that described for the preparation of 2-chloroadenosine 5′-phosphoromorpholidate was carried out in which 2-chloroadenosine was substituted for 2-chloro-AMP. Chromatography of the water-soluble reaction product in *i*-Pr_2O-EtOH-H_2O (25:10:saturated)[17] on a cellulose column separated 2-chloroadenosine and the fluorescent material. Evaporation of fractions containing the latter product gave a crystalline solid, 2-morpholinoadenosine, which was recrystallized from water as needles: mp 245–246°; λ_{max} (0.1 *N* HCl) 260 nm (ε 15,700), 299 (7800); λ_{max} (0.1 *N* NaOH) 261 nm (ε 13,000), 284 (8000). *Anal.* ($C_{14}H_{20}N_6O_5$) C, H, N.

Guinea Pig Taenia Coli Preparation. Strips of taenia coli were dissected and mounted in organ baths in modified Krebs solution containing hyoscine as described.[6] Muscle activity was recorded with an isotonic lever writing on a smoked drum. ATP and triphosphate analogs were added to the bath to give concentrations of 0.10–10 μ*M*. Dose–response curves were plotted for six different concentrations over this range, each point representing the mean of at least four observations. Molar potencies were obtained from the antilog of the difference between the pD_2 values for the analog and ATP.[18]

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References

(1) A. N. Drury, *Physiol. Rev.*, **16**, 292 (1936).
(2) E. Mihich, D. A. Clarke, and F. S. Philips, *J. Pharmacol. Exp. Ther.*, **111**, 335 (1954).
(3) R. E. Nichols and E. S. Walaszek, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **22**, 308 (1963).
(4) J. Axelsson and B. Holmberg, *Acta Physiol. Scand.*, **75**, 149 (1969).
(5) G. Burnstock, G. Campbell, D. Satchell, and A. Smythe, *Brit. J. Pharmacol.*, **40**, 668 (1970).
(6) G. Burnstock, D. G. Satchell, and A. Smythe, *ibid.*, **46**, 234 (1972).
(7) D. G. Satchell and G. Burnstock, *Biochem. Pharmacol.*, **20**, 1694 (1969).
(8) C. Su, J. A. Bevan, and G. Burnstock, *Science*, **173**, 336 (1971).
(9) G. Burnstock, *Pharmacol. Rev.*, **24**, 509 (1972).
(10) G. Gough, M. H. Maguire, and F. Penglis, *Mol. Pharmacol.*, **8**, 170 (1972).
(11) F. Michal, M. H. Maguire, and G. Gough, *Nature (London)*, **222**, 1073 (1969).
(12) J. Angus, L. B. Cobbin, R. Einstein, and M. H. Maguire, *Brit. J. Pharmacol.*, **41**, 592 (1971).
(13) J. G. Moffatt, *Can. J. Chem.*, **42**, 599 (1964).
(14) G. Gough, M. H. Maguire, and F. Michal, *J. Med. Chem.*, **12**, 494 (1969).
(15) D. E. Hoard and D. G. Ott, *J. Amer. Chem. Soc.*, **87**, 1785 (1965).
(16) Z. Stransku, *J. Chromatogr.*, **10**, 456 (1963).
(17) J. A. Carbon, *J. Amer. Chem. Soc.*, **86**, 720 (1964).
(18) E. J. Ariens and J. M. von Rossum, *Arch. Int. Pharmacodyn.*, **60**, 275 (1957).

Methotrexate Analogs. 2. A Facile Method of Preparation of Lipophilic Derivatives of Methotrexate and 3′,5′-Dichloromethotrexate by Direct Esterification†,[1]

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Recent years have witnessed an expanding interest in the clinically advantageous pharmacological properties of "prodrugs" or drug derivatives exhibiting the phenomenon of latentiation.[2] The synthesis[3] of a series of lipophilic long-chain alkyl esters of the antitumor agent 1-β-D-arabinofuranosylcytosine (ara-C) with superior therapeutic properties as immunosuppressive and antileukemic agents in experimental animals[4] provides but one notable illustration of this approach to drug design. While latentiation was achieved in this instance *via* esterification of an alcohol function in the parent molecule, the converse strategy of esterifying a carboxy group with a long-chain alcohol appears to have received sparse attention in the literature. In the work reported here, some potential latent derivatives of the second type were prepared, in the form of a series of heretofore undescribed alkyl esters of 4-amino-4-deoxy-N^{10}-methylpteroylglutamic acid (amethopterin, methotrexate, MTX), another cancer chemotherapeutic agent in widespread clinical use.[5,6] The structures of eight such MTX esters are shown in Table I, together with six others derived from 3′,5′-dichloro-4-amino-4-deoxy-N^{10}-methylpteroylglutamic acid (3′,5′-dichloromethotrexate, DCM).‡ The latter were viewed with particular interest because of the possibility that their enhanced lipophilicity might favor passage across the blood-brain barrier and thereby lead to agents useful for the treatment of tumors of the central nervous system.

Esters of both MTX and DCM were obtained in excellent yield *via* a facile procedure for direct esterification adapted from the early work of Hutchings and coworkers[8] who esterified folic acid in 65% yield by reaction with 0.1 *N* HCl in anhydrous methanol at room temperature for 24 hr. In the present study, esterification was achieved *via* any of four modifications of this method, the choice of variant being dictated mainly by the chemical reactivity and water miscibility of the particular alcohol selected. Examples of each procedure are given in the Experimental Section and yields are presented in Table I. A surprising and significant aspect of the findings reported here is the apparent stability of the 4-amino group of MTX under moderately strenuous acid conditions.

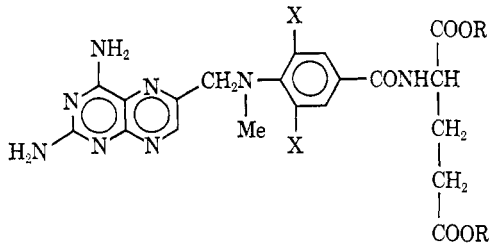
Esterifications proceeded rapidly at room temperature when either MTX or DCM was stirred in low-molecular-weight primary alcohols containing 0.25 *N* HCl (methods A and C). Reaction mixtures tended to remain heterogeneous with MTX but became homogeneous almost immediately when DCM was used, in accord with the expected enhancement of lipophilicity by 3′,5′-dichloro substituents. Although uniform reaction times of 24 or 48 hr were generally employed for the sake of convenience, thin-layer chromatography revealed disappearance of the starting material after a much shorter time. For example, in one experiment involving the dibutyl ester **5**, work-up after only 6 hr produced a yield not significantly different from that obtained with other primary alcohols after longer intervals.

With secondary alcohols or long-chain primary alcohols such as 1-octanol, reactions at room temperature gave poor yields. Two procedural variants were developed in order to surmount this problem. In the first of these (method B), involving for example the diisopropyl ester **4**, water generated during the esterification was removed continuously by azeotropic distillation with benzene, the reaction being run in a Soxhlet apparatus containing molecular sieves in the thimble. This method was successful with 1-pentanol but failed with 1-octanol, apparently because hydrogen chloride is sparingly soluble in boiling mixtures of benzene and 1-octanol and therefore is easily lost. Accordingly, for the synthesis of esters **12** and **13**, another variant (method D) was devised which involved

† This investigation was supported by Research Grant C6516 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

‡ A referee has kindly called the author's attention to a recent abstract describing MTX and DCM esters as substrates for liver aldehyde oxidase; see Wolpert, *et al.*[7]

Table I. Physical Constants of 4-Amino-4-deoxy-N^{10}-methylpteroylglutamate Esters



Compd no.	R	X	Mp, °C	% yield	Method[b]	Tlc data[a] Solvent 1	Solvent 2	Partition coeff[b]	Formula	Analyses
1	C_2H_5	H	132–135	75	A	0.58	0.62	0.55	$C_{24}H_{30}N_8O_5$	C, H, N
2	C_2H_5	Cl	237–241 dec	82	A	0.67	0.69	0.88	$C_{24}H_{28}Cl_2N_8O_5$	C, H, Cl, N
3	n-C_3H_7	H	130–133	80	A	0.57	0.63	0.69	$C_{26}H_{34}N_8O_5$	C, H, N
4	i-C_3H_7	H	150–153	69	B	0.56	0.64	0.77	$C_{26}H_{34}N_8O_5 \cdot 0.5H_2O$	C, H, N
5	n-C_4H_9	H	134–135	66	C	0.58	0.64	0.88	$C_{28}H_{38}N_8O_5$	C, H, N
6	n-C_4H_9	Cl	219–221 dec	82	C	0.68	0.73		$C_{28}H_{36}Cl_2N_8O_5$	C, H, Cl, N
7	i-C_4H_9	Cl	217–221 dec	76	C	0.65	0.79		$C_{28}H_{36}Cl_2N_8O_5$	C, H, Cl, N
8	n-C_5H_{11}	H	140–145	74	B	0.62	0.68	0.87	$C_{30}H_{42}N_8O_5$	C, H, N
9	i-C_5H_{11}	H	144–146	69	C	0.62	0.72	0.89	$C_{30}H_{42}N_8O_5$	C, H, N
10	i-C_5H_{11}	Cl	209–213 dec	75	C	0.69	0.85		$C_{30}H_{40}Cl_2N_8O_5$	C, H, Cl, N
11	2-C_5H_{11}	Cl	220–225 dec	63	D	0.74	0.88		$C_{30}H_{40}Cl_2N_8O_5 \cdot 0.5H_2O$	C, H, Cl, N
12	n-C_8H_{17}	H	131–134	57	D	0.65	0.79	0.87	$C_{36}H_{54}N_8O_5$	C, H, N
13	n-C_8H_{17}	Cl	184–190 dec	56	D	0.85	0.92		$C_{36}H_{52}Cl_2N_8O_5$	C, H, Cl, N
14	$EtOCH_2CH_2$	H	122–125	76	A	0.55	0.77	0.57	$C_{28}H_{38}N_8O_7 \cdot 0.5H_2O$	C, H, N

[a] Tlc performed on silica gel (Eastman 6060 sheets, with fluorescent indicator) using 4:1 $CHCl_3$–MeOH (solvent 1) and 5:3:1 n-BuOH–AcOH–H_2O (solvent 2); R_f values given were determined by measurement from the origin to the leading edge of the spot. [b] See Experimental Section.

heating in 1-octanol at 55–60° for 5–6 hr, without the use of benzene or molecular sieves. This procedure also worked well with secondary alcohols, as evidenced by the preparation of the bis(1-methylbutyl) ester **11**.

The ready solubility of MTX and DCM esters in various organic solvents such as chloroform greatly facilitated their purification, inasmuch as unesterified or monoesterified material could be removed by extraction into aqueous $NaHCO_3$ solution and the fully esterified fraction could be chromatographed on silica gel. The product isolated after column chromatography was of analytical purity and did not require recrystallization. Because esterification of MTX proceeds in high yield and chromatography of the esters is rapid and can be performed efficiently on a large scale, this technique may constitute a useful alternative to existing methods of purification of MTX *via* ion-exchange chromatography.[9]

Some estimate of the lipophilic character of MTX and DCM esters of increasing alkyl chain length was gained initially by comparison of the thin-layer chromatographic mobilities of these compounds and subsequently by direct determination of partition coefficients (see Experimental Section), which provide a truer measure of lipohilicity since they do not involve differences in adsorptive properties. As indicated in Table I, R_f values for the MTX derivatives vary from 0.58 for the diethyl ester **1** to 0.65 for the dioctyl ester **12** in 4:1 $CHCl_3$–MeOH (solvent 1) and from 0.62 to 0.79 in 5:3:1 BuOH–AcOH–H_2O (solvent 2). A similar pattern can be observed among the 3′,5′-dichloro derivatives, although the R_f values are consistently higher because of the enhanced lipophilic character of these substances. Of some interest is the fact that the bis(2-ethoxyethyl) ester **14** of MTX shows less mobility than the dipentyl ester **8** in solvent 1 but greater mobility in solvent 2. These results are consistent with the expectation that, for two esters of comparable chain length, replacement of CH_2 by O should confer greater solubility in polar solvents and lower solubility in nonpolar solvents.

As indicated in Table I, partition coefficients for the MTX esters vary from 0.55 for the diethyl ester **1** to 0.88 for the dibutyl ester **5** but remain essentially constant as the length of the alkyl chain is increased beyond four carbons. The high partition coefficient of 0.88 for the diethyl ester **2** of DCM and the low partition coefficient of 0.57 for the bis(2-ethoxyethyl) ester **14** of MTX corroborate the thin-layer chromatographic data cited above. One additional noteworthy aspect not revealed by tlc but evident from partition coefficient data is that chain branching in the alkyl moiety (compare especially the dipropyl and diisopropyl esters **3** and **4**) appears to enhance lipophilicity.

Biological Results. Evaluation of the growth-inhibitory properties of the MTX and DCM esters synthesized in the course of this work was carried out *in vitro* with the folate-dependent microorganism *Streptococcus faecium* (ATCC 8043) as previously described.[10] With the noteworthy exception of the dioctyl esters **12** and **13**, which were inactive even at a concentration of 1 μg/ml, the esters of MTX and DCM generally showed ID_{50} values below 0.03 μg/ml at a folate concentration of 0.001 μg/ml. Under the same assay conditions, unesterified MTX and DCM had ID_{50} values of 0.002 and 0.01 μg/ml, respectively.

Several of the esters have been assayed *in vivo* against two transplantable murine leukemias in ascitic form: L1210 leukemia in BDF/1 hybrid mice and P1534 leukemia in DBA/2 inbred mice. Intraperitoneal injections were given at regularly spaced dose levels chosen on the basis of prior determination of acute toxicities. Compounds were suspended in 10% Tween 80, and animals received four daily injections beginning on the first day after tumor implantation. At an optimal dose of 32 mg/kg (qd 1–4), the diethyl ester **1** of MTX produced a 51% extension of survival in L1210 leukemic mice. The dibutyl ester **5** of MTX prolonged the life span of L1210 leukemic mice by 25% at an optimal dose of 2.5 mg/kg (qd 1–4) while extending the survival of P1534 leukemic animals by 44% at an optimal dose of 5 mg/kg (qd 1–4). Another member of the series, the dibutyl ester **6** of DCM, caused a 66% increase in survival of L1210 leukemic mice at 62.5

mg/kg (qd 1-4). The survival increases observed with these compounds were comparable to those seen with MTX under similar conditions.

Preliminary data from a study of the direct binding of the MTX and DCM esters to a preparation of dihydrofolate reductase from *Lactobacillus casei* (ATCC 7469) indicate that they are bound as much as 1000 times less tightly than the parent drug.§ This finding suggests that the antibacterial activity of the esters referred to above may be accounted for on the basis that, upon entry into the cell, some free MTX or DCM is released as a consequence of intracellular esterase activity. According to this view, the esters represent transport forms of MTX and DCM and may not be very active in their own right. Their biological activity in a cell culture assay presumably reflects the net effect of several variables, including the rate of passage of the ester across the cell membrane and its subsequent enzymatic hydrolysis inside the cell. In whole animals, this picture may be complicated further by the possibility of serum esterase hydrolysis (*i.e.*, even before entry into target cells). Similar considerations have been advanced in a brief report on the antitumor effects of MTX and its dimethyl ester.[11] More detailed studies aimed at elucidating some of these problems and also dealing with questions of schedule dependency, optimal route and vehicle of administration, and range of tumor specificity (including evaluation against resistant lines and intrathecally implanted tumors) will be reported separately.

Experimental Section

Melting points (uncorrected) were determined in Pyrex capillary tubes by means of a Mel-Temp apparatus (Laboratory Devices, Inc., Cambridge, Mass.) at a heating rate of 2°/min. Column chromatography was performed on silica gel powder (Baker 3405, 60-200 mesh) without prior activation. Thin-layer chromatography was carried out on silica gel sheets (Eastman 6060, with fluorescent indicator), likewise without activation, using 4:1 $CHCl_3$-MeOH or 5:3:1 *n*-BuOH-AcOH-H_2O as the solvent. Unless otherwise specified, solutions were dried over anhydrous granular Na_2SO_4. Where analyses are specified only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of theoretical values. Methotrexate and 3′,5′-dichloromethotrexate were supplied by Lederle Laboratories, Division of American Cyanamid Co., through the courtesy of the Cancer Chemotherapy National Service Center, National Cancer Institute.

Methotrexate Diethyl Ester (1) (Method A). A suspension of methotrexate (4.0 g, 0.0088 mol) in 600 ml of 0.25 *N* HCl in absolute EtOH was stirred at room temperature for 72 hr. After evaporation to dryness under reduced pressure, the residue was treated with 0.25 *M* $NaHCO_3$ (1 l.) in order to dissolve any unreacted methotrexate, and the esterified product was extracted into $CHCl_3$ (3 × 500 ml). The combined $CHCl_3$ extracts were rinsed to neutrality with saturated NaCl solution (500 ml) and distilled H_2O (500 ml), dried, and evaporated. Column chromatography of the semisolid orange residue on silica gel (200 g) using 95:5 $CHCl_3$-MeOH as the eluent yielded a minor fast-moving band, which was discarded, and a major band from which was isolated on solvent evaporation a granular yellow-orange solid (3.3 g). Pulverization of this material in a mortar afforded 1 as a bright yellow, analytically pure microcrystalline powder.

Methotrexate Diisopropyl Ester (4) (Method B). Methotrexate (1.0 g, 0.0022 mol) was added to a mixture of 0.25 *N* HCl in *i*-PrOH (300 ml) and dry benzene (200 ml) in a 1-l. round-bottom flask fitted with a Soxhlet extractor containing molecular sieves (Linde 3A) in the thimble. The mixture was stirred under reflux for 4 hr, after which time it was cooled, treated with decolorizing carbon, and concentrated to near dryness on a rotary evaporator. Addition of 0.4 *M* $NaHCO_3$ (300 ml) to the residue, extraction with $CHCl_3$ (3 × 300 ml), washing to neutrality with distilled H_2O, drying, and evaporation yielded a viscous orange-colored gum which was purified by column chromatography on silica gel (100 g). A small quantity of fast-moving material was removed by elution with $CHCl_3$ and the product was obtained with 95:5 $CHCl_3$-MeOH. Evaporation left a glassy residue which yielded a bright yellow powder (0.82 g) on trituration with Et_2O and petroleum ether (bp 30-60°).

Dichloromethotrexate Dibutyl Ester (6) (Method C). 3′,5′-Dichloromethotrexate (2.0 g, 0.0038 mol) was dissolved in 0.25 *N* HCl in BuOH (300 ml) and the solution was allowed to stand at room temperature for 48 hr. Extraction with 0.4 *M* $NaHCO_3$ (2 × 150 ml), washing to neutrality with saturated NaCl solution, rinsing with distilled H_2O, and solvent evaporation by means of a rotary evaporator connected to a Dry Ice-acetone-cooled receiver and vacuum pump left a clear yellow glass. Column chromatography on silica gel (70 g) with 95:5 $CHCl_3$-MeOH as the eluent gave a trace of fast-moving material which was discarded and a main band consisting of the desired product. Evaporation of pooled $CHCl_3$-MeOH eluates and trituration of the glassy residue with petroleum ether (bp 30-60°) afforded a transparent gel which was broken up with a spatula, collected by suction filtration, and dried to a bright yellow granular solid (2.0 g) of analytical purity.

Dichloromethotrexate Dioctyl Ester (13) (Method D). A mixture of 3′,5′-dichloromethotrexate (2.0 g, 0.0038 mol) and 300 ml of 0.50 *N* HCl in 1-octanol was stirred at 55-60° for 5 hr. Extraction of the dark solution with 0.25 *M* $NaHCO_3$, rinsing to neutrality with saturated NaCl solution and distilled H_2O, dilution with a large volume of $CHCl_3$, drying, and solvent take-off (H_2O aspirator to remove the $CHCl_3$ and vacuum pump with Dry Ice-acetone in the receiver to remove the 1-octanol) afforded an amber-colored semisolid residue which was purified by column chromatography on silica gel (75 g). Elution with $CHCl_3$ alone gave a small amount of fast-moving material which was discarded, and elution with 95:5 $CHCl_3$-MeOH yielded the desired ester as a yellow glass. Trituration of this material with Et_2O and petroleum ether (bp 30-60°) produced the characteristic transparent yellow gel, which was broken up with a spatula, suction filtered, and dried under reduced pressure to a bright yellow microcrystalline powder (1.6 g).

Partition Coefficients. Because of the virtual insolubility of the MTX and DCM esters in H_2O, a two-phase system was prepared by shaking H_2O (25 ml), DMF (25 ml), and 1-octanol (50 ml) thoroughly in a separatory funnel and allowing the two layers to separate and equilibrate for 1 hr. The lower layer (40 ml) and upper layer (60 ml) were assumed to represent the "aqueous phase" (mixture A) and "lipid phase" (mixture B), respectively. A 2.0-2.5-mg sample of ester was dissolved in 3.0 ml of mixture A (with gentle warming on the steam bath if necessary), a 0.5-ml aliquot was removed and diluted to 5.0 ml with 95% EtOH, and the optical density (E_1) at 375 nm was determined in a standard 1-cm quartz cell. The remaining 2.5-ml solution was shaken for 60 sec at 35° with 2.5 ml of mixture B, the layers were allowed to separate, and another 0.5-ml aliquot of the lower layer was removed and diluted to 5.0 ml with 95% EtOH. The optical density (E_2) at 375 nm was recorded again, and a partition coefficient (P) was calculated on the basis of the formula $P = (E_1 - E_2)/E_1$ where E_1 was the optical density of the "aqueous phase" (mixture A) before partition and E_2 was the optical density of the same phase after partition. The values derived in this manner are shown in Table I.

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References

(1) M. Chaykovsky, A. Rosowsky, and E. J. Modest, *J. Heterocycl. Chem.*, **10**, 425 (1973) (paper 1).
(2) C. J. Cavallito in "Medicinal Chemistry," 3rd ed, A. Burger, Ed., Wiley-Interscience, New York, N. Y., 1970, p 235.
(3) D. T. Gish, R. C. Kelly, G. W. Camiener, and W. J. Wechter, *J. Med. Chem.*, **14**, 1159 (1971).

§ Enzyme binding studies are being performed on a collaborative basis by Dr. Roy L. Kisliuk, Department of Biochemistry, Tufts-New England Medical Center, Boston, Mass. The results of this investigation will be reported separately.

(4) G. D. Gray, F. R. Nichol, M. Mickelson, G. W. Camiener, D. T. Gish, R. C. Kelly, W. J. Wechter, T. E. Moxley, and G. L. Neil, *Biochem. Pharmacol.*, **21**, 465 (1972).
(5) S. Farber, R. Toch, E. M. Sears, and D. Pinkel, *Advan. Cancer Res.*, **4**, 1 (1956).
(6) R. B. Livingston and S. K. Carter, "Single Agents in Cancer Chemotherapy," Plenum, New York, N. Y., 1970, pp 130–172.
(7) M. K. Wolpert, D. Farquhar, and D. G. Johns, Abstracts of Papers, 5th International Congress on Pharmacology, San Francisco, Calif., July 23–28, 1972, p 255.
(8) B. L. Hutchings, E. L. R. Stokstad, N. Bohonos, N. H. Sloane, and Y. SubbaRow, *J. Amer. Chem. Soc.*, **70**, 1 (1948).
(9) V. Oliverio, *Anal. Chem.*, **33**, 263 (1961).
(10) G. E. Foley, R. E. McCarthy, V. M. Binns, E. E. Snell, B. M. Guirard, G. W. Kidder, V. C. Dewey, and P. S. Thayer, *Ann. N. Y. Acad. Sci.*, **76**, 413 (1958).
(11) A. J. Eisenfeld, H. G. Mautner, and A. D. Welch, *Proc. Amer. Ass. Cancer Res.*, **3**, 316 (1962).

Book Reviews

Perspectives in Membrane Biophysics. Edited by D. P. Agin with 22 contributors. Gordon and Breach, New York, London, and Paris. 1972. 317 pp. 16 × 23.5 cm. $12.50.

This book was conceived as a tribute to K. S. Cole and presents a collection of 16 papers by scientists who have made significant contributions to the study of membrane properties. The format of the volume is pleasing; regrettably, some of the articles contain many typographical errors. The individual presentations vary greatly in length and in scope. Five papers (papers 1, 2, 4, 5, and 7) concern findings on squid axons. They cover the results of direct measurements of the influx of Na ions during the action potential, recent models of active ion transport, biionic potentials in perfused axons, a model for the mechanism of inactivation of the sodium conductance, and the temperature dependence of excitability. Paper 3 is a broad discussion of electrogenic phenomena with emphasis on nonlinear voltage–current relations. Paper 6 presents studies of the relationship between structure of macrocyclic compounds and their capacity to function as ion carriers across artificial membranes. Four papers (papers 8, 10, 11, and 15) are mainly theoretical. One stresses the need for further studies of membrane capacity under different experimental conditions. The other papers present a model of ion transport across the membrane in terms of conformational changes in the protein subunits of the channels, a mathematical treatment of ion flow in an electrolyte–membrane–electrolyte system, and an extension of a theoretical method by Cole to calculate membrane current variations with membrane potential at any point in a fiber. Paper 9 describes a rapid voltage-clamp data processing system. Paper 12 concerns ion flux ratio determinations under nonsteady state conditions. Paper 13 is a review of ionic conductances at synapses. Paper 14 is a comprehensive review of pharmacological data obtained on axonal and endplate membranes. Paper 16 points out similarities and differences in the development of ideas and methods in research on nerve and muscle. Anyone who is interested in this area will find the discussions, which clearly indicate some of the major open problems, stimulating.

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Antiarrhythmic Agents. By Arthur J. Moss and Robert D. Patton. Charles C Thomas, Springfield, Ill. 1973. xi + 161 pp. 16 × 23.5 cm. $10.00.

Although this book was written primarily for the clinician, it should also be useful to teachers of pharmacology, clinical pharmacy, and medicinal chemistry. The first chapter is a concise review of the electrophysiologic properties of cardiac tissue while the second is a very brief description of arrhythmogenic mechanisms. A five and one-half page discussion of the general principles of pharmacodynamics completes the first section of the book.

The second section consists of ten chapters, each of which is devoted to a single antiarrhythmic drug. The drugs covered in this section are: quinidine, procainamide, lidocaine, diphenylhydantoin, propranolol, bretylium, atropine, digitalis glycosides, potassium, and isoproterenol. Five or six pages are devoted to each drug and the discussion of the individual agents is divided into the following subheadings: introduction (including a description of chemical structure), electrophysiologic properties, pharmacologic considerations (absorption, metabolism, excretion), therapeutic uses, and adverse drug reactions. The authors have restricted their discussion of the drugs as much as possible to those aspects relating to antiarrhythmic activity. For example, the general utility of atropine as an anticholinergic agent and the anticonvulsant properties of diphenylhydantoin are mentioned only in passing. The use of digitalis as an inotropic agent is not covered. Thus, the scope of the "therapeutic use" sections is quite limited.

The third section of the book, called "general therapeutic considerations," consists of a chapter which compares the electrophysiologic properties, pharmacodynamics, adverse reactions, and costs of the ten drugs described in the second section along with a chapter on the management of refractory arrhythmias. The chapter devoted to comparison of the agents is a well-written summary and contains tables which greatly facilitate comparison of electrophysiologic properties and side effects.

The book contains 426 references, a substantial number of which were published in the years 1969–1971. In spite of the brevity of this book, the authors appear to have accomplished their goal of summarizing the current state of knowledge about the principles which underlie antiarrhythmic drug therapy and the agents which are most frequently used in the management of patients with cardiac arrhythmias.

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Advances in Immunology. Vol. 15. Edited by F. J. Dixon and Henry G. Kunkel. Academic Press, New York, N. Y. 1972. 329 pp. 23 × 15 cm. $18.50.

This most recent addition to the Advances in Immunology series contains chapters by a number of distinguished authors. In the first chapter, "The Regulatory Influence of Activated T Cells on B Cell Responses to Antigen," D. Katz and B. Benacerraf discuss the interaction of B and T cells in the immune response, the mechanism of T cell regulation of B cells, and the functions of the B and T cells in such immune phenomena as tolerance, anamnesis, and cellular immunity. E. Unanue's chapter on "The Regulatory Role of Macrophages in Antigenic Stimulation" is timely considering the intense interest over the past couple of years concerning the role of macrophages in the immune response. The author stresses the need for improvement of and experience with tissue culture methodology to clarify the cellular interactions be-