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BURGER AWARD ADDRESS

Toward More Selective Antiarthritic Therapy[1]

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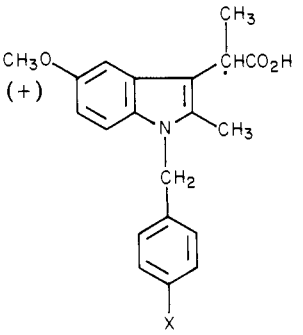
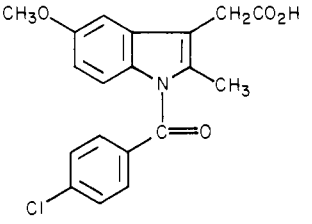
Arthritis is a family of joint diseases affecting 7% of the total population worldwide. Its impact on the well-being of patients and socioeconomic loss is generally recognized. The search for antiarthritic therapy, to relieve the swelling, redness, pain, and fever associated with inflamed joints and to restore impaired physical functions, dates back to antiquity. Aspirin and sodium salicylate have been in wide use for almost a century. However, modern chemical and pharmacological research to discover more effective and better tolerated antiarthritic drugs only fluorished in the late 1940's after the dramatic antirheumatic effect of corticosteroids was serendipidously discovered with a synthetic sample of hydrocortisone.[2] A decade of intensive steroid research during the 1950's produced many corticosteroid analogues with increasing potency. However, attempts to reduce their hormonal and metabolic side effects were generally disappointing. Following this, a search for nonsteroidal antiinflammatory agents was initiated in our laboratories.

At that time, our understanding of the inflammatory process in arthritis was admittedly meager. It was shortly before the publication of Dr. Burger's first issue of the *Journal of Medicinal Chemistry* and there was no chapter on antiinflammatory agents in the first edition of "Burger's Medicinal Chemistry". Therefore, our approach was essentially a pharmacological one. Extending his previous research on dexamethasone and other corticosteroids, my colleague, Dr. Charles Winter, adapted the classical cotton pellet granuloma assay for the screening of nonsteroidal compounds.

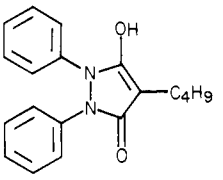
Since serotonin was considered as a potent inflammatory mediator at that time, various indole derivatives prepared in our laboratory which might in some way affect serotonin metabolism naturally became attractive screening candidates. From this humble and naive beginning, our indole lead was discovered.[3a] Soon afterwards, our interest in indoleacetic acids was further encouraged by the clinical report that an abnormal tryptophan metabolism was observed in rheumatic patients.[3b] In the course of an extensive effort, we went through two clinical candidates, MK-555 (1) and MK-410 (2). These two prototypes assured us that the antiinflammatory activity of indole derivatives seen in our rat assay indeed had clinical significance. The chemical structures of MK-555 and MK-410 also demonstrated two interesting structure–activity features. First, the preferential activity of the dextrorotary sinister enantiomers was found later to be generally true for many antiinflammatory arylpropionic acids.[4] Second, the biological and physical properties of *p*-methylthio and *p*-methylsulfinyl groups observed in this study[5] were successfully applied to the design of sulindac almost 10 years later. Our study culminated with the discovery of indomethacin (3) in 1961; its potency in animal models was 20–80 times our reference, phenylbutazone (4), and 2–300 times our original lead.[6–8]



1 (MK-555), X = Cl
2 (MK-410), X = CH_3S



3 (indomethacin)



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The discovery and clinical usage of indomethacin stimulated a wide interest in the study of nonsteroidal com-

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(2) L. H. Sarett, A. A. Patchett, and S. L. Steelman, *Prog. Drug Res.*, **5**, 13–153 (1963).
(3) (a) T. Y. Shen, *Int. Congr. Ser., Excerpta Med.*, **No. 82**, 13–20 (1965). (b) I. M. Bett, *Ann. Rheum. Dis.*, **21**, 388 (1962).
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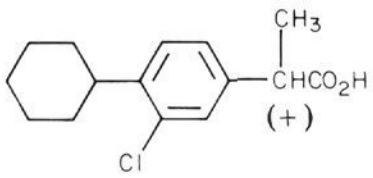
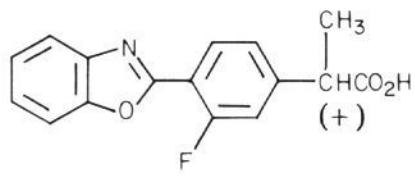
pounds with similar properties. The hypothetical receptor contour we postulated in 1964 became a useful working model for the search for other active structures.[6] Independent from studies in other laboratories, we also found that biphenyl[9] and cyclohexylphenylacetic acids[4] are active compounds. The discovery of a potent clinical candidate, MK-830 (**5**), which was 10–20 times more active than in-



5 (MK-830)



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domethacin in animal assays, clearly demonstrated the antiinflammatory potential of substituted phenylpropionics. As we all know, a large family of new agents [including a benzoxazolyl analogue (**6**) we studied[10]] commonly designated as nonsteroidal antiinflammatory drugs (NSAIDS), have since been developed in the past 2 decades.[11,12]

Meanwhile, indomethacin quickly became a much needed reference standard for laboratory scientists to unravel the mode of action of NSAIDS. More than a dozen of possible biochemical mechanisms, e.g., uncoupling of oxidative phosphorylation, were suggested. However, their in vivo significance was undermined by the high level of drug concentrations needed to show inhibition in vitro and/or by the lack of structure specificity as compared with in vivo experiments. By 1971, it was reported by Vane and co-workers in London that indomethacin and aspirin are capable of inhibiting the biosynthesis of prostaglandins (PGs) at their therapeutic concentrations.[13] Prostaglandins have been shown to modulate some important cellular and vascular changes in inflammation. The inhibition of their biosynthesis suppresses inflammation, pain, and fever.[14] We also found that the in vitro inhibitory activity of various indomethacin and phenylpropionic acid analogues closely parallel their in vivo/clinical potency, including the stereospecificity for a (*S*)-(+) configuration in arylpropionic acids.[15] Knowing cyclooxygenase as a biochemical target for NSAIDS, with computer simulation, we were able to refine the hypothetic receptor contour mentioned earlier by considering the possible mechanism of arachidonic acid oxidation.[16] The resulting contour readily accommodates space-filling models of arachidonic acid and NSAIDS, as well as the X-ray projections of indomethacin analogues. Further investigations also showed that many cyclooxygenase inhibitors used as antiinflammatory agents are nonequivalent biologically.[17–19]

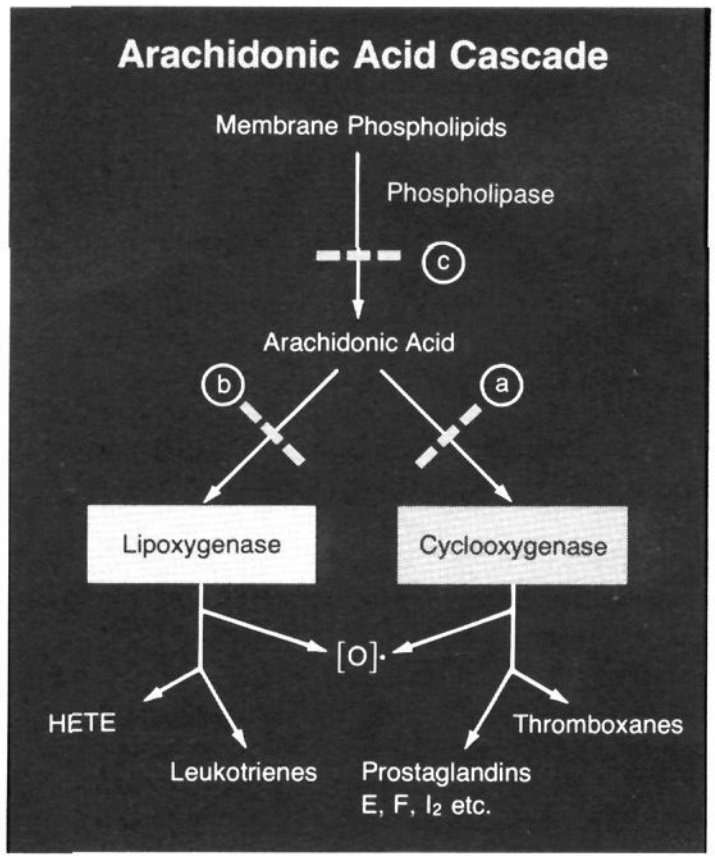

Figure 1. A schematic presentation of the arachidonic acid cascade and various groups of oxygenated metabolites: (a) inhibition of cyclooxygenase, e.g., by aspirin and indomethacin; (b) inhibition of lipoxygenase(s); (c) inhibition of phospholipase(s) to block the release of arachidonic acid from its membrane storage.

As we shall see later, they may differ in several enzymatic and pharmacokinetic aspects which influence their overall clinical efficacy and safety.

The recognition of indomethacin as a potent inhibitor of PG production in vivo enabled many scientists to use it as a highly sensitive probe to determine the roles of prostaglandins in various physiological systems.[20] These experiments not only clarified many biological concepts but also helped to explain some of the common side effects of NSAIDS, such as gastrointestinal irritation and interference with renal functions. In addition, they also provided a rationale for physicians to use indomethacin, and other cyclooxygenase inhibitors, to treat various prostaglandin-mediated disorders, such as primary dysmenorrhea,[21] patent ductus arteriosus,[22] and Bartter's syndrome.[23] Thus, as often happened in science, the discovery of indomethacin as a clinically useful new drug also provided a valuable research tool to advance our basic knowledge in a much broader scope.

The development of indomethacin and other NSAIDS marked some progress in mimicking the antiinflammatory activity of corticosteroids without their serious side effects. However, the gastrointestinal and idiosyncratic CNS side effects of indomethacin still left room for further improvement. Since there are no adequate animal models, even today, to evaluate the transient CNS effect of indomethacin, we decided to use a medicinal chemical approach to resolve this problem by studying its analogues and isosteres. The 5-(dimethylamino) analogue, MK-825 (**7**), showed different pharmacodynamic properties and was

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(10) T. Y. Shen, J. P. Li, and C. P. Dorn, U.S. Patent 4046905 (1977).
(11) R. A. Scherrer and M. W. Whitehouse, Eds., "Antiinflammatory Agents", Academic Press, New York, San Francisco, and London, 1974.
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(13) J. R. Vane, *Nature (London), New Biol.*, **231**, 232 (1971).
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(22) M. A. Kehmann, A. M. Rudolph, and N. H. Silverman, *N. Engl. J. Med.*, **295**, 530 (1976).
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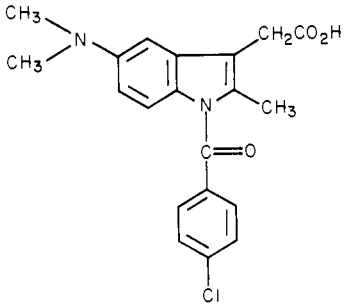
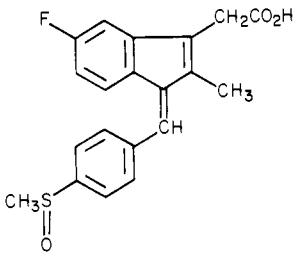
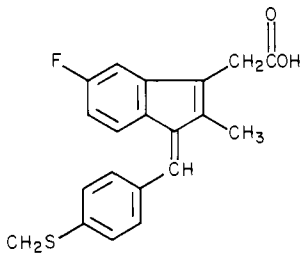


7 (MK-825)



8 (sulindac)

effective in man, but it was still not tolerated better.[8] Among various types of isosteres investigated, promising antiinflammatory–analgesic activities were found with the indene isosteres, which possess similar molecular configuration and electronic properties as indomethacin.[16] It was gratifying to learn that an indene isostere of indomethacin was less irritating to the gastrointestinal tract and free from central nervous system side effects in man. The potency and the pharmacodynamics of this indene isostere was significantly improved by the introduction of a 5-fluoro and a *p*-methylsulfinyl group in the molecule to give sulindac (**8**).[24-27] Extensive metabolic and pharmacological experiments established that sulindac is a new type of reversible prodrug.[28] Sulindac is an inert compound per se. In the body it is converted to a biologically active sulfide metabolite (**9**) which is comparable to indomethacin



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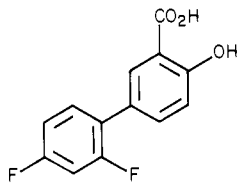

10 (diflunisal)

both in animal assays and as an inhibitor of prostaglandin synthesis in vitro. The sulfide also has an unusual capacity to react with the destructive oxygen radicals generated in the arachidonic acid cascade in a stoichiometric manner[29] (Figure 1). In contrast with many irreversible prodrugs used previously, sulindac and its sulfide are reversibly interconvertible in different tissue compartments. This regenerating process plus enterohepatic recyclization of sulindac sustain the level of the active sulfide in vivo. The active sulfide has a long serum half-life of 16 h and a tendency to concentrate in inflamed tissue. Thus, only a twice-daily dosage of sulindac is required in treating arthritis. The inertness of sulindac minimizes the local disturbance of the gastrointestinal tract both during oral administration and in the course of enterohepatic recirculation. Its gastric tolerance was shown recently by a series of clinical gastroscopic examinations.[30] Preliminary clinical experiments further suggested that sulindac, presumably through the differential tissue distribution of its sulfide metabolite in the kidney, may have less effect on renal prostaglandin synthesis and less interference with some renal functions than other NSAIDS.[31] In man, therapeutic doses of sulindac do not antagonize the prostaglandin-dependent renin release and the action of loop diuretics. Apparently, the pharmacodynamics of a reversible prodrug renders sulindac a more selective, long-acting, and potent antiarthritic drug.

Knowledge in prostaglandin biochemistry also contributed to the development of a new salicylate derivative, diflunisal, 5-(2,4-difluorophenyl)salicylic acid (**10**), in our laboratories.[32] The search for a superior aspirin with higher potency, better tolerance, and a longer duration of action has interested many laboratories in the past.[4] After an extensive investigation of 500 salicylic acid analogues in our animal assays, the presence of a hydrophobic 2,4-difluorophenyl group at the C_5 position was found to enhance both the potency and the duration of action of salicylates. The *O*-acetyl group in aspirin is traditionally associated with its improved analgesia and tolerance over sodium salicylate. However, in recent years, the *O*-acetyl group in aspirin was shown by several investigators to be chemically reactive toward nucleophiles in biopolymers, such as serum proteins, immunoglobulins,[33] and the key enzyme cyclooxygenase,[34] involved in the biosynthesis of prostaglandins. In fact, the preferential transacetylation of the platelet enzyme by aspirin irreversibly impairs the function of platelets and increases the tendency of bleeding. For this reason, in our selection of a new salicylate for clinical study, we have purposely avoided any structure with an *O*-acetyl group in order to achieve more selective action. Our final choice, diflunisal, is approximately four times more potent than aspirin as an analgesic–antiinflammatory agent with a long duration of action of 12 h.[35] It has only a weak and reversible effect on platelet function at therapeutic doses.[36] In contrast with aspirin, diflunisal is a reversible inhibitor of cyclooxygenase. Since the *O*-acetyl derivative of diflunisal is incapable of acetylating the enzyme and since diflunisal, like indomethacin, protects cyclooxygenase from acetylation by aspirin only partially, it seems reasonable to assume that the mode of binding of diflunisal is different from that of aspirin.[35] In an extensive gastroendoscopic study in man, diflunisal was shown to be less irritating to the stomach than most NSAIDS.[29]

A contributing factor to potential GI irritation is the tissue distribution of drugs. As shown by radioautography, many acidic drugs have a tendency to accumulate in the stomach wall soon after oral absorption.[37] This process

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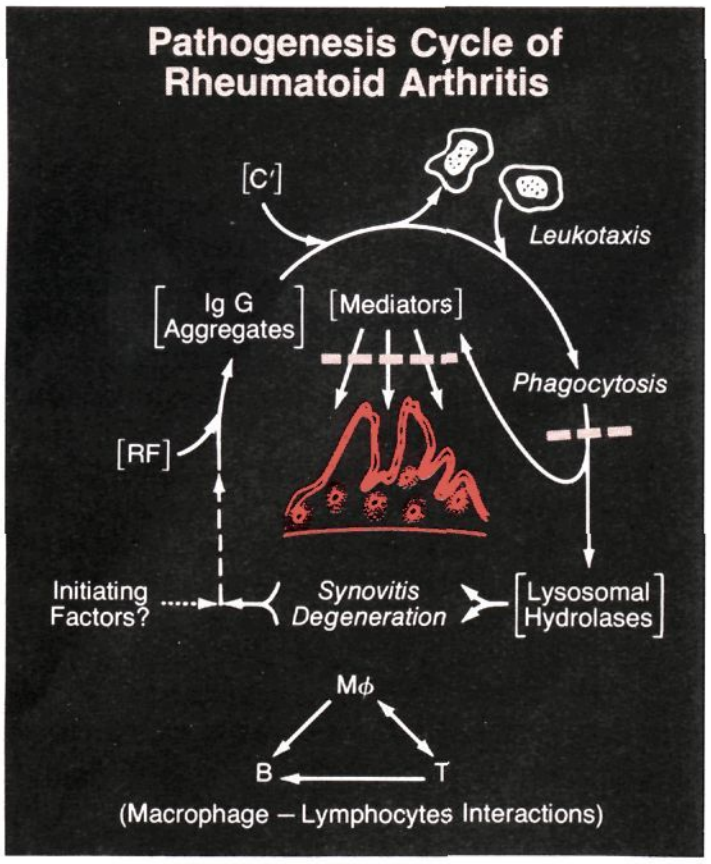
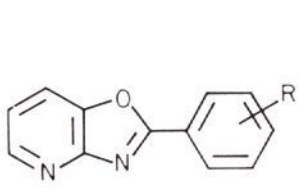


Figure 2. The current concept of the pathogenesis cycle of rheumatoid arthritis: RF = rheumatoid factor, C′ = complement, MØ = macrophage, T and B = lymphocytes. The initiating factor(s) remains to be elucidated.

is clearly influenced by the physicochemical properties of the drug. Preliminary experiments indicated that the GI tolerance to diflunisal may be partly attributable to its permeability characteristics resulting in low concentration in the GI tissue.[38] It is also clear that nonacidic compounds generally have less tendency to accumulate in the stomach tissue. Indeed, many nonacidic antiinflammatory agents[39,40] have shown better GI tolerance in animal models. Some of them, such as a group of 2-substituted phenyloxazolopyridines (**11**),[41] are comparable to indomethacin in potency, both in the carrageenan paw edema assay and in cyclooxygenase inhibition.



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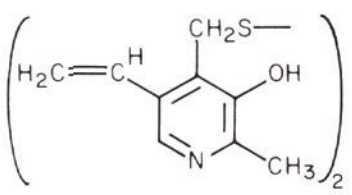

12 (MK-159)

The development of sulindac and diflunisal illustrate the application of biochemical knowledge and pharmacodynamics in improving the efficacy, duration of action, as well as safety of NSAIDS. Looking ahead, the search for new classes of antiarthritic drugs, specifically designed for rheumatoid arthritis and osteoarthritis, respectively, will continue to progress.[42] Mechanism of action considerations will play an increasingly important role in the discovery of new antiarthritic drugs.[43] We already have a group of slow-acting antirheumatic drugs (SAARDS), such as D-penicillamine, gold, and antimalarials, which are more effective than NSAIDS but, unfortunately, are limited in clinical utility by their severe toxicities. Nevertheless, these are clinical leads challenging medicinal chemists and biologists alike to develop new antirheumatic assays and to synthesize more effective but less toxic new agents. The mechanism of action of SAARDS is still poorly understood. As we recall, the pathogenesis cycle of rheumatoid arthritis involves several immunological aspects: abnormal immunoglobulin production, complement fixation, leukocyte infiltration and proliferation, phagocytosis and release of lysosomal enzymes (Figure 2). Superimposed upon this cycle is the triangular relationship of macrophage and T and B lymphocytes. Based on this concept, various cellular and in vivo immunological models are being explored as potential antirheumatic approaches. In our laboratory, a 5-mercaptopyridoxine analgoue, the disulfide of 5-vinyl-4-mercaptopyridoxine, MK-159 (**12**), which inhibits the action of dermal lymphokine and adjuvant arthritis was studied several years ago.[44] Agents capable of regulating the release of prostaglandins, lysosomal hydrolases, and other antiinflammatory mediators can also be evaluated in a macrophage system.[45,46] It is of further interest to note that indomethacin and cyclooxygenase inhibitors have a moderate capacity to restore the depressed cellular and humoral immune responses in laboratory systems and in man.[47] The recognition of a feed-back regulatory role of prostaglandins on macrophages and T lymphocytes illustrates a convergence of pharmacological and immunological approaches to more selective and less cytotoxic immunoregulators.[48]

In the biochemical area, continued elucidation of the arachidonic acid pathway and the chemotactic process will offer new therapeutic targets. For example, the recent characterizations of the action of phospholipases[49,50] and the lipoxygenase metabolites, especially the leukotrienes,[51] have generated a great deal of interest. Nonsteroidal compounds capable of inhibiting phospholipases or both lipoxygenase and cyclooxygenase may block the entire arachidonic acid pathway and achieve a higher ceiling of antiinflammatory effect than current NSAIDS (Figure 1).

Since most cellular and humoral responses are cell-surface phenomena, the design and synthesis of drugs with more specific actions will be facilitated by quantitative biophysical characterizations and structure–function analysis of determinants, receptors, and membrane enzymes. For example, the well-defined peptide chemoattractant f-Met-Leu-Phe,[52] the macrophage stimulant tuftsin,[53] and the binding region of complement C5a and

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their membrane receptors[54] may provide rational targets for inhibitor design. With the recent advances in membrane technology, the molecular interaction of NSAIDS with model membrane systems can readily be studied by NMR, ESR, membrane potential, and other physical measurements.[55,56] It may be noted that in QSAR analysis only the physical properties of drug molecules themselves have been generally measured and evaluated. Such membrane information may provide a set of physicochemical parameters to denote actions on drug targets and to augment our QSAR analysis of new membrane regulators. With this new knowledge and new concepts, we are optimistic that more selective and superior NSAIDS and SAARDS will continually evolve in the next 2 decades to alleviate the sufferings of arthritic patients.

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(55) S. S. Fan and T. Y. Shen, in press.
(56) S. B. Hwang and T. Y. Shen, in press.

Finally, an association of various arthritis disorders with the genetic HLA determinants of patients, e.g., rheumatoid arthritis and ankylosing spondylitis with HLA antigens DW4 and B27, respectively, has been observed recently. These associations may be related to the influence of genetic determinants on specific immunological events, such as antigen processing and the interactions between macrophage and T lymphocytes. In this light, one may hope that progress in immunogenetics and selective immunoregulation in the near future would bring forth better understanding of pathogenic mechanisms, early diagnosis of susceptible patients, and open the possibility of selective and preventive medicine for this family of degenerative and debilitating diseases. We are deeply appreciative to Dr. Burger's dedicated educational and research effort in medicinal chemistry in the past 50 years. Looking ahead, medicinal chemical research may further advance in the coming half century through chemotherapy to preventive medicine, from enzyme inhibition to phenotypic regulation. Selective antiarthritic therapy based on immunogenetics may constitute such an opportunity.

Communications to the Editor

Potential Organ- or Tumor-Imaging Agents. 21. Acyl-Labeled Esters of Cholesterol[1]

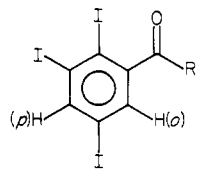
Sir:

Radioiodinated derivatives of cholesterol[2-4] have been widely used as imaging agents for the diagnosis of a variety of adrenal disorders in humans.[5] More recently, esters of radioiodinated cholesterol have also been shown to selectively accumulate in adrenals as well as ovaries.[6,7] In these, as well as other studies to date, it has been assumed that the appropriate γ-emitting radionuclide (^{131}I, ^{75}Se,[8] ^{123m}Te[9]) must form a part of the cholesterol molecule, either by direct attachment to the steroid nucleus or by incorporation into the side chain, in order to achieve target-organ specificity.

This communication was prompted by our observation that cholesteryl oleate radiolabeled in the potentially hydrolyzable acyl moiety accumulated in target tissues at 0.5 h with approximately the same degree of selectivity noted for cholesteryl oleate radiolabeled in the steroid nucleus (Table I). As a consequence of this finding, the synthesis of cholesteryl esters bearing radioiodine on the acyl moiety was undertaken. We report here the synthesis and tissue distribution for one of these radiolabeled esters, cholesteryl 2,3,5-tri[^{125}I]iodobenzoate (**3**).

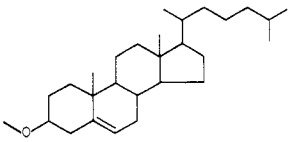
Table I. Distribution of Radioactivity at 0.5 h After Intravenous Administration of Radiolabeled [^{14}C]Cholesteryl Oleate to Rats

	% administered dose/g of tissue ± SEM	
tissue	4-^{14}C (n = 3)	oleate 1-^{14}C (n = 4)
adrenal cortex	3.852 ± 0.084	2.448 ± 0.128
blood	5.781 ± 0.276	5.196 ± 0.097
liver	1.569 ± 0.261	1.236 ± 0.141
ovary	2.815 ± 0.388	2.088 ± 0.158
thyroid	0.446 ± 0.036	0.344 ± 0.017



1, R = -OH

2, R = 

3, R = 

Cholesteryl 2,3,5-triiodobenzoate (**3**) was prepared from 2,3,5-triiodobenzoic acid (**1**) in dry THF by means of the imidazolide (**2**):[10] yield 65%; mp 199-200.5 °C; TLC R_f

(1) Presented in part before the Third International Symposium on Radiopharmaceutical Chemistry, St. Louis, MO, June, 1980.
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