These studies on the mechanism of suppression of blood clearance are being continued in the hope that they will contribute ultimately to a better understanding of the factors involved in blood clearance itself.

Addendum: After this manuscript was submitted for publication, a colleague called the author's attention to a paper by D. Weisberger (*Proc. Soc. Exptl. Biol. Med.*, 29, 445 [1931]). Dr. Weisberger observed a prolonged *Streptococcus viridans* bacteremia in rabbits sensitive to horse serum, whereas normal rabbits cleared the blood rapidly. He did not study the mechanism of the phenomenon.

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The Use of Ion Exchange Resins with Flavonoid Compounds[1]

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Ion exchange resins have been found to be of much value in our laboratory in the isolation, separation, and purification of flavonoid compounds. Examples of such applications to be described include the isolation and purification of homoeriodictyol from yerba santa and the removal of quercetin from commercial samples of rutin.

Pyrex columns, $2'' \times 48''$, were packed with a 2-in. layer of glass wool, followed by 2 in. of purified sea sand, and then filled to a height of 36 in. with a slurry of Amberlite IRC-50(H) synthetic cation exchange resin.[2] The columns were downwashed with 4 liters of 2% HCl, backwashed with distilled water, and finally downwashed with distilled water until the washings were neutral.

Isolation and purification of homoeriodictyol. Five hundred g of powdered yerba santa leaves[3] was successively extracted with three 10-gal portions of boiling distilled water in 2-hr periods. The last extract gave only a very weak test for flavonoid materials. The extracts were filtered through a flannel bag and passed through $2'' \times 48''$ glass columns packed with Amberlite IRC-50(H) cation exchange resin. Five gal of the extract was passed through each column at a rate of 2 gal/hr.

[1] This research was supported in part by grants-in-aid from the Office of Naval Research (Project NR-059 226) and the Atomic Energy Commission (Project AT-(40-1)-235).

[2] The Resinous Products Division, Rohm & Haas Company.

[3] S. B. Penick and Company.

The columns were washed with distilled water as long as any color was removed (about 4 liters/column). The flavonoid material was then eluted by washing each column with approximately 1½ liters of ethyl alcohol. The alcohol solutions were combined, concentrated to 3½ liters in a flash evaporator, diluted with 2½ liters of water, and set in the refrigerator. A brownish-colored tarry mass of crude homoeriodictyol (5,7,4′-trihydroxy-3′-methoxyflavanone) separated from the solution on standing.

The solution was filtered, and the tarry residue extracted with 1,200 ml of ethyl ether. The ether solution was decanted from a small amount of undissolved black solid and shaken with four 50-ml portions of 10% ammonium carbonate solution (*1*). This treatment removed a considerable amount of acidic tarry materials. The homoeriodictyol was then precipitated as the insoluble sodium salt by extracting the ether solution with five 90-ml portions of 10% sodium carbonate solution. The sodium homoeriodictyate was collected by filtration of the sodium carbonate solution and washed with a small quantity of distilled water.

The sodium salt was dissolved in 3 liters of distilled water and passed through a column of Amberlite IRC-50(H) resin. A bright-yellow band of homoeriodictyol formed at the top of the resin bed as the complex was decomposed by hydrogen-sodium exchange. As further solution was passed through the column, the band broadened in width. The column was washed with 3 liters of distilled water, by which time the filtrate was clear and colorless. The homoeriodictyol was then eluted from the column with ethyl alcohol. The alcohol solution was concentrated to approximately 600 ml and then diluted with an equal volume of distilled water. A pale-yellow precipitate of homoeriodictyol separated on standing in the refrigerator. Yield 14.0–14.5 g, or 2.8–2.9%.

Paper partition chromatography (*2*) of the final product revealed no evidence of contamination by eriodictyol or other flavonoids. Mixed chromatograms and mixed melting point determinations with authentic homoeriodictyol substantiated the purity and identity of the product.

Purification of rutin. Commercially available rutin (3,5,7,3′,4′-pentahydroxyflavone-3-rhamnoglucoside)[4] usually contains a small quantity of quercetin (3,5,7,-3′,4′-pentahydroxyflavone). Paper partition chromatography of the rutin used in this experiment indicated the quercetin content was of the order of 1%.

Two g of rutin was dissolved in 3 liters of boiling water, and the resulting solution was quickly cooled to room temperature. The solution was passed through an ion exchange column packed with Amberlite IRC-50(H) and followed by 1 liter of distilled water. A light-yellow band of color extended ⅓ of the distance down the column. The water wash was allowed to drain from the column until level with the top of the resin bed. Three liters of 20% isopropyl alcohol–80% water was then passed through the column at a flow rate of 60–70 ml/min. The isopropyl alcohol–

[4] S. B. Penick and Company.

water solution slowly moved the yellow band of color down the column. The alcohol solution was followed through the column by distilled water in order to flush the last of the alcohol through the resin bed. The alcohol–water eluate was concentrated at reduced pressure to 600 ml, and the sample set in the refrigerator overnight to allow precipitation to occur. The precipitate was collected and dried at 110° C. One and eight-tenths g of rutin was recovered from the column.

Paper partition chromatography of the recovered rutin revealed no fluorescent zone of quercetin in contrast to the easily detectable zone of quercetin in paper chromatograms of the original sample. The quercetin was subsequently recovered from the column by elution with 95% ethyl alcohol. Paper partition chromatography of the concentrated ethyl alcohol fraction revealed a small amount of rutin present along with the quercetin.

Preliminary studies indicate promising possibilities for expanding the applicability of ion exchange resins to flavonoid compounds through the use of ion exchange resins other than the Amberlite IRC-50. The flavonoids may also be adsorbed on the hydrogen form of Amberlite IRC-50 from solutions of their sodium, potassium, lead, or aluminum salts. Metal ion–hydrogen ion exchange occurs on the resin bed, and the adsorbed flavonoid may be subsequently eluted with alcohol.

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Alteration of Immunological Response in Malignancy: Decline of Proteus Agglutinin[1]

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In a recent study (*1*) focused on the course of nonspecific antibody in malignancy, we observed a fall in the *Proteus agglutinin* of chicken sera throughout the development of a Rous sarcoma. This finding, supported by literature indicating a lessened incidence of antibody in malignant disease, stimulated our interest and provoked our current investigation of antibodies to *Proteus* in human sera under normal and neoplastic conditions, of which we are now submitting a preliminary report.

Proteus agglutinin was selected as particularly convenient for our purpose since we found it to be quite common to normal adults. For all titrations described below the antigen used was a *Proteus* vaccine prepared from the OX19 strain according to our method described elsewhere (*1*). Before testing, the stock solution of antigen was diluted 10–15 times with saline, depending upon trial tests necessary to determine the dilution that would give agglutination with normal but not with cancer sera. As a normal control we used a pooled sample of sera from individuals with no apparent disease, and for a negative control, serum from a patient with a proved malignancy.

The actual test was done in small tubes (1.2 cm × 10.1 cm) stoppered with cork to prevent evaporation. To each tube containing 0.1 ml of inactivated serum (56° C for 30 min), whether undiluted or in dilution, we added 0.1 ml of our antigen. The tubes were incubated at room temperature and read with the aid of a binocular microscope (10 × and 23 ×) at several time intervals over a 2-hr period. We observed that the human sera which agglutinate with *Proteus* antigen give a strong reaction when used undiluted or diluted 1–10 times. Most normal adults give a strong positive reaction, whereas infants and children fail to react at all. The latter finding corresponds to our results with the sera of chicks less than 1 month old, and merely reflects the slow development of antibody in the young.

TABLE 1

INCIDENCE OF *Proteus agglutinin* IN NORMAL HUMAN SERA

Group	Positive agglutination	No agglutination
Infants to 1 yr	1	13
Children 1–5 yrs	7	3
Donors (normal)	41	2
Pregnant women	14	1

Table 1 lists various normal groups and for each group the number of sera tested that agglutinated with the *Proteus* antigen contrasted with the number that did not. In infants under 1 year 1 out of 14 agglutinated, whereas in children from 1 to 5 years, 3 of 10 gave a positive reaction. It is of particular interest that in the adult groups 95% were found to have agglutinins to *Proteus*, and this includes 14 of the 15 pregnant women tested.

To continue our study we obtained sera from the Clinics and Tumor Registry of the Grace–New Haven Community Hospital and had them tested for *Proteus* agglutination by a technician. When the clinical diagnoses were received 3–4 months later the results were compared with the findings of the clinicians and are summarized in Table 2 according to a diagnosis of malignancy[3] or nonmalignancy, omitting 6 cases where the final diagnosis was questionable.

From Table 2 it may be noted that the sera from patients with various nonmalignant diseases gave an

[1] This investigation was supported by grants from the Division of Grants and Fellowships of the National Cancer Institute (USPHS), the American Cancer Society, the Jane Coffin Childs Memorial Fund for Medical Research, and the Damon Runyon Fund for Cancer Research.

[2] National Cancer Institute special research fellow.

[3] Established by biopsy.