liver. "Red-leg" infection was prevented by the use of sulfathiazole.

Eight to 10 frogs were placed in 8- by 8-in. glass jars that contained about ½ in. of tap water, which was changed weekly or as required. The temperature was recorded daily. At the time intervals indicated in Table 1 the frogs were injected in the dorsal lymph sac with 1 ml of an aqueous solution of chorionic gonadotropin containing 10, 20, 30, or 40 IU. Before each injection the urine of the frogs was checked to insure that it was sperm-free. Each frog's urine was checked for the presence of sperm at 1, 1½, and 3 hr after the injection.

The positive frogs in groups 1, 2, 4, 6, and 7 were returned to their respective experimental environments after the results of their injection were established; the positive frogs in groups 3 and 5 were kept at room temperature, with and without illumination, respectively, for approximately 5 days to insure elimination of all liberated spermatozoa (*9, 11*). The negative frogs of groups 3 and 5 were kept at room temperature for about 6 hr before they were returned to the refrigerator.

The frogs in groups 1, 4, and 7 were kept in an incubator and those in groups 2 and 6 were kept in an unheated, unventilated room.

Illumination was supplied from a common, unfiltered, 100-w light bulb. Feeding was accomplished by forcing a small piece of beef liver (1 to 2 g) down the animal's esophagus.

Table 1 summarizes the results of all the experiments performed. The concentration is expressed in International Units (1 IU = 0.1 mg). The day of injection was computed from the first day on which the experimental environment was imposed. The frogs in group 7 were the same as those in group 4. After these animals had been maintained in total darkness for 74 days, they appeared to be permanently sterile, for they failed to respond positively to 40 IU of chorionic gonadotropin after 65 days of exposure to constant illumination (group 7).

Figure 1 presents the dose-response curves for groups 1, 2, 3, 4, and 5. The percentage responding positively is plotted against the concentration of chorionic gonadotropin injected. It is evident from this figure that light has a more pronounced effect on the sensitivity than temperature.

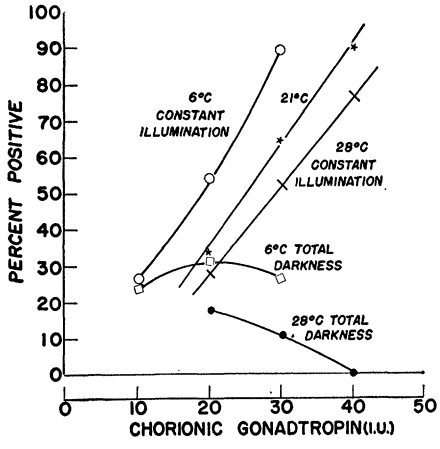

Fig. 1. Dose-response curves for groups 1, 2, 3, 4, and 5.

Comparison of groups 2 and 6 in Table 1 reveals that nutrition also affects the sensitivity. However, work in progress here indicates that nutritional effects are negligible when the animal is maintained at refrigerator temperature.

The following recommendations are made on the basis of these findings: (i) For maximum sensitivity to exogenous chorionic gonadotropin, male *R. pipiens* should be maintained at refrigerator temperature, under constant illumination, and in a state of good nutrition. (ii) All frogs should be screened, after having been thus maintained for a 2-wk period, to establish their sensitivity to the hormone and to eliminate females and refractory males. (iii) Under these conditions we have observed that 50 and 100 percent respond to 20 and 40 IU of chorionic gonadotropin, respectively.

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Unique Electron Exchange Polymer

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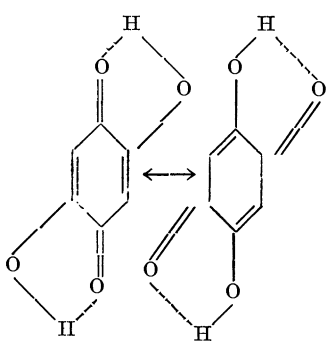
The field of electron exchange resins was initiated by Cassidy when he showed that polyvinylhydroquinone had reversible redox properties (*1*). His first product was readily soluble in many common organic solvents, indicating a low degree of polymerization. However, on copolymerization of vinylhydroquinone and divinylbenzene, he obtained a cross-linked, insoluble, infusible redox resin, which was shown to be useful for electron exchange experiments in columns (*2*).

Manecke measured the oxidation potentials of the redox polymers resulting from the condensation of

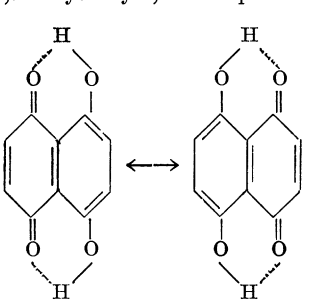
the polyphenols hydroquinone, catechol, and pyrogallol with formaldehyde (*3*). The values obtained for the polymers were close to those of related polymethylated polyphenols. For example, the E_0 of the polymer prepared by condensing hydroquinone, phenol, and formaldehyde in the ratio of 1:1:2 was 0.70 v. The corresponding value for tetramethylhydroquinone was 0.69 v. Similarly, the values obtained by Cassidy and his coworkers for ethylhydroquinone, vinylhydroquinone, and polyvinylhydroquinone (later shown to be mostly dimer and trimer) were 0.64, 0.67, and 0.68, respectively.

In considering other polyphenols that could be condensed with aldehydes to form redox polymers, certain tetrahydroxy derivatives seemed attractive because of greater potential and capacity (*4*). These are the isomers in which the four hydroxy groups are present as adjacent pairs on a benzene ring or are juxtaposed on a polyaromatic ring system so that a hydrogen-bonded dihydroxyquinone results as the first oxidation step. Some examples are 1,2,4,5-tetrahydroxybenzene, 1,2,3,4-tetrahydroxybenzene, 1,-4,5,8-tetrahydroxynaphthalene, and 3,4,3′,4′-tetrahydroxy biphenyl.

The resonance symmetry of the intermediate hydrogen-bonded dihydroxyhydroquinone stage, as is shown in the structural formulas for two of the examples cited, should increase the stability of this oxidation state in relation to the tetrahydroxy and diquinone states. Hence, when such a material is in



2,5 Dihydroxy 1,4 benzoquinone



5,8 Dihydroxy 1,4 naphthoquinone
(naphthazarin)

either of the extreme reversible oxidation states, it should have better oxidation and reduction potentials than some other polyphenol-quinone couple that does not possess such a stabilized intermediate state. This idea can obviously be extended to more complex systems having more than four hydroxy groups.

The values for the oxidation potentials of the couples 1,4,5,8-tetrahydroxynaphthalene-naphthazarin and naphthazarin-naphthodiquinone are 0.37 and 0.93 v, respectively (*5*). One might expect slightly lower values for a polymer of this material and formaldehyde because alkylation usually decreases the oxidation potential. Disregarding this and other minor effects, it was deemed possible to prepare a resin that could oxidize or reduce any couple whose oxidation potential lies between these values.

In order to test this hypothesis, a naphthazarin-formaldehyde polymer was prepared (*4*). Five grams of naphthazarin was dissolved in a mixture of 500 ml of glacial acetic acid and 50 ml of concentrated sulfuric acid. Excess zinc dust was added and the mixture was refluxed until the color of the solution changed from red to a pale grayish green. Excess trioxane was then added and the refluxing was continued for 5 hr. The resulting black precipitate was filtered, and then boiled with several portions of glacial acetic acid, 1 normal aqueous hydrochloric acid, and water in order to remove any low-molecular-weight polymers, other organic reaction products, zinc dust, and zinc salts. The polymer, when dried in a vacuum, was brown.

An attempt was made to determine the oxidation potentials of the oxidation states of this polymer by potentiometric titration with ceric sulfate. However, our experience was similar to Cassidy's and Manecke's. A slow downward drift of potential resulted after each addition of oxidant solution. We found that at least 10 hr were needed to half-titrate a given sample, and even then we were never sure that the potentiometric readings were equilibrium values. Cassidy concluded that the titration of his copolymers should be considered as "semiquantitative" for similar reasons. Qualitative experiments, however, proved that either member of the couples I^-, I_2; $Fe(CN)_6^{4-}$, $Fe(CN)_6^{3-}$; and Fe^{2+}, Fe^{3+} could be oxidized or reduced, depending on the state of the resin. The standard electrode potentials of these couples are -0.54, -0.69, and -0.77 v, respectively.

The qualitative tests demonstrating the oxidation properties of the resin were run as follows. Approximately 1 g of resin was treated with excess acidified ceric ammonium sulfate solution. After thorough mixing for a few minutes, the excess oxidant solution was removed by centrifugation. The polymer was then washed with successive portions of distilled water until ceric ion was absent from the final washing. The washed polymer was then treated with a ferrous sulfate solution. One could see the immediate formation of a yellow color. The solution was centrifuged, and the centrifugate was treated with thiocyanate. The deep red color showed the presence of ferric ion. The resin was then washed by centrifugation with excess water until apparently free from

ferric ion. However, it was found that this treatment was insufficient for the removal of all the ferric ion from the resin. It seems that in the naphthazarin state the resin functions as an ion exchanger. It was necessary to treat the resin with 3 normal hydrochloric acid to effect complete removal of ferric ion. This cycle was repeated five times in all for the oxidation of ferrous ion. In the same manner, the oxidation of iodide ion and ferrocyanide ion was proved.

The reducing properties of the resin were shown in a similar way. Approximately 1 g of resin was treated with excess sodium hydrosulfite and was then washed free of excess reductant. A solution of ferric chloride was added to the reduced polymer. The centrifugate showed the absence of ferric ion by test with thiocyanate. Addition of hydrogen peroxide to this test mixture gave a deep red color, showing the presence of ferrous ion. The polymer was regenerated by treatment with 3 normal hydrochloric acid and successive water washes. This cycle was also repeated five times in all. The reduction of ferricyanide and iodide ions was similarly demonstrated.

In view of the fact that ferric ion was picked up by the resin when it was presumably in the naphthazarin oxidation state, several other ions were tested for exchangeability. It was found that Ni^{2+}, Cu^{2+}, and Co^{2+} were readily picked up and eluted.

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Alterations in Serum Properdin Levels Following Injection of Zymosan

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The discovery and isolation of properdin, a new serum protein that participates in many immune reactions, has been described recently (*1*). Properdin acts in conjunction with complement or complement-like substances and requires Mg^{++} for its activity (*1*). Available evidence suggests that this system plays an important role in natural immunity.

Zymosan (*2*), the insoluble cell-wall residue from yeast, combines with properdin *in vitro*. This provided the basis for the isolation of properdin (*1*). Experiments directed toward a better understanding of the role of the properdin system in experimental infections and other disease states have now shown that the injection of zymosan causes marked alterations in the serum properdin levels of laboratory animals (*3*).

A series of experiments has been conducted in which healthy 12- to 16-g CF1 female mice, and 175 to 200-g Wistar rats were injected intravenously or intraperitoneally with various doses of a boiled saline suspension of zymosan (*4*). Changes in the properdin content of the blood were followed by doing titrations (*1*) on pools of at least six serums obtained from groups of animals sacrificed at various times before and after injection. The serum samples were frozen and stored at −70°C in a mechanical deep freeze until the last sample had been obtained. Properdin titrations were then done on all the samples with the same reagents on the same day. Some of the samples were also titrated for complement and for C'3.

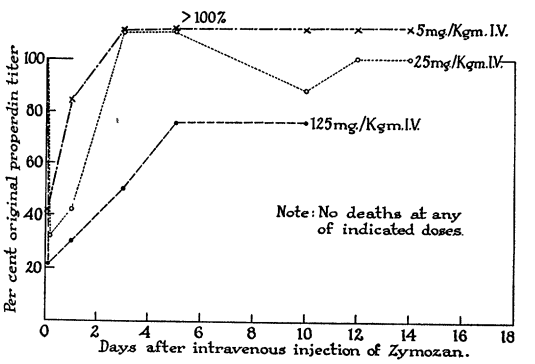

Fig. 1. The serum properdin levels in mice following intravenous injection of zymosan.

The results of a typical experiment with mice (Fig. 1) show that the intravenous injection of small doses of zymosan caused a rapid fall in properdin titer within 1 to 2 hr, followed, after 2 to 14 days, by a marked rise in titer 200 to 300 percent above normal level. Large doses of zymosan caused a greater fall in the properdin levels, which slowly returned to 75 percent of the normal level after 6 to 10 days. It is, therefore, possible either to decrease or increase the properdin concentration in the serums of mice by injection of suitable doses of zymosan. The intraperitoneal injection of zymosan produced similar, although slower and more prolonged, changes in the properdin titers. A more detailed report of these experiments is in preparation. Rats and rabbits behaved similarly to mice in their reaction to zymosan. In contrast to the marked changes in properdin following zymosan injection, no changes were observed in C'3 titers. This result is the opposite of that expected on the basis of *in vitro* experiments which showed that the zymosan-properdin complex inactivates C'3 at 37°C (*1*).

The observation that serum properdin levels in animals may be increased or decreased by injection of zymosan under controlled conditions now offers a means for studying the role of the properdin system in experimental infections and other disease states. Indeed, Derrick Rowley, at the Wright-Fleming Institute of Microbiology, London (personal communication), recently found that the injection of zymosan