range within a well defined chemical configuration.[1,4,5,6] Such sensitivity patterns have been perfectly identical in different persons and therefore may be regarded as essential in the mechanism of the sensitization. In the group of procaine sensitive patients, for instance, 3 cases have been reported in which the sensitivity was restricted to those p-aminobenzoic acid derivatives which contain a tertiary amino nitrogen in the side chain.[4,6] The case herein described is similar to 3 cases reported by Schwarzschild[1] in which the sensitivity pattern was restricted to the alkyl esters of p-aminobenzoic acid. Schwarzschild reported in one case that the methyl ester of p-aminobenzoic acid caused considerably less reaction than the ethyl, propyl and isobutyl esters.

A repeatedly encountered feature of these group sensitivities is that maximum sensitivity is found not with the actually sensitizing compound but with one of the related homologues.[4,6] This was the case in the present observation. The patient was sensitized to the butyl ester but was 100 times more sensitive to the propyl ester with which he apparently never had contact before. The sensitivity patterns studied so far in eczematous reaction can be well explained by Pauling's theory of antibody formation.[7]

Summary. A case is reported with eczematous hypersensitivity restricted to alkyl esters of p-aminobenzoic acid. No reaction was obtained with substances deviating from this basic structure in the ring or in the side chain.

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Mydriatic Activity of Some New Synthetic Anticholinergic Esters.*

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Numerous publications during recent years have indicated that mydriasis and cycloplegia may be brought about by drugs that are primarily anticholinergic in action. Nyman[1] determined the relative mydriatic activity in man of various alkaloidal and synthetic substances. Scopolamine, l-hyoscyamine and atropine methyl-nitrate exceeded the activity of atropine whereas synthetic agents such as trasentine, euphthalmine and di-n-butylcarbaminoylcholine sulfate (dibutoline) are distinctly less active. Fromherz[2] found that the diphenylglycolic acid ester of γ-diethylamino-β,β-dimethylpropanol would produce maximum mydriasis in rabbits when instilled into the conjunctival sac in a concentration of 0.2%. Swan and White[3] have described the mydriatic activity of dibutoline

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TABLE I.
Spasmolytic Action of Anticholinergic Drugs.

Drug	Structure	Spasmolytic action against acetylcholine induced contractures (Isolated segments of rabbit ileum)
612	β-Diethylaminoethyl di-α-thienylacetate HCl	3 M*
1565	β-Diethylaminoethyl di-α-thienylglycolate HCl	50 M
606	β-Diethylaminoethyl phenyl-α-thienylacetate HCl	4 M
600	β-Diethylaminoethyl phenyl-α-thienylglycolate HCl	60 M
1597	β-Diethylaminoethyl cyclohexyl-α-thienylacetate HCl	17 M
623	β-Diethylaminoethyl cyclohexyl-α-thienylglycolate HCl	100 M
Trasentine	β-Diethylaminoethyl diphenylacetate HCl	1 M
109	β-Diethylaminoethyl diphenylglycolate HCl	33 M
Atropine sulfate		50 M

* Dilution of the drug in parts per million.

and recommend a concentration of 7.5% for clinical use. Ing, Dawes and Wajda[4] determined the mydriatic activity in mice of a large number of new synthetic compounds and found several to be more potent than atropine. However, since these drugs were given intraperitoneally, direct comparison cannot be made with results obtained in other investigations of mydriatic substances wherein the material was applied externally to the eye.

Lands, Nash and Hooper[5] described the pharmacology of a series of anticholinergic esters containing a few drugs with marked mydriatic effects. The synthesis of additional mydriatic drugs has permitted this laboratory to carry out further experiments in an effort to develop synthetic substitutes for atropine and homatropine. A portion of the results obtained is described in this communication.

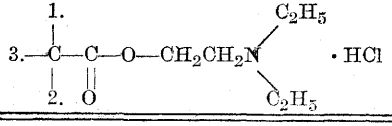
Results. All drugs were screened for anticholinergic activity by determining the dilution required to abolish acetylcholine contractures in isolated intestinal segments of the rabbit (Magnus), as previously described.[5] Results obtained are shown in Table I. Esters of acetic acid, disubstituted by phenyl, cyclohexyl, or α-thienyl groups, or combinations thereof, were tested for anticholinergic activity. Disubstitution in the acetate portion by unlike groups, such as phenyl/α-thienyl (No. 606) or cyclohexyl/α-thienyl (No. 1597), in the group of compounds tested, gave compounds which were more spasmolytic than either the corresponding diphenyl (trasentine) or di-α-thienyl analogues. The esters of disubstituted hydroxyacetic acid tested were from 6 to 33 times as spasmolytic (anticholinergic) as the nonhydroxy compounds. The most active compound, β-diethylaminoethyl cyclohexyl-α-thienylglycolate HCl (No. 623) exceeded atropine sulfate in spasmolytic potency.

Mydriatic action was determined in albino rabbits selected at random from our colony. The drugs, dissolved in distilled water, were instilled directly into the conjunctival sac; the eyes were held closed for one minute and upon release the excess drug was allowed to drain away. The pupillary response to strong direct illumination (100-watt electric light bulb with reflector held near the eye) was determined before and at intervals after drug instillation. The results obtained are shown in Table II. In general, mydriatic potency parallels spasmolytic potency. The disubstituted acetates, trasentine, No. 612, No. 606 and No. 1597 produce incomplete mydriasis when used at a concentration of 0.5 to 2.0%. The corresponding glycolates are effective mydriatics. No. 623 produces definite mydriasis in rabbits at a concentration of 0.02%. A 0.1% solution produces maximum mydriasis lasting more than an hour with some mydriasis lasting more than 7 but less than 24 hours. With a 1.0% solution there was prolonged mydriasis, corneal anesthesia and moderate irritation of the conjunctival

[4] Ing, H. R., Dawes, G. S., and Wajda, Isabelle, *J. Pharm. Exp. Therap.*, 1945, **85**, 85.

[5] Lands, A. M., Nash, V. L., and Hooper, K. Z., *J. Pharm. Exp. Therap.*, 1946, **86**, 129.

TABLE II.
Mydriatic Action of Anticholinergic Drugs.



Drug	Structure 1.	2.	3.	Conc. %	Mydriatic action	Dur. of recorded mydriasis, min.	Dur. of any mydriasis, hours	Toxicity approx. LD_{50}*
612	α-thienyl	α-thienyl	H	1.00	none			500
1565	α-thienyl	α-thienyl	OH	0.10	+, ++	8	8	
606	phenyl	α-thienyl	H	1.00	+	60	1	320
				2.00	++	60-180	>6	
600	phenyl	α-thienyl	OH	0.05	+++, ++++	30-60	>7	135
				0.10	++++	45-60	>7	
1597	cyclohexyl	α-thienyl	H	0.50	++, +++	20	1	
623	cyclohexyl	α-thienyl	OH	0.02	+++	60-120	>5	225
				0.10	++++	60-120	>7, <24	
Trasentine	phenyl	phenyl	H	1.00	+	120	2-4	194
				2.00	++	120	>3	
109	phenyl	phenyl	OH	0.02	+++	120	>5	110
				0.05	++++	45-60	>5	
Atropine sulfate				0.001	+++	100	>7	240
,, ,,				0.005	++++	120-180	>7	

* All drugs were dissolved in distilled water and administered intraperitoneally to mice weighing 14-21 g. The test animals were obtained from our own colony, and both colony and test animals were housed in an air-conditioned laboratory. Deaths occurring during the 72 hours following injection were recorded. At least 30 animals were used for the determination of the LD_{50} of each drug described.

TABLE III.
Salts of β-Diethylaminoethyl Cyclohexyl-α-thienylglycolate.

Salt	M.P., °C	Formula	Analysis: Nitrogen calcd.	Nitrogen found	Halogen calcd.	Halogen found	Solubility in water 25°C—%	Mydriatic activity 0.02% solution
Hydrobromide	184-185	$C_{18}H_{30}O_3NSBr$	3.33	3.34	19.01	19.03	< 1.0	++, +++
Bitartrate	118-123	$C_{22}H_{35}O_9NS$	2.86	2.77			7.0	++, ++++
Nitrate	79- 82	$C_{18}H_{30}O_6N_2S$	*				100.0	++, +++
Methobromide	174-176	$C_{19}H_{32}O_3NSBr$	3.22	3.08	18.39	18.25	>30.0	+++, ++++
Hydrochloride	†						1.0	++, +++

* Sulfur analysis, calcd. 7.97, found 7.97.
† Blicke and Tsao.[8]

membranes. None of the synthetic drugs described here is more mydriatic than atropine sulfate.

Because of limited solubility of the hydrochloride, other salts were prepared and tested. Results obtained are shown in Table III. The nitrate is completely soluble in water and equals the hydrochloride in its mydriatic potency. A 1.0% aqueous solution of this salt is slightly irritant to the conjunctiva but causes no demonstrable damage to either the cornea or conjunctiva. Solutions at a concentration of 2.0 and 5.0%, made isosmotic to tears by adding glycerine, were instilled into rabbit eyes daily for 5 days. At the end of the test period the eye was bathed with sodium fluorescein and the cornea examined for damage. Irritation was slight with the 2.0% solution and there were only a few fluorescent areas on the corneal surfaces. These were small, being about 1-3 mm in diameter. They disappeared rapidly after treatment was discontinued. The 5.0% solution, similarly applied, caused edema and hyperemia of the conjunctiva and nictitating membrane and by the 5th day of treatment there was a large amount of mucoid discharge and cloudiness in the corneas of the treated eyes. This cloudy area covered half of the corneal surface from the inferior corneal margin upward and was strongly fluorescent when bathed with sodium fluorescein. Recovery was rapid after medication was discontinued and by the 4th day no evidence of corneal damage or irritation could be found.

Gilman *et al.*[6] have described significant local anesthetic effects for β-diethylaminoethyl diphenylglycolate HCl and related structures. The local anesthetic effect of a few of the drugs in our series has been determined in albino rabbits. The drugs were dissolved in distilled water to make a 1.0% solution of the hydrochloride salts and instilled directly into the conjunctival sac of the rabbit eye, as described above for the determination of mydriatic action. The medial surface of the cornea was touched lightly with a blunted pencil at 2- to 5-minute intervals after drug application. The absence of the wink reflex was taken as an indication of anesthesia. The results obtained were: No. 606—20 min.; No. 600—18 min.; No. 109—5 to 20 min.; No. 623—43 min. Gilman *et al.*[6] have reported that No. 109 and trasentine, in a 2.0% solution, cause corneal anesthesia for 18 and 21 minutes. No. 606 and 600 appear to have comparable anesthetic effects. No. 623 is distinctly more anesthetic than the above drugs.

Discussion. Lands *et al.*[5] have shown that β-diethylaminoethyl acetate has a cholinergic action on the isolated intestinal segment. Anticholinergic action was observed in those compounds containing an hydroxyl and phenyl, cyclohexyl or α-thienyl groups on the acetate portion of the ester. High spasmolytic potency against acetylcholine induced contractures was associated with high mydriatic potency. Both actions appear to be the result of "blocking" at the cholinergic receptors of the effector cells. Direct comparison of relative effects on these two types of cells is difficult because of the differences in methods used for studying and evaluating the actions described here. The determination of mydriasis was by direct instillation of drugs into the conjunctival sac. The actual concentration of drug in the aqueous humor would be influenced greatly by the rate at which it passed across the corneal barrier and by the rate of absorption from the aqueous humor. On the other hand, the isolated intestinal segments were bathed directly by a known concentration of drug. However, it should be noted that drugs with low potency for the intestinal segment were also poor mydriatics (No. 612, 606 and trasentine).

Blicke and Kaplan[7] have reported results obtained by Dr. John G. Beall with a large group of mydriatic drugs synthesized by them. They report that a 2.0% solution of the γ-dimethylamino-β, β-dimethylpropanol esters of mandelic, β-phenyl-α-hydroxypropionic, β-phenyl-β-hydroxypropionic and β,β-diphenyl-β-hydroxypropionic acids are with-

[6] Gilman, A., Goodman, L., Thomas, J. M., Hahn, G. A., and Prutting, J. M., *J. Pharm. Exp. Therap.*, 1942, **74**, 290.

[7] Blicke, F. F., and Kaplan, H. M., *J. Am. Chem. Soc.*, 1943, **65**, 1967.

out mydriatic action whereas the esters of the corresponding tropic and benzilic acids are strongly mydriatic. The importance of the structure of the acid portion of the molecule is similarly emphasized in our series. The mydriatic and spasmolytic potencies of the β-diethylaminoethyl glycolates described here were much greater than those found for the corresponding acetates. Although two of the drugs described by us (Nos. 600 and 623) have spasmolytic action exceeding that of atropine, none is as strongly mydriatic as atropine. The reasons for this discrepancy are not readily apparent.

Summary. The β-diethylaminoethyl esters of di-α-thienyl-, diphenyl-, phenyl-α-thienyl- and cyclohexyl-α-thienylglycolic acids are anticholinergic drugs with significant mydriatic action. Of these, the cyclohexyl-α-thienylglycolic acid ester (No. 623) is the most mydriatic substance. The nitrate salt of this ester is very soluble in water and causes only small damage to sensitive tissues when used daily for several days at a concentration of 1.0 or 2.0%. It is suggested that this substance may have clinical applications as a mydriatic and cycloplegic agent.

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Enzyme Studies on Human Blood. I. Proteolytic Activity Associated with a Fraction of Plasma.*

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The demonstration of the proteolytic enzyme activity of the blood serum and plasma has attracted the attention of many workers since Dastre[1] originally inferred such an activity in serum. Delezenne and Pozerski[2] reported the proteolytic activity of serum which had been shaken with chloroform. Tagnon[3,4,5] studying this problem more thoroughly found that this enzyme is an euglobulin. Tillett and Garner[6] extracted from cultures of hemolytic streptococci a substance capable of dissolving fibrin clots. Milstone[7] later showed that this material did not act on highly purified fibrinogen and that a plasma euglobulin fraction was necessary for the "fibrinolysis." Christensen[8,10] and Christensen and MacLeod[9] found that the substance isolated from the streptococcic culture acted as an activator ("streptokinase") transforming the inactive plasma enzyme ("plasminogen") into "plasmin." They believe that this "plasmin" differs from trypsin but is identical with the chloroform-activated enzyme. Ferguson *et al.*[11] employing "fibrinogenolysis" as an assay technic of

* The protein fractions and the dried whole plasma were obtained through the courtesy of Dr. E. J. Cohn. They were prepared in collaboration between the Department of Physical Chemistry, Harvard Medical School, Boston, Mass., and the Division of Biologic Laboratories of the Massachusetts Department of Public Health from blood collected in collaboration with the American Red Cross.

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2 Delezenne, C., and Pozerski, E., *C. R. Soc. biol.*, 1903, **55**, 690.

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