aggressive reaction patterns that in time gave way to increased passivity and malaise; by the time of gross autopsy, the animals had lost a considerable amount of body weight and showed definite signs of nephro- and hepatotoxicity. Compounds *5* and *9* were similar to *6*.

Compound *7* showed some symptomatology of disorientation at 31.6 and 10 mg./Kg. The animals were very jittery with increased motor activity and mydriasis. This compound may have some psychosomimetic activity. At high doses, it produced clonic convulsions preceded by Straub tail erection. It has a pattern of activity similar in many respects to pentylenetetrazol. Compound *10* showed considerable resemblance to *7* with regard to hyperreflexia, but also could be considered to be somewhat like *1*.

This gross pharmacologic screen has established the general lack of promazine-like activity in the *1–12* series of compounds.

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Complex Interaction of Starches with Certain Drug Pharmaceuticals

By M. WAFIK GOUDAH* and EARL P. GUTH

A study has been made of the complexing behavior of potato and arrowroot starch with a number of pharmaceutical compounds. By means of the solubility method of analysis, it has been found that the starches form complexes in solution with benzoic acid, salicylic acid, *p*-hydroxybenzoic acid, *m*-hydroxybenzoic acid, *p*-aminobenzoic acid, methyl *p*-hydroxybenzoate, ethyl *p*-hydroxybenzoate, ethyl *p*-aminobenzoate, and propyl *p*-hydroxybenzoate. No evidence of complex formation was detected between the starches and caffeine. It is postulated that attractive forces and inclusion formation are responsible for the interaction observed.

Starch, one of the most widely distributed naturally occurring organic compounds, is mainly composed of polysaccharides of the glucan type. The view now is accepted generally that the majority of starches contain molecules which can be classified according to one of two quite different structural patterns (1). One type is a linear polymer, and the polymeric bonds are substantially 1-4 α-glucosidic linkages. This linear fraction is called amylose. The other type has a branched structure due to connection of another chain of glucose units to the primary chain by a 1-6 glucosidic linkage. This nonlinear fraction is called amylopectin. When the spatial distribution of the chain of glucose units in amylose is considered, it is found to assume a spiral form. Support for this helical concept comes from X-ray, ultracentrifuge, viscometric, and other studies of the amylose–iodine complex. Results indicate that the period of each spiral is six glucose units, and in the amylose–iodine complex, it has been shown that the iodine atoms are situated in the core of helically oriented amylose molecules (2). Amylopectin, most likely, has a large and ramified structure with short linear branches. This concept of structure is supported by the results of studies employing enzymatic degradation (3).

Separation of amylose and amylopectin can be made by adding to a starch dispersion certain agents such as butanol, nitropropane, nitrobenzene, and thymol, which form complexes with amylose and cause it to precipitate in semicrystalline form. The amylose complex is collected by centrifugation, and the amylose is regenerated by adding hot water or ethanol. Amylopectin then is isolated from the mother liquor by precipitation with alcohol or by freeze-drying (4–6).

Starch is used in many pharmaceutical preparations. It is used extensively as an absorbent in "weeping" types of dermatosis. It is applied

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to the skin as dusting powders, pastes, and ointments for this purpose (7). Starch paste, 10% w/w, is often a tablet binder when rapid disintegration is expected. Starch is also used as a disintegrator and as a diluent in tablets. Although starch has been used widely in pharmaceutical formulations, no investigations of the possibilities of complex reactions by this macromolecule appear to have been made. This study was undertaken to investigate the possible complex formation between starches and a number of pharmaceutical compounds. These compounds were chosen partly because of pharmaceutical use as preservatives and partly because their molecular structure might give some insight concerning the structural factors favoring complex formation.

EXPERIMENTAL

Reagents.—Potato starch (Mallinckrodt Chemical Works); arrowroot starch, (Penick and Co.); benzoic acid (analytical reagent) m.p. 122–122.5°; salicylic acid (analytical reagent) m.p. 157–159°; *p*-hydroxybenzoic acid (recrystallized) m.p. 213–215°; *m*-hydroxybenzoic acid (recrystallized) m.p. 198–200°; *p*-aminobenzoic acid (recrystallized) m.p. 185–186°; methyl *p*-hydroxybenzoate (recrystallized) m.p. 125–126°; ethyl *p*-hydroxybenzoate (recrystallized) m.p. 115–116°; propyl *p*-hydroxybenzoate (recrystallized) m.p. 94–95°; ethyl *p*-aminobenzoate N.F., m.p. 88–90°; and caffeine U.S.P., m.p. 234–235°, were utilized.

Apparatus.—The necessary apparatus comprised a constant-temperature water bath (set at 30° ± 0.5°) with rotating spindle, a 125-ml. capacity bottle with gum rubber stoppers, and a Beckman DU spectrophotometer.

PROCEDURE

Preparation of Starch Sol.—Starch, 16.2–24.3 Gm., was weighed accurately into a beaker, smoothed into a slurry with distilled water, then poured slowly with stirring into a flask of hot water at 90–95°, stirred for 5 min., then transferred to an autoclave and heated at a pressure of 19–21 p.s.i. for 3 hr. The resulting sol was stirred then until cooled to room temperature. The sol then was transferred to a volumetric flask and distilled water added to volume of 500 ml. Autoclaving insured complete dispersion of the starch granules and markedly decreased the viscosity. During this heating process, it is recognized that the starch molecule is deaggregated into amylose and amylopectin. Since the helical structure of these compounds is unaltered and since the entire starch entity is used in pharmaceuticals, no attempt was made to separate the two components. Therefore, throughout this paper references are made to the individual starches rather than to the deaggregated components. All starch sols were discarded 24 hr. after preparation.

Solubility Studies.—The solubility method of Higuchi and Lach (8) was employed in determining the extent and the nature of possible complexing reactions between the compounds and starch.

Excess quantities of the drug were weighed accurately into a 125-ml. bottle together with varying amounts of starch sol, then water was added to a final volume of 60 ml. The bottles were closed, then agitated in a constant-temperature water bath at 30° until the system reached equilibrium. The excess solids were allowed to settle while the bottles remained in the water bath. A 1-ml. aliquot of the clear supernatant liquid was withdrawn and appropriately diluted for spectrophotometric analysis. To avoid any error due to the viscosity of the sols, each pipet was calibrated to deliver all of its contents by washing the pipet several times with distilled water.

Method of Analysis.—The spectrophotometric analysis was determined for each of the drugs studied at the following wavelengths: benzoic acid, 272.5 mμ; salicylic acid, 298 mμ; *p*-hydroxybenzoic acid, 251 mμ; *m*-hydroxybenzoic acid, 290 mμ; *p*-aminobenzoic acid, 278 mμ; *p*-hydroxybenzoate, 256 mμ; ethyl *p*-hydroxybenzoate, 256 mμ; propyl *p*-hydroxybenzoate, 255 mμ; ethyl *p*-aminobenzoate, 283 mμ; and caffeine, 272 mμ. The absorbance of the starch sol was negligible at dilutions of 1:500. At lesser dilutions, the absorption of the starch sol was corrected for by determining the absorbance of a series of dilutions of the starch sol at the wavelength of the drug. The specific extinction coefficient of the sol obtained was used to calculate the absorbance of the starch sol. This value was subtracted from the absorbance obtained for the drug starch sol mixture.

RESULTS

In the solubility method of analysis, complex interaction is evidenced by either a decrease or an increase in the solubility of the complex. Figures 1 and 2 represent the interaction of the organic acids with potato starch. Figures 3 and 4 represent interactions with arrowroot starch. The interaction of

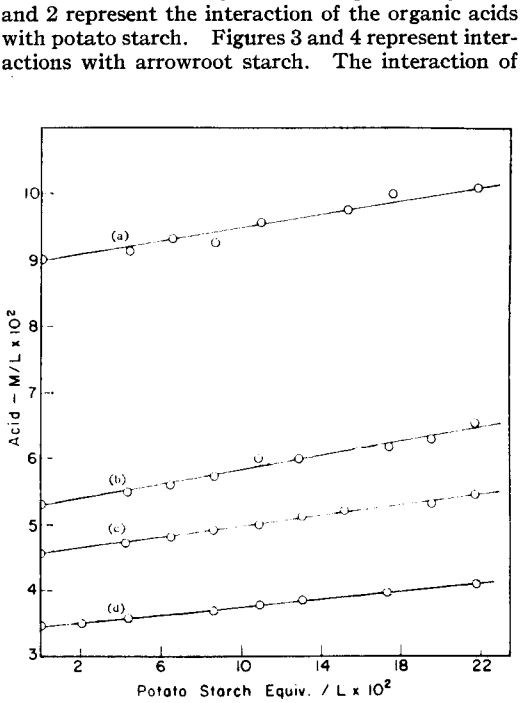

Fig. 1.—Key: (a) *m*-hydroxybenzoic acid; (b) *p*-hydroxybenzoic acid; (c) *p*-aminobenzoic acid; (d) benzoic acid.

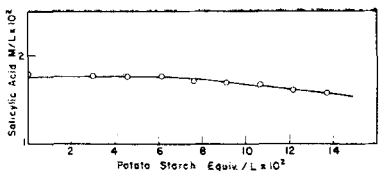

Fig. 2.—Solubility behavior of salicylic acid in presence of potato starch in water at 30° C.

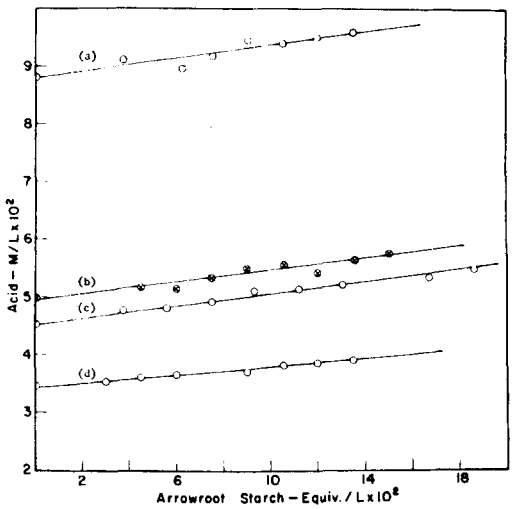

Fig. 3.—Key: (a) *m*-hydroxybenzoic acid; (b) *p*-hydroxybenzoic acid; (c) *p*-aminobenzoic acid; (d) benzoic acid.

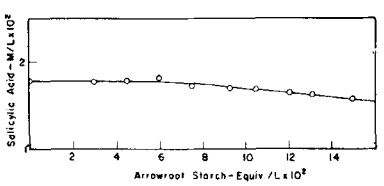

Fig. 4.—Solubility behavior of salicylic acid in presence of arrowroot starch in water at 30° C.

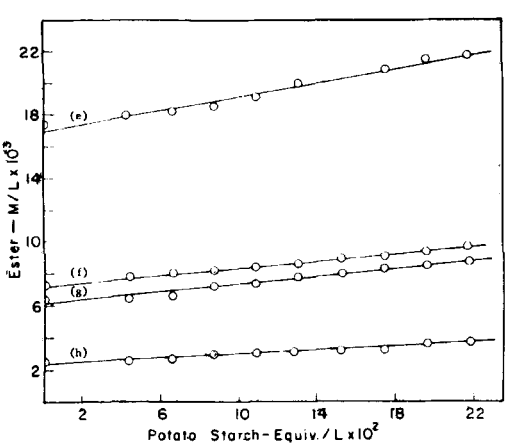

Fig. 5.—Key: (e) methyl *p*-hydroxybenzoate; (f) ethyl *p*-aminobenzoate; (g) ethyl *p*-hydroxybenzoate; (h) propyl *p*-hydroxybenzoate.

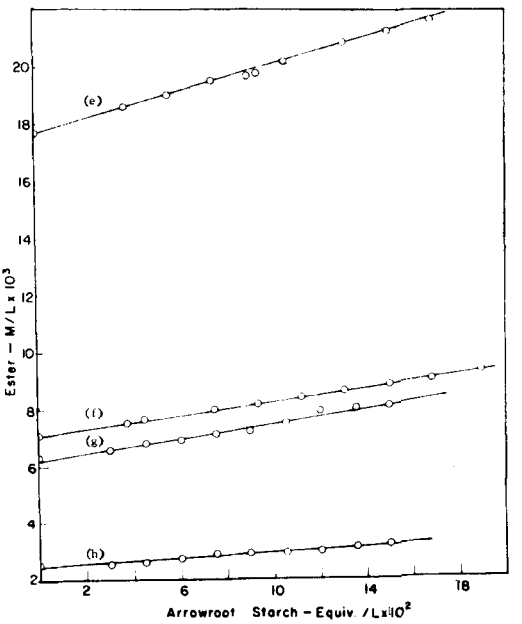

Fig. 6.—Key: (e) methyl *p*-hydroxybenzoate; (f) ethyl *p*-aminobenzoate; (g) ethyl *p*-hydroxybenzoate; (h) propyl *p*-hydroxybenzoate.

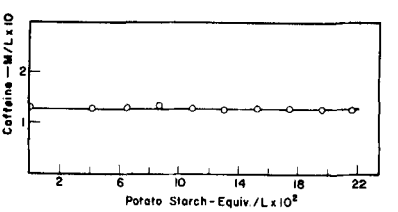

Fig. 7.—Solubility behavior of caffeine in presence of potato starch in water at 30° C.

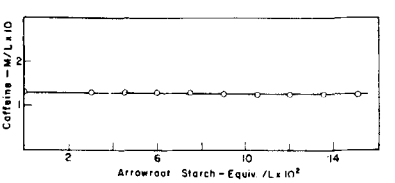

Fig. 8.—Solubility behavior of caffeine in presence of arrowroot starch in water at 30° C.

the esters with potato and arrowroot starch is shown in Figs. 5 and 6, respectively. Caffeine did not evidence complex interaction with either potato or arrowroot starch, as shown in Figs. 7 and 8. Except for caffeine, all drug chemicals studied showed a definite interaction with both potato and arrowroot starches. Benzoic acid, *p*-hydroxybenzoic acid, *m*-hydroxybenzoic acid, *p*-aminobenzoic acid, methyl *p*-hydroxybenzoate, ethyl *p*-hydroxybenzoate, ethyl *p*-aminobenzoate, and propyl *p*-hydroxybenzoate associated with the starches to form soluble complexes. The straight-line plots obtained show that the increase in solubility of the acid is a function of the concentration of the starch.

The method for comparing the slope of the interaction isotherms, used by Lach and Cohen (9), is used as a measure of the relative complexing tenden-

TABLE I.—SLOPES OF ISOTHERMS OF INTERACTIONS OF THE DRUG CHEMICALS WITH THE STARCHES

Drug	Potato Starch Slope × 10^2	Arrowroot Starch Slope × 10^2
m-Hydroxybenzoic acid	5.55	5.86
p-Hydroxybenzoic acid	5.48	5.29
p-Aminobenzoic acid	4.20	5.10
Benzoic acid	3.31	3.41
Methyl *p*-hydroxybenzoate	2.15	2.40
Ethyl *p*-hydroxybenzoate	1.23	1.28
Ethyl *p*-aminobenzoate	1.08	1.15
Propyl *p*-hydroxybenzoate	0.536	0.473
o-Hydroxybenzoic acid	Insoluble complex	Insoluble complex
Caffeine	No interaction	No interaction

cies of the drugs. The slopes of the interaction plots are shown in decreasing order in Table I.

Certain plots obtained through solubility data provide information concerning both stoichiometry and formation constant of the complex formed. Where no plateau region is obtained, stoichiometry cannot be determined because the concentration of free drug present in solution is an invariant. Only salicylic acid interaction plots showed a plateau region indicative of formation of an insoluble complex. The stoichiometric ratio of the salicylic acid–starch complex formed in the plateau region can be calculated from the phase diagrams. Analysis of this region in the salicylic acid–potato starch diagram (Fig. 2) indicates that 21 equivalents of starch react with one molecule of salicylic acid. The same stoichiometric ratio of 22:1 was obtained for the salicylic acid–arrowroot starch interaction (Fig. 4). This value is regarded as an approximation because of the complexity and heterogenicity of the starch chemical composition. No attempt was made to calculate the formation constant of the complex due to lack of information on the exact molecular weight of the starches.

DISCUSSION

The drug chemicals interacted in the same manner with the two starches tested. This indicates that inherent in the starch structure is the liability to form complexes with polar drug pharmaceuticals. The mechanism of interaction of the drug chemicals tested with the starch sols can be elucidated by considering the chemical structure of the starch molecules as well as the chemical structure of the drugs. Because of the helical structure of the starch molecules and the relatively large open space within the molecule, it might be expected that one factor for complexation is the formation of monomolecular inclusion compounds where one molecule of the host encloses one or more molecule of the guest. Attractive forces, *i.e.*, hydrogen bonding, is another important factor to be considered as part of the complexation mechanism. The multiplicity of hydroxyl and carboxyl groups on the starch molecule enables it to interact with polar drugs.

Data for the relative complexing tendencies of the drugs tested are in support of a mechanism that includes both attractive forces and inclusion formation as the one responsible for complexation.

Meta- and *p*-hydroxybenzoic acids showed the greatest slope and therefore the greatest degree of interaction. This is definite proof that hydrogen bonding is an important factor in the complexation mechanism. This is further substantiated by the fact that *p*-aminobenzoic acid has a degree of interaction intermediate between benzoic acid and the hydroxybenzoic acids. *p*-Aminobenzoic acid has less tendency toward hydrogen bonding than the hydroxy acids because of the weak electrophilic nature of amino hydrogen contrasted to the hydroxyl hydrogen.

The importance of inclusion formation as an additional factor for the interaction can be appreciated when the degree of interaction of starch with the esters is examined (Table I). A decrease in the slope with an increase in molecular weight is observed. This is attributed to special filling—as the ester chains become longer and the molecule therefore larger, less inclusion of the ester takes place. The failure of caffeine to interact with starch sols is probably due to its large stereochemical configuration, which would be difficult to fit in the voids of the helical starch molecule.

The relative complexing tendencies of the drugs and the mechanism postulated for the interaction is in close agreement with the results of Lach and Cohen (9) on the complex interaction of the Schardinger dextrins. The Schardinger dextrins are a series of homologous oligosaccharides obtained from the breakdown of starch by the action of bacillus macerans amylase.

Pharmaceutical complexation is an important factor that must be considered in formulations. The complex interaction of the starches with benzoic acid and *p*-hydroxybenzoic esters is expected to decrease their preservative activity, which is primarily a function of the unbound molecules (10).

The ability of starch to form complexes with drugs should not be overlooked when starch is an ingredient of the formula.

SUMMARY AND CONCLUSIONS

Potato starch and arrowroot starch were found to form complexes with benzoic acid, hydroxybenzoic acid, *p*-aminobenzoic acid, ethyl *p*-aminobenzoate, and esters of *p*-hydroxybenzoic acid. Salicylic acid was the only drug that formed an insoluble complex with both starches. Caffeine did not interact with the starches tested. The mechanism of interaction of the drug chemicals with the starch sols is believed to be a combination of inclusion formation and attractive forces, such as hydrogen bonding, dipole-dipole interactions, and other attractive forces.

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