The fifth (10S) was treated for 37 days and during this time was for the most part anestrus although there were occasional days on which cornified cells in good number were encountered. Seven rats were treated with 1 cc of the B-complex mixture daily for 20 to 46 days. While on the supplement, these rats showed a tendency to remain in anestrus but the response was for the most part incomplete (Fig. 2). Fifty mg para-aminobenzoic acid daily in addition to the other B vitamins did not improve the response of one rat (10S) showing partial anestrus.

The livers of the untreated rats on the B-deficient diet showed no histological evidence of damage except in one case where a slight fatty change was demonstrable.

Discussion. The impairment of estrone inactivation which was observed in rats receiving a diet low in protein and high in fat may be assumed to have resulted from the accompanying injury to the liver. The onset of functional disability appeared after an interval when severe histological changes are known to make their appearance. It would appear that the method should be useful in the study of experimental cirrhosis. Reversal of the estrus reaction by the administration of large doses of yeast indicates that curative measures may be very nicely assessed in the living animal.

In confirmation of the work of Biskind and Biskind, vitamin B-complex deficiency was found to interfere with hepatic inactivation of estrone. The alteration produced by the B-deficient diet, however, was neither so constant in occurrence nor so solidly sustained as that which followed the cirrhosis-producing diet and the effects of therapy were not so easily judged. There appeared to be a strain difference in response. Sherman rats were very susceptible while a local strain of hooded rats was relatively resistant. Sprague-Dawley rats were intermediate. The B vitamins known to be required by the rat were not adequate for the complete reversal of the effect when given in ordinary maintenance doses. Segaloff and Segaloff,[5] however, in somewhat similar experiments recently reported that large doses of riboflavin and of thiamine were effective.

Summary. Liver damage produced in rats by diets low in protein and high in fat was consistently reflected in the living animal by impairment in ability to inactivate estrone. The curative effect of large doses of yeast could be demonstrated.

Vitamin B-complex deficiency was usually accompanied by a similar but less constant hepatic impairment and without anatomical damage to the liver. There appeared to be a strain difference in susceptibility. A mixture of known vitamins was only partially effective in abolishing the effect of the diet, while yeast was distinctly and more consistently effective.

We are greatly indebted to Miss Ethel Buchwald for technical assistance.

[5] Segaloff, A., and Segaloff, A., *Endocrinol.*, 1944, **34**, 346.

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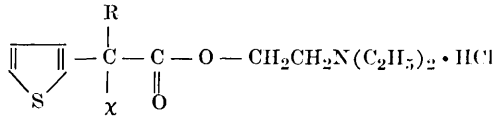
Spasmolytic Activity of Diethylaminoethyl Esters of Substituted α-Thienylacetic and α-Thienylglycolic Acids.

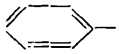
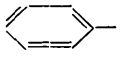
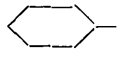
A. M. LANDS AND V. LORAINE NASH.

From the Pharmacological Research Laboratory, Frederick Stearns and Company, Detroit, Mich.

During recent years a number of reports have appeared which described the pharmacologic activity of a variety of synthetic products which were developed in an effort to obtain an effective antispasmodic substance that would be relatively free of the undesirable side actions of atropine. These substances may be conveniently divided into two groups: (1) amines, such as Perparine, Cyverine, or Sestron, and (2) basic esters of arylaliphatic

TABLE I.
Antispasmodic Activity.
Rabbit Jejunum



Compound No.	R	χ	Isolated rabbit jejunum: acetylcholine*	Isolated rabbit jejunum: $BaCl_2$†	Intact rabbit jejunum: Dose—i.v. mg/kg	Intact rabbit jejunum: Response	Intact rabbit jejunum: Duration min
612		H	1-4 M(illion)	1-200,000	0.20-2.00	+, ++	3-45
606		H	5 M	200,000-400,000	0.10-2.00	0, +++	0-90
600		OH	60 M	200,000-400,000	0.05-1.00	++, ++++	10-45
623		OH	80 M	500,000	0.01-0.10	+++, ++++	6-135
β-diethylaminoethyl diphenylacetate‖			1 M	200,000	0.20-2.00	0, +++	0-45
β-diethylaminoethyl cyclohexylphenylacetate‡			3 M	500,000	0.01-0.02	+	Transient
β-diethylaminoethyl fluorene-9-carboxylate§			2 M	200,000	0.50-1.00	++	13-16
Atropine Sulfate			50 M		0.10-0.20	++++	60
Papaverine				200,000			

* Maximum dilution that will cause complete contracture. Usually 1-1M, 2M.
† Maximum dilution that will cause complete contracture. Usually 1-10,000.
‡ Graham and Lazarus.[7]
§ Pavatrine, *cf* Lehmann and Knoefel.[8]
‖ Trasentin.

acids, such as Trasentin or Syntropan. The first group exerts its action primarily on the smooth muscle cell; the second is effective largely by virtue of the ability to diminish or abolish cholinergic activity in organs composed mostly of smooth muscle. It has been previously pointed out by Tainter,[1] Alles and Feigan,[2] Blicke and Zienty,[3] Blicke and Burkhalter,[4] and Warren *et al.*,[5] that the substitution of the thiophene for the benzene ring in various pharmacologically active molecules yielded compounds that are, in a pharmacologic sense, equivalent. Accordingly, Blicke and Tsao[6] have prepared a series of basic esters of substituted α-thienylacetic and α-thienylglycolic acids (α-thienylhydroxyacetic acid) which were tested by us for their antispasmodic activity and toxicity. Chemical formulas of these compounds are indicated in Table I.

Toxicity. Albino mice weighing between 15 and 20 g were used as test animals. They were housed at a relatively constant temperature of 75°F and a relative humidity of 35%. The animals were kept under these conditions for at least 7 days before being used for tox-

[1] Tainter, M. L., *Quart. J. Pharm. and Pharmacol.*, 1930, **3**, 584.

[2] Alles, G. A., and Feigan, G. A., *J. Pharm. and Exp. Therap.*, 1941, **72**, 267.

[3] Blicke, F. F., and Zienty, M. F., *J. Am. Chem. Soc.*, 1941, **63**, 2945.

[4] Blicke, F. F., and Burkhalter, J. H., *J. Am. Chem. Soc.*, 1942, **64**, 477.

[5] Warren, M. R., Marsh, D. G., Thompson, C. R., Shelton, R. S., and Becker, T. J., *J. Pharm. Exp. Therap.*, 1943, **79**, 187.

[6] Blicke, F. F., and Tsao, M. U., *J. Am. Chem. Soc.*, in press.

icity determinations. The drugs were dissolved in distilled water, keeping the amount of fluid to be injected at the smallest volume that could be accurately measured. Survival was recorded for 72 hours. The results obtained are shown in Table II. Compound No. 612, the di-α-thienylacetate, was found to be the least toxic (500 mg/kg approximately); No. 600 (phenyl-α-thienylglycolate) the most toxic (131 mg/kg). Saturation of the benzene ring to produce the corresponding cyclohexyl-α-thienylglycolate significantly reduced the toxicity. The corresponding acetate was not available for testing. Graham and Lazarus[7] have reported that basic esters of acetic acid with hydrogenated phenyl groups are considerably less toxic than those containing phenyl groups, but did not indicate the source of their information. However, in their own investigations, the cyclohexyl analog of Trasentin (Trasentin –6H) was found to be somewhat more toxic than Trasentin. The influence of the hydroxyl group of glycolic acid is interesting. The toxicity of compound No. 600 is approximately twice that of the corresponding reduced acid. Similarly, Lehman and Knoefel[8] have reported that diphenylglycolate of β-diethylaminoethanol to be twice as toxic as Trasentin. In view of the above, it is interesting to note that No. 623, the cyclohexyl analog of No. 600, is significantly less toxic than this latter substance.

TABLE II.
Toxicity of Antispasmodic Compounds.
(According to the method of Behrens.)

Compound No.	LD_{50}* mg/kg
612	500 approx.
606	260
600	131
623	225
β-diethylaminoethyl diphenylacetate	194
β-diethylaminoethyl cyclohexylphenylacetate	240†
β-diethylaminoethyl fluorene-9-carboxylate	320‡
Atropine Sulfate	240

* Intraperitoneal injection into albino mice.
† Graham and Lazarus.[7]
‡Lehmann and Knoefel.[8]

[7] Graham, J. D. F., and Lazarus, S., *J. Pharm. Exp. Therap.*, 1940, **69**, 331.

[8] Lehmann, G., and Knoefel, P. K., *J. Pharm. and Exp. Therap.*, 1942, **74**, 274.

Action on the Gastro-intestinal Canal. Antispasmodic action was determined on isolated segments of rabbit jejunum and colon according to the method of Magnus. Contracture was induced by acetylcholine or $BaCl_2$ and the maximum dilution of antispasmodic that would cause 75% relaxation within 2 minutes was determined. The average results obtained are shown in Table I. The glycolates were found to be much more active than the acetates against acetylcholine contractures. No. 623 was found to be the most active, being significantly greater than the corresponding acetate, No. 600, and approximately equal to atropine, when differences in molecular weight are considered (atropine sulfate, M.W. 676.80; No. 623, M.W., 375.95). The activity of No. 623 against $BaCl_2$ induced contractures of the jejunum is about 2.5 times that of papaverine. All were about equally effective against $BaCl_2$ contractures of the colon.

Antispasmodic activity of these substances on the intact intestine of anesthetized rabbits was determined by the method of Barbour. Results are shown in Table I. As above, No. 623, the cyclohexyl-α-thienylglycolate was found to be most effective in reducing tonus. Distinct responses were obtained with doses of 0.01 to 0.10 mg/kg (Fig. 1).

Action on the Uterus. Segments of the isolated rat uterus were tested according to the method of Magnus. Results obtained are shown in Table III. Compounds No. 623 and 600 were found to be more active in relaxing acetylcholine contractures than was No. 606. In a few instances, effects on the motility of the intact uterus were determined by the method of Barbour. Doses of these substances that are effective in relaxing the intact jejunum usually depress uterine motility (Fig. 2).

Other Actions. The effect of these antispasmodic drugs on the vascular response following the intravenous injection of acetylcholine was determined in dogs anesthetized with Na pentobarbital. Acetylcholine was injected in an amount that would cause a 40-60 mm Hg. fall in carotid blood pressure.

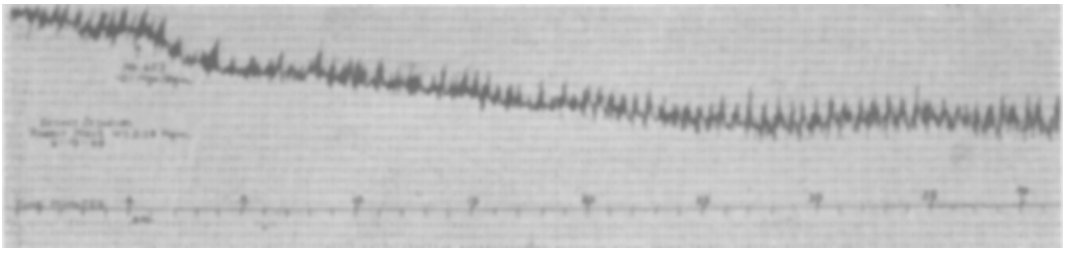


FIG. 1.
Intact rabbit jejunum. At ↑, Comp. No. 623, .01 mg/kg, was injected intravenously.

TABLE III.
Antispasmodic Action on Isolated Colon and Uterus.

	Rabbit colon		Rat uterus	
Comp. No.	Acetylcholine (1-1 M)	$BaCl_2$ (1-10,000)	Acetylcholine (1-10,000)	$BaCl_2$ (1-10,000)
606	1- 1 M (illion) 2 M	1- 200,000- 500,000	1- 200,000- 500,000	1- 100,000
600	20 M	200,000- 500,000	1 M- 4 M	100,000
623	40 M	500,000	4 M- 20 M	100,000 200,000
β-diethylaminoethyl diphenylacetate	500,000	200,000- 500,000	200,000	200,000
β-diethylaminoethyl fluorene-9-carboxylate	1 M	200,000- 500,000	500,000- 1 M	100,000
Atropine Sulfate	40 M		4 M- 20 M	
Papaverine		500,000		

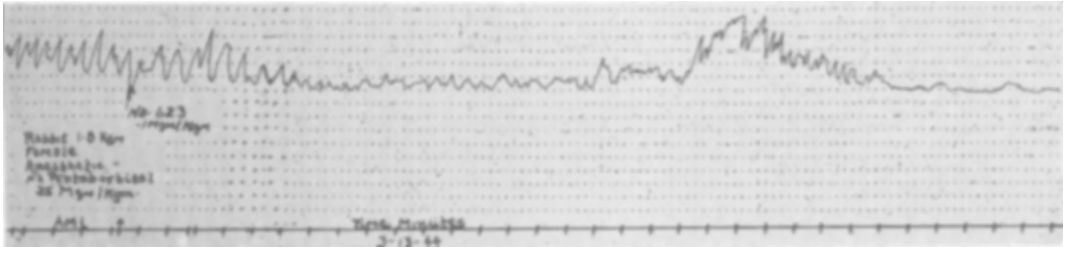


FIG. 2.
Intact rabbit uterus. At ↑, Comp. No. 623, .1 mg/kg, was injected intravenously.

No. 623, in intravenous doses of 0.025-0.10 mg/kg, reduced the acetylcholine fall by 78-100%; No. 600 in the same amounts 56-80%; Nos. 606 and 612 in amounts up to 1 mg/kg either had no effect or caused only a small and transient reduction. With compound No. 623, some effect on the vascular response to acetylcholine was noted for 53 to more than 150 minutes; with No. 600 for 17 to 146 minutes. Comparisons with the action of atropine sulfate suggest that No. 623 is probably less active, and No. 600 definitely less active, in depressing the vascular response to acetylcholine.

TABLE IV.
Vascular and Mydriatic Activity of Antispasmodics.

	Vascular action			Mydriasis		
Comp. No.	Dose, mg/kg	Max. depression,* %	Duration depression, min.	Conc. %	Mydriasis	Duration, hr
612	.5 -1.0	0- 30	6- 20	1.0		—
606	.25 -1.0	0- 28	0- 9	1.0	Maximum	2
600	.05 -2.0	56- 80	17-146	1.0	"	1¼-4½
623	.025-2.0	78-100	53-150	.1	"	8-24
β-diethylamino-ethyl diphenyl-acetate	.5 -1.0	0- 15	0- 5	1.0	Slight	1½
β-diethylamino-ethyl cyclo-hexylphenyl acetate	1.0	—	14	More active than Trasentin†		
β-diethylamino-ethyl fluorene-9-carboxylate				1.0	Maximum	8
Atropine Sulfate	.01 - .025	50-100	35-165	.1	"	8-24

* % reduction in response to acetylcholine.
†Graham and Lazarus.[7]

The effect of the antispasmodic compounds on salivary secretion was determined in rabbits by the method described by Graham and Lazarus.[7] Pilocarpine in doses of 20-50 mg was subcutaneously injected in the flank region of various rabbits and after 15 minutes, a spasmolytic amount of the antispasmodic drug was injected into the marginal ear vein. The effect on the subsequent secretion of saliva was determined and compared with that secreted in a control period without the antispasmodic. Compound 612 was not used here because of an insufficient amount of the substance. No. 606 was without any definite effect whereas Nos. 600 and 623 definitely depressed, but did not abolish, salivary secretion.

Mydriatic and local anesthetic activity were determined in albino rabbits. The drug was instilled into the palpebral sac, the eyelids held closed for 1 minute then released and the excess fluid allowed to drain away. The size of the pupil was determined under direct illumination. Mydriasis obtained is shown in Table IV. As in previous tests, No. 623 was found to be the most potent substance. Maximum mydriasis was observed when these substances were given in dilutions of 0.01%. In 1% solution, No. 612 was without effect and No. 606 induced mydriasis lasting 2 hours. Compound No. 600, as a 1% solution, caused mydriasis which lasted for 1-5 hours. Anesthesia was determined by touching the cornea with a blunted pencil. Both Nos. 606 and 600 induced moderate anesthesia of the cornea. A 1% solution produced anesthesia lasting 12 to 60 minutes.

Summary. The β-diethylaminoethyl esters of some substituted α-thienylacetic and α-thienylglycolic acids have been investigated in the experimental animal and found to possess important antispasmodic properties.

The α-thienylglycolic acid esters are significantly more active than the former but also have greater effect on other cholinergically controlled structures, thus resembling atropine more than do the acetates. The antispasmodic activity of the cyclohexyl-α-thienylglycolate (No. 623) is comparable to that of atropine; the phenyl-α-thienylacetate (No. 606) appears to be somewhat more effective than β-diethylaminoethyl diphenyl acetate and equal to diethylaminoethyl fluorene-9-carboxylate. Compound No. 606 is relatively free from undesirable actions on other autonomic organs. Except for the phenyl-α-thienylglycolate (No. 600), toxicity of these substances is not unfavorable.