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Review Article

Thiophene Compounds of Biological Interest

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THE CONCEPT OF preparing thiophene analogs of biologically active compounds has stimulated workers in pharmaceutical chemistry so that it now appears that at least one thiophene analog has been prepared for every important therapeutic group containing a benzene nucleus. It has been hoped that the thiophene analog would possibly be as active as the parent compound or that the thiophene analog would, by virtue of its similar chemical structure, combine with the receptor and—if not eliciting a response of its own—serve effectively as a competitive inhibitor. Thus, when a thiophene analog is prepared, it is conceivable that such an agent may either intensify, mimic, or antagonize the physiological activity of the parent substance. Many of the thiophene compounds are more toxic than their benzene analogs and, consequently, less effective medicinal agents. There are, however, some exceptions: several thiophene derivatives are employed clinically.

Excellent summaries of the literature up to 1959 are available (1–5). In this review particular emphasis will be placed on knowledge gained since 1959. To present a more comprehensive understanding of the role of thiophene in pharmaceuticals, mention of some of the earlier work will be included.

The electronic structure of thiophene must be very similar to that of benzene, pyrrole, and furan. Each carbon atom must be in an approximately trigonal state of hybridization with a $2p$ orbital perpendicular to the plane of the ring. These orbitals interact laterally with the two $3p$ electrons provided by the sulfur atom to form the π-bonds. As the energy difference between $3p$ and $3d$ is not great, it has been suggested (6) that the sulfur-carbon bonds in thiophene do not consist of pure p-orbitals, but that some of the $3d$ orbitals of the sulfur atom were used. When pd hybridization of the sulfur orbitals occurs, three pd (2) hybrid orbitals are formed, two of which have the correct symmetry and energy to conjugate with the carbon atoms. The third hybrid orbital is high in energy and is unoccupied in the ground state (7). No agreement has been reached, however, on the importance of pd hybridization of the sulfur atom. This hybridization is considered by some workers as an essential feature in the electronic structure of thiophene, making the sulfur atom similar to a CH=CH group, and being responsible for some of the differences between thiophene, furan, and pyrrole; whereas others have suggested that most of the properties of thiophenes may be accounted for without invoking pd hybridization at all (8). Mangini and Zauli (8*b*) even doubt the basic validity of the Longuet-Higgins approach (7*a*).

The structural similarity between thiophene and benzene is also reflected in that both form "sandwich" compounds with certain metals. For example, thiophene forms thiophene-chromium-tricarbonyl (9), and numerous similar compounds are known in the benzene series.

In addition to the established use as anti-

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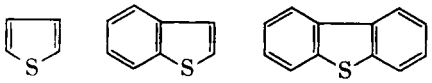
histamines (10), certain thiophene derivatives have shown (1, 5, 11–16) substantial activity as pressor compounds, local anesthetics, hypnotics, analgesics, anticholinergics, antispasmodics, anticonvulsants, tuberculostats, antibacterials, antimalarials, and germicides. Many of these products have biological and pharmacological properties markedly different from the benzene analogs.

Both biochemical and pharmacological studies have shown that the "theoretical equivalence" of a thiophene and a benzene nucleus—which is easy to establish if only similarities are mentioned and dissimilarities are disregarded—is by no means universal. The general conclusion to be drawn on determination of the biological properties of thiophene (I) and its higher ring homologs, thionaphthene (II) and dibenzothiophene (III), is that these compounds usually possess activities similar to those of benzene, naphthalene, and anthracene but less pronounced and often more toxic (1, 5). Comprehensive studies of the



I II III

biological properties of thiophene and its higher ring homologs have been limited by the low solubility of such compounds in aqueous media. However, excellent historical reviews of the biological properties of the simpler thiophene derivatives have been compiled by Blicke (1) and Martin-Smith (5).

THIOPHENE DERIVATIVES AS BIOLOGICAL ANTAGONISTS

The best known of the thiophene antimetabolites are the two isomeric β-thienylalanines which are antimetabolites of the essential amino acid, β-phenylalanine. The "antiphenylalanine" properties of thienylalanine have been investigated with *Saccharomycea cerevisiae*, *Escherichia coli*, *Streptococcus faecalis*, and *Lactobacillus arabinosus*. It was observed (17) that only the L isomer, and not the D, inhibited the growth of *S. cerevisiae*, *E. coli*, and *L. delbrueckii*, and that the inhibition of growth of rats produced by the DL, D, or L isomer is counteracted by phenylalanine (18–20). Of the two isomers, the 3-thienyl isomer appears to be a more active antagonist than the 2-thienyl isomer (21–23). For *S. cerevisiae*, strain 139, the growth inhibitory properties of the 3-thienyl isomer were about twice those of β-2-thienylalanine, and for *E. coli* the 3 isomer was about one-third more effective. The activity was reversed by phenylalanine.

Since no systematic study had been made of the tissue changes brought about by feeding 2-thienylalanine, Kaufman, *et al.* (24), studied the pathologic changes induced by β-2-thienylalanine. A purified synthetic diet of the 2 isomer in amounts of 1 and 2% was fed to rats for 4 weeks. Marked loss of weight was noted in all these animals. In addition to the expected loss of weight, the pyloric glands of the stomach and Brunner's glands of the duodenum were shrunken, and the cytoplasm was vacuolated. The acini of the submaxillary glands were atrophied; kidneys were enlarged and the proximal convoluted tubules were damaged; there was spermatogenic arrest at the spermatid level. The ductus epididymis was narrowed, the epithelium was atrophic, and the glands contained a decreased amount of secretion. There was a decrease in the number of mature lymphocytes of the spleen and of the thymus; the skin was hyperkeratotic, and the hair follicles were atrophic. Thinning of the epiphyseal plate was observed, with a decrease in growth of bone. Less marked changes were noted in the heart, pituitary gland, thyroid, adrenals, and the liver. Hruban and Wissler (25), using β-3-thienylalanine, reported changes in the pancreas, liver, spleen, and thymus which resembled in some respects the changes described by Kaufman for β-2-thienylalanine.

More recently, work has been directed toward the study of the biological activity of simple peptides (26) formed from the β-thienylalanines, and there has been interest in the simple amino acids themselves as potential antiviral (27) and anticancer agents (28). Generally, the toxicity for rats (29) and the growth stimulation and inhibition for *E. coli* (26*a*, *b*, *d*) are no more pronounced than with the corresponding simple amino acids; in some cases the thienyl peptides are less effective. Dipeptides of β-2-thienylalanine competitively inhibited the utilization of dipeptides of phenylalanine (26*d*, 30). Such inhibition studies have contributed additional evidence to previous proposals of a special role of peptides in the growth of microorganisms (31, 32) and indicate the possibility of specific sites of utilization of dipeptides.

Growth studies with *Lactobacillus arabinosus* (33) showed that L-leucyl-L-phenylalanylglycine was more effective than phenylalanine or the related dipeptide in reversing the toxicity of L-leucyl-β-2-thienyl-L-alanylglycine; this was accepted as evidence for possible utilization of tripeptides without previous hydrolysis to the free amino acids or the intermediate dipeptides. To pursue studies of the role of tripeptides in

metabolism, Dunn (34) prepared four tripeptide analogs containing β-2-thienylalanine. Tripeptides of phenylalanine were more active than phenylalanine in reversing the toxicity of β-2-thienylalanine in *E. coli*, strain 9723. Tripeptides of β-2-thienylalanine were comparable to free β-2-thienylalanine as growth inhibitors for *E. coli* when tested in the salt-glucose medium; and when tested in the presence of analogous tripeptides of phenylalanine, they were many times as toxic as free β-2-thienylalanine. The toxicity of the tripeptides of β-2-thienylalanine was reversed noncompetitively by phenylalanine and its dipeptides. The results are interpreted as indicating that tripeptides of β-2-thienylalanine compete with tripeptides of phenylalanine in a specific way and that their route of utilization is independent of the route followed by the possible hydrolytic products.

It has been shown that although structural analogs of amino acids added to a culture in the exponential growth phase inhibit normal multiplication of the organism, they do not cause an immediate cessation of growth and protein synthesis (35). Incorporation of structural analogs of amino acids, *e.g.*, *p*-fluorophenylalanine, β-2-thienylalanine, and norleucine, in bacterial proteins during linear growth of the organism has been observed (36). It is suggested that structural analogs of amino acids which can be incorporated into bacterial proteins during the linear growth phase do not act by inhibiting protein biosynthesis, but rather by promoting the synthesis of proteins which are abnormal with respect to structure and activity.

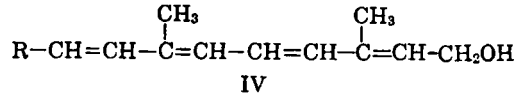
Comparison (37) of isotopic thienylalanine and fluorophenylalanine with respect to their participation in peptide and in protein synthesis reveals that the former amino acid was incorporated about 20 times more readily into gramicidin S by *Bacillus brevi* than the fluoro compound. In protein synthesis, fluorophenylalanine was more than five times more extensively utilized than was the thienyl derivative. Wolfe and Hahn (38) reported that β-2-thienylalanine was strongly activated by an *E. coli* extract but was not utilized for net protein synthesis by this organism.

3-Thienylalanine has also been reported (39) to have an effect on protein formation. The incorporation of labeled amino acids into serum and tissue proteins of β-3-thienylalanine–treated rats was compared with control rats. Suppression of the labeled amino acid uptake was found in all of the proteins analyzed, with a significant difference between the degrees of suppression. The γ-globulin and albumin among the serum proteins were less affected than the β- and α-globulins. Intestinal proteins showed the least suppression.

Moderate growth inhibition has been observed in several transplanted tumors (Murphy-Storm lymphosarcoma) by feeding β-3-thienylalanine in diets lacking phenylalanine (40). Diets of β-3-thienylalanine fed to male albino rats have also been noted to inhibit the growth of transplanted Jensen and Walker tumors, but no regression of the tumors occurred (41). These authors report that β-3-thienylalanine potentiates the inhibitory action of triethylene amine on tumor growth.

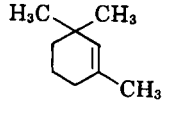
When β-3-thienylalanine is fed to rats for an appropriate time before and after antigen injection, it markedly inhibits antibody response (42). Added to antibody-synthesizing tissue culture system, it did not inhibit antibody synthesis. It appears that unless β-3-thienylalanine is fed during the induction period, its inhibitory effect will not be manifested. The activity is probably in inhibiting the setting up of the antibody-forming mechanism.

Thiophene derivatives as antimetabolites are not solely limited to the antiamino acids. Acheson, *et al.* (43), have synthesized some thiophenes (IV) related to vitamin A, although no thiophene derivative closely analogous to vitamin A appears to have been achieved.

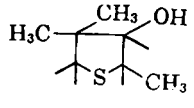


IV

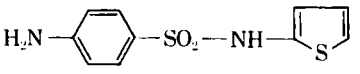
R (vitamin A) =



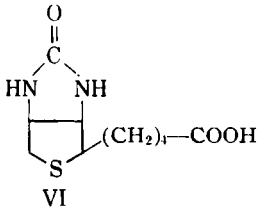
R (thiophene relative) =



Many modifications of the sulfanilamide molecule, an antimetabolite to folic acid-requiring bacteria, have been synthesized; among these are compounds containing a thiophene ring (5). 2-Thiophenesulfonamide (V), a carbonic anhydrase inhibitor, has been used to determine the role of carbonic anhydrase in the bicarbonate excretion from salivary glands and the mechanism of ionic excretion (44). There is also a considerable amount of literature on biotin (VI) (vitamin H) and structurally related compounds such as norbiotin, homobiotin, and biotin sulfone, which act as antimetabolites (5, 45).



V



VI

ANTIHISTAMINES

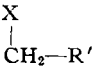
Thiophenes are most extensively used as antihistaminics. The literature on these compounds is so voluminous that only a brief consideration can be given herein. Blicke (1) records 26 thiophene derivatives which are analogous in structure to tripelennamine, while Martin-Smith (5) has observed that there are over 100 publications dealing with 2-thienyl isosteres of tripelennamine. In addition to the Blicke and Martin-Smith articles, reviews of antihistaminic drugs, in which the relationship of the various thiophene derivatives to other drugs of this class is shown, are available (10, 46). Some of the most important antihistamines possessing thiophene rings are included in Table I (5). Chlorothenylpyramine (No. XI, Table I) is also analogous to chlorprophenpyridamine. Being twice as active and possessing one-half the acute toxicity (54, 56), the 5-halogenated thiophenes (No. XI and XII, Table I) were more active than tripelennamine. When equal doses of the drugs were administered, chlorothenylpyramine protected against histamine shock twice as long as tripelennamine (61). Thiophene isosteres of other well established antihistamine drugs have also been prepared (50, 58, 62–66). However, the thiophene compounds have not shown marked superiority as antihistamines to compounds of other chemical groups, and the relative potency of the isosteres has varied according to the biological test system employed.

Campaigne and co-workers (67) studied the synthesis and reactions of 3-substituted thiophenes and prepared four *N*-substituted dimethylaminoethyl pyridines containing the 3-thienyl and halogen-substituted 3-thienyl nucleus. The 3-thienyl compounds all possessed activities about equal to those of the 2-thienyl analog (methapyrilene) and tripelennamine. The unsubstituted derivative (thenyldiamine; compound X, Table I) is more potent than the chloro and bromo substituted analogs (48, 53). This difference between the increased activity on halogenation in the 2-thienyl series and the lack of this in the 3-thienyl analogs is attributed to the difference in the position of the halogen atoms, being 5 in the former and 2 in the 3-thienyl series (48).

In common with other antihistamines, the thiophene derivatives show local anesthetic activity (68), possess antifungal action (69), prolong the blood clotting time (70), exert cardiodepressant activity (71), possess antipyretic activity in some species (72), possess some oxytocic activity, and demonstrate, to a greater or lesser degree, atropine-like activity.

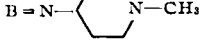
Methapyrilene is reported to possess hypnotic activity approximately equal to that of phenobarbital (73) and to potentiate the effect of other hypnotic drugs (74). The nicotinates of methapyrilene and thenyldiamine have been reported to be useful sedatives (75). Methapyrilene was found to possess quinidine-like activity in experimental cardiac arrhythmias in dogs (76). In atrial arrhythmias, it was much stronger than quinidine. For ventricular tachycardia resulting from acute mycocardial infarction, it was more effective than quinidine in suppressing the ectopic activity. In addition, methapyrilene was found to possess anti-

TABLE I.—ANTIHISTAMINIC THIOPHENE COMPOUNDS



No.		R	R′	X[a]	Ref.
VII	(Antergan)	Phenyl	Phenyl	A	...
VIII	(Tripelennamine)	2-Pyridyl	Phenyl	A	...
IX	(Methapyrilene)	2-Pyridyl	2-Thienyl	A	(47–51)
X	(Thenfadil)	2-Pyridyl	3-Thienyl	A	(48, 52, 53)
XI	(Chloromethapyrilene) (chlorothen)	2-Pyridyl	2-(5-Chlorothienyl)	A	(54, 55)
XII	(Bromothen)	2-Pyridyl	2-(5-Bromothienyl)	A	(56)
XIII	(Methaphenilene) (Diatrin)	Phenyl	2-Thienyl	A	(50, 57)
XIV	...	2-Pyrimidyl	2-Thienyl	A	(58)
XV	...	2-Lepidyl	2-Thienyl	A	(59)
XVI	...	2-Lepidyl	2-(5-Chlorothienyl)	A	(59)
XVII	...	Phenyl	2-Thienyl	B	(60)

[a] A = $N—(CH_2)_2—N(CH_3)_2$.



veratrinic activity. Results are available which indicate that antiarrhythmic activity is common to a large number of different antihistaminic compounds and is not restricted to specific structural types (77). However, only diphenhydramine and methapyrilene exhibit high activity against both types of arrhythmias. In a study of the effect of antihistamines on experimental cardiac arrhythmia, Agotsuma (78) reports that tripelennamine was most effective, followed by methapyrilene, diphenhydramine, and chlorprophenpyridamine. Methapyrilene and its chloro derivative (chlorothenylpyramine) have also been reported (79) to be more effective than quinidine when tested for ability to protect the canine heart from spontaneous ventricular fibrillation during progressive hypothermia. The isomeric carboxy methapyrilenes of 2- and 3-thiophene have been prepared, and the observed antihistaminic activity and the soporific effects are described (80).

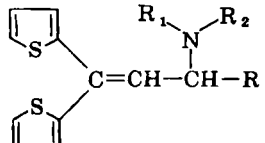
Finally, methapyrilene has also been employed in ophthalmology (81).

ANALGESICS

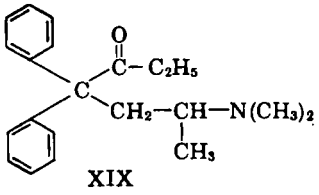
In the course of an investigation of the biological properties of a series of 3-tertiaryamino-1,1-di-(2-thienyl)but-1-enes prepared as analogs of the 3,3-diphenylallylamines which were known to have atropine-like, antihistaminic, and local anesthetic properties (82), the important discovery was made that the thiophene compounds—in addition to having similar activity to the compounds on which they were modeled—also possessed pronounced analgesic activity (83).

Unfortunately, the compounds of this series are addictive as are other potent analgesics.

The potent analgesics derived from 3-tertiary amino-1,1-di-(2-thienyl)-1-butene (XVIII; thiambutene) have been studied especially by



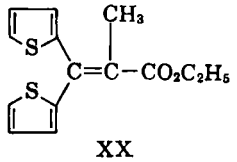
XVIII



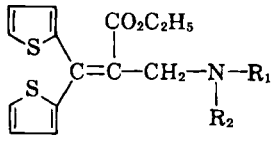
XIX

Japanese workers (85–88). Thiambutenes can be represented by the general formula (XVIII) and can thus be regarded as cogeners of methadone (XIX), in which the ketone side chain has been omitted, with the introduction of a double bond and the benzene rings replaced by thiophene rings. The double bond is not necessary for analgesic activity, and the corresponding saturated 1,1-di-(2-thienyl)-3-tertiaryaminobutanes are also potent analgesics, although somewhat weaker than the unsaturated compounds (89). It has been shown that it is the enantiomorph related to D-alanine which is the analgesically active optical isomer (90).

The thiambutene analgesics have attracted considerable attention in Japan where modified syntheses have appeared (91). Methods for the detection of thiambutenes have been described (92). The Reformatsky reaction between di-2-thienyl ketone and ethyl α-bromopropionate, followed by dehydration, gave compound XX. Bromination of the methyl group of XX with *N*-bromosuccinimide, followed by reaction with excess secondary amide, gave XXI which shows combined analgesic and antitussive properties (93). It is claimed that 3-piperidyl-1,1-di-(2-



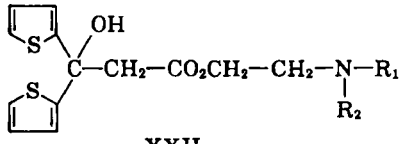
XX



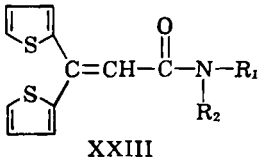
XXI

thienyl)but-1-ene is a potent antitussive as measured on the dog (94). The same compound was later reported to be very effective by oral administration to adults (95).

The Reformatsky reaction has also been used for the preparation of 2-aminoethyl 3,3-diaryl-3-hydroxypropanoates XXII and their dehydrated products (96). The propenamides (XXIII) have also been prepared for pharmacological evaluation (97). In 1-methyl-3-bis(2-thienyl)-



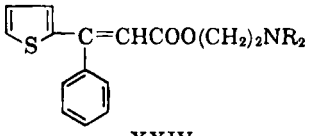
XXII



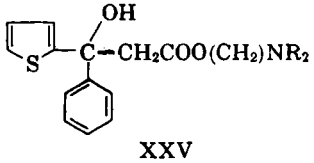
XXIII

methylenepiperidine, a potent nonnarcotic antitussive has been found (98). It is claimed to be more potent than codeine and shows no analgesic activity. 3-Piperidino-1,1-bis(2-thienyl)-1-butene has been tested clinically as an antitussive (99).

In a study of structure-activity relationships in antitussive agents, a working hypothesis—that the introduction of a piperidino group into a compound showing any action on the central nervous system can produce antitussive activity if the activity has been latent, or strength if such activity is already manifest—has been presented (100). For compounds of type XXI, XXIV, and XXV, some of which show thiambutene-like



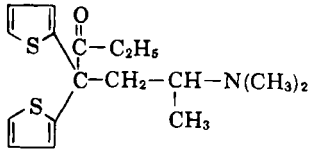
XXIV



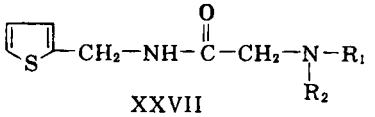
XXV

structure and weak analgesic activity, the piperidino compounds are definitely superior to the other antitussive activity (101).

The thiophene analogs of methadone XXVI and isomethadone have been prepared and shown to be active analgesics (102). Heterocyclic acetamides



XXVI

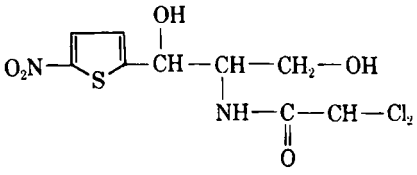


XXVII

of the type XXVII have been prepared for evaluation of their analgesics and antipyretic activity (103). Luts and Nobles have prepared the 3-thienyl analog of *d*-propoxyphene (104). Preliminary studies indicate that some of the 3-thienyl analogs combined sedative and stimulating properties in an unusual manner.

ANTIMICROBIAL AGENTS

Because of the complex chemical structure of many of the antibiotics, the synthesis of antibiotic isosteres has been somewhat limited. In the case of the penicillins, the important discovery that addition of a variety of organic acids or their derivatives to the culture medium resulted in their incorporation into new "biosynthetic" penicillins (105) provided a route for the easy production of several thiophene-containing penicillins (106). The thiophene analog of the naturally occurring antibiotic chloramphenicol (XXVIII) has been synthesized (107), as have been similar structures (5). The antibacterial



XXVIII

activity of all was much lower than that of the natural antibiotic (108).

An observation that nitrofurans possessed antibacterial activity encouraged the preparation of the thiophene isosteres. Thus 3-(5-nitro-2-thienyl) acrolein and 2-bromo-3-(5-nitro-2-thienyl) acrolein have been reported to be very active against *S. aureus* and *E. coli* (109) and to have a low toxicity to the tissues of the host. Cyanonitrovinylthiophenes have been reported to inhibit growth of bacteria and fungi (110).

Of the many nitrothiophenes prepared (111), it has been determined that the presence of the double bond as in the acrolein derivatives appears necessary for pronounced antibacterial activity and that the presence of a nitro group is not essential for effective antibacterial activity. However, it has been observed (111*a*) that the optimum position for the nitro group in the thiophene ring was the 5-position. Many chalcone derivatives of nitrothiophene carboxaldehydes (112) as well as hydantoin, thiohydantoin, and piperidine derivatives (113) have been tested, but these compounds exhibited little or no activity as bactericides. In addition, certain 5-chloro-2-thienyl-β-dialkylaminoethyl ketones have been reported to exhibit antibacterial activity (114).

In a comparative study of *in vitro* cellular toxicity of some isosteric derivatives with benzene and thiophene rings, Aurousseau (115) has noted that derivatives of thiophene have generally higher bacteriostatic potency than analogs of benzene. Only sodium thenoylacrylate and the sodium and ammonium salts of thenoic acid are slightly less active. The salt of thenoic acid with a bactericidal cation, 8-quinolinol, is more powerful than its analog. Synergism was observed in the cases of sodium thenoylacrylate, nitrothiophene, nitroacetothienone, and their homologs. Similarly, it has been demonstrated that some compounds, *e.g.*, DL-2-thienylalanine, with little or no antimicrobial activity when used

alone, act as synergists when mixed with certain antibiotics (116). Such synergism might make feasible increased therapeutic ranges of the more toxic antibiotics.

The main chemical classes of drugs showing high antitubercular activity are the sulfones, thiosemicarbazones, and certain acid hydrazides. Thiophene derivatives of each group have been prepared. The sulfone isosteres (117) possess slight antitubercular and antistreptococcal activity. All the thiosemicarbazone thiophene isosteres are reported to be active against *Mycobacterium tuberculosis* (118–123). The thiosemicarbazone of 4-(2-thienyl)-3-buten-2-one has been reported capable of completely inhibiting the *in vitro* growth of *M. tuberculosis* in relatively low concentration (124). Preliminary experiments with the 3 isomer have indicated that it has even higher activity. The hydrazine derivatives of the thiophene isosteres have been found to have an order of activity somewhat less than that of isonicotinylhydrazide (11, 125–128). The condensation products of these hydrazides with a large number of aldehydes and ketones have been investigated for the possibility of their blocking of the free amino group (11, 128). Also prepared for testing as potential tuberculostatic compounds were the condensation products of isonicotinylhydrazide and various aldehydes and ketones of the thiophene series (129).

Other compounds found to have antitubercular action were 1-*p*-thenoylphenyl-3-methyl-2-thiourea (130), which was reported to be very active against tuberculosis in mice, 6-(2-thienyl)-3-mercapto-1,2,4-triazin-5-one (131), and the thioamide of 2-thenoic acid (132). 2-Thiothenamide did not surpass thioisonicotinamide in antitubercular activity.

Recently a technique which employs a thiophene derivative has been developed for differentiation of *M. tuberculosis* into human and bovine types (133). By means of niacin test and tests of resistance against 2-thenoylhydrazine and 2-furoylhydrazine, *M. tuberculosis* strains can be divided into human and bovine types.

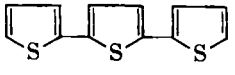
Many of those compounds exhibiting antibacterial activity are reported to possess fungicidal activity (134). Antifungal activity has also been reported in many other types of thiophene derivatives, especially those containing mercury (135). The antimicrobial activities of $(RC{\equiv}C)_2Hg$ and $RC{\equiv}CHgCl$ (R = 2-thienyl) were recently studied and found effective against both fungi and bacteria (136). 2-Thiophenemercurichloride has also found use as a plant bactericide by inhibiting the growth of imochibyo (a disease of rice) bacteria (137). Several 3-thienylrhodamines (138) and a thienyl benzimidazole derivative (139) are reported to exhibit fungistatic activity. 2-(2-Thienylthio)-ethylpyridine is reported (140) to have no insecticidal or bactericidal properties, but has mild fungicidal activity. 3,3,4,4-Tetrachlorohydrothiophene-1,1-dioxide is used as a fungicide (141). Fuerst and Higgins (142) have presented data on the inhibition of *Neurospora crassa* by a group of thiophenes. The phenomenon of colonization induction of *Neurospora* was observed with L-sorbose and by the thiophene derivatives. Some thiophenes were fungicidal while others were fungistatic.

Thiosemicarbazones of many substituted thiophene carboxaldehydes were synthesized as potential antiviral agents (143), while relatively few compounds containing thiophene rings have been prepared and tested for their toxicity to protozoan parasites. 2-Thiopheneglyoxal has been found only moderately active against Newcastle disease virus and influenza virus in embryonated eggs (144). Recently, certain β-amino ketones (Mannich bases) have been prepared (145) for which 2-acetyl thiophene was the ketone employed and 3-azabicyclo[3.2.2.]nonane served as the amine moiety. Results of preliminary screening (145*b*) indicated that significant antimicrobial activity against certain Gram-positive and Gram-negative bacteria, fungi, and protozoa is possessed by these compounds. Indications are that the thiophene isosteres are not as active as the benzene analogs.

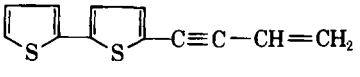
PESTICIDAL AGENTS

Thiophene compounds related to DDT have been examined for insecticidal properties (1, 5). For effectiveness against house flies, the thiophene isosteres and related thiophene derivatives have been disappointing, but they are reported effective against cockroaches and mosquito larvae, and a variety of other insects.

The interesting nematicidal activity of 2,2′-5′,2″-terthienyl (XXIX) (146) (also called α-terthienyl) and of 5-(3-buten-1-ynyl)-2,2′-bithienyl (XXX) (147), prompted Uhlenbroek and Bijloo (148) to investigate more systematically the nematicidal properties of polythienyls and related compounds. The compounds exhibiting highest activity are recorded in Table II.

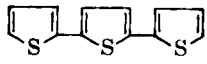
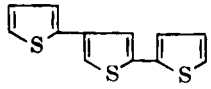
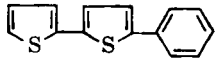
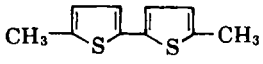
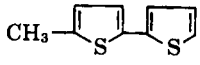
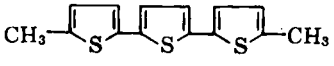
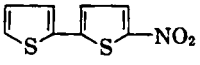
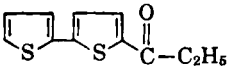


XXIX



XXX

TABLE II.—NEMATICIDAL THIOPHENE COMPOUNDS

Compd. No.		Structure
XXIX	2,2′-5′,2″-Terthienyl	
XXX	2,2′-4′,2″-Terthienyl	
XXXI	5-Phenyl-2,2′-bithienyl	
XXXII	5,5′-Dimethyl-2,2′-bithienyl	
XXXIII	5-Methyl-2,2′-bithienyl	
XXXIV	5,5″-Dimethyl-2,2′-5′,2″-terthienyl	
XXXV	5,5′-Dichloro-2,2′-bithienyl	
XXXVI	5-Nitro-2,2′-bithienyl	
XXXVII	5-Propionyl-2,2′-bithienyl	

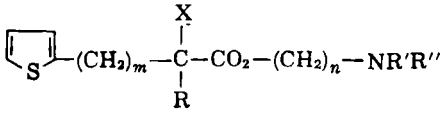
The consequences of the following structural variation were studied in particular: (*a*) isomeric changes, (*b*) substitution of one or more thiophene rings in polythienyls by benzene rings, (*c*) introduction of methyl groups into polythienyls, and (*d*) introduction of other substituents into polythienyls. The conclusion is drawn that any compound with very high nematicidal activity can be considered to be a derivative of 2,2′-bithienyl. However, 2,2′-bithienyl itself and a number of derivatives show little or no nematicidal activity. Therefore, proper substitution may also play an important part. In addition to their effectiveness against nematodes, the polythienyls are effective herbicidal agents against many noxious plants (149).

Several of the halogenated tetrahydrothiophene-1,1-dioxide compounds may be employed in nematicidal and other pesticidal application (150). An advantage is their ease of use, for no water seal is required. They may be used as dry powder with filler or as a liquid when wetting agents are added.

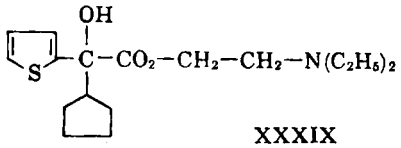
The nematicidal action of thenyl isothiocyanate has been demonstrated by its action against *Panagrellus redivivis* in water and *Meloidogyne incognita* var. *acrita* in soil (151).

ANTISPASMODICS

Earlier investigations have shown that powerful antispasmodic activity occurs in various basic esters of α-substituted thienylglycolic acids,



XXXVIII



XXXIX

thienylacetic acids, and thienylpropionic acids of the general type XXXVIII (5). One such compound, the 2-diethylaminoethyl ester of α-cyclopentyl-(2-thienyl)glycolic acid (XXXIX), is employed clinically in the form of its salt. The most recent work is concerned with the basic alkyl esters of α-(2-cycloalkenyl)-2-thienylacetic acids, which have been pharmacologically evaluated in the form of their acid addition and quaternary ammonium salts (152, 153). Several have been found to possess anticholinergic activity of a high order.

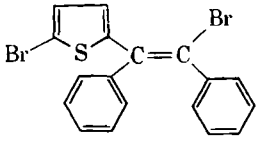
β-(α-Phenyl-α-3-thenylacetoxy)ethyldimethylsulfonium bromide was synthesized from 3-methylthiophene in an effort to determine the potentiality of this isostere of thiospasmin as an

antispasmodic agent (154). This agent was markedly less effective as an antispasmodic than is the cyclohexyl analog of thiospasmin. The compound appeared to potentiate the action of serotonin on the isolated rat uterus.

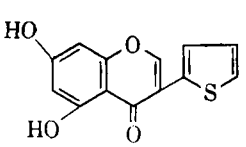
The main choleretic principle of the plant *Curuma domestica* was identified (155) as *p*-tolylmethylcarbinol. The isosteric alkyl-2-thienylcarbinols and alkyl(5-methyl-2-thienyl)-carbinols have been prepared (156) and reported to display choleretic activity in rats.

ESTROGENIC AGENTS

Many stilbene-like thiophene compounds have been prepared for a study of estrogenic activity, especially by Buu-Hoi, *et al.* Thiophene derivatives of nonhydroxylated stilbene types showed no significant activity (157), whereas weak estrogenic activity was found in 5-acetyl, 5-propionyl- and 5-benzoyl-2-(-stilbenzyl)thiophene (158). 1-Bromo-1,2-diphenyl-2-(5-bromo-2-thienyl)ethylene (XL) was found to inhibit body growth and to produce extensive testicular atrophy in male rats (159). It has been suggested that this compound deserves clinical trial in mammary cancer. 1 - Bromo - 1 - phenyl - 2 - (*p* - methoxyphenyl)-2-(5-bromo-2-thienyl)ethylene and 1-bromo-1-(*p* - chlorophenyl) - 2 - (*p* - methoxyphenyl - 2-(5-bromo-2-thienyl)-ethylene were reported to



XL



XLI

exert a true estrogenic action in rats (160), 1-Bromo-1,2-diphenyl-2-(5-bromo-2-thienyl)ethylene did not exhibit true estrogenic activity, although it did inhibit release or action of the follicle-stimulating hormone.

A thiophene analog of an isoflavone (XLI) showed no activity (161).

THIOSTERS OF CARCINOGENIC HYDROCARBONS

In the review article by Martin-Smith (5), the interest in compounds containing thiophene rings is reported to be derived largely from the ideas inherent in the receptor theory of drug action, the theory of biological antagonism, and the concept of bioisoterism. A new theory, which has gained favor in recent years and which also incorporates many of the principles of the three concepts mentioned above, is based on quantum mechanics. For example, although one would not ordinarily think of diagnosing diseases in a human patient by means of quantum mechanics, such a technique has been developed for differentiation between obstructive jaundice and nonobstructive jaundice.

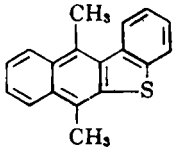
Schmidt (163) was the first to use wave mechanical methods to study the mechanism of the action of carcinogenic substances. He concluded that in order for an aromatic hydrocarbon to be carcinogenic, it was necessary that there exist in the molecule a region rich in π electrons, the density of which exceeded a certain threshold value. Svartholm (164) proposed to associate the carcinogenic properties of hydrocarbons with the reactivity of addition across one of the bonds. To explain an antagonism phenomenon which they had observed, Lacassagne, Buu-Hoi, Daudel, and Radali (165) imagined that carcinogenic hydrocarbons must be able to form an addition complex with the substrate which controls cellular division. This addition complex was assumed to be effective in producing a cancer only if it could take place at certain well defined points in the cell.

Several theoretical studies, of which summaries are available in recent literature (166), have shown that hydrocarbons or derivatives that are carcinogenic have a bond with high bond order. As this property determines the rate of certain addition reactions to this bond, it is tempting to assume that the addition of the hydrocarbon through one of its bonds to certain cellular constituents is an important step in the production of cancer. A more recent report states that it seems improbable that there was a clear relation between the total amount of a substance which was fixed to the cellular proteins and its carcinogenic power (167). However, it remained probable that a necessary condition for a substance to be carcinogenic was that a substantial amount was fixed by one of its bonds to the protein.

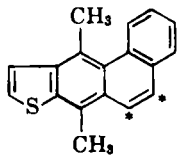
Many of the polycyclic hydrocarbons that have proved to be carcinogenic are phenanthrene derivatives suitably substituted by alkyl groups or with fused aryl rings attached to phenanthrene. Some heterocyclic analogs of these carcinogenic hydrocarbons, such as acridine, carbazole, and thiophene derivatives, have also proved to be carcinogenic (168). From these facts, Robinson proposed that the essential structural features for carcinogenicity in the polycyclic hydrocarbons may be an activated phenanthrene bridge (the 9,10 double bond in phenanthrene shown by asterisks in formula), which needs to be unsubstituted (169).

In an attempt to determine what effect, if any,

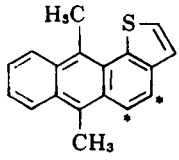
the 9,10 double bond might contribute towards carcinogenic activity, Robinson and Tilak have synthesized thiophene isosteres of carcinogenic hydrocarbons, in which the sulfur atom in the thiophene ring replaces the key phenanthrene bridge. A review of the work in this field, which contains a considerable amount of unpublished data, has been presented by Tilak (170). Interestingly, compound XLII was only slightly active



XLII

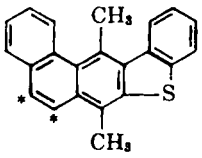


XLIII

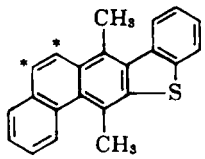


XLIV

when painted on mice and inactive by subcutaneous injection when compared to the carcinogenic compounds XLIII and XLIV. The



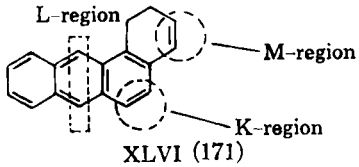
XLV



XLV*a*

inactivity of XLII was attributed to the absence of the phenanthrene bridge, since high activity again emerged in the case of XLV and XLV*a*.

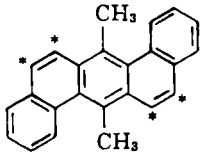
Since the first step in carcinogenesis involves a reaction of the carcinogen and a cellular receiver probably of an electrophilic nature, Pullman (166*a*) postulated that the "K-region" should be sufficiently active and that the "L-region" (*meso* position in anthracene residue) should be inactive (by substitution, *e.g.*, by methyl groups). The more carcinogenic hydrocarbons are generally



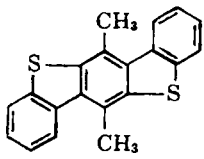
XLVI (171)

more strongly bound to proteins by the "K-region," while weaker carcinogenic or inactive hydrocarbons were bound through the "L-region" or by neither. For example, the electronic charge of the "K-region" of a series of benzoacridines was reported (172) to correlate directly with a tumor index (per cent of animals developing tumors per time in days for tumor appearance). There are exceptions which are difficult to explain on the basis of the Pullman hypothesis.

The synthesis of thiosters of carcinogenic hydrocarbons in which the "K-region" is replaced by isosteric substitution with thiophene has been continued (173). Many of the thiosters remain to be tested. Some unexpected results were observed among those which have been tested. The two phenanthrene bridges (PB's) in 9,10-dimethyl-1:2,5:6-dibenzanthracene (XLVII) have been replaced stepwise as in XLV*a* (173, 174) and XLVII*a* (175). Compound XLV*a* is a much more powerful carcinogen than XLVII (168*b*), and the high activity

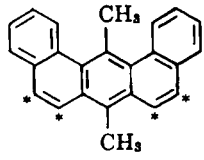


XLVII

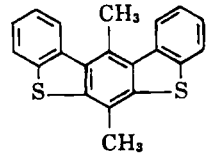


XLVII*a*

is attributed to a single activated PB instead of two competitive PB's as in XLVII (173). The key thioster XLVII*a*, wherein both the PB's are removed, proved highly carcinogenic, contrary to the expectation that it would be inactive (176). When one of the two PB's in 9,10-dimethyl-1:2,7:8-dibenzanthracene (XLVIII) was replaced, a highly active carcinogen XLV resulted as expected (177). However, the key thioster

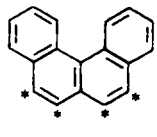


XLVIII

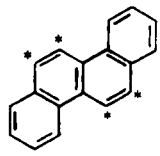


XLVIII*a*

XLVIII*a*, wherein both the PB's were removed (178), also proved to be highly carcinogenic contrary to its expected inactivity (176). The thioster of 3:4-benzphenanthrene (XLIX) and chrysene (L) have proved inactive (168*b*, 176), although the thiosters may contain one or two of the original phenanthrene bridges. The key thiosters of XLIX and L, in which both PB's

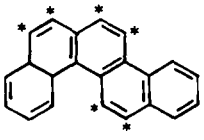


XLIX

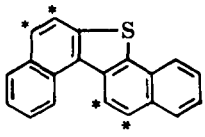


L

are replaced by sulfur, have also proved non-carcinogenic. The thioster (LI*a*) of the weakly carcinogenic 1:2,5:6-dibenzphenanthrene (LI) has given a highly toxic initial reaction when given subcutaneously to mice (178).



LI



LI*a*

Tilak (170) has observed that the interrelation between carcinogenicity and the structure of polycyclic hydrocarbons and their thiosters does not appear to be simple. The carcinogenicity of some of the thiosters which do not contain a phenanthrene bridge cannot be fitted into the "K-region" hypothesis, and it seems likely that the sulfur atoms in the condensed thiophenes have their own characteristic reactivity towards a biological substrate.

Metabolic studies (179) of dibenzothiophene have provided experimental support for the suggestion that the sulfur atom is implicated in metabolism. When fed to rats through a standard diet, 1-hydroxydibenzothiophene-5,5-dioxide[1] was isolated. The sulfur atom in thiosters, like the "K-region" in the corresponding hydrocarbon, is oxidized *in vivo* and is responsible for the carcinogenicity of these compounds.

As noted earlier for the β-thienylalanine, some thiophene derivatives possess the ability to retard the growth of various tumors. Thiophene-2,5-carboxylic acid in repeated doses inhibited the growth of Yoshida sarcoma in rats and the growth of a fibrosarcoma in mice (180). *In vitro* studies with a C^{14}-labeled glucose suggested that certain thiophene compounds interfered with the hexose monophosphate pathway. β-Chloroethylamino derivatives of thiophene were found quite toxic and inhibited the growth of tumors (sarcoma "45," Ehrlich's tumor) only slightly (181). Uptake and incorporation of glycine-1-C^{14} by Ehrlich ascites carcinoma cells was inhibited by α-amino-2-thiophene acetic acid (thienylglycine) (182).

MISCELLANEOUS

The earlier literature of thiophene chemistry indicates a potential use of thiophene isosteres as anticonvulsants (1, 5). A large series of 5-(2-thienyl) substituted hydantoins, including some bearing 3-alkyl substituents and 1,3-dialkyl substituents have been prepared (183), and some have been found to possess the same order of activity as 5,5-diphenylhydantoin. The anticonvulsant properties of 5-phenyl-5-(2-thienyl) hydantoin have been well established and compare directly with those of 5,5-diphenylhydantoin (184). It was clinically used for a time (185) on account of its favorable therapeutic ratio, since although less active than 5,5-diphenylhydantoin, it is also less toxic. 5,5-Di(2-thienyl) hydantoin is claimed to be active as an anticonvulsant (186).

Thiophene derivatives have been studied quite extensively for local anesthetic activity (1, 5). When a series of dialkylaminoalkyl esters of 3-thenoic acid were compared with the 2-isomers (67*c*), only γ-di-*n*-butylaminopropyl-3-thenoate was found to have significant local anesthetic activity. The activity of this compound was slightly less than that of the corresponding 2 isomer. A study of the influence of isosterism of acids on activity of salts and esters endowed with local anesthetic or analgesic properties has recently appeared (187). For procaine salts, the 2-thenoate was less potent, quicker acting, and of shorter duration of action than the benzoate, but both were more potent local anesthetics than the hydrochloride salts. The benzylurethan of glycol and the 2-thenylurethan of glycol were both local anesthetics, but much weaker than procaine hydrochloride. The thenyl isostere was more potent and also more toxic than the benzyl isostere. For morphine salts, the 2-thenoate acted more slowly, but the action was of longer duration than that of the benzoate. The (2-thenoyl)-acrylic acid salt of morphine was slightly more potent than the benzoylacrylic acid salt.

The discovery that certain β-haloethylamines possessed powerful adrenergic blocking action inspired a mild interest in thiophene compounds of this type (188). Of the thiophenes studied *N*-thenyl-*N*-benzyl-2-chloroethylamine was the most active (189). The antiadrenaline and antinoradrenaline activity of *N*-benzoyl-*N*-2′-halogenoethyl 2-thenylamines has been studied (190).

The radiation protective action of *N*-phenylamidines of thiophene carboxylic acid in white rats has been studied (191). The radioprotective effect of the *N*-phenylamidine of 2-thiophenecarboxylic acid has been reported (192) dependent on the dose used. The radioprotective effect began to be manifest at doses of 60 mg./Kg. Below these doses there was a sharp decline in the radioprotective capacities. At 60 mg./Kg. the percentage of survivals among the female animals was 60, while that among the males was 35. The difference between the protected male and female

[1] Tilak (170) reported earlier that 2-hydroxydibenzothiophene-5,5-dioxide was isolated during the metabolic studies of dibenzothiophene. Undoubtedly a typographical error has been made in one of these reports.

rats indicates a possible significance of female sex hormones. The radiosensitivity of various transplanted tumors is claimed to be increased by pretreatment with 2,5-dicarbethoxy-3,4-dihydroxythiophene (193). This agent inhibited the uptake of oxygen by homogenates of various mouse tumor tissues and of normal liver, kidney and spleen, but not of brain.

The diuretic activity of some thiophene derivatives has been studied recently (194). 2,4-Disulfonamidothiophene, 2,4-disulfonamido-5-chlorothiophene, and 2,4-disulfonamido-5-methylthiophene showed notable diuretic effect. However, at doses above 250 mg./Kg. they showed neuromuscular effects characterized by a degree of hypotonicity and ataxia and accompanied by respiratory depression. The diuretic activity of oral doses of 12.5 and 50 mg./Kg. was determined after 5 hours; the effect of daily doses was studied at the end of 3 and 6 weeks. Marked increases over the levels for controls were observed in the volume of urine, sodium, potassium, and chlorine excreted 5 hours after ingestion.

N'-Substituted-*N*-thiophenesulfonyl ureas have been reported to cause a significant lowering of the blood sugar level when administered intravenously as determined on rabbits (195).

A series of compounds containing thiophene rings which are isosteres of the less important biological active compounds, or else which have had very little work done on them is summarized by Martin-Smith (5). This series includes agents which act as vasoconstrictors, choleretics, antithyroid agents, vasopressors, sedatives, antimitotic substances, vasodilators, muscle relaxants hypotensive agents, and heart and respiratory regulators.

Recently 2-[3-(2-methylbenzothienyl)methyl]-2-imidazoline has been used to increase arterial blood pressure (196).

CONCLUSIONS

In the search for drugs with a higher degree of potency and fewer toxic side effects, considerable attention has been devoted to compounds which possess thiophene rings. From a voluminous amount of work very few new drugs of clearly superior clinical desirability have been produced. As a result, one can notice a certain slackening interest in the preparation of thiophenes for pharmacological studies. However, these results should serve as a guide to future research, and not as a sign that negative conclusions invalidate future work. As noted earlier (5), the work on thiophenes has made valuable contributions to the study of antimetabolites: it has shown in general the biological similarity of the phenyl and thienyl groups; and it has shown that the position of substitution of the thiophene ring is important (3-thienyl derivatives usually being more active and less toxic than their 2 isomers). The data accumulated should contribute to the development of newer theories which may lead to better understanding of drug action and biochemical processes.

In this connection, one can mention the use of thiophene and its homologs as starting compounds for the preparation of aliphatic amino acids (197). Long chain amino acids, both straight and branched, with a variable number of carbon atoms between the amino and carboxyl groups were prepared by synthesis of the corresponding thiophene derivatives and subsequent Raney nickel reductive desulfurization. The significant importance of the aliphatic amino acids and the opportunities offered by this method should be obvious, particularly regarding metabolic studies, since it would appear feasible for desulfurization to occur under biochemical conditions, as well as oxidation of the thiophene molecule. Studies have shown that there was increase of neutral sulfur in the blood, but no increase in the sulfate ion concentration (198).

It would appear advantageous to study thiopene derivatives more as individuals and less in regard to their isosteric relationship to some known biologically active agent. For example, 2,2'-5',2''-terthienyl (148) is reported to exhibit very high nematicidal activity, while the phenyl isostere (*p*-terphenyl) gives negative results. Also, the thiosters of the carcinogenic polycyclic hydrocarbons, in many cases, exhibited carcinogenic activity considerably higher than that of the phenyl isomer (170). Thus, while the thiophene isosteres may not exhibit activity comparable to the benzene isostere from which they were modeled, they may display a unique action (biological activity) of their own which will be entirely different from that of the benzene isomer.

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Research Articles

Gastrointestinal Factors in Aspirin Absorption

A Quantitative Study

By EDWARD B. TRUITT, Jr., and ANN M. MORGAN

Three gastrointestinal factors influencing aspirin absorption have been selectively adjusted for quantitative measurement of their effect on the rate of salicylate absorption in humans and dogs. These are (a) gastric emptying, (b) aspirin dissolution rate, and (c) intragastric pH. Other factors such as tablet disintegration time, aspirin particle size, and intersubject variability have been eliminated or reduced where possible. The major portion of the salicylate in the blood during the first 20 minutes comes from the stomach. Impedance of gastric emptying by the use of atropine and placing the subjects in a left lateral position did not eliminate the higher plasma salicylate levels produced by the inclusion of the buffer antacids in aspirin tablets. The major effect of these antacids appears to be an acceleration of aspirin dissolution. This has been confirmed in this study by an *in vivo* demonstration of more rapid absorption from solutions of aspirin than from tablets. Aspirin in solution has a lower pH alone than with the inclusion of buffering antacids. Selective adjustment of the degree of aspirin and sodium salicylate ionization through the use of various buffers provided a method of study of the influence of intragastric pH on the rate of aspirin absorption. Support was obtained from dog and human studies in favor of the pH partition hypothesis that salicylates are absorbed more rapidly at low pH values.

MANY INVESTIGATIONS of salicylate absorption have been made because of the ease with which salicylic acid can be measured in body fluids. Despite this, some factors regulating this membranous transfer have only recently been measured under physiologic conditions in man. One such factor is the direct transfer of salicylate from the stomach into the blood circulation (1, 2).

The claim of Paul, *et al.* (3), that certain antacids increased the gastrointestinal absorption rate of aspirin has received considerable examination. Despite a number of studies showing no significant differences (4–7), quite a few tests using adequate numbers and crossover design to reduce intersubject variability have shown clearly

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