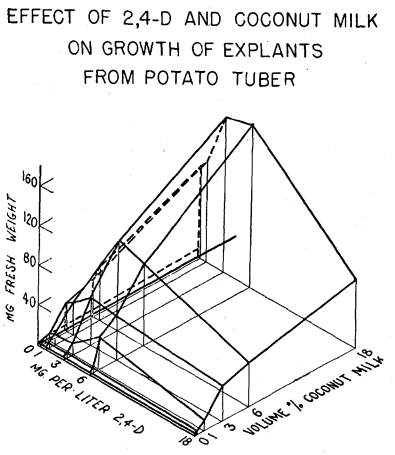

FIG. 2.

the growth in the basal medium supplemented by 0, 0.1, 1, 3, 6, and 18 mg/liter of 2,4-D. The interaction of the CMF with 2,4-D is shown by the growth in media containing both supplements in all the possible combinations of the dosages shown above.

The conclusions are self-evident from Fig. 1, which shows a representative culture harvested after 5 weeks from each treatment. Table 1 describes the growth in terms of the mean fresh weight of the cultures. In Fig. 2 these data are shown plotted on isometric paper. Occasional cultures that were not viable (for unknown reasons) were omitted when calculating the means. (Even including these, however, does not appreciably affect the results.)

Briefly, the conclusions are these. At zero, or very low, concentrations of coconut milk, the effect of 2,4-D on growth is small, and the specific effect of its concentration is also small. At zero, or very low, concentrations of 2,4-D, the effect of coconut milk is negligible at all concentrations. Cultures, however, in contact with *both* 2,4-D and the CMF grew, and the solid surface depicted in Fig. 1 shows that the optimum concentration of 2,4-D is of the order of 6 mg/liter. At this concentration of 2,4-D the tissue shows the maximum response to concentration of the CMF, and even at 18% by volume of coconut milk the limit of response has not been reached.

One further point should be stated. The experiment described in Table 1 and in Figs. 1 and 2 lasted some 5 weeks. For a relatively large part of this period (10–14 days) the tissue made but little growth. The significance for the future growth of this lag, or induction, period has yet to be investigated.

The implications of this work are clear. Whereas CMF alone may be a powerful tool in stimulating some cells into active growth, it is of no avail in some cases. At least in some such cases, the reason is that the cells cannot respond to CMF because their growth is also limited in some manner that requires the intervention of 2,4-D, or a similarly acting substance. Since the response to CMF is so clearly a function of the concentration of 2,4-D and vice versa, these two distinct stimulants appear to act synergistically on the cells of the potato tuber. Understanding of these relations may be expected to shed light both on the mechanism of the CMF on cells and also on the mechanism of the action of 2,4-D.

In view of the reported (*6*) but disputed (*7*) effect of onion juice to increase the growth-promoting activity of auxin or 2,4-D, experiments were conducted to test this further. Potato cultures were grown with combinations of onion extract and 2,4-D added to the medium. The results obtained clearly indicated that onion juice could not substitute for coconut milk. Also at relatively small concentrations (juice of 0.1 g of onion tissue/100 ml of culture medium) onion juice inhibits the growth of carrot even in the presence of the CMF.

The combined action of CMF and 2,4-D on cells of the potato furnishes a system of growing, randomly proliferating, cells that may be contrasted with the cells of the resting tuber, or with the metabolically active cells at the surface of a tissue slice which have, however, only a more limited ability to grow. When these contrasts are described in terms of protein synthesis, salt accumulation, and respiration, we should know more about the physiological characteristics of growing and nongrowing cells.

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Factors Involved in Blood Clearance of Bacteria[1]

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It was recently reported (*1*) that the injection of an antigen into a rabbit previously immunized to that antigen was followed in 15–30 min by a pronounced reduction of the hemolytic complement of the serum. Also observed were the previously noted phenomena of the so-called negative phase, including decreases in specific antibody (*2*), granulocytes, and platelets (*3*), and in the coagulability of the blood (*4*). Evidence was presented (*1*) to support the concept that the decrease of complement was due to the fixation of complement by an *in vivo* complex of extracellular antigen and extracellular antibody. It was suggested that this specific reaction might be applied to the experimental reduction of complement *in vivo* in order

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TABLE 1

EFFECT OF INJECTION OF AN ANTIGEN INTO AN IMMUNIZED RABBIT ON SUBSEQUENT BLOOD CLEARENCE OF SHIGELLA

Rabbit No.	Time (p.m.)	Treatment	Organisms/ml blood	Complement titer (in ml)*	Polymorphonuclear cells (%)	Lymphocytes
4 A	2:00	Bled	—	0.6	45	50
Normal	2:01	1 ml *Shigella* IV	—	—	—	—
	2:02	Bled	21,000	0.61	45	50
	2:17	"	0	0.6	42	50
	2:32	"	0	0.6	42	50
67 immunized to BGG†	2:30	Bled	—	0.52	30	65
	2:35	1 ml 2% BGG IV	—	—	—	—
	2:50	Bled	—	4.1	0	98
	2:51	1 ml *Shigella* IV	—	—	—	—
	2:51½	Bled	18,000	—	—	—
	3:06	"	15,000	4.0	5	95
	3:21	"	10,000	2.5	15	85
	3:36	"	8,000	1.2	30	62
	4:06	"	400	0.5	41	59

* Complement titer = ml 1 : 10 rabbit serum required to hemolyze 50% of sensitized cells; the larger the figure, the smaller the concentration of complement in the sample.

† BGG = Bovine gamma globulin.

to determine the effect of such a reduction on host resistance. The present study is concerned with the effect of the reinjection of an antigen on the clearance from the blood of an antigenically unrelated particulate antigen (two species of bacteria).

The phagocytosis of bacteria by reticuloendothelial cells (*5*) represents a fundamental mechanism whereby the host deals with bacteria that have gained access to the circulation. This phenomenon was selected for study for two reasons: (1) it would appear to furnish an *in vivo* phagocytic system that could be easily studied and quantitated, and (2) clearance occurs rapidly and, therefore, lends itself well to study in conjunction with the rapidly occurring *in vivo* reductions of complement, granulocytes, and platelets.

The basic experiment consists in the comparison of the number of bacteria removed from the circulating blood of a normal rabbit and from the blood of a rabbit immunized against bovine gamma globulin and injected with the globulin 15 min before the inoculation of bacteria. Both the antigen and the bacteria were inoculated intravenously into the marginal ear vein. Blood cultures were made by taking up 0.1 ml of blood into a tuberculin syringe by cardiac punctures at appropriate intervals and spreading the blood on tryptone agar plates either directly or after dilution with saline. Parallel samples were taken for blood counts, smears, and determinations of the hemolytic complement activity (*6*) of the serum. The organisms selected for the clearance observations included the gram-positive *Micrococcus candidus* and the gram-negative *Shigella paradysenteriae flexneri*.

Table 1 illustrates the representative findings of such an experiment. The normal animal rapidly reduced the number of circulating bacteria. The immunized animal, which received homologous antigen shortly before injection of the bacteria, accomplished this reduction much more slowly and incompletely. The results were similar with the *Shigella* and *Micrococcus*. Thus far the interference with clearance of bacteria has been noted in 22 immunized rabbits that were given antigen before inoculation.

One other feature of these experiments is worthy of note. It was occasionally found that rabbits immunized with bovine globulin, which had not been inoculated with the *Shigella,* nevertheless possessed "natural" or spontaneously acquired agglutinins for this organism in low titer. When the "negative phase" was induced in such rabbits, and *Shigella* then introduced intravenously, the animals cleared the circulation of the bacteria as rapidly and completely as did the normal rabbits.

The mechanism of the interference with blood clearance is not known at this time. It would, of course, be most tempting to link the inhibition of clearance with the drop in complement, but alternative explanations are possible. Indeed, this inhibition may not even be related to any of the associated phenomena of the early "negative phase." The data obtained to date tend to eliminate specific bacterial antibody and coagulability of the blood as factors involved in the delayed blood clearance. Repeated serological tests have shown that most of the rabbits did not possess agglutinins against the bacteria; when such spontaneous antibodies were found, as previously noted, injection of the antigen did not affect clearance of the organisms from the blood.

To determine the role of coagulability of the blood, heparin was injected intravenously to simulate the rise in this substance believed to occur during anaphylactic shock (*7*). Up to 20 mg of heparin had no effect on the clearance. The effect of heparin on the suppression of clearance following reinjection of an antigen has not been determined.

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.

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[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.