TABLE I. Effect of Detergents (Duponol, Aerosol OT) on Stimulation of Endogenous O_2 Uptake of Intact Embryos Due to DNP (Diapause Embryos in Ringer).

DNP	Duponol, %	% of control O_2
5×10^{-5} M	—	196
"	.01	172
"	.05*	100
"	.10	60
—	.05	100
	Aerosol OT	
5×10^{-5} M	—	196
"	.002	196
"	.020*	100
"	.10	49
—	.020	100

* Concentration completely antagonizing stimulating effects of DNP.

(10-minute hydrolysis) of the intact diapause embryo drops to approximately 30% of the untreated control. Such a result indicates that in this reaction of DNP phosphorylation is markedly interfered with. Concentrations of 0.025% aerosol OT which completely antagonize the stimulating action of DNP cause no appreciable change in labile phosphorus of the intact embryo. It would seem, therefore, that perhaps some surface activity of the detergents seriously interferes with the stimulating action of DNP on the intact embryonic cells.

Other reagents and conditions which might possibly affect the stimulating action of DNP on the intact embryo have also been tested. Among them the following produced no effects: B.A.L., methylene blue, succinate, fructose, 1.6 diphosphate, ATP. Subjection of the intact embryo to high centrifugal forces so as to stratify without beaking the cells produced no change in the stimulating action of DNP. Intact embryos soaked in 5×10^{-5} M DNP for 1½ hours, then washed 3× in Ringer, show no effects of the DNP on their endogenous O_2 uptake. Homogenates of embryos in DNP when added to intact embryos produce stimulation of O_2 uptake similar to that by DNP alone. Apparently DNP is held loosely by the embryonic cells and can readily be washed out.

In *summary*, one might reasonably assume that in addition to the stimulating action of DNP and its effect upon the phosphorylating system of the intact living embryonic cell, surface reactions may also play an important part in its reactions.

1. Simon, E. W., *Biol. Rev.*, 1953, v28, 453. (contains rather complete bibliography)

2. Bodine, J. H., and Boell, E. J., *J. Cell. and Comp. Physiol.*, 1938, v11, 41.

3. Clowes, G. H., and Krahl, M. E., *J. Gen. Physiol.*, 1938, v20, 145.

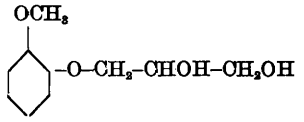
4. Sumner, J. B., *Science*, 1944, v100, 413.

Received January 28, 1954. P.S.E.B.M., 1954, v85.

Effect of O-Methoxyphenylglycerol Ether (Resyl) on Spinal Reflex Arcs. (20869)

JURG A. SCHNEIDER AND ALFRED E. EARL. (Introduced by Robert Gaunt.)
(With the technical assistance of Robert Moore.)
From the Research Department, Ciba Pharmaceutical Products, Inc., Summit, N. J.

Recent studies indicated that o-methoxyphenylglycerol ether (Resyl) is of specific therapeutic value in the treatment of tetanus and as a muscle relaxant in anesthesia(1-6). This guaiacol derivative is chemically and pharmacologically related to Myanesin(2). However, according to Ginzel, Tschabitscher, and Wayand(5) it has less hemolytic effects than Myanesin in therapeutic doses, which is considered to be an advantage in its clinical use. An electrophysiological

Resyl: 

evaluation of the action of Resyl on spinal reflexes was conducted in view of the apparent

TABLE I. Comparison of Equally Effective Doses of Resyl and Myanesin after Intravenous Administration on a Polysynaptic Reflex Arc in the Cat. The reduction in amplitude of contraction of the anterior tibialis muscle was taken as a criterion. Note that 3 times the dose of Resyl was necessary to cause approximately the same quantitative effect as Myanesin.

Drug	Cat	Date	Stimulus (6/min.) Volts	Duration, ms.	Dose, mg/kg	Dose ratio	Reduced amplitude of contraction of tibialis ant. muscle, %	Avg reduction of amplitude, %
Resyl	F	10/23	1.5	1.0	75	3	90	71.5
	M	11/5	.7	1.6	75		53	
Myanesin	F	11/4	.3	.1	25	1	93	77
	F	11/4	1.2	.1	25		79	
	M	11/5	.7	1.5	25		59	

clinical value of the drug.

Methods and materials. Ten cats weighing 2-3.5 kg were employed in this study. Dial in a dose of 70 mg/kg i.p. was used as an anesthetic in 3 of the animals. Five of the cats were decerebrated and 2 were pithed under ether anesthesia, with the ether being discontinued after destruction of the brain. The pithed animals were maintained under artificial respiration. The *effect of Resyl and Myanesin* on a polysynaptic reflex arc was tested by studying the action of these preparations upon the contractions of the anterior tibialis muscle after stimulation of homolateral afferent fibers in the cut tibial portion of the sciatic nerve. Single square wave impulses (0.6-1.2 vs; 0.11-1.0 ms; 6/min.) were applied. In two of these experiments the contralateral peroneal nerve was prepared, cut centrally and stimulated, thus eliminating the reflex arc. The blood pressure was recorded with a mercury manometer. In a second group of experiments the spinal cord of cats was prepared and both dorsal and ventral roots, usually of the S_1 segment, were cut on one side. The whole area was then submerged in mineral oil. Two platinum electrodes were applied to the dorsal root which was stimulated by single square wave impulses (0.4-1.2 vs; 0.03 ms; 4/min.). Action potentials were recorded from the corresponding ventral root with platinum electrodes. The action po-

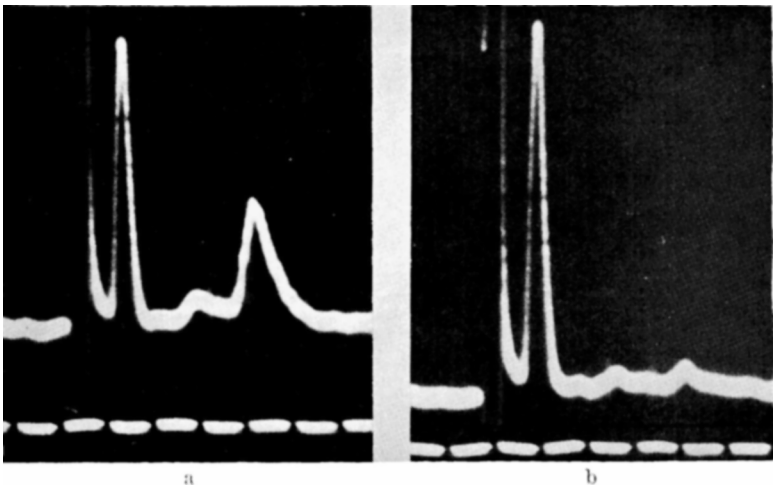


FIG. 1. Cat, decerebrated, 2.2 kg, F. Oscillographic record of action potentials taken from a ventral root of a cat spinal cord after dorsal root stimulation with single square wave impulses (0.8 v., 0.01 ms., 4/sec.). Time interval 2 ms. One and eight-tenths millisecond following the onset of the stimulus a monosynaptic spike occurred. Following the monosynaptic spike (latency 4.5-12 ms.) the polysynaptic potential presented itself. a. Control. b. 4 min. after intravenous injections of 75 mg/kg of Resyl. Note the disappearance of the polysynaptic spikes in b.

TABLE II. Effect of Resyl on Height of Monosynaptic and Polysynaptic Responses to Dorsal Root Stimulation in the Cat. Frequency/min., 4. The reduction of the height of the polysynaptic spikes after 75 mg/kg i.v. was statistically significant (P>0.01). For comparison 2 experiments with Myanesin were tabulated.

				Stimulus (4/min.)		Ht of potentials before drug		Ht of potentials after drug			Effect			Recovery of polysynaptic response		
Date	Anesthesia	Drug	i.v. dose, mg/kg	Volts	Duration, ms.	Monosynaptic, mm	Polysynaptic, mm	Min. after drug	Monosynaptic, mm	Polysynaptic, mm	Monosynaptic, mm	Polysynaptic, mm	Polysynaptic, %	mm	%	In min.
7/21/53	Dial, 0.07 g/kg	R	50	.5	.03	16	4	4	16	.5	0	—3.5	— 87	2	50	85
9/ 2	D*	R	50	.5	"	18	7	3	18	5	0	—2	— 28	7	100	25
9/ 1	D	R	50	.8	"	28	7	4	27	5	—1	—2	— 28	6	86	25
7/15	Dial, 0.07 g/kg	R	75	.8	"	10	12	7	6	2	—4	—10	— 83	3	35	100
9/ 2	D	R	75	.5	"	18	6	2	14	0	—4	—6	—100	5	83	55
9/28	D	R	75	.8	.01	13	6	4	18	4	+5	—2	— 33	6	100	55
10/ 2	D	R	75	1.1	"	14	5	6	22	1	+8	—4	— 80	4	75	45
10/16	D	R	75	.9	"	11	4	4	15	1	+4	—3	— 75	3	75	55
9/ 1	D	M	50	.8	.03	20	9	11	7	0	—9	—2	—100	8	72	55
9/ 2	D	M	50	.5	"	16	5.5	2	10	0	—6	—5.5	—100	4	73	50

* D = Decerebration; R = Resyl; M = Myanesin.

tentials were amplified by a DC preamplifier and fed into the amplifier of a Dumont double beam oscilloscope and photographed with a Grass recording camera. *Toxicity* studies were done in rats and the LD_{50} was calculated according to the method of Behrens(7). All injections were *administered intravenously* and doses of Resyl mentioned refer to milligrams of o-methoxyphenylglycerol ether.

Results. The contractions of the tibialis muscle after stimulation of the central end of the cut tibial portion of the sciatic nerve was markedly reduced after doses of 50-75 mg/kg of Resyl. The contractions following stimulation of the peripheral part of the cut peroneal nerve of the contralateral side was not affected by the same doses of Resyl. The injection of Resyl in the doses mentioned above regularly produced a transient fall in blood pressure. A comparison of the effect of Resyl and Myanesin indicated that the latter is about 3 times more active on a milligram per milligram basis (Table I).

The action potentials recorded from the ventral roots after stimulation of the corresponding dorsal root showed a typical monosynaptic spike (latency approximately 1.5 ms) which was followed by polysynaptic potentials (latency 2.5-15 ms) (Fig. 1a). The injection of 75 mg/kg of Resyl markedly reduced or completely abolished the polysynaptic responses (Fig. 1b), whereas, the monosynaptic spike was unchanged, slightly decreased or augmented. The height of the polysynaptic potentials was significantly reduced ($P < 0.01$) following 75 mg/kg of Resyl.

Fifty mg/kg of Myanesin uniformly abolished the polysynaptic response in contrast to the same dose of Resyl which failed to produce a significant reduction ($P > 0.1$).

The results of these studies are summarized in Table II.

In some of the experiments the height of the monosynaptic spike was increased after Resyl while in others a definite decrease was observed. However, these changes were not statistically significant.

The toxic manifestations of both Resyl and Myanesin in rats after intravenous injections consisted of dyspnea, ataxia, paralysis and hematuria. The incidence of hematuria was greater after toxic doses of Myanesin than after Resyl. The calculated LD_{50} for Resyl was 360 mg/kg as compared to 133 mg/kg for Myanesin.

Discussion. These results show that the clinical findings of Ginzel and Wayand(3,4),

Ginzel, Tschabitscher, and Wayand(5) and of Bowers(6) with o-methoxyphenylglycerol ether in the treatment of tetanus could be explained on the basis of inhibition of polysynaptic reflexes. These authors found that side reactions such as hemolysis, anemia and hemoglobinuria which are frequently produced by the i.v. injection of Myanesin were not produced by the i.v. administration of o-methoxyphenylglycerol ether, although the same well-known pharmacological effects on spinal reflexes described for Myanesin by Berger and Bradley(8) and by Henneman, Kaplan and Unna(9) could be obtained.

It can be stated with certainty that Myanesin is the more potent drug on a milligram per milligram basis, its potency being probably about 3 times that of Resyl. On the other hand the LD_{50} of Myanesin in rats is about 3 times lower than the LD_{50} of Resyl. Although higher doses of Resyl are required to obtain quantitatively equal effects as with Myanesin the therapeutic ratio of both drugs is about the same.

Summary. 1. Resyl (o-methoxyphenylglycerol ether) has been found to block effectively the transmission of impulses through the internuncial units of spinal reflex arcs in cats in doses of 75 mg/kg. 2. On a mg/mg basis Myanesin is about 3 times more active than Resyl, however, on the basis of the ratio of activity to toxicity Resyl and Myanesin appeared to be equally effective.

1. Ginzel, K. H., Mayrhofer, O., and Chott, F., *Wien. Klin. Wschr.*, 1949, v61, Nr 44.
2. Ginzel, K. H., *Subsidia Medica*, 2, Dec. 1949.
3. Ginzel, K. H., and Wayand, E., *Oesterreich. Aerzteztg*, 1951, v4.
4. ———, *Wien. Klin. Wschr.*, 1951, v101, 435.
5. Ginzel, K. H., Tschabitscher, H., and Wayand, E., *Klin. Med.*, 1951, v6, 161.
6. Bowers, A. G., *Calif. Med.*, 1953, v78, 468.
7. Behrens, *Arch. Exp. Path. und Pharmak.*, 1929, v140, 237.
8. Berger, F. M., and Bradley, W., *Brit. J. Pharmacol.*, 1946, v1, 265.
9. Henneman, E., Kaplan, A., and Unna, K., *J. Pharmacol. Exp. Ther.*, 1949, v97, 331.

Received January 29, 1954. P.S.E.B.M., 1954, v85.

Effect of Carboxylic and Amino Acids on Fibrinolysis Produced by Plasmin, Plasminogen Activator and Proteases.* (20870)

STEN MÜLLERTZ. (Introduced by T. Astrup.)
From the Biological Institute of the Carlsberg Foundation, Denmark.

It was reported recently that citrate increases the fibrinolysis induced by streptokinase-activated human globulin(1). This agent(2) and the globulin fraction isolated from spontaneously active human blood(3) were later found to be potent activators of plasminogen. Both preparations produce fibrinolysis chiefly by activation of the plasminogen present in the fibrin substrate. In the present paper bovine plasmin and a number of other proteases were compared with the plasminogen activators to determine whether the increased fibrinolysis depends on a modified enzymatic effect on fibrin or on activation of plasminogen in the substrate. A number of chemical compounds were studied in an effort to find a possible relationship between increased fibrinolysis and chemical structure.

Methods and materials. The following plasminogen activator preparations were used: *Streptokinase activated globulin ("S-activator")*: Normal human globulin was activated by streptokinase and subsequently lyophilized(4). Buffer solutions were prepared, in which the globulin concentration was 1/20 of the original concentration in serum. *Spontaneously active globulin ("Spontaneous activator")*: A lyophilized globulin fraction was prepared from spontaneously active blood obtained from human corpses after sudden anoxaemic death(5). Buffer solutions were

* Supported by a grant from the Josiah Macy, Jr. Foundation, New York, to Dr. Tage Astrup.