Complexation of Ammonium Salts with Phenols

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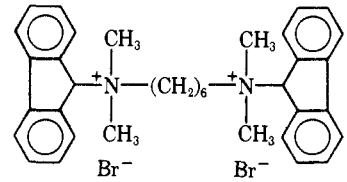
A variety of phenols and quaternary ammonium salts in aqueous solutions form complexes that vary considerably in stability and solubility. Certain diquaternary ammonium bromide salts yield nonhydrated crystalline complexes with phenol with retention of bromide ions, or with increasing pH, loss of the inorganic anion. Complexes also have been prepared from hydrophobic group substituted diquaternary ammonium compounds and β-naphthol, pyrocatechol, and pyrogallol. An analogous, but amorphous, complex was obtained from a hydrophobic-substituted monoquaternary salt and phenol. Quaternary ammonium derivatives also bind strongly to the phenol-containing polypeptide, polytyrosine. The phenolic group of tyrosine units in polypeptide structures may be able to serve as a bonding receptor for biochemically or pharmacologically active ammonium compounds.

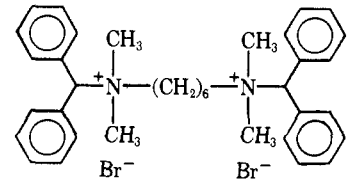
Organic quaternary ammonium salts of biochemical and pharmacological interest usually are assumed to undergo at least ion-exchange bonding interactions with some anionic group of a biochemical receptor. Carboxylic and phosphoric acid moieties are the most often implicated of these anionic groups. Simple phenolic compounds and certain quaternary ammonium salts in aq solutions form crystalline complexes, the stoichiometry of which depends on the particular phenol and on the pH. These complexes can form under physiologically compatible conditions. The phenolic group of tyrosine in biopolymers is another possible receptor for ammonium compounds.

It has been known that phenols and many proton-acceptor amines form complexes, particularly in inert organic solvents. In sufficiently concentrated solutions, and appropriate pH, amines and phenols in aq solutions also form less soluble complexes or liquid phases. We observed that many quaternary ammonium salts and phenol in concentrated aq solutions, particularly in the pH range 7.5–10, form amorphous precipitates or separate oily phases. By selecting diquaternary ammonium salts bearing appropriate hydrophobic substituents, it was possible to isolate stable, crystalline complexes of these ammonium derivatives and phenols. These complexes were less soluble in H_2O than their individual components and could be recrystallized from H_2O. Two types of complexes were obtained, one in which the inorganic ions of the ammonium salt were retained, the other (at higher pH) in which the inorganic ion was displaced. The complexes could be prepared as anhydrous products and are not H_2O-containing clathrates.

A quaternary ammonium salt found to be convenient for the preparation of crystalline complexes with phenols in aq solutions was hexafluorenium bromide (I) or hexamethylene-1,6-bis(9-fluorenyldimethylammonium) dibromide. This diquaternary salt became commercially available for use as a muscle relaxant in surgery.[2-4] Aq solutions of I and phenol in a variety of proportions, but conveniently in molar concentrations of 0.01 and 0.05, respectively, with no pH adjustment



I



II

(about 5.6) promptly yielded a crystalline precipitate, soluble in hot H_2O but much less soluble in the cold. The product was dried at 25–35° *in vacuo* to yield a sharply melting, stable complex comprised of one mole of I, two of phenol, and no H_2O. This composition was further verified spectrophotometrically. Other hexamethylene and decamethylene α,ω-bisquaternary ammonium salts with smaller hydrocarbon substituents or tetrabutylammonium Br^- yielded only oily precipitates with phenol at relatively high concentrations. To assess whether I favored complexation as a result of the large coplanar, hydrophobic 9-fluorenyl groups, the biphenylmethyl analog II was investigated. Although crystal separation occurred more slowly and in smaller yield, II also formed a product containing 2 molecules of phenol. The total hydrophobic mass in I and II need not be coplanar for formation of these crystalline complexes; however, the planarity of individual phenyl rings may facilitate hydrophobic interaction with the phenolic aromatic ring.

Efforts to form complexes were extended to other phenols. β-Naphthol and I formed an analogous complex containing 2 molecules of the naphthol. The di- and trihydric phenols, pyrocatechol and pyrogallol, did not form precipitates with I unless the pH was raised to above 7 with aq NH_4OH. With pyrocatechol the composition of the complex formed with I changed with increase in pH with a gradual displacement of Br^-. At a pH of about 10, phenol, pyrocatechol, and pyrogallol formed sharply melting complexes with I in which Br– had been displaced, and that contained 6, 4, and 4 molecules of the phenolic component, respectively.

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(2) C. J. Cavallito, A. P. Gray, and E. E. Spinner, *J. Amer. Chem. Soc.*, **76**, 1862 (1954).

(3) F. F. Foldes, N. R. Hillmer, R. E. Molloy, and A. P. Monte, *Anesthesiology*, **21**, 50 (1960).

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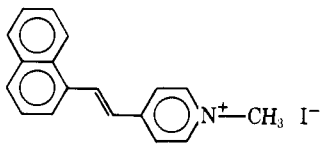
Other phenols, including α-naphthol, hydroquinone, resorcinol, and 2,3-naphthalenediol, yielded precipitates with I under comparable conditions, but these were not investigated further at this time.

Interest in the phenolic group as a possible biochemical bonding receptor for certain pyridinium quaternaries has been expressed recently from a different perspective.[5] Uv spectral data showed evidence of some interaction between III and phenol in dilute aq solutions. With a rise in pH to above 7, III and phenol formed a less soluble product. This reaction with III appears to bear some resemblance to the interaction of



III

I and phenol at elevated pH. Biochemical interest in III is related to its activity as an inhibitor of the enzyme choline acetyltransferase[6] and to its interaction with DNA[7].

A complex of III and phenol was isolated, but with difficulties associated with the need to work with dilute solutions (limited solubility of III), the noncrystalline nature of the complex, and conditions of drying.

The observed interaction with simple phenols led to the investigation of the binding propensity of III with tyrosine and certain derivatives that might serve as models for the Tyr moiety of polypeptides. Since the very low solubility of the homopolymers did not permit comparison of complex formation under the same conditions as used with phenols, their effect on the uv absorption spectra of the quaternaries was used to estimate the extent of interactions. By this method a strong binding of either cation I or cation III with poly(Tyr) was noted. A maximum binding of one molecule of III per approximately 6 Tyr residues was achieved in these experiments. Under identical conditions organic cations I and III were considerably less bound by poly(histidine) or poly(phenylalanine). Figure 1 illustrates the marked effect of poly(Tyr) on the spectrum of solutions of I and the comparatively small effects of poly(His) and poly(Phe). Spectra obtained with III showed similar decreases in absorbance with poly(Tyr) and poly(Phe); poly(His), however, exhibited a somewhat greater affinity for III than for I. Relatively little interaction was detected with L-Tyr, L-TyrGly, Gly-L-Tyr, and *N*-acetyl-L-Tyr hydrazide.

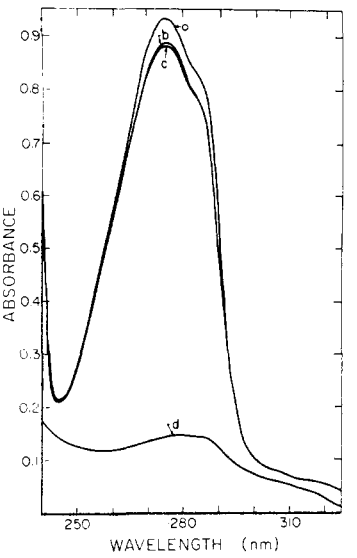


Figure 1.—Effect of polyamino acids on spectrum of hexafluorenium bromide (I). A solution of I (5 ml) at 50 μ*M* in 0.1 *M* phosphate buffer (pH 7.4) was stirred for 3 days at 5° in the presence of 1 mg of each homopolymer. After centrifugation at 5000*g* to remove insoluble polymer, the spectra of the supernatants were recorded (Cary 15); (a) control with no added polymer; (b) poly(L-phenylalanine); (c) poly(L-histidine); (d) poly(L-tyrosine).

Discussion

Interactions between quaternary ammonium compounds and phenols may be of wider occurrence than has been appreciated. The limited aq solubility and crystallinity of the phenolic complexes of I may facilitate their isolation and the recognition of a more general phenomenon. This may be so, particularly, with the type of complex of I–phenol obtained below pH 7. X-Ray crystallography and other physical techniques may help establish the spatial structure of these complexes. However, some speculations can be made as to the nature of the interacting forces among the components.

In aq solutions of a quaternary ammonium salt such as I, the Br^- and the cationic charges concentrated on the onium N centers are bonded by nondirectional electrostatic interactions. In nonaqueous media, halide ions from quaternary ammonium salts and proton donors show evidence of H bonding to the anion.[8,9] Phenols as the unionized species can be excellent H donors. Tetra(*n*-heptylammonium) iodide in a variety of aliphatic alcohols or phenol as H donors showed evidence of H-bonding complexing involving the iodide ion.[10] Phenol showed the largest equilibrium constant for a presumed 1:1 H-bonded complex of ROH and the onium iodide. In the complex of I·di-Br^- with 2 molecules of phenol, the phenol may be held by a combination of H bonding to Br^- and hydrophobic and/or van der Waals bonding to the fluorene group. Dimensional features are favorable for such dual interaction. One molecule of phenol for each of the two fluorenylammonium bromide moieties would be involved. The hydrophobic character of the onium substituent may further favor H-bonding interaction of phenol with Br^- by locally excluding competing H_2O molecules to more closely approximate complexing conditions in nonaqueous media. The quaternary ammonium salt substituted with a large hydrophobic mass may provide a unique local environment for reactions in aq media. A cationic charge is centered on a N atom which cannot

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(8) A. Allerhand and P. von Ragué Schleyer, *J. Amer. Chem. Soc.*, **85**, 1233 (1963).

(9) M. J. Blandamer, T. E. Gough, and M. C. R. Symons, *Trans. Faraday Soc.*, **60**, 488 (1964).

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participate in H bonding. Propensity for solvation further is diminished by the hydrophobic hydrocarbon substituents. This places the cationic charge and the counterion in a local environment of low dielectric, similar to conditions in an "inert" solvent.

As the pH is raised in solutions of I and phenols, the phenolate anion concentration is increased. The pK_a values for phenol and for the first OH of pyrocatechol are 10 and 9.5, respectively.[11] The extent of displacement of Br^- is far greater than expected from simple ion exchange of competing concentrations of phenolate anions. In the complexes formed from I and phenols at pH 10, more phenolic molecules are bonded to the diquaternary salt than in the complexes formed at pH 5.6 which retain Br^-. The complexes formed between I and phenols with loss of halogens presumably contain two phenolic moieties in the formal equivalent of phenolate ions. The additional phenol units may be held by a combination of H bonding with phenolate and hydrophobic interactions. Hydrophobic interactions also may favor stacking of the aromatic rings. With pyrocatechol and pyrogallol, considerable Br^- displacement was evident in the products formed in the pH range of 7 to 8. Displacement of Br^- may be facilitated by H bonding of the halide ions of I with phenolic OH to weaken the electrostatic bonding energy of the halide ion with the onium center. The polyhydric phenols, which can provide phenolic OH and phenolate anion within the same molecule appear to displace Br^- more readily than does phenol.

Although these complexes are of interest from the standpoint of the physical chemistry of complex formation, they also may have biochemical significance. The phenolic group of Tyr may be capable of serving as a binding site in a protein receptor structure for ammonium compounds. The high affinity of I and III for poly(Tyr) illustrates the marked enhancement of bonding which may occur when a simple molecular entity is incorporated in an appropriate polymeric structure. This can be important in considering the significance of simple complexations to reactions of natural biopolymers. Physiologically involved catechol derivatives are another target for possible interactions with quaternary salts, particularly if one of the components is attached to a biopolymer carrier.

Experimental Section

Experimental conditions described in the preparation of the complexes are those which readily yielded stoichiometrically reproducible compositions as reflected by elemental analysis and physical characteristics. Yields of isolated products were of secondary concern, but were sufficient to indicate the principal direction of product formation. Analyses were performed by M-H-W Laboratories, Garden City, Mich.

Complexes from Hexamethylene-1,6-bis(9-fluorenyldimethylammonium) Dibromide (I). A. With Phenol.—Twenty milliliters each of aq solns of 264 mg of I (0.02 *M*) and 188 mg of phenol (0.1 *M*) were combined. Crystals sepd that could be dissolved by heating and pptd in better yield by cooling with ice. The pH was 5.6–5.8 and not adjusted. The white crystals were filtered off, washed with small vols of ice H_2O, and dried *in vacuo* at 25° for 3 days or at 35° for 1 day to yield 280 mg of product, mp 122–123°. *Anal.* (Br) found: 18.1%; (I) calcd: 24.1%. A complex of I with 2 molecules of phenol would be $C_{48}H_{54}Br_2N_2O_2$, calcd: C, 67.74; H, 6.39; Br, 18.8. Found: C, 67.59; H, 6.19. Such a composition represents an 82% yield. One added molecule of H_2O would require calcd: C, 66.34; H, 6.49. Drying for 2-hr intervals at 80° *in vacuo* resulted in slight but continuing wt losses, presumably of some phenol.

B. With Phenol, pH > 7.—When reaction A was carried out with an increase in pH up to pH 10 by addn of 5% aq NH_4OH soln, immediate pptn occurred of a less sol white material. The sepd, dried product contained only about 1% Br. It was recrystd from hot H_2O containing some phenol and NH_4OH to pH 10. The product obtained and dried *in vacuo* had mp 137–139°, did not contain Br, and had an N content of 2.43%. A compn consisting of 1 mole of the diquaternary dication, 2 of phenolate anions, and 4 of phenol, or $C_{12}H_{76}N_2O_6$, would require calcd: C, 81.19; H, 7.19; N, 2.63. Found: C, 80.89; H, 7.21. The yield of 250 mg accounts for 60% of the I dication.

Treating the quaternary salt with twice the previous concn of phenol (10:1) to provide an excess, yielded 380 mg (90%) of product, mp 139°. *Anal.* found: C, 81.62; H, 7.32. Slow recrystn from hot 0.015 *M* aq phenol soln, pH 10 (NH_4OH), did not change the mp measurably. *Anal.* Found: C, 80.54; H, 7.39; N, 2.50. Recrystn from distd H_2O results in reduced recovery of the product.

C. With β-Naphthol.—I (0.02 *M*, 132 mg, 10 ml) and 10 ml of warm 0.027 *M* (40 mg) β-naphthol were combined and cooled to room temp to yield a cryst product. A theoretical deficiency of the β-naphthol and nonrefrigeration minimized contamination by uncombined naphthol. The ppt was filtered off, washed with H_2O, and dried *in vacuo* at 80° for 2 hr to yield 90 mg of white product, mp 111–112°. *Anal.* calcd for 1 mol of I and 2 of naphthol: $C_{56}H_{58}Br_2N_2O_2$: C, 70.72; H, 6.15. Found: C, 70.67; H, 5.92.

D. With Pyrocatechol.—Combining 20-ml solns each of 264 mg of I (0.02 *M*) and 220 mg of pyrocatechol (0.1 *M*), pH 5.45, without adjustment, and cooling to 5°, yielded no ppt. If dil NH_4OH was added, a ppt began to form near pH 7. Sepn of the prepn showed it to be nonhomogeneous, with a Br content of 7.8%. A complex of I with 2 moles of pyrocatechol, or $C_{48}H_{54}Br_2N_2O_4$, would have 18.1% Br. On increasing pH, Br was further displaced. At pH 10, a reproducible product was obtained using either a 5:1 or 10:1 molar ratio of pyrocatechol to I. The white compd was recrystd from a hot dil soln of pyrocatechol adjusted to pH 10 with NH_4OH, filtered, washed with H_2O, and dried *in vacuo* at 25 or 35°, mp 150–152°. The loss of Br and an N analysis of 2.65% suggested a compn of 1 mol of I dication and 4 mols of pyrocatechol with loss of 2 H, or $C_{60}H_{64}N_2O_8$. *Anal.* calcd: C, 76.58; H, 6.85; N, 2.98. Found for prepns from 5:1 and 10:1 molar proportions, respectively: C, 76.89, 76.62; H, 6.84, 6.89. Yields of 300 and 310 mg represent 90% of theory.

E. With Pyrogallol.—As with pyrocatechol, pyrogallol did not yield a ppt unless the pH was raised with dil NH_4OH. The first material sepg on raising pH above 6 was nonhomogeneous and had 6.85% Br. An adduct of I with 2 mols of pyrogallol would have 17.5% Br. Twenty milliliters each of aq solns of 264 mg of I (0.02 *M*) and 252 mg of pyrogallol (0.1 *M*) were combined and bubbled with N_2 for 10 min. Under N_2, 5% aq NH_4OH was added to raise the pH to 10. The white ppt formed was filtered off by suction, washed quickly with cold H_2O, and dried *in vacuo* at 25° for 3 days (or 35° for 1 day); yield 340 mg, mp 171–173° dec. Loss of Br and a found N content of 2.56% suggested a compn of 1 mol of I dication and 4 mols of pyrogallol (analogous to pyrocatechol complex). Elimination of 2 H would provide $C_{60}H_{64}N_2O_{12}$. *Anal.* Calcd: C, 71.70; H, 6.41; N, 2.78. Found: C, 72.80; H, 5.93. The yield represents 85% of theory. Although the C anal. is less satisfactory than that of the other complexes, the pyrogallol prepn was not recrystd to avoid oxidn and darkening. No other proportion of components can accommodate the analyses.

Complex from Hexamethylene-1,6-bis(diphenylmethyldimethylammonium) Dibromide (II) and Phenol.—Carrying out the prepn as with procedure A and replacing I with II yielded only a few crystals after several hr at 5°. Combining 5-ml vols with 133 mg of II (0.04 *M*) and 94 mg of phenol (0.2 *M*), respectively, in H_2O (twice preceding concns) gave no immediate ppt (in contrast with I), but cooling to 5° overnight yielded 70 mg of white crystals, dried *in vacuo* at 25° for 2 days, mp 136–138°. *Anal.* for a compn comprised of 1 mol of II and 2 of phenol, or $C_{48}H_{58}Br_2N_2O_2$, calcd: C, 67.42; H, 6.84; Br, 18.71. Found: C, 67.67; H, 6.65; Br, 18.53. Recovered yield was 41%.

Absorption Spectra of Complexes.—The two complexes of

(11) Merck Index, 8th ed, Merck and Co., Inc., Rahway, N. J., 1968.

phenol-I obtained by procedures A and B were dissolved in 0.1 *M* Na phosphate buffer at pH 7.4. Uv spectra were recorded and found to be nearly identical with spectra of solns of the 2 components having molar ratios of I to phenol of 1:2 and 1:6, respectively, in agreement with the compn indicated by elemental anal. of the isolated complexes.

Complex from *N*-Methyl-4-(1-naphthylvinyl)pyridinium Iodide (III) and Phenol.—A soln of 37 mg of III in 100 ml of H_2O (1 m*M*) was poured slowly, with rapid stirring, into 500 ml of 0.67 *M* aq phenol adjusted to pH ~8 with NH_4OH. Glass-distd H_2O and pink light illumination were used in the operations. An amorphous ppt formed immediately, and the pH was raised to ~10 with NH_4OH. The ppt was collected by centrifugation at 3°, and dried to constant wt *in vacuo* over P_2O_5 and mineral oil (3 days) to yield 14 mg of an amorphous, yellow product. The limited aq soly of III in the cold required use of dil solns, and the physical nature of the product contributed to losses during isolation.

The complex did not contain iodide. Uv spectrophotometric anal. of the product in 0.1 *M* Na phosphate buffer, pH 7.4, showed it to contain 3 mols of phenol for each III cation. The compn was calcd by detg the concn of III cation from its ϵ_{max} at 377 nm, where phenol does not absorb, and the ratio of absorbances at 377 nm and 270 nm; both III cation and phenol absorb at 270 nm. The 3:1 ratio of phenol to monocation is equiv to the 6:2 ratio found with dicationic I (preparation B).

Interactions of Quaternary Salts with Polypeptides. Poly(L-tyrosine) (mol wt ~4950), poly(L-histidine) (mol wt ~8750), and poly(L-phenylalanine) (mol wt ~7000) were purchased from Miles-Yeda, Ltd. In a typical expt, 1 mg of the polymer was added to 5 ml of a 50 μM buffered aq soln of a quaternary compd, either at pH 7.4 (0.1 *M* Na phosphate) or pH 10 (0.1 *M* Na_2CO_3). These were covered and stirred magnetically for 3 days at 5°. The resulting polymeric suspensions then were centrifuged at 5000*g* and 3° (International refrigerated centrifuge). Absorption spectra of supernatants were obtained using a Cary Model 15 recording spectrophotometer. These were compared with spectra of the original soln of the ammonium compd, which had been treated in a similar manner in the absence of polymer. With poly(Tyr), 85-95% of the test compd was removed from soln at pH 7.4 or 10, whereas poly(Phe) removed only 5-12%. Poly(His) showed a greater affinity for III then for I, combining with about 26% of the former and only 5% of the latter. The π-electron-deficient pyridinium system in III and electron-donor propensity of unprotonated histidine imidazole moieties may contribute to bonding by charge-transfer interaction.[5,6]

Nonspecific Inhibition of Enzymes by Organic Contrast Media

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The activities of a variety of enzymes were measured in the presence of several organic roentgenographic contrast media. The compounds were found to inhibit all of the enzymes tested, and for each compound, the concentrations producing 50% inhibition fell in a relatively narrow range, resulting in a nearly constant order of effectiveness. The order of effectiveness as inhibitors correlates well with the order of binding to serum albumin and appears to parallel the hydrophobic natures of the compounds. The results are interpreted as implying a very general property of proteins: the ability to bind small molecules in a nonspecific way.

This laboratory has been interested for some time in the interactions of organic roentgenographic contrast media with enzymes and other proteins in an effort to understand the mechanisms by which these compounds exert their physiologic effects.[1] Similarity of some symptoms of contrast media toxicity with those produced by ACh led to a study of the inhibition of AChE by contrast media.[2] Other enzymes which might be effected *in vivo* by contrast media were investigated and when a pattern of inhibitory effectiveness began to emerge, the study was expanded to include a variety of different types of enzymes encompassing a wide range of molecular weights and catalytic specificities, without regard to their physiologic significance. In this communication, we report on the inhibition of the enzymes lysozyme, β-glucuronidase (two types), alcohol dehydrogenase, and glucose 6-phosphate dehydrogenase, and we discuss an apparently general relationship between inhibitory strength and structure.

Almost uniquely in medicine, contrast media as clinically employed are present locally in blood vessels and in some tissues in very high concentrations. Concentrations of injectable media are of the order of 0.3 to 1 *M*. In angiography as much as 0.2 mole might be injected. Very high concentrations, therefore, occur at the site of injection into a vessel and until the bolus has passed the first capillary bed. Excretion occurs *via* the kidney or the bile-forming system of the liver, and these organs are presented with high concentrations of the compounds. Oral cholecystographic agents do not appear in high concentrations in the blood, but they are concentrated in bile by way of the liver.

Contrast media are, of necessity, relatively nontoxic, but they are employed in such large doses that mild toxic effects are a common clinical experience, and severe reactions sometimes occur. Consequently, although for some of the compounds relatively high concentrations are required to effect enzymic activity, the findings are relevant to the physiologic situation.

Material and Methods

The contrast media tested were iopanoate (**1**), iodipamide (**2**), diatrizoate (**5**), and iothalamate (**6**), which were obtained as the commercially available pharmaceuticals, and acetrizoate **4** and **3**, which are experimental compounds. The structures are shown in Chart I.

Alcohol dehydrogenase and bacterial β-glucuronidase were products of Sigma Chemical Co., hen's egg lysozyme was obtained from Worthington Biochemical Corp., and bovine liver β-glucuronidase was obtained from Nutritional Biochemicals Corp. Glucose 6-phosphate dehydrogenase was prepared as follows. Erythrocytes of freshly collected heparinized human blood were washed twice with 0.15 *M* NaCl and then lysed with 9 vol of a soln contg 0.01 mg of triphosphopyridine nucleotide and 0.5 μl of 2-mercaptoethanol/ml. The

(1) E. C. Lasser, *Radiology*, **91**, 63 (1968).
(2) E. C. Lasser and J. H. Lang, *Invest. Radiol.*, **1**, 237 (1968).