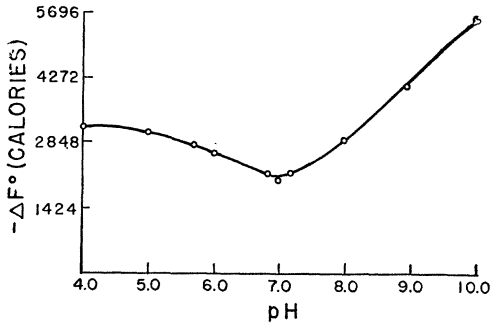

FIG. 1. Apparent "phosphate bond energy" of *p*-nitrophenyl phosphate.

tion constants of phosphoric acid, respectively. Similarly, E_t, k'_E and k''_E have corresponding meanings with respect to nitrophenyl phosphate and A_t and k_A with respect to nitrophenol.

As an approximation, concentrations are employed instead of activities. The concentration of water is assumed to be that of pure water. Following the convention of Lewis and Randall (*5*), the standard state of water is taken as pure water. pK values are not corrected to reaction temperature from their values as given in the literature. The following values were employed:

$$pK'_P = 1.97 \ (6), \ pK''_P = 6.82 \ (6), \ pK'_E = 2^3, \quad pK''_E = 5.7 \ (7), \ pK'_A = 7.18 \ (8).$$

The standard free energy of the above reaction is given by

$$\text{I} \quad \Delta F^\circ = -RT\ln\frac{(P_t)\ (A_t)}{(E_t)} - RT\ln \left(\frac{(H^+)}{(H^+)+K_A}\right)\left(\frac{\frac{(H^+)^2}{k'_E\,k''_E}+\frac{(H^+)}{k''_E}+1}{\frac{(H^+)^2}{k'_P\,k''_P}+\frac{(H^+)}{k''_P}+1}\right)$$

It is not necessary to know the actual concentrations of P_t and E_t but only their ratio which, as a sufficiently good approximation, is equal to total radioactivity of the reaction mixture divided by the radioactivity found in the nitrophenyl phosphate. The value found in the reaction mixture was 2.135×10^6 cpm. In Expts A and B the nitrophenyl phosphate contained 732 and 1,064 cpm, respectively. The average ΔF° is $-1{,}615$ cals. This value refers to the reaction as written and is independent of pH.

It is common practice to evaluate the so-called free energy of hydrolysis of phosphate esters by the use of the "apparent equilibrium constant" (e.g., see Alberty *et al.* [*9*]), in which only the total concentrations of the reactants are considered. Such an expression corresponds to the first term in the right side of equation I. The "apparent ΔF°" is proportional to the r'P of Dixon (*10*). Fig. 1 shows the dependence of the "apparent ΔF°" on pH. The latter value is only moderately high at pH 4, namely, $-3{,}180$ cal; it drops slowly to a minimum of about $-2{,}200$ cal at pH 7.0, and then increases to higher values at the rate of $-1{,}424$ cal/pH unit.

The efficacy of a donor depends upon enzyme specificity with regard to the donor phosphate, the acceptor and its phosphate, as well as on energy considerations. Thus "bond" energies are only permissive in the matter of transferability. In the present case the "apparent ΔF°" values do happen to accord with our observation that efficacy of transfer in the acid region increased with decreasing pH (*1*).

[3] The primary dissociation constant of *p*-nitrophenyl phosphate is too low to permit its determination with a glass electrode. It is reasonable to assume that it is lower than k'_P, and by analogy with other phosphate esters the value cited above is probably of the right magnitude. However the difference in ΔF° occasioned by this uncertainty is inconsequential in the pH range for which calculations are shown.

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Reactions of Dibenzodixanthylenes

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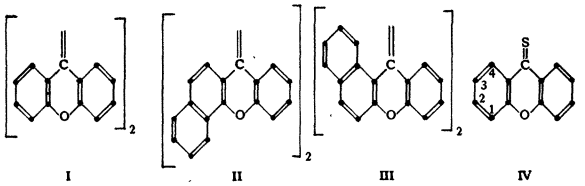
Dixanthylene (I) is almost colorless at low temperatures, but acquires a bluish-green color on heating, and the hot solutions—for example, in anisole—are also bluish-green (*1*). The authors have studied the thermochromic behavior of the two dibenzodixanthylenes (II and III) and have found that (II) is pale-yellow at room temperature, green when molten, mp, 345° C, gives a bluish-green solution in warm anisole, and in general exhibits remarkable thermochromic behavior. (III), which forms yellowish-green crystals, melts at 295° C, forming an olive-green melt. Its solutions in anisole are more weakly thermochromic to the naked eye than solutions of (II). In both cases, (II) and (III), the reversibility of the thermochromic phenomenon is the same as in the case of dixanthylene (*2*).

(II) and (III) have been obtained by the action of copper bronze on 1: 2-benzoxanthone or 3: 4-benzo-



xanthone ketochloride, respectively (*3*). (II) has also been prepared by the action of copper bronze on 1:2-benzoxanthione (IV), which may be prepared by the action of thioacetic acid on 1:2-benzoxanthone ketochloride; similar reactions are known in the case of xanthione (*4*).

When dixanthylene (I) is heated with sulfur, xanthione is formed (*3*). Similar reactions were carried out with (II) and (III), and thus 1:2-benzoxanthione (IV), mp, 141°, and 3:4-benzoxanthione, mp, 148°, were obtained, respectively. When (II) and (III) are treated with oxalyl chloride, followed by the action of water, 1:2-benzoxanthone and 3:4-benzoxanthone are obtained, respectively (*5*).

A full report describing the previous reactions, the action of lithium aluminum hydride, the Grignard solutions on 1:2-benzoxanthone and 3:4-benzoxanthone, respectively, and the action of ethereal diazomethane on 1:2-benzoxanthione and 3:4-benzoxanthione, together with photochemical behavior of 9-aryl-1:2-benzoxanthen and 9-aryl-3:4-benzoxanthen toward oxygen (*6*) and the photo-action of *p*-benzoquinone (*7*) and oxygen (*6*), respectively, on 1:2-benzoxanthen will be published soon.

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Method for Obtaining Large Yields of Human Platelets as a By-Product of Blood Collection[1]

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Clinical and experimental use of platelets has been limited by the expense and impracticality of obtaining them in quantity. The development of methods for storing blood (*1–4*) and isolating its constituents (*5, 6*) has permitted mass collection of human blood and detailed study of its fractions, including the formed elements (*7*). Among the latter, probably platelets are the least well understood, but the most important in thrombocytopenic conditions accompanied by bleeding. Recently the use of ion-exchange resin for the purpose of rendering blood incoagulable by fixing calcium ions (*8, 9*) proved, fortuitously, to cause partial disappearance of platelets from the blood. This apparent obstacle to the eventual harvesting of platelets by centrifugation for laboratory study and clinical use was turned to advantage when elution of the resin proved to be an efficacious means of salvaging quantities of platelets (*10*).

Blood-collecting and -transfusion sets (*9*) made of translucent plastic material (polyvinyl chloride acetate copolymer) and containing a column of ion-exchange resin (sulfonated polysterene divinyl benzene copolymer[2]) for making the blood incoagulable by decalcification were used. Needles were coated with tris (2-hydroxyethyl) dodecylamine.[3] The column of resin, contained in 28-mm plastic tubing, was suspended from the donor needle on the afferent end by small-caliber tubing and was led to the blood receptacle by similar tubing on the efferent end. The ion-exchanger consisted of 50 g of Dowex-50 beads on the sodium cycle. The resin was washed with saline solution and kept moist. Platelet suspensions were received in clean silicone-lined[4] flasks or bottles. The eluting fluid was unbuffered solution of 0.085% NaCl in distilled water. Ordinary 50-ml glass syringes equipped with metal adapter tips were used to deliver the physiologic saline eluting solution.

The usual blood donation unit of 500 ml was collected by means of the described blood-collecting set (*9*). The resin container was then cut free at both ends, leaving a few centimeters of tubing attached at each end. The contents of the resin container were then washed with the physiologic saline solution, simply by introducing the adapter tip of a syringe filled with saline solution into the open end of the snugly fitting afferent tube, forcing the saline through the resin container, and catching the washings in a silicone-lined bottle. Approximately 10 ml of saline solution was added at a time, and the resin and saline were manipulated by kneading the contents of the container from the outside with the fingers, to aid in freeing whatever blood elements were attached to the resin beads. This process, and straight flushing with portions of saline solution, were alternated as necessary.

In order to obtain platelets in concentrations equivalent to that of whole blood, for purposes of counting, each resin column was washed with 500 ml of saline solution, the original blood volume. Elutions were made, also, with smaller quantities, for purposes of concentrating the platelets. Platelet counts were made by the direct method, using 3.8% sodium citrate solution as a diluent (*11*). Sealing the tubing at both ends of the resin container, dielectrically (*9*), would simplify recovery of platelets under sterile conditions if that were desirable.

Washing of the resin with sufficient saline solution to make up the original blood volume resulted in high yields of platelets that had been filtered out by the resin. A series of resin columns, through each of which 500 ml of whole blood had been passed once, were eluted with 500 ml of saline solution. The average concentration of recovered platelets was approximately 119,000/mm^3, or about 40% of the total normal

[1] Work done in the laboratories of the Harvard Medical School in cooperation with the Blood Characterization and Preservation Laboratories of the Bussey Institution of Harvard University, University Laboratory of Physical Chemistry, and the American National Red Cross.

[2] Dowex-50, Dow Chemical.
[3] A-15, Armour and Company.
[4] Dri-Film 9987, General Electric.