexanone for solvent; numbers refer to minutes of milling.

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.