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Pharmacological Manipulation of Complement System

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I. Introduction

COMPLEMENT* is a powerful effector system involved in the body's immunological defenses. In normal individuals complement pathways are activated only when exposed to foreign invaders such as bacteria and other antigens. After the foreign material has been removed from the body by complement-mediated processes, activation of complement ceases. In a wide variety of diseases, however, such as immune complex diseases, autoimmune diseases and immunodeficiency diseases, e.g., hereditary angioneurotic edema (HANE) and C3b-inactivator deficiency, the disease state is associated with the activation of complement. In some diseases complement is activated for a comparatively short time (e.g., in HANE) whereas in others there is prolonged or indefinite activation. In some diseases complement activation is local, in others systemic; in some vascular and in others extravascular. In some diseases the whole chain is activated, in others only a few components are activated (e.g. in HANE). In some the classical pathway is activated, in others the alternative pathway. In almost all complement-mediated diseases, the otherwise advantageous biological activities of complement fragments become detrimental, resulting in tissue injury and disease.

It is believed that the inhibition of complement by pharmacological means is likely to arrest the disease process. For example, if, in a given disease, complement is consumed via the alternative pathway, inhibition of early step(s) of this pathway by pharmacological means will not only protect the patient from the pathological effects of alternative pathway activation, but will also provide C3 and later components to the patient thus making the classical pathway from C1 to C9 intact. Similarly, if, in a given disease, complement consumption is due to the activation of the classical pathway, inhibition of early steps of this pathway will probably not only protect the patient from the pathological effects of classical pathway activation, but will make C3 and later components, and thus the whole alternative pathway up to C9, available to the patient. From this point of view,

* The nomenclature used for the proteins of the classical (Bull. W.H.O. **39:** 935–938, 1968) and alternative pathway (Bull. W.H.O. **59:** 489–491, 1981) is that of the World Health Organization. Other abbreviations used have been defined at the time of their first usage.

various laboratories are concerned with the modulation of complement activity by synthetic compounds. An attempt has been made in this review to assemble the disjointed literature regarding the synthetic inhibitors of complement. Toxic as well as relatively less toxic complement inhibitors have been included in this review with the belief that once the types of structures that can inhibit complement components are known, attempts can be made to design and develop less toxic analogues. A description of the classical and alternative pathways has been omitted as very good reviews on these subjects have been published recently (107, 117, 118). A review on earlier literature on complement inhibitors with special reference to aromatic amino acids was presented by Becker (25) and excellent reviews have been published recently by Johnson (83), Reynard (124), and Patrick and Johnson (116).

II. Inhibitors of the Classical Pathway

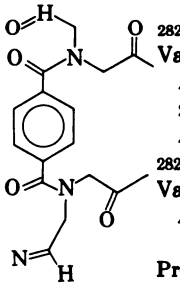
A. *Polypeptides*

It is generally agreed that the C1 fixing site on IgG resides in the C_H2 domain although there seems to be controversy over the exact region within this domain that fixes complement. Several groups of workers have proposed different regions as possible complement fixing sites (34, 40, 41, 119). Polypeptides resembling these proposed C1 fixing sequences have been synthesized and have been shown either to activate or inhibit C1 (34, 84, 85, 100, 119).

Some synthetic polypeptides with strong complement inhibiting or consuming activity are listed in table 1. Some of these peptides cause activation of complement while others cause inhibition. As is described below, with some peptides inhibition at lower concentrations and activation at higher concentrations has been reported. More studies are required to ascertain inhibition or activation of complement by these peptides. Johnson and Thames (84, 85) have shown that 2 mg of peptides 1 and 2 (see table 1) can remove 100% and 47% CH50 units from 1 ml of human serum, respectively. Prystowsky and coworkers (119) have shown that peptide 3 can inhibit 50% of C1 hemolysis at 1.5×10^{-4} M and is about half as active as monomeric 7S human IgG on a molar basis and essentially as active on a site basis. Peptide 4 was slightly less effective. Peptides 5 and 6 removed 50% of C1 at 7×10^{-8} and 4.2×10^{-6} M and caused destruction of C2 at 1.8×10^{-6} and 2.14×10^{-5} M, respectively. Compound 5 caused C3 conversion at 7×10^{-5} M whereas compound 6 was inactive in this respect. It appears that peptides 5 and 6 have a twofold role: at lower concentration they bind to C1 and cause inhibition and at higher concentrations they cause activation leading to C2 destruction and C3 conversion (34). Peptide 7 was 42% and 26% as active as monomeric IgG and Fc fragment of IgG, respectively, in inhibiting C1 binding to immunoglobulins on molar basis. Peptide 8 was about 100% and 65% as active as monomeric IgG and Fc fragment, respectively (100).

TABLE 1

Some synthetic polypeptides that inhibit or consume complement.

	Peptide*	Reference
1.	Glu-Trp-Tyr-Glu-Arg-Gly	84, 85
2.	Z-Asn-Trp-Tyr-Val	84, 85
3.	^{275} form | Phe-Asn-Trp-Tyr-Val-Asp-Gly-Val-Gln-Val- ^{285} ^{290} His-Asn-Ala-Lys-Thr-Lys	119
4.	^{281} ^{290} Gly-Val-Gln-Val-His-Asn-Ala-Lys-Thr-Lys	119
5.	^{227} ^{281} Lys-Ala-Asp-Trp-Tyr-Val-Asp-Gly	34
6.	^{277} ^{281} Trp-Tyr-Val-Asp-Gly	34
7.	^{282} Val-Gln-Val-His-Asn-Ala-Lys-Thr-Lys- ^{292} Pro-Arg-OH	100
8.	 ^{282} Val-Gln-Val-His-Asn- Ala-Lys-Thr-Lys-Pro- ^{292} Arg-OH ^{282} Val-Gln-Val-His-Asn- Ala-Lys-Thr-Lys- ^{292} Pro-Arg-OH	100

* 273–283 residues of human IgG: Val-Lys-Phe-Asp-Trp-Tyr-Val-Asp-Gly-Val-Glu; 484–494 residues of human IgM: Val-Phe-Val-Glu-Trp-Met-Glu-Arg-Gly-Glu-Pro.

Takada and coworkers (141) have shown significant inhibition of $C\bar{1}s$ esterolysis by tripeptides glutathione and leupeptin (acetyl-leucyl-arginal) at 10 to 40×10^{-3} M and 3×10^{-5} M, respectively. Both peptides inhibited $E\overline{AC142}$ formation and total complement activity. Leupeptin is, however, not a specific inhibitor of $C\bar{1}s$; it has been shown to inhibit many other enzymes as well (43, 44, 97).

In guinea pigs, passive cutaneous anaphylaxis was inhibited by about 50% at 25 μg of leupeptin per site. Histamine reaction was also shown to be affected by leupeptin and this effect may be partially responsible for the inhibition of passive cutaneous anaphylaxis reaction (61). Intraperitoneal administration of leupeptin, however, did not cause appreciable suppression of experimental allergic encephalomyelitis (EAE) in Lewis rats (37b).

TABLE 2
*Complement-consuming abilities of polyanions.**

Compound with Molecular Weight >30,000 Daltons	Moles of Amino Acid Bound/Gram of Polymer	Percentage of CH50 Removed by 2000 μg	No. of CH50 Units Removed/Milligram Material
Human IgG1			30.7
1. $(Tyr\text{-}Glu\text{-}Ala\text{-}Gly)_nGly$	Equimolar	100	15.4
2. $(Phe\text{-}Glu\text{-}Ala\text{-}Gly)_nGly$	Equimolar	0	
3. $(Trp\text{-}Glu\text{-}Ala\text{-}Gly)_nGly$	Equimolar	25	
4. $(Tyr\text{-}\gamma\text{-}Glu\text{-}Ala\text{-}Gly)_nGly$	Equimolar	32	
5. $(Tyr\text{-}Glu\text{-}Gly\text{-}Gly)_nGly$	Equimolar	30	
6. $(Tyr\text{-}Gly\text{-}Ala\text{-}\beta\text{-}Ala)_nGly$	Equimolar	100	
7. $(Ala\text{-}Glu\text{-}Ala\text{-}Gly)_nGly$	Equimolar	0	
8. $(Glu)_n$	Equimolar	0	
9. $(Glu\text{-}Ala)_n$	Equimolar	0	
10. $(Glu\text{-}Ala\text{-}Tyr_4)_n$	$(3.6{:}6.7{:}0.25) \times 10^{-3}$	0	
11. $(Glu\text{-}Ala\text{-}Tyr_{10})_n$	$(3.4{:}6.3{:}0.61) \times 10^{-3}$	0	
12. (Maleic acid-vinyl methyl ether)$_n$	0	0	
13. (Maleic acid-Tyr-methyl ether)$_n$	1.44×10^{-3}	100	58.9
14. (Maleic acid-Phe-vinyl methyl ether)$_n$	1.40×10^{-3}	100	43.5
15. (Maleic acid-His-vinyl methyl ether)$_n$	1.10×10^{-3}	0	
16. (Maleic-acid-Leu-vinyl methyl ether)$_n$	120×10^{-3}	0	
17. (Maleic acid-Pro-vinyl methyl ether)$_n$	1.77×10^{-3}	0	
18. (Maleic acid-Gly-vinyl methyl ether)$_n$	1.70×10^{-3}	0	
19. (Maleic acid-Lys-vinyl methyl ether)$_n$	1.67×10^{-3}	0	
20. (Maleic acid-Trp-Gly-vinyl methyl ether)$_n$	1.35×10^{-3}	100	
21. (Maleic acid-styrene)$_n$	0	100	87.0
22. (Maleic acid styrene-Tyr)$_n$	0.75×10^{-3}	100	105.3

* Adapted from Johnson (83).

B. Polyanions

A series of synthetic polyanions, some of them polypeptides, was investigated by Johnson (83) for their anticomplementary activity. Compounds possessing aromatic moieties and anionic groups consumed complement (table 2). Compound 1 has a polypeptide backbone and an aromatic tyrosyl group is adjacent to the anionic glutamyl residue, 22 has a carbon backbone with aromatic and anionic moieties in randomly arrayed positions. Both of these compounds consumed complement. If an aromatic tyrosine group is removed from 1 and replaced by alanine, as in 7, the complement-consuming activity is lost. The importance of an aromatic residue and an anionic carboxyl group near it could also be visualized by comparison of 12 with 13, 14, and 20. Compound 12 lacks such aromatic moieties and also lacks complement-consuming activity. Both compounds 13 and 1, which are analogous in the sense that both have tyrosine and carboxyl groups, consumed complement. Compounds 10 and 11 did not consume complement perhaps because their sequences are random and the tyrosine moiety may not be close enough to the anionic carboxyl group of glutamic acid. Similarly other comparisons between the compounds listed in table 2 clearly indicate that polyanions with aromatic and anionic groups close to each other can consume complement. It is clear from table 2 that compound 1 possesses half the capacity of human IgG to remove complement. From these studies it is not clear whether consumption of complement by these polyanions is associated with activation of complement sequence or that they simply interact with one or more components without activating others.

Lauenstein and coworkers (93) studied the effect of polyanions—carrageenan, polyvinyl alcohol sulphate, polyethene sulphate, heprinoid "Bayer", dextran sulphate, and heparin—on complement system. All six of these substances inhibited complement. Their effect was directed against C1 and C2. Carrageenin, which is a sulphated polysaccharide, was the most powerful inhibitor of C1. Borsos and coworkers (36) showed that carrageenin inhibits hemolytic activity of complement by interfering with the capacity of C1 to interact with EA. Carrageenin interacted with C1 and removed it from fluid phase. It had no effect on C1-esterase. In vivo, however, the degree of inhibition of complement by carrageenan was not appreciable and increasing the concentration of carrageenan caused an increase in bleeding time. Earlier studies by Davies (50) showed that intravenous injection of this polyanion (5 mg/kg) diminishes total hemolytic complement activity by 99%. Raepple and coworkers (120) studied the affect of eight polyanions on fluid phase and bound $C\bar{1}$ as well as on purified C4 and C2. They had no affect on C4 and C2 but were strong inhibitors of $C\bar{1}$. These polyanions were dextran sulphate, polyvinyl sulphate, polyethanol sulphate, heparin, polyinosinic acid, pentosan polysulphoester sp. 54, suramin, and chondroitin sulphate. Dextran sulphate, polyvinyl sulphate, and polyethanol sulphate were the strongest inhibitors. The inhibitory effect was due to

interaction of these polyanions with C1q but not with $C1\bar{s}$. Heparin at a concentration of 0.3 μg/ml interfered with C1q binding of immune complexes and at a concentration of 100 μg/ml inhibited $C1\bar{s}$-mediated consumption of C4 and C2 but was without affect on $C1\bar{s}$-mediated esterolysis of *p*-tosyl-L-arginine-methyl ester (TAME) (120, 139), thereby indicating possible interference in binding of $C1\bar{s}$ with C4 and C2. Loos and coworkers (98) have shown that dextran sulphate, polyvinyl sulphate, polyethanol sulphate, heparin, and polysulfoester sp. 54 prevented the uptake of C2 by EAC4b. This effect was due to sequestration of Mg^{++} by these polyanions. Walb and coworkers (148) have demonstrated that microgram quantities of pentosan polysulfoester sp. 54 inhibit most reaction steps except C1 activation. At a concentration of 10 to 100 μg/ml it inhibited C4 and C2 utilization by $C\bar{1}$ (27, 98, 120). Low molecular weight dextran was similarly inhibitory at high concentrations (30% inhibition at 10 mg/ml) (39). Polyanethol sulphate has been shown to inhibit $C1\bar{r}$ (109).

Baker and coworkers (19) investigated several polyanions and polycations for their ability to influence the formation of $E\overline{C567}$ complex from $\overline{C56}$, C7, and E. six of the 11 polyanions tested, including polyanethol sulphate, heparin, and dextran sulphate, inhibited this reaction. Heparin (2 μg/ml) also inhibited $\overline{C567}$ complex-induced lysis. On the other hand, five of the polycations, including polybrene, protamine, and polyornithine, potentiated the formation of $E\overline{C567}$. The inhibition was reminiscent to that shown by $\overline{C567}$ INH and potentiation seemed to involve neutralization of $\overline{C567}$ INH.

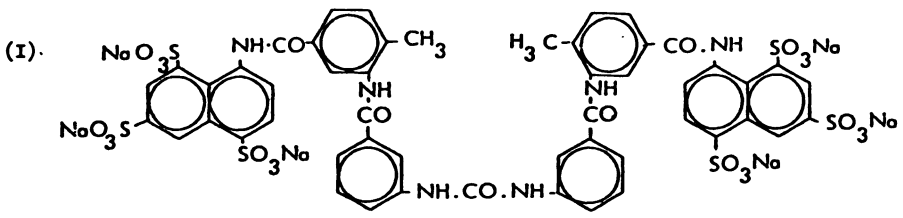
Interaction of heparin with protamine in fresh human serum in amounts far smaller than those required for complement inhibition by either agent alone, induced consumption of complement (121). This depletion was dependent on time, temperature, pH, divalent cations, and serum concentrations and was similar to that induced by antigen-antibody interaction.

Polylysine has been shown to strongly inhibit immune hemolysis at very low concentrations (61). Fletcher and Lin (61) have shown 50% blocking of binding of C1q to immune aggregates at about 5×10^{-3} M. These results are similar to those of Hughes-Jones and Gardner (80). Another small polypeptide (compound A) with 50% lysine content had 100-fold greater inhibitory activity. In vivo effects of polylysine and compound A were similar. Both inhibited passive cutaneous anaphylaxis reaction in guinea pigs to the same extent at the same concentration. Both compounds were toxic and compound A perturbed the fixation of I^{125}-IgG to kidney tissues in experimental glomerulonephritis.

Suramin (I) has been shown to be an efficient inhibitor of the complement system (59, 62). Inhibition of total complement activity by suramin was rapid and reversible (75% at 5×10^{-5} M). It inhibited the interaction between EA and $\underline{C1}$ (at 1×10^{-4} M), $EAC\overline{1}$ and C4 (at 5×10^{-4} M), $EAC\underline{14}$ and C2 (at 5×10^{-4} M), and $EAC\underline{\overline{142}}$ and C3 to C9 (in microgram quantities). In other studies suramin was shown to inhibit interaction between $EAC\overline{1-7}$ and C8 (125, 137). Suramin also reacts irreversibly with bound C3 thereby preventing the subsequent action of C3b-inactivator on C3 (91).

Asghar and coworkers (6) studied the affect of suramin on complement-mediated tissue injury by using the Arthus reaction in rabbits as a model. They studied the influence of suramin administration (14 mg/kg) on several aspects of the Arthus reaction, namely development of erythema, accumulation of leukocytes, deposition of complement, and destruction of endothelium and its basal lamina. Prior administration of suramin only slightly affected the development of erythema; only a 20% reduction in size was noted. As regards accumulation of leukocytes at the site of the Arthus reaction, these authors devised a new technique for quantification of cellular inflation. They used a Leitz-Wetzler microscope connected to a computerized area integrator (PDP 11/60, Tektronix 4010-1) (figure 1) and studied H and E stained sections of skin biopsies. Red light emerging from a light source was made to fall on the border (or a defined area) of a biopsy section seen directly under the microscope and the movement of light source for the completion of one round of the border (or a defined area) of a section was recorded and the computer gave the area of the section (or of the defined part of the section). Similarly, with higher magnification, the area of each patch of infiltration was measured within the section (or defined part of the section). The results were obtained in terms of square micrometers (μm^2) and the extent of infiltration was calculated by dividing the sum of area of infiltration by the area of section (or a defined part of it).

By using this technique, Asghar and coworkers (6) demonstrated approximately 65% inhibition of cellular infiltration from a prior injection of suramin. These



Suramin sodium

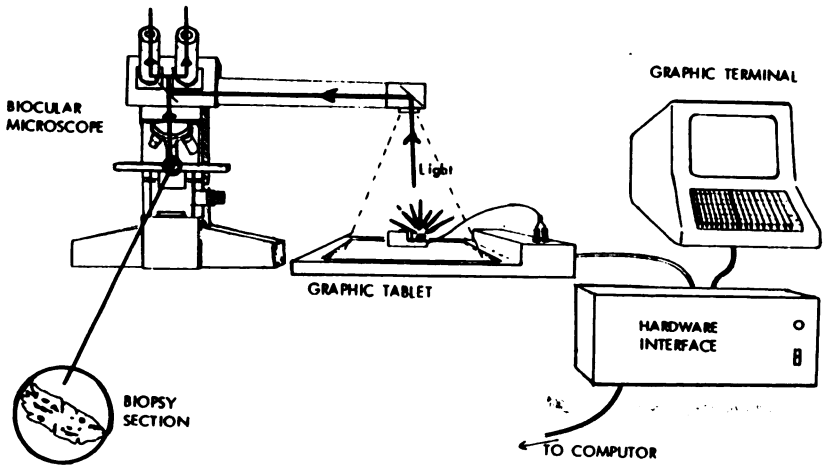


FIG. 1. Computerized area integrator used for quantification of cellular infiltration at the site of the Arthus reaction. For details, see text.

experiments were carried out on pilot scale with five rabbits; the same rabbits were used without and with drug treatment. Recently a statistical model has been developed for these experiments and will be described in detail elsewhere.

With an immunofluorescence technique, these workers showed some reduction but not complete absence of complement deposits due to suramin treatment. Electron microscopic studies (6) showed that the Arthus reaction caused damage to the venules as evidenced by discontinuities in endothelial linings, degradation of endothelial remnants, and destruction of basal lamina (figure 2, A and B). In an Arthus reaction created after suramin treatment, none of the venules showed frank destruction that could be found in an Arthus reaction without prior drug treatment and the endothelium and its basal lamina remained almost intact (figure 2, C and D).

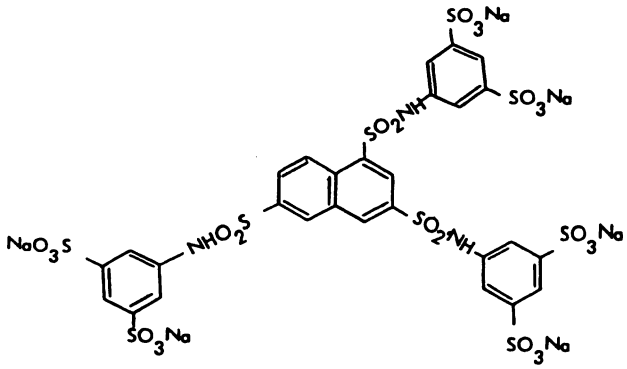
Suramin has also been shown to be an effective inhibitor of passive cutaneous anaphylaxis in guinea pigs and the proteinuria manifestation of experimental glomerulonephritis (EGN), while not affecting antibody fixation to tissue or the histamine-mediated skin reaction (61).

The results obtained by Asghar and coworkers (6) and Fletcher and Lin (61) do suggest that effective inhibitors of complement, like suramin, may be of value in controlling immune-complex-mediated tissue injuries in disease states. The argument may be raised that the observed affect of suramin on tissue injury may be due to its ability to inhibit a variety of enzymes other than those of complement system (59, 151). However, since many other inhibitors of complement behave like suramin in suppressing complement-mediated tissue injury, the inhibition of complement as a cause of suppression of tissue injury remains a strong possibility.

In humans, suramin has been used to prevent attacks of edema in patients with HANE who virtually lack natural inhibitor of first component of complement (37). In one of the two patients, attacks of edema could be prevented with suramin.

Conrow et al. (46) recently synthesized a novel group of polyanionic compounds that are strikingly active against complement. These include 5,5′,5″-(1,3,6-naphthalene triyl tris (sulfonylimino))—(1,3-benzene disulfonic acid) hexa sodium salt, represented by the title polysulphonic acid, compound II b (II).

(II)



Compound II b

Compound II b was 30 times more active than suramin in inhibiting human $C\overline{1}$ and four times more active in inhibiting lysis of $EAC\overline{142}$ by C3 to C9. It was eight times more effective than suramin in inhibiting human alternative-pathway-mediated lysis of mercaptan-treated human erythrocytes in the presence of cobra venom factor (CVF). Compound II b was five to 30 times more active than suramin in suppressing hemolytic activity in undiluted guinea pig serum. In vivo, where pharmacology may be superimposed upon intrinsic activity, II b is substantially more effective than suramin. An intraperitoneal injection of 200 mg/kg of compound II b to guinea pigs weighing 300 g resulted in no detectable complement in 2 hours.

C. *Polynucleotides*

Several polynucleotides were investigated by Yachnin (152, 153) for their complement-inhibiting ability. Polyinosinic acid (Poly I) (III) was the most potent inhibitor of total complement activity; 7.3×10^{-9} M phosphate of

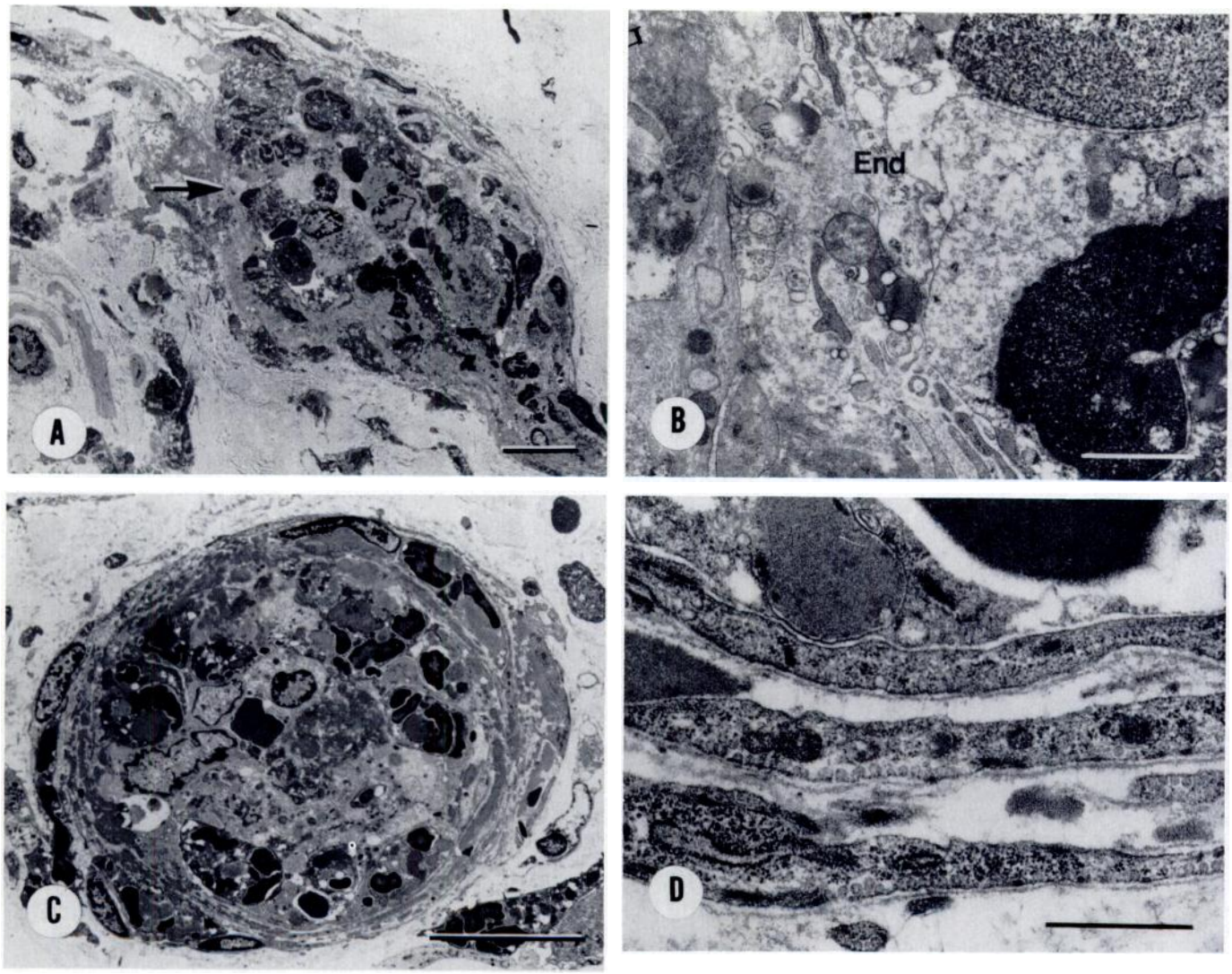
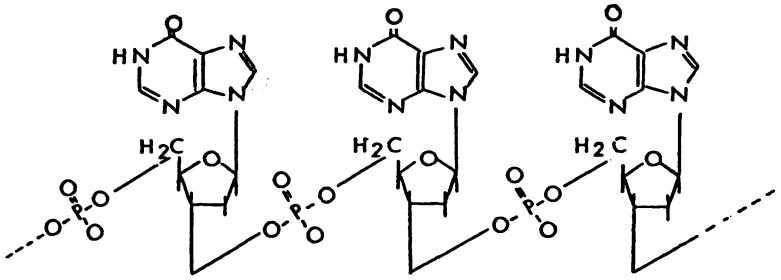


FIG. 2. A. (2188-25) Venule in uninhibited Arthus reaction that is damaged at the site of leukocyte accumulation, whereas other area (upper left) has remained largely intact. Note the presence of cells and debris in perivascular connective tissue, giving the venules a frayed appearance. The area indicated by the arrow is enlarged in the next figure. Bar represents 10 μm. ×1100. B. (2188-35) Detail of the vessel wall at the site of leukocyte accumulation in the preceeding figure. Parts of degenerating leukocytes are visible in right half of the micrograph. Some cell remnants are recognizable as fragments of the degenerated endothelial lining. No basal lamina is visible. Bar represents 1 μm. ×17,000. C. (2152-6) Accumulation of leukocytes and proteinaceous deposits in venule at the Arthus reaction inhibited by suramin. Note the smooth vascular contour. Area at bottom is enlarged in the next figure. Bar represents 10 μm. ×2300. D. (2152-8) Details from the vessel illustrated in the preceding figure. Note that the cells making up vascular wall are intact despite intercellular deposition of homogeneous material. Bar represents 1 μm. ×22,000.

Poly I inhibited one 50% hemolytic unit. Polyguanylic acid (Poly G) was a less effective inhibitor; 5.4×10^{-8} M phosphate of Poly G inhibited one CH50 unit. Polyadenylic acid (Poly A), polyuridylic acid (Poly U), polycytadylic acid (Poly C), and a wide variety of mammalian and bacterial RNA as well as calf thymus DNA had no affect on complement activity. Mononucleosides were also devoid of inhibitory activity.

Further studies by Yachnin and coworkers (154, 155) suggested that Poly I selectively inhibits C1q by preventing its attachment to sensitized sheep erythrocytes (EA). It even facilitated the detachment of $C\overline{1}$ from $EAC\overline{1}$ complex but it did not dissociate subunits of C1. Among other synthetic polyribonucleotides, only polyriboguanylic acid and mixed polymers of inosinic acid and uridylic acid or guanylic acid and uridylic acid having more than 80% inosinic acid or guanylic acid contents possessed inhibitory activity. Poly I incorporated into a double-stranded helix with Poly A does not inhibit C1. The effect of Poly I on C1q was reversed by appropriate concentrations of Poly A. In one of the later reports by Yachnin and coworkers (155) it was shown that Poly I can inactivate C4 but only in the presence of a source of C1, thus indicating that poly I resembles aggregated γ-globulins or antigen-antibody complexes, in its action on complement. High molecular weight Poly I was more anticomplementary than low molecular weight (51). Inactivation of whole complement at 10 to 40 μg/ml has been shown by Poly I, Poly G, Poly AG (A:G, 1.2:1), Poly Uz (poly 2′-azido-2′-desoxyuridylic acid), Poly CCl,

(III)



Polyinosinic acid

(Poly 2′-chloro-2′-desoxycytidylic acid), and Poly dCz (Poly-2′-Azidodesoxcytidylic acid).

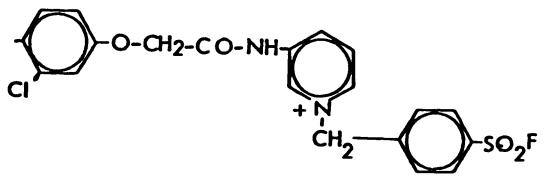
Intravenous injections of Poly I (10 μmol of P/rat) into Wistar rats gave a precipitous decline in complement activity in vivo (156). After 2 hours, activity returned to more than 50% of the preinjection level. Intravenous lower doses (5 μmol of P/rat) and intraperitoneal doses as high as 15 μmol of P/rat failed to cause a consistently significant fall of complement activity, although partial inhibition was occasionally seen.

Renk and Hoffman (122) obtained a factor from Ehrlich ascites tumor cells that was capable of inhibiting complement. The partially purified material inhibited the lysis of EAC$\bar{1}$ and EAC14. A slow inhibition of fluid phase C1 was also demonstrable. RNA from mouse tissues, yeast, and *Escherichia coli* also inhibited complement. RNA-rich, partially purified, tumor cell extract was capable of precipitating C1q.

D. Pyridinium Sulphonylfluorides, Benzamidines, and Guanidines

Baker and Hulbert (17, 18) studied a series of pyridines quaternized with fluorosulphobenzyl bromide related to IV in structure. They inhibited whole guinea pig complement. Many of these compounds were strong irreversible inhibitors of C1 and correlation between inhibition of C1 and whole complement by analogues of IV strongly suggested that the main site of action was C1. More recently Bing and coworkers (31) have shown that the site of action was the esterase activity associated with C1.

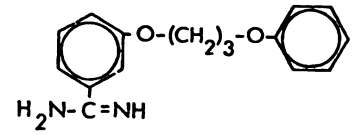
(IV)



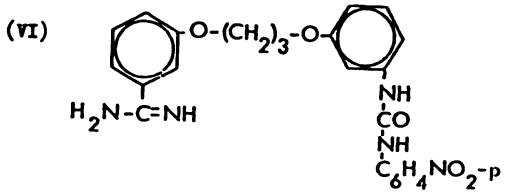
Removal of the SO_2F moiety from IV resulted in loss of its inhibitory activity indicating that an SO_2F group was necessary for inhibition.

Benzamidine was a weak inhibitor of complement (15). Introduction of *m*-phenoxypropoxy substituent (V) increased its inhibitory activity sixfold (15) which was further enhanced 400-fold by substitution of *m*-(*p*-nitrophenyl urea) on the phenoxy moiety as in VI. Since V and VI were even stronger reversible inhibitors in contrast to IV, Baker and Cory (13) thought that compounds of type VI but bearing an SO_2F terminal group (as in VII), could prove to be irreversible and more potent inhibitors. Indeed compound VII namely *m*-[*m*-(*p*-fluorosulphonylphenylureido)phenoxypropoxy] benzamidine was a stronger inhibitor than VI at lower concentrations although at higher concentrations VI was stronger. Glovsky et al. (69) showed that VI inhibits whole complement at 1.5×10^{-4} M, blocks C2 and C5 utilization at 1.8×10^{-4} M, and C1 fixation at 1.3×10^{-4} M. Compound VII and its close derivatives inhibited C1$\bar{s}$ (31, 32). Close derivatives of compound VII inhibited C$\bar{1}$s-induced vascular leakage in guinea pigs (2).

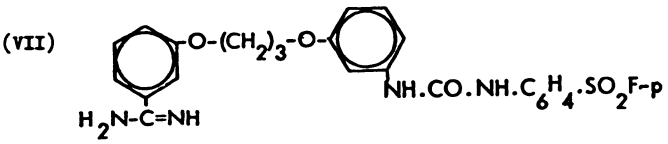
(V)



(VI)

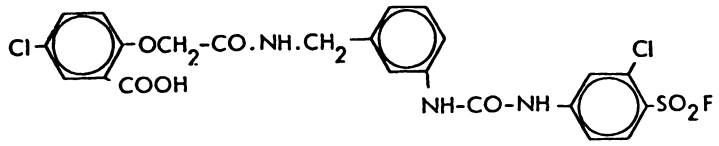


(VII)



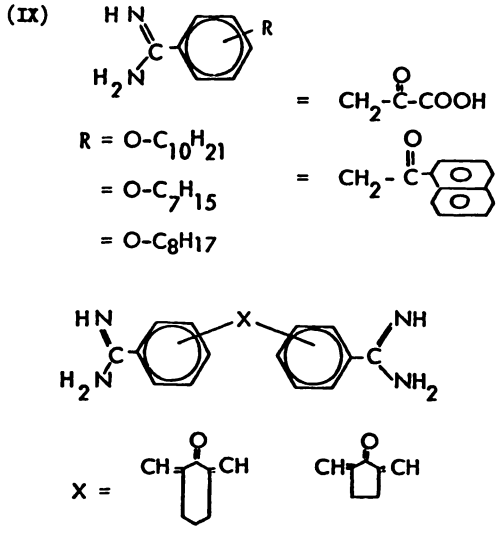
Many analogues of structures IV to VII and that of VIII were studied for their effects on C1 and whole complement. All of them inhibited total complement and most of them inhibited C1 (2, 11–18, 31, 32, 57, 69). Many of these diverse arylsulphonylfluorides inactivate complement at 1.5×10^{-5} M to 1.0×10^{-3} M concentrations. The structure activity relationship for arylpyridinium sulphonyl fluorides of type IV and arylamidine sulphonyl fluorides of type VII has been studied by Hansch and Yoshimoto (73) and Hansch et al. (74). (For finer details readers may refer to the original articles.)

Benzamidine inhibited whole complement at 3×10^{-3} M (15) and C1 at 2×10^{-3} M (30). N-substitution on benzamidine resulted in loss of inhibitory activity and

(VIII) 

meta substitution realtive to para increased the activity (13, 14, 65, 69). The structure activity relationship for inhibition of total complement by benzamidines has been studied by Hansch and Yoshimoto (73).

Hauptmann and Markwardt (76) have shown the inhibition of total complement activity by benzamidine and its *p*-chloro, *p*-amino, aminophenyl ester, and ketone derivatives. They also studied the effects of various diphenyldiamidine derivatives on the complement system. Complement-mediated hemolysis was inhibited by various derivatives to different extents. Diphenyldiamidines were found to be relatively potent inhibitors. Hydrophobic substituents enhanced the inhibitory activity. Structures of some of the strong inhibitors found in this study are given below (IX)

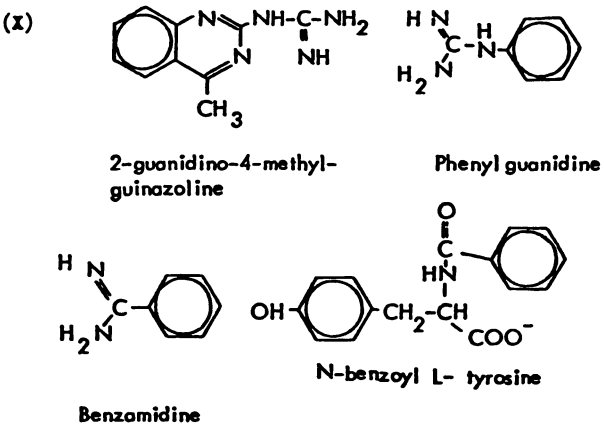
(IX) 

Asghar and coworkers (7) showed that certain diphenyl diamidines consisting of two amidinophenyl residues linked in meta- or paraposition by a molecular bridge were strong competitive inhibitors of C1r̄ and C1s̄ (table 3). They also inhibited the overall generation of C1s̄ when added to a system containing three subunits of C1 and Ca^{++}. Their results not only supported the suggestion of Bing (29) that C1s̄ possesses anionic and hydrophobic regions at the active site but also suggested similar regions in the active site of C1r̄.

All the diphenyldiamidines listed in table 3, but not monophenyldiamidines, interacted with the B-determinant of C3 in a manner to render it incapable of reacting with anti-B-determinant antiserum (4) (figure 3). The interaction was reversible. A concentration of 6×10^{-4} M strongly inhibited complement-mediated hemolysis. Since a close relationship exists between the B-determinant of C3 and its hemolytic and biological activities, interaction of diphenyldiamidines with this determinant together with inhibition of C1r̄ and C1s̄ might contribute to the inhibition of total hemolytic activity of complement. Despite the inhibitory activity of diphenyldiamidines against C1, C3, and total complement, pentamidine has been shown to be inactive in suppressing the Frossman reaction (115).

Asghar and coworkers (6) studied the effect of 2-hydroxystilbamidine (50 mg/rabbit) on several aspects of the Arthus reaction, namely, development of erythema, accumulation of leukocytes, deposition of complement, and destruction of endothelium and its basal lamina. The size of erythema was slightly reduced (15%). In a pilot study carried out in detail with reproducible results, there was a 50% reduction in cellular infiltration as measured by the computerized area integrator technique described above for suramin. There was appreciable reduction in complement deposition at the Arthus reaction site as seen by immunofluorescence technique (figure 4). The endothelium and its basal lamina were completely protected by administration of 2-hydroxystilbamidine prior to creation of the Arthus reaction, as seen by electron microscopy. The results were similar to those obtained with suramin (figure 2).

Bing (29, 30) has shown competitive inhibition of C1s̄ by a variety of guanidines, amidines, and other aromatic compounds. The structures of some of the strongest inhibitors found in this study are given below (X).

(X) 

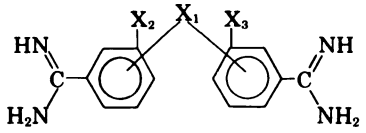
A guanidino derivative, *p*-nitrophenyl guanidinobenzoate, has been shown to suppress EAE in Lewis rats (37b).

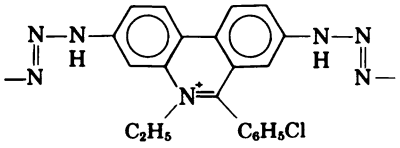
E. Anthranilates

Three anthranilate type anti-inflammatory agents, niflumic acid, flufenamic acid, and mefenamic acid, (XI)

TABLE 3

*Inhibition of esterolytic activities of $C1\bar{r}$ and $C1\bar{s}$ by diphenyldiamidines.**



Compounds	X_1	X_2	X_3	Ki (M)	
				$C1\bar{r}$	$C1\bar{s}$
Amicarbalide	—NH—CO—NH—	***	***	3.5×10^{-5}	1.8×10^{-4}
Propamidine	—O—$(CH_2)_3$—O—	H	H	3.7×10^{-4}	1.6×10^{-4}
Stilbamidine	—CH=CH—	H	H	1.15×10^{-4}	8.8×10^{-5}
Pentamidine	—O—$(CH_2)_5$—O—	H	H	1.05×10^{-4}	8.5×10^{-5}
2-Hydroxystilbamidine	—CH=CH—	OH	H	6.0×10^{-5}	6.0×10^{-5}
Dibromopropamidine	—O—$(CH_2)_3$—O—	Br	Br	1.1×10^{-4}	3.0×10^{-5}
M & B 4596		***	***	3.2×10^{-5}	**

* Adapted from Asghar et al. (7).

** Strong inhibition but irregular kinetic behaviour.

*** Two benzamidines linked to x_1 in meta position.

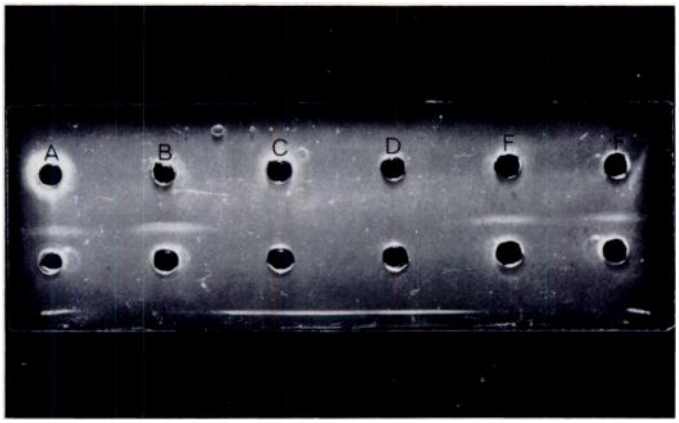
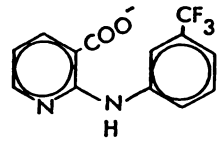


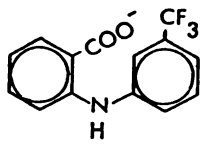
FIG. 3. Inhibition of interaction of B-determinant of C3 with the anti-B-determinant by diphenyldiamidines in agar gel. Lower row: all the wells contained anti-B-determinant. Upper row: A, normal serum; B, purified C3; C, normal serum plus pentamidine; D, C3 plus pentamidine; E, normal serum plus pentamidine washed on ultrafilter and reconstituted; F, C3 plus pentamidine washed on ultrafilter and reconstituted. Pentamidine concentration was 6×10^{-3} M. Contents of wells A to D were pre-incubated at 37°C for 90 min. and so were those of E and F before washing on ultrafilter. The slides were incubated at room temperature for 3 days and photographed without washing. Note that pentamidine caused inhibition of formation of precipitin line (C and D) and its removal caused reappearance of line (E and F). Similar results were obtained with all diphenyldiamidines listed in table 3. Inhibition of antigen-antibody reaction was specific for B-determinant-anti-B-determinant interaction. Reproduced from Asghar and Cormane (4).

have been studied by many workers for their inhibitory activity against human and guinea pig complement (75, 81, 89, 113). Phlorizin, a known complement inhibitor that inhibits total complement at 0.3 to 5×10^{-3} M by altering C3 utilization (134), was also included in one of these studies for comparison purposes. Flufenamate was most inhibitory showing significant inhibition of human complement at 5×10^{-5} M, whole guinea pig complement at 7 to 9×10^{-4} M, and terminal component (C3 to C9) activity at 1×10^{-4} M. $EAC\overline{14}$ formation from C1 and $EAC\overline{4}$ was partially inhibited by flufenamate at 1.8×10^{-3} M but not by niflumate. Both C2 cleavage and $EAC\overline{142}$ formation was decreased considerably by flufenamate at 9×10^{-4} M, while niflumate was a weak inhibitor and phlorizin was ineffective. This effect of flufenamate on C2 was not due to Mg^{++} or Ca^{++} chelation nor due to its affect on $EAC\overline{142}$ stability. $EAC\overline{1423}$ formation from C3 and $EAC\overline{142}$ was partially inhibited by flufenamate and strongly inhibited by phlorizin, but niflumate was a weak inhibitor of this reaction.

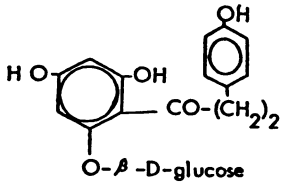
(XI)



Niflumate



Flufenamate



Phlorizin

All of these compounds at a concentration of 9×10^{-4} M inhibited $EAC\overline{1-5}$ formation from C5 and $EAC\overline{1-3}$ and increased the lability of $EAC\overline{1-5}$ at 25° C, but flufenamate was the most effective.

Kohler and Martinez (89) have shown that flufena-

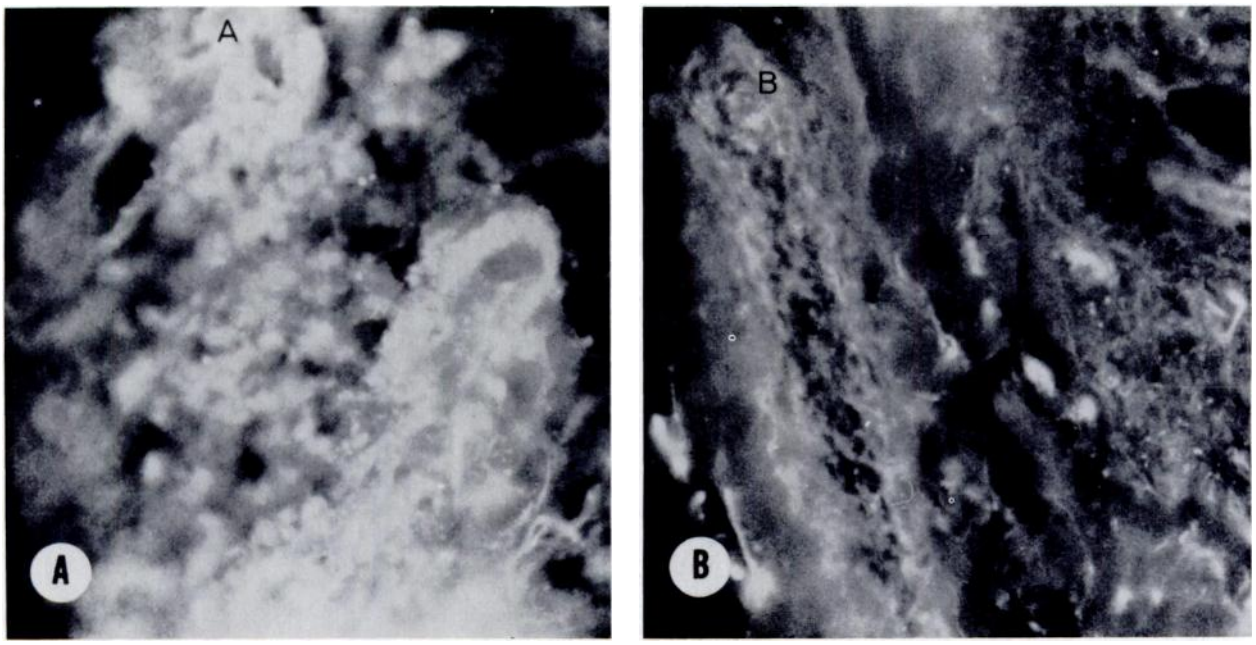
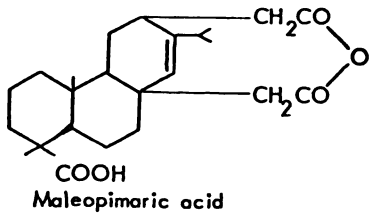
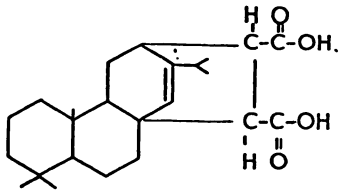


FIG. 4. Complement (C3) deposition at the Arthus reaction site as seen by the immunofluorescence technique. A. Deposition of C3 at the site of antigen challenge in immunized rabbit. B. Deposition of C3 at the site of Arthus reaction in same rabbit the next day; 2-hydroxystilbamidine (12 mg/kg) was administered intramuscularly half an hour before antigen challenge. Biopsies were taken 6 hours after antigen challenge.

(XII)



Maleopimaric acid



Fumaropimaric acid

mate (5 mg/mouse) can inhibit the Arthus reaction when given 10 minutes to 8 hours before antigen challenge. This effect persisted for 24 hours. The inhibition of the Arthus reaction appeared to be due to multiple effects of drugs, one of which was C3 inactivation as measured by radial immunodiffusion 4 to 8 hours after drug administration.

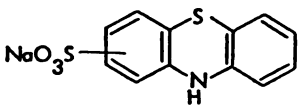
F. Levopimaric Acid Derivatives

Maleopimaric acid, fumaropimaric acid, and related compounds (XII) inhibited complement in vitro (9, 68, 70). Maleopimaric acid in the concentration range of 0.8 to 7.0×10^{-3} M inhibited the formation of $\overline{EAC1}$ from EA and C1 and dissociated $\overline{C1}$ and $\overline{EAC1}$. This action was not due to chelation of Ca^{++}. The formation of $\overline{EAC142}$ was also depressed by maleopimaric acid. The inhibition was greater than could be accounted for by the action of maleopimaric acid on $\overline{C1}$. Maleopimaric acid did not dissociate C2a from $\overline{EAC142}$ cells nor did it act by chelating Mg^{++}. Maleopimaric acid inhibited the generation of EAC1–5, EAC1–6, and EAC1–7 cells from appropriate purified component and intermediate cells and dissociated the preformed C567 complexes.

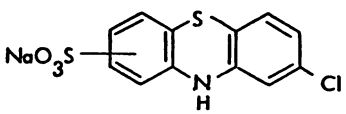
Fumaropimaric acid, at a concentration of 7.0×10^{-3} M, inhibited the chemotactic activity of C5–6 complexes generated in rabbit serum. EAC1–3 formation from $\overline{EAC142}$ cells and C3 and EAC1–8 and EAC1–9 formation from $\overline{EAC1}$–7 and C8 and C9, respectively, were not inhibited by maleopimaric acid. Immune adherence was also not affected.

Fumaropimaric acid, given repeatedly over 3 to 4 days (0.3 to 0.6 g/kg) reduced serum complement levels (9). It also suppressed complement-dependent systemic Frossman, cutaneous Frossman, and reverse passive Arthus

(XIII)



Phenothiazine sulphonate



Chlorophenothiazine sulphonate

reactions (70). In the case of the passive Arthus reaction, fixation of antigen-antibody complexes and C3 in the vessel wall was not affected and yet there was total inhibition.

G. *Phenothiazines*

Phenothiazine sulphonate and chlorophenorthiazine sulphonate (XIII) have been shown to interact with the B-determinant of C3 in a manner to render the B-determinant incapable of interacting with anti-B-determinant antiserum (5). The interaction was reversible in the case of phenothiazine sulphonate and irreversible with chlorophenothiazine sulphonate (figure 5). A- and D-determinants of C3 and antigen-antibody reactions in general were not affected. Total hemolytic activity of complement was also strongly inhibited by these compounds (50% inhibition at about 1×10^{-4} M). Other compounds tested in this study were promazine, triflupromazine, promethazine, quinacrine, and chloroquine, none of which showed any influence on the B-determinant or total complement activity.

Asghar and coworkers (6) studied the effects of chlorophenothiazine sulphonate on the Arthus reaction by techniques described above for suramin. Prior administration of chlorophenothiazine sulphonate (10 to 50 mg/kg of body weight) intramuscularly to rabbits had no affect on the size of erythema developed during the Arthus reaction. Chlorophenothiazine sulphonate (90 mg) administered intra-arterially caused a decrease in the size of erythema by about 20% and also had a remarkable influence on the composition of the infiltrate, eosinophils being present in appreciable numbers whereas macrophages and polymorphonuclear leukocytes were almost absent. Oral administration of chlorophenothiazine sulphonate 1 hour before antigen challenge did not inhibit the extent of infiltration nor did it influence the composition of cellular infiltrate. Chlorophenothiazine sulphonate administration (100 mg) directly into the stomach caused a 55% decrease in infiltration. It also caused protection of the endothelium and its basal lamina as seen by electron microscopy as did suramin (fig. 2).

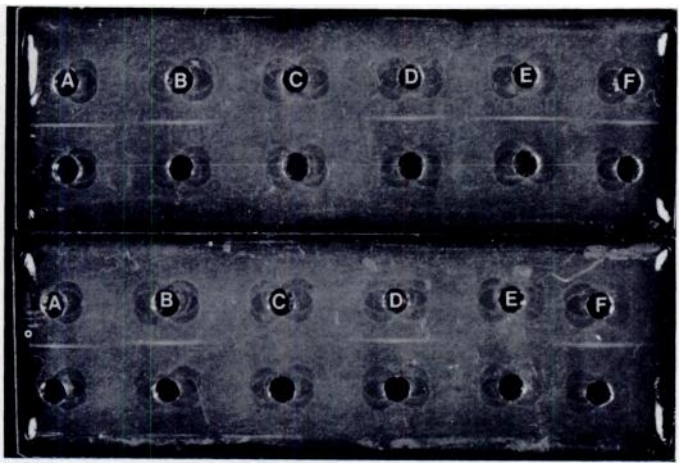


FIG. 5. Inhibition of interaction of B-determinant of C3 with anti-B-determinant in agar gel by phenothiazine and chlorophenothiazine sulphonates. Upper slide. A, upper, C3; lower, anti-B-determinant; B, upper, C3; lower, anti C3c; C, upper, C3 + phenothiazine sulphonate; lower, anti-B-determinant; D, upper, C3 + phenothiazine sulphonate; lower, anti-C3c; E, upper, C3 plus phenothiazine sulphonate subsequently washed on ultrafilter; lower, anti-B-determinant; F, upper, C3 plus phenothiazine sulphonate subsequently washed on ultrafilter; lower, anti-C3c. The concentration of phenothiazine sulphonate was 6×10^{-3} M. Lower side. The contents of the wells were the same as that of the upper slide except that in wells C to F chlorophenothiazine sulphoate was used instead of phenothiazine sulphonate. Note that precipitation line formation between B-determinant and anti-B-determinant was inhibited by phenothiazine sulphonate (upper slide, compare wells A and C) but the inhibition was reversed by its removal (upper slide, compare wells C and E). The inhibition by chlorophenothiazine sulphonate (lower slide, compare wells A and C) and was not reversed by washing the incubation mixture on ultrafilter (compare wells C and E). These compounds did not inhibit the formation of precipitin line between C3 and anti-C3c. Reproduced from Asghar and Kammeijer (5).

In another study with eight phenothiazines, Mao et al. (102) observed inhibition of total hemolytic activity at 0.27 to 2.2×10^{-3} M. Thioridazine and chlorpromazine were inhibitory at 2.7×10^{-4} M and 2.2×10^{-3} M, respectively. These agents suppressed the utilization of C2 and C4.

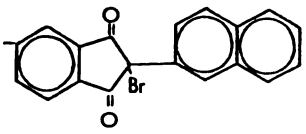
Asghar and coworkers (6) studied the effect of chlorpromazine administration (50 mg/rabbit) on several aspects of the Arthus reaction. In a pilot study with five rabbits, prior administration of chlorpromazine caused a 50% decrease in the size of the erythema developed during the Arthus reaction and a 50% decrease in cellular infiltration as measured by the newly developed technique utilizing the computerized area integrator described above. Prior administration of chlorpromazine also caused protection of the endothelium and basal lamina of blood vessel walls at the Arthus reaction site to the same extent as that caused by suramin (fig. 2) as seen by electron microscopy.

Recently in our clinic, chlorpromazine has been shown to very efficiently suppress human immune complex vasculitis.

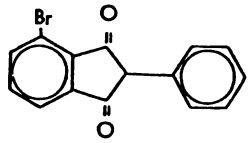
H. *Phenylindandiones*

Rosini and Mazzoncini (126) studied the effect of 2,5-dibromo-2-(β-naphthyl)-indan-1,3-dione (XIV) and 4-bromo-2-phenyl-indan-1,3-dione (XV) on the complement system in vitro and in vivo. Both compounds were antiinflammatory and devoid of anticoagulant activity. Compound XIV inhibited 100% of hemolytic activity at a concentration of 3.2×10^{-4} M whereas compound XV caused 100% inhibition at 6.6×10^{-4} M in vitro. The authors concluded from their recomplementation studies that XIV blocks the activation of C1 whereas XV blocks the activation of C4.

(XIV)



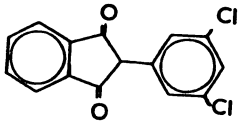
(XV)



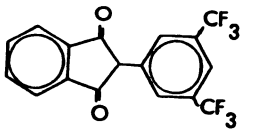
A single oral dose of 50 mg/kg bodyweight of XIV was effective in keeping complement inhibited in vivo for 2 hours in rabbits. Compound XV was less effective.

A recent study by Asghar and coworkers (8) has shown that substitution of two *t*-butyl, isopropyl, trifluoromethyl, or chloride groups at the 3 and 5 positions of the phenyl ring of phenylindandiones drastically enhanced the complement-inhibiting activity and this activity of 3,5-substituted derivatives was due to their interaction with C5. The strongest of these inhibitors was 3,5-dichlorophenylindandione (XVI) which was required in a concentration of 1.6×10^{-5} M to obtain a Z/Z_0 value of 0.5 whereas 3,5-di(trifluoromethyl) phenylindandione (XVII) required 4.0×10^{-5} M for the same effect (Z and Z_0 refer to the number of lytic sites per cell in presence and absence of inhibitor, respectively).

(XVI)



(XVII)



I. Amino Acids and Their Derivatives

ϵ-Aminocaproic acid (EACA) (XVIII), at concentrations ranging from 1.25 to 20×10^{-2} M, inhibited intrinsic activation of C1 without influencing preformed $C\bar{1}$ (136). This effect was reversible and dependent on time and temperature. α-Aminocaproic acid and valeric acid were without effect indicating the importance of the position of $-NH_2$ group (143). Decarboxylation of EACA caused an increase in inhibitory activity. EACA at a concentration of

(XVIII) $NH_2 \cdot CH_2 \cdot (CH_2)_4 \cdot COOH$

ϵ -aminocaproic acid (EACA)

1.2×10^{-2} M, did not inhibit $\overline{C1s}$ or $C1\bar{r}$ (7). However, Tamura et al. (142) have shown that three analogues of EACA, XVIII a, b, c, inhibit $C1\bar{s}$ esterolysis at 3 to 50×10^{-5} M and effective inhibition of $C1\bar{r}$ was observed by XVIII a at 4.4×10^{-6} M. Muramatu and coworkers (108) have shown that aromatic ester analogues of EACA at 1×10^{-3} M inibit activation as well as action of C1.

NH_2—C(=NH)NH X COO Y COOZ

	X	Y	Z
(XVIIIa)	1,4-C_6H_4	1,4-$C_6H_4CH_2$	$CH_2CON(CH_3)_2$
(XVIIIb)	1,4-C_6H_4	1,4-C_6H_4	$CH_2CON(CH_3)_2$
(XVIIIe)	$(CH_2)_5$	1,4-C_6H_4	C_2H_5

EACA inhibits plasminogen activation (141) and serum carboxypeptidase (at 1.0 M) (144) and thus can indirectly influence complement activation and biological activities of fragments of components generated during complement activation (60).

Passive cutaneous anaphylaxis in guinea pigs was inhibited to approximately the same degree by EACA as by similar amounts of suramin (500 mg/site) or gold thiomalate. EGN was shown to be slightly inhibited at relatively high concentrations (200 mg/kg) without affecting IgG binding (61). EACA also caused suppression of EAE (37b). trans-4-Aminomethylcyclohexane-carbolic acid, which is a stronger inhibitor of complement than EACA, caused more effective suppression of EAE than EACA in Lewis rats (37b). EACA has been shown to be effective in controlling attacks of edema in patients with HANE (71) and has also been shown to cause rapid improvement in patients with relapsing multiple sclerosis of recent onset and mild disease and in patients with acute encephalitis (3).

Several natural amino acids have been shown to inhibit complement though at quite high concentrations. Cysteine and homocysteine inhibit C1s, $\overline{C142}$ generation and total complement activity at 10 to 40×10^{-3} M (141). Aspartic acid, glutamic acid, and lysine inhibited binding of C1q to immune complexes at 2 to 5×10^{-3} M (1). Inhibition of generation of C4 site by L-tyrosine and L-tryptophan was observed by Shimada and Tamura (133) and Baba and Tamura (10) at concentrations in the order of 10^{-3} M.

Since several proteolytic activities are generated during complement activation, the synthetic substrates of these activities or the analogues of the substrates can competitively inhibit the proteolytic and hemolytic activities of the generated enzymes, again at quite high concentrations. Thus $C1\bar{s}$ was inhibited by TAME (133, 138). Conversion of C1s to $C1\bar{s}$ by $C1\bar{r}$ was significantly inhibited by N-acetyl arginine at 4.3×10^{-2} M and by TAME at 6×10^{-3} M (109). The C423 enzyme was inhibited by glycyl-L-tyrosine and other derivatives of aromatic amino acids (21, 48). Acetyl tyrosine ethyl ester (ATEE) at 2×10^{-3} M inhibited generation of $\overline{EAC423}$ from $\overline{EAC42}$ and C3 by about 50% (134). N-benzyl-arginine ethyl ester, a substrate of $C1\bar{r}$, inhibited total complement at 2×10^{-3} M. About 50% inhibition of the generation of C4 site was observed with 1.1×10^{-3} M of carbobenzoxy-L-phenylalanine. Substitution of an hy-

droxyl group resulted in the increase of inhibitory activity, indicating the importance of a phenolic hydroxyl group.

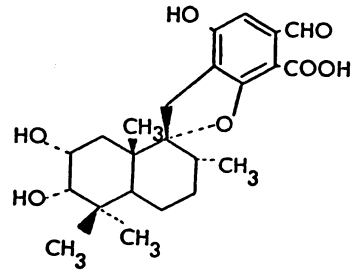
J. Diamines

Several diamines dissociate the activated C1 from the antigen-antibody complexes probably by chelating Ca ions necessary for binding of C1q with C1r and C1s (49). Cinnarizine inhibited both pathways at 7.0×10^{-4} M perhaps through the chelation of Mg^{++} (53, 54). 2,5-Diaminododecane and 2,5-diaminotoluene and several diamines inhibited C1q binding to insolubilized IgM at 10×10^{-3} M (135). Aniline inactivated complement at 2.6×10^{-3} M (10) although primary amines at a concentration of 3.0×10^{-3} M usually do not inhibit complement. Allan and coworkers (1) have shown that 1,4-diaminobutane and 1,5-diaminopentane inhibit C1q-fixation to immune complexes appreciably at a concentration of 8.5×10^{-4} M.

K. Other Organic Compounds of Diverse Structure

Hong and coworkers (79) purified a monocarboxylic acid derivative (K-76 COOH) (XIX) of K-76 from culture filtrates of *Stachybotrys complementi* nov.sp. K-76 and studied its affect on the complement system. At 5 to 20 $\times 10^{-4}$ M it inhibited the generation of EAC1–5 from EAC1–3 and C5 and accelerated the decay of EAC1–5. It also caused some inhibition of the reactions of C2, C3, C6, C7, and C9 with their respective preceding cellular intermediates. K-76 COOH had no affect on the reactions of C4 and C8 with EAC$\overline{1}$ and EAC1–7, respectively. It increased the generation of EAC$\overline{14}$ from EAC$\overline{4}$ probably by inhibiting transfer or turnover of C1. It did not affect the stability of EAC$\overline{142}$ or its generation time. K-76 COOH inhibited immune adherence only at high concentrations.

(XIX)



K-76 COOH

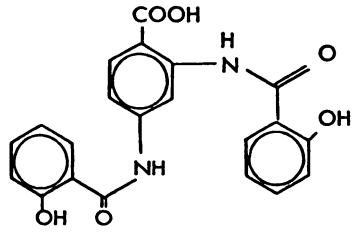
(6,7-dihydroxy-2,5,5,8a-tetramethyl-1,2,3,4a,5,6,7,8,8a-decahydronaphthalene-1-spiro-2'-(7'-carboxyl-6'-formyl-4'-hydroxy-2',3'-dihydrobenzofuran)

K-76 COOH strongly inhibited hemolysis caused by the alternative pathway of complement activation. This inhibition did not appear to be due to interference of early steps in the alternative pathway as K-76 COOH had little affect on the consumption of C3 on treatment of serum with zymosan. It is likely that K-76 COOH may complex with C5 molecules or cause structural alterations of C5.

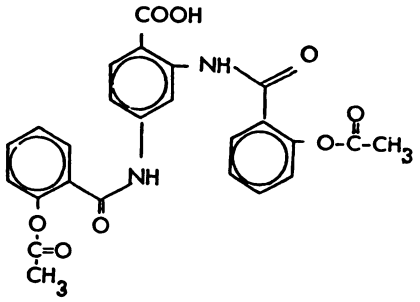
K-76 COOH has also been shown to cause dose-dependent inhibition of degradation of cell bound as well as fluid phase C3b by C3b inactivator and β_1H (78). It inhibited C3b-inactivator but not β_1H or C3b. Suppression of immune adherence, manifestation of conglutinin reactivity, and reaction between conglutinin and EAC1-3b′ were all inhibited by K-76 COOH. EAC1–3b cells did not form rosettes with Daudi or Raji cells, but after treatment with C3b-inactivator and β_1H, they became reactive with Daudi cells and this change was inhibited by the drug. EAC1–3b cells when treated with β_1H can form rosettes with Raji cells and this rosette formation is enhanced by C3b-inactivator. This enhancement was also inhibited by K-76 COOH.

Inhibition of C5 and C3b inactivator by K-76 COOH has been exploited to prepare EAC1–3b, EAC4b3b, EAC1–3bP, and EAC4b3bP with either human or guinea pig complement components for titration of various complement components such as C5 and factor B (77).

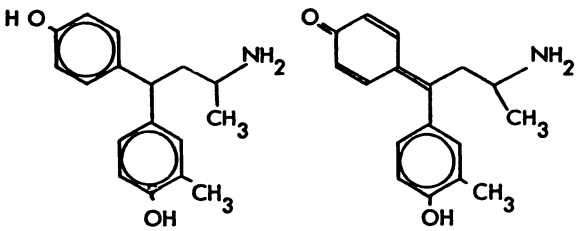
(XX)



(XXI)



(XXII)



(Compound 1935)

2,4-Bis(2-hydroxybenzamido)benzoic acid (XX) and its diacylated derivative (XXI) were found to inhibit complement (55, 114). Compound XX inhibited both pathways at 1.25 to 5.0×10^{-4} M. Compound XXI which is readily metabolized to compound XX suppressed the Arthus reaction in guinea pigs and proteinuria in experimental nephrotoxic rats (100 to 500 mg/kg).

The histamine-releasing agent 1935 (XXII) inhibited whole complement at 1.2×10^{-4} M in vitro and lowered serum complement levels in rats (67). Compound RMI 9563 (XXIII), an analogue of tilerone, suppressed total complement activity by 50% at 3×10^{-4} M, probably acting on C1s̄, and assembly of C3-convertase (103). It also inhibited the passive Arthus reaction (10 to 30 mg/kg). These results have been confirmed by Doherty (56) who also showed inhibition by tilerone (XXIV) but to a lesser extent. The inhibitory activity was much reduced in undiluted serum, presumably due to protein binding. This phenomenon has been noted with other complement inhibitors as well. No complement inhibition could be detected in vivo following a near lethal dose of RMI 9563 (25 kg/kg i.v.). These results indicated that complement inhibition may not be the mechanism by which these compounds exert their anti-inflammatory activity.

Di-isopropylfluorophosphate and phosphonate esters of general structure given below (XXV); which are specific inhibitors of serine esterases, irreversibly inhibited the activity of C1s̄ (96). They also inhibited C1 and whole complement activity (24, 72).

Copper chlorophylin was shown to inhibit the complement system at a stage of the reaction between $\overline{EAC1-3}$ and C5 (99). By virtue of its ability to inhibit complement it also inhibited anaphylaxis (42).

Gold sodium thiomalate inactivated $\overline{C1}$ and C1s̄ at 1 to 5×10^{-5} M (130). As described in the next section, it also inhibits the alternative pathway. Gold thiomalate inhibited passive cutaneous anaphylaxis reaction in guinea pigs (61). The effect appeared to be due to histamine and C1-inhibition and probably effects on other systems.

Polymyxin B, several hours after intravenous administration (1 mg/kg), suppressed rat serum complement level by 50% (67). In vitro 50% inhibition was achieved with 5×10^{-4} M polymyxin B.

Vitamin B_6-type compounds, pyridoxine, pyridoxal, pyridoxamine, and pyridoxal-5-phosphate inibited C1q-fixation at 4.5×10^{-4} M (1).

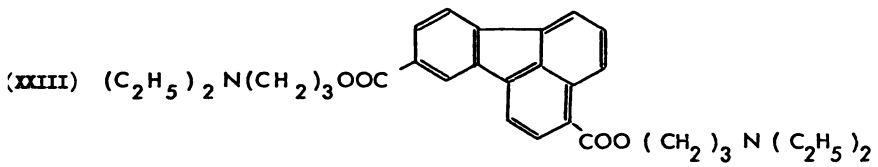
Human complement in the fluid phase has been shown to be very easily inactivated by atopic allergens. The complement-consuming power of allergens was directly related to the number of sugar-blocked lysine residues per mole, independent of the nature of the carrier molecules. These observations led Berrens and Liempt (26) to investigate the anticomplementary activity of sugar-protein compounds. These authors showed that conjugates of β-lactoglobulin and reducing sugar prepared by the Maillard reaction acquire the capacity of inactivating hemolytic complement in human serum. The complement-inactivating capacity was related to the mean number of (lysine)-ϵ-amino-1-deoxy-2-ketose residues incorporated. These authors, however, did not investigate whether the inactivation of complement system by conjugates of β-lactoglobulin and reducing sugar is due to consumption of complement or due to simple blockade of its activity.

In the absence of C9, certain sugars, e.g. glucose, sucrose, certain disaccharides, and trisaccharides, inhibited lysis of $\overline{EAC1-8}$ cells (88).

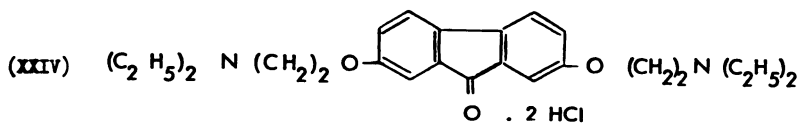
A low molecular weight (500 daltons) inhibitor of both classical and alternative pathways has been isolated recently from normal human serum and urine by Baker and Osofsky (20). Formation of $\overline{EAC142}$ from $\overline{EAC14}$ and guinea pig C2 was blocked, but lysis of $\overline{EAC142}$ was not suppressed.

Besides the above-mentioned compounds, many drugs can inhibit total complement at relatively high concentrations. Furosemide and ethacrynic acid caused total inhibition of hemolytic activity of complement at concentrations 4.2×10^{-3} M and 3.2×10^{-3} M, respectively (55). Hydrocortisone succinate and 6-methylprednisolone inhibited human and guinea pig complement in the fluid phase. The inhibition was dependent on time of preincubation and appeared to be at the C1, C4, C2, and C3 levels (66).

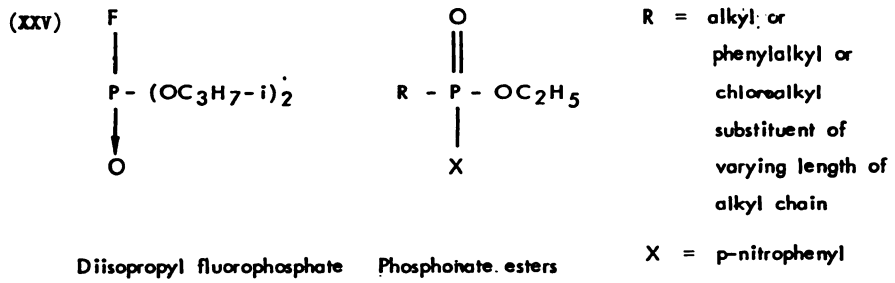
Recently, Brandslund and coworkers (37a) have shown that 90% to 95% of patients with seropositive, classical rheumatoid arthritis with active disease had raised plasma C3d levels. Six of the patients were treated with prednisolone with a starting dose of 30 mg/day decreased



(RMI 9563)



Tilorone (RMI 1008)



by 5 mg/day to a maintenance dose of 5 mg/day from days 5 to 10. The treatment resulted in immediate symptomatic relief and a prompt decrease in C3d levels. This indicates that steroids inhibit complement activation and this inhibition is linked to clinical improvement.

Jobin and Gagnon (82) showed that several inhibitors of aggregation of human platelets inhibited hemolytic activity of guinea pig complement. Hydroxyphenylbutazone and sulfinpyrazone were the most potent, inhibiting 87% of hemolytic activity at 1.8 and 3.0 $\times 10^{-3}$ M, respectively. Chloroquine and hydroxychloroquine caused about 80% inhibition at 5 $\times 10^{-3}$ M, vanillin at 8 $\times 10^{-3}$ M, colchicine at 10 $\times 10^{-3}$ M, and vanillic acid at 13 $\times 10^{-3}$ M. They inhibited the hemolysis of EAC142 cells by EDTA-complement as did salicylaldoxime. Warfarin, collacin, and plaquenil were effective in inhibiting complement at 3, 4, and 5 to 10 $\times 10^{-3}$ M, respectively (150).

Minta et al. (103a) have shown that treatment of fresh human serum with indomethacin, sulindac, phenylbutazone, and oxyphenbutazone inhibited both classical and alternative pathway activities in a dose-dependent manner with a 50% inhibition at 1.3 $\times 10^{-2}$, 2.8 $\times 10^{-3}$, 5.3 $\times 10^{-3}$, and 4.3 $\times 10^{-3}$ M, respectively. Aspirin had a very weak complement-inhibitory activity. Sulindac, phenylbutazone, and oxyphenbutazone were shown to form complexes with C5, thereby inhibiting the interaction between C3b and C5 and the cleavage of the latter into phlogistic fragments.

L. Inorganics

Treatment of human C3 with hydroxylamine (2.5 $\times 10^{-1}$ M) or hydrazine at physiological pH and ionic strength resulted in loss of its hemolytic activity which was associated with appearance of a single sulphydryl group in the C3d domain of α-chain (140).

Sodium cyanate has been shown to affect C3, C5, C6, C7, and C3b-inactivator and to inhibit serum complement irreversibly at 5 $\times 10^{-2}$ M (129). Sodium azide was shown to inhibit diluted complement at 7.5 to 60 $\times 10^{-3}$ M (131).

Zn^{+} inhibited all components of the classical pathway except C5 and C9 and thus whole complement (at 2.5 $\times 10^{-5}$ to 5 $\times 10^{-4}$ M 12% to 55% inhibition). In vivo, a concentration of 1 $\times 10^{-4}$ was achieved which caused decreased levels of complement and suppression of the reverse passive Arthus reaction (104).

M. Potentiators of Natural C1 Inhibitor

So far we were discussing the inhibitors of the complement system, but it is also worthwhile to mention here the studies aimed at increasing the activity of natural inhibitors by pharmacological means. Bauman and Brockman (23) have recently shown that heparin, compound XXVI, and compound XXVII potentiate the interaction of C1-inhibitor and C1. Noninhibitory concentrations of XXVI and XXVII in the presence of

(XXVI) 5,5′,5″-(1,3,6-naphthalenetriyl-tris-(sulphonylimino))tris-(1,2,3-benezene tricarboxylate) sodium

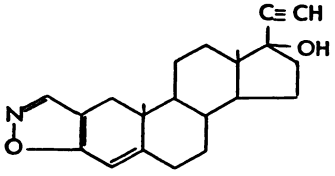
(XXVII) 5,5′,5″-(1,3,6-naphthalenetriyl-tris(sulphonylimino))tris-(1,3-benzenedisulphonate) sodium

noninhibitory concentrations of C1-inhibitor protect C4 from activated C1s (activated C1 + EDTA). In whole serum compounds XXVI and XXVII at 2.5 to 5.0 $\times 10^{-6}$ M protected C4 from destruction by $C\bar{1}$. In vivo, these compounds inhibited complement and protected guinea pigs from fatal Forssman shock. Heparin (2 to 200 μg/ml) inhibited complement by potentiating C1-inhibitor activity in serum as well as in the purified state (110, 123). Previous studies have shown that the sulphonic acid azo dye, chlorazol fast pink, inhibits serum complement through potentiation of C1-inhibitor (22, 23).

N. Drugs That Can Induce Natural C1 Inhibitor Production

An androgen derivative, danazol XXVIII, has been shown to induce increased production of C1-esterase inhibitor in patients with HANE. HANE is an autosomal dominant disorder characterized by attacks of episodic edema and is associated with decreased functional levels of C1-esterase inhibitor. Approximately 85% of the patients have lowered antigen levels of a normal inhibitor protein and 15% have normal or elevated levels of a functionally abnormal protein.

(XXVIII)



DANAZOL

Danazol treatment caused increased production of normal C1 inhibitor in both groups of patients (63, 64). Raised C1-inhibitor levels caused an increase in C4 levels. The minimum effective dose of danazol has been shown to vary from 100 to 400 mg/day (128). Among its side effects only menstrual irregularities have been described at this dose range.

Similar increases in normal functional C1-estrase inhibitor and C4 levels have been reported after treatment with other natural and synthetic androgen derivatives such as methyl testosterone (132), fluoxymesterone, and oxymetholone (127).

III. Inhibitors of the Alternative Pathway

A. *Polypeptides*

Lesavre et al. (95) studied three hexapeptides (XXIX, a, b, and c) which mimic the partial sequence of factor B surrounding the bond which is cleaved by factor D. These peptides were not substrates for factor D but they inhibited factor D enzyme activity.

(XXIXa) NH_2-Gln-Lys-Arg-Lys-Ile-Val-COOH

(XXIXb) NH_2-Glu-Lys-Arg-Lys-Ile-Val-COOH

(XXIXc) NH_2-Pyr-Lys-Arg-Lys-Ile-Val-COOH

XXIX b and XXIX c caused 50% inhibition of fluid phase C3-convertase at 3×10^{-5} M and 3×10^{-4} M, respectively. XXIX a was ineffective at 1×10^{-3} M. Factor D-dependent fluid-phase activation of factor B was inhibited 50% by about 3×10^{-4}, 8×10^{-4}, and 1×10^{-3} M concentrations of XXIX b, XXIX c, and XXIX a, respectively. XXIX b was also tested for cell-bound C3/C5 convertase inhibition and was found to cause 50% inhibition at 1×10^{-4} M. The experimental design of these authors was such, however, that it is not possible to say whether these peptides inhibit the above-mentioned convertases, their formation, or both. These polypeptides did not inhibit the classical and alternative pathways in whole serum up to a concentration of 10^{-3} M, probably due to cleavage of these polypeptides by serum proteases.

Caporale and coworkers (43, 44) have demonstrated the inhibition of C3 convertase, CVFBb, by leupeptin (Ac-Leu-Leu-Arginal) with Boc-Leu-Gly-Arg-Amino-methylcoumarin as substate. Tripeptide glutathione also inhibited alternative pathway (141).

B. *Polyions*

Burger et al. (39) have shown that several polyanions namely DS 500 (dextran sulphate mol. wt. 5×10^5 daltons) zymosan, inulin, DNP_{60}-HSA, carrageenan, levan, and lipopolysaccharide (LPS) induce activation of C3 and factor B in C4-deficient guinea pig serum and that this activation was inhibited by low molecular weight (<10,000) polyanions, namely DS 5 (mol. wt. 5×10^3) in a dose-dependent manner. CVF was also inhibited by these polyanions. Addition of Mg^{++} did not restore the C3-activating effect of CVF or other activators. Non-substituted dextran or monomeric glucose-6-sulphate did not inhibit alternative pathway activation by the above-mentioned activators. The preformed enzyme CVFB was only partially inhibited by polyethylene sulphonic acid (mol. wt. 4.8×10^3) (PES 4.8). The formation of zymosan-bound C3bB was inhibited by PES 4.8. These data indicate that low molecular weight polyanions interfere with early steps in the alternative pathway.

High molecular weight sulphated polyanions (>10^4 daltons) were previously shown to be the activators of the alternative pathway, but with sulphated sephadex (SS), Bitter-Suermann and coworkers (33) have shown that they do not behave like zymosan. They appear to absorb β_1H (750 μg SS/ml of guinea pig plasma removes 95% β_1H) and thereby cause exclusive fluid phase activation of C3 and factor B in guinea pig and human C2- and C4-deficient sera without C5 cleavage. Low molecular weight sulphated polyanions do not activate C3 but possess an inhibitory effect caused by an as yet unknown mechanism.

Pentosan polysulphoester (SP54) (10 to 100 μg/ml) has been shown to inhibit C3bB formation (28). Heparin in microgram quantities inhibited the formation of C3bB and prevented β_1H-mediated decay dissociation of this enzyme (149). Poly-L-lysine (50,000 daltons) (87) at concentrations of the order of 10^{-8} M was more active in preventing the formation of cell-bound or fluid phase C3bBb than in preventing generation of C3bB. It does not alter the stabilizing effect of properdin. Like polylysine, polyberene also interfered with the cleavage of B by D. The authors concluded from their studies that relative inhibitory action of polycations was dependent on the density of postively charged groups on the molecules.

C. *Amino Acids and Their Derivatives*

EACA at a concentration of 1M enhanced spontaneous C3 cleavage observed during incubation of serum at 37°C, believed to be due to the alternative pathway (147). At lower concentrations it inhibits rather than enhances C3 cleavage in the serum. At concentrations exceeding 0.5 M, the inhibition is overcome by enhancement.

Reactions involving zymosan and CVF were likewise depressed by EACA. The inhibitory effect was due to inhibition of assembly of C3 convertase of alternative pathway. The enhancing effect of higher concentrations of EACA on C3 cleavage in serum was explained by its observed inhibition of C3b inactivator. At lower concentrations, the effect of EACA on C3b inactivator was less significant and therefore its inhibitory effect on C3 cleavage by the alternative pathway predominates. Vallota (144) has shown inhibition of C5-convertase formation by EACA at 1.0 M.

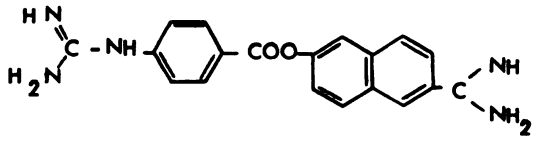
Cystein and homocysteine have been shown to be inhibitors of both pathways (141).

Conversion of factor B to factor $\bar{B}$ by factor D was inhibited by acetyl-L-lysine methyl ester and N-α-acetyl-L-lysine methyl ester (52).

D. Amidines and Guanidines

Propamidine was shown to inhibit alternative pathway by its affect on C5 turnover (145, 146) and perhaps, also, by its interaction with the B-determinant of C3 (4).

(XXX)



6-amidino-2-naphthyl-4-guanidinobenzoate

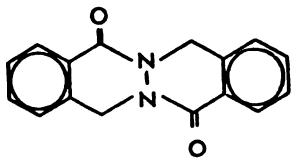
6-Amidino-2-naphthyl 4-guanidinebenzoate (XXX) has been shown to bind specifically to the Bb-fragment of factor B or CoVFBb (80a). It is a noncompetitive inhibitor of the esterolysis of L-leucyl-L-alanyl-L-arginine naphthyl ester by factor B and CoVFBb. It also inhibits the hemolytic activity of factor B, the C3-convertase activity of CoVFBb and the factor-B-cleaving activity of factor D. The concentration of XXX causing 50% inhibition of these activities was 10^{-5} to 10^{-4}. Like other guanidines described earlier, XXX was also an inhibitor of $C1\bar{r}$, $C1\bar{s}$, and total classical pathway hemolytic activity.

E. Other Diverse Structures

Gold sodium thiomalate (AuTM) was shown to inhibit the alternative pathway by interfering with the interaction of C3b, factor B, and factor D to generate $C3b_nB$ (38). The inhibition was dependent on the presence of properdin. When the complex was formed in the absence of factor D to generate $C3b_nB$, a higher concentration of the inhibitor was required for inhibition. This suggested that AuTM inhibits not only the assembly of this complex but, also, the presentation of C3bB to factor D. It had no irreversible effects on cell bound C3b, fluid phase factor B, factor D, or properdin, or on the stability of preformed $C3b_nB$, $C3b_n\bar{B}$ or $C3b_nP\bar{B}$. It was most active in inhibiting convertase formation on cellular intermediates having the lowest number of C3b and requiring development with the highest B concentration suggesting the binding site on C3b for B as the target of AuTM action. The inhibition of $C3b_n\bar{B}$ formation by AuTM is concentration-dependent and occurs at molar concentrations comparable to those observed in the plasma of patients undergoing treatment of rheumatoid arthritis. AuTM is a reversible inhibitor of $C3b_nB$ formation and can thus modulate a critical step in the entire sequence.

Glucocorticoids inhibit complement activation by lipopolysaccharide in a dose-related manner in vitro and the dose that inhibits endotoxin (1 mg)-induced complement activation by 50% in 1 ml of plasma was 13.5 to 16 moles for hydrocortisone, prednisone, and dexamethasone. In a recent study, Packard and Weiler (115a) studied the effect of two glucocorticosteroids and three steroids which lacked glucocorticoid activity on the activation of alternative amplification pathway. These steroids were 5β-cholanic acid-3α,7α-diol N-(carboxymethyl)amide sodium, prednisolone-21-phosphate, testosterone sulphate, 17-β-estradiol-3-sulphate, and hydrocortisone-21-sodium phosphate. Each of the above steroids inhibited the generation of $EAC\bar{4}3B\bar{P}$ and $EAC\bar{4}3\bar{B}$. 5 β-Cholanic acid-3-α 7-α-diol N-(carboxymethyl)amide was the strongest inhibitor which inhibited $EAC\bar{4}3B\bar{P}$ formation strongly at a concentration range of 3.0 to 6.0 $\times$ 10^{-4} M and $EAC\bar{4}3\bar{B}$ formation at 3.0 to 12 $\times$ 10^{-4} M. All these steroids also inhibited fluid phase consumption of factor B in presence of C3 and D. The strongest inhibitor of consumption of factor B was 17-β-estradiol-3-sulphate which inhibited the reaction strongly at a concentration of 1.7 $\times$ 10^{-3} M. No correlation between glucocorticoid activity and inhibition of generation of $EAC\bar{4}3\bar{B}$ or $EAC\bar{4}3B\bar{P}$ was observed.

(XXXI)



Diftalone

Diftalone (XXXI), which inhibited the classical pathway, also inhibited the alternative pathway at 5 $\times$ 10^{-4} M (114). 2,4-Bis(2-hydroxybenzamido)benzoic acid was shown to inhibit both pathways in the concentration range 1.25 to 5.0 $\times$ 10^{-4} M (55, 114). Cinnarizine inhibited both pathways perhaps through the chelation of Mg^{++} (53, 54).

Complestatin, a low molecular weight (about 800 daltons) product of *Streptomyces lavendulae* that yields D-4-hydroxyphenylglycine, D-3,5-dichloro-4-hydroxyphenylglycine, and an acid chromophore compound on acid hydrolysis, inhibited the alternate pathway in microgram quantities (86). It inhibited the interaction of cell bound C3b with factor B and factor D to form $C3b_n\bar{B}$ and this inhibition was not influenced by properdin nor was it due to metal ion chelation. The decay of preformed $C3b_nB\bar{P}$ on the erythrocyte surface was not altered by complestatin. The inhibition of the alternative pathway of complement was due to the ability of complestatin to bind to factor B reversibly. This was evident from the fact that 1) it blocked Mg^{++}-dependent uptake of factor B by cell bound C3b in a dose-dependent manner, and 2) the function of inhibited factor B could be restored by dilution.

A thermostable (56°C, 30 min) low molecular weight (<1500 daltons) inhibitor of the alternative pathway has been isolated from normal human urine (111, 112). Its chemical structure has not yet been established but amino acid analysis showed only the precent of aspartic acid and NH_3 in amounts less than 10% of the weight.

Carbohydrates could not be demonstrated. Its extinction coefficient ($E^{1\%}_{1cm}$) was 0.05 at 280 nm and 13 at 230 nm. When this material was added to the mixture of EAC3, B and D, it inhibited C3-convertase formation in fluid phase on sheep erythrocytes as well as on zymosan particles. It also inhibited $\overline{CVF \cdot B}$ formation by factor D when simultaneously present with D. In contrast to β_1H, it had no affect on the C3b binding of B. A similar substance was also found in normal human serum. A similar low molecular weight inhibitor of cleavage of factor B by D has been isolated from human serum and urine by Baker and Osofsky (20). However, these authors have reported that the molecular weight of this inhibitor is about 500 daltons and that it inhibits the classical pathway C3-convertase formation as well.

In a recent study, Baker et al. (20a) reported that the serum-derived low molecular weight inhibitor blocks alternative pathway C3/C5 convertase formation but not their activities. It inhibits the binding of factor B to C3b and activation of C3bB complex by factor D.

Iodipamide methylglucamine (20 mM) probably induces polymerization of C3 and factor B thereby causing inactivation of the complement system in vivo and in vitro (58). No evidence for the iodipamide-methylglucamine-induced proteolytic cleavage of these components could be gathered.

Polymyxin B was shown to interact with endotoxin and to prevent activation of the alternative pathway by endotoxin (106).

Diisopropylphosphofluoridate and phenyl methyl sulphonylfluoride inhibited factor D (52).

Fishelson and Müller-Eberhard (60a) studied the effect of metal ions of the lanthanide series on formation and stabilization of C3/C5 convertase of the alternative pathway of complement. Binding of factor B to EC3b in presence of 4.0×10^{-5} M gadolinium (Gd^{+++}) was two to three times greater than in presence of 1×10^{-3} M Mg^{++}. Binding of factor H and of properdin to EC3b was partially inhibited by Gd^{+++}. At a concentration of 1.0×10^{-5} M, Gd^{+++} completely inhibited Mg^{++}-dependent enzyme activation by factor D. The preformed enzyme was not inhibited; instead it was stabilized at 4.0 to 10×10^{-5} M. Similar enzyme stabilization was observed with terbium, ytterbium, dysprosium, and lanthanum. The Gd^{+++}-stabilized enzyme was less susceptible to control by factor H and properdin than the unstabilized enzyme. Gd^{+++} also protected surface-bound C3b from being cleaved by factor I.

IV. Discussion and Conclusion

It has been known for a long time that if an animal is depleted of complement by means of complement-depleting agents such as aggregated γ-globulins, CVF, or zymosan, it cannot elicit complement-mediated in vivo reactions such as the Arthus reaction (45). Animals depleted of complement fail to develop EAE (105) and experimental myasthenia gravis (94) after immunization with myelin basic protein(s) and acetyl choline receptor, respectively. These observations indicate that interception of complement in complement-mediated diseases can cause suppression of disease processes. But since the complement-depleting agents mentioned above cannot be administered in clinical situations, several workers thought that perhaps low molecular weight complement inhibitors can also intercept complement and thereby prevent complement-mediated disease processes. The growing realization that the activation of complement is associated with disease states of various organs such as kidney, skin, brain, and eye has led to increased efforts to find compounds that can inhibit complement activity in vitro and in vivo and can suppress complement-mediated diseases. Thus a large number of compounds have been shown to inhibit the classical pathway and a considerable number to inhibit the alternative pathway in vitro. Some inhibitors have been shown to inhibit complement levels in vivo and some have been shown to suppress experimental models of complement-mediated diseases such as the Frossman reaction, the Arthus reaction, and glomerulonephritis. Although these studies have not reached a stage where complement inhibitors could be frequently used in clinical situations, there are examples that show their usefulness in complement-mediated human diseases. EACA (71) and suramin (37) have been used for the treatment of HANE. Chlorpromazine has been shown in our laboratory to suppress human vasculitis. EACA has been shown to cause rapid improvement in patients with relapsing multiple sclerosis of recent onset and mild disease and in patients with encephalitis (3). Certain complement inhibitors such as sodium gold thiomalate and sulphonated polysaccharides have been used to treat rheumatoid arthritis (90).

An argument against the use of low molecular weight complement inhibitors in clinical situations may be that the inhibition of complement will deprive the patient of the complement system which is one of the important immunological defense systems and will weaken the immunological defenses of the patient. In order to answer this argument, one has to look closely at different clinical situations. In the case of HANE in which C1 is believed to be activated extravascularly due to deficiency of natural C1 inhibitor, trauma such as dental surgery can precipitate an attack that lasts about 3 days. If a patient has to undergo dental surgery he can be maintained on a low molecular weight synthetic inhibitor of C1 for few days before, during, and after surgery. Such a treatment is likely to protect the patient from the attack of edema and such short-term therapy appears to be very practicable and desirable. In case of dengue fever, the patient gets life-threatening shock due to complement consumption (35). Complement is absent anyway during the shock due to its consumption and treatment with a low molecular weight complement inhibitor is likely to keep complement in a dormant state and thus prevent vascular

permeability and loss of plasma proteins. There are instances where an autoimmune disease is initiated after trauma (101) due to exposure of a protein which was otherwise hidden within the tissue structure and was not exposed to the immune system. The exposure of such a protein to the immune system initiates antibody production, complement-mediated tissue destruction, and further exposure of otherwise hidden protein to immune system. Complement inhibitors by virtue of their ability to prevent complement-mediated tissue destruction are likely to prevent tissue destruction and further exposure of antigen to the immune system. Such interception of antigen exposure to the immune system is likely to cause eventual disappearance of antibody and suppression of autoimmune disease caused by the above-mentioned reasons. In many heart, lung, liver, brain, gastrointestinal, eye, skin, kidney, and infectious diseases with proven involvement of complement system in tissue destruction, complement inhibitors can perhaps protect the organs while the underlying cause of the disease is being treated by other means. Since many of the autoimmune diseases occur with exacerbations, complement inhibitors given at a very early stage of exacerbation can perhaps prevent organ or tissue damage by suppressing exacerbations.

It is apparent from this review that no appreciable amount of work has been done on complement inhibitors. Many of the known complement inhibitors are quite toxic and most of the inhibitors require unrealistically high concentrations to inhibit complement in vivo. Many of them with acceptable toxicity levels have not yet been tested for their effects on experimental models and on human diseases. Thus large scale screening programs are required to find relatively safe, strong, specific inhibitors of early components of the classical and alternative pathways. The most effective inhibitors so obtained must be tested for their ability to suppress complement-mediated in vivo reactions and experimental models of complement-mediated human diseases. If suitable compounds that can suppress disease in model systems are eventually discovered, they may be subjected to clinical trial after exhaustive safety testing in experimental animals. It is certain, however, that the present state of knowledge is not sufficient to proclaim that the complement system is ready for pharmacological manipulation for controlling human diseases.

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