effective than sulfapyridine and distinctly superior to sulfanilamide and to sulfanilylguanidine against the colon-typhoid-dysentery group.

Feeding the several compounds in the diet to mice, caused, with a single exception, a significant reduction of the number of coliform organisms normally present in the intestinal excreta. Sulfanilamide, under the conditions of this test, proved to be ineffective in lowering the fecal *B. coli* count of the animals.

13432

Mechanism of the Effect of Epinephrine on Bioluminescence of the Firefly.

G. A. EMERSON AND MARJORIE JEAN EMERSON.

From the Department of Pharmacology, West Virginia University School of Medicine, Morgantown.

There is general agreement[1-4] that flashing of fireflies is regulated by innervated tracheal end-cells which normally occlude tracheoles leading to the light organ. The effect of epinephrine may be interpreted[1] as dilatation of the occluding musculature to permit constant access of O_2 to photogenic areas with consequent prolonged luminescence. Use was made of this action to demonstrate[5] that tensions of ether vapor above the minimal lethal tension will extinguish the constant bright glow of fireflies treated with epinephrine although ether vapor itself causes a constant glow in untreated fireflies exposed to sublethal tensions. Enough specimens were captured from a swarm of the lampyrids, *Photuris pennsylvanica*, during the summer of 1941, at Suncrest, W. Va., to permit the present limited further study.

A recent note by Chakravorty and Ballentine[6] mentions the effect of hydrosulfide on luciferase-oxidized luciferin. Accordingly, 0.01-0.02 cc of 1% freshly prepared NaHS was injected intraäbdominally in 10 fireflies. A moderately bright glow developed immediately and persisted for several minutes, gradually diminishing. Since most available solutions of epinephrine contain 0.1-0.2% $NaHSO_3$, a

[1] Creighton, W. S., *Science*, 1926, **63**, 600.

[2] Gerretsen, F. C., *Biol. Zentralbl.*, 1922, **42**, 1.

[3] Harvey, E. N., *Physiol. Rev.*, 1924, **4**, 639.

[4] Snell, P. A., *Science*, 1931, **78**, 372; *J. Cell. Comp. Physiol.*, 1932, **1**, 37.

[5] Emerson, G. A., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 36.

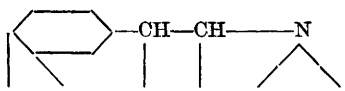
[6] Chakravorty, P. N., and Ballentine, R., *J. Am. Chem. Soc.*, 1941, **63**, 2030.

commercial grade of bisulfite was injected in concentrations of 0.1-1%. With the stronger concentration, a moderately bright luminescence appeared for a short time in 8/10 fireflies and a bright glow in 2. With the weaker solution, a very weak glow developed in 10/10, lasting about 5 minutes. Pure 0.1% epinephrine HCl, prepared from Eastman epinephrine, caused the same constant bright glow lasting several hours in 30/30 fireflies as was noted with commercial 10^{-8} epinephrine. Contaminants in commercial epinephrine solutions are not responsible for the prolonged bright luminescence of fireflies treated with these solutions.

Further corroborative evidence is desirable in support of Creighton's thesis[1] that epinephrine acts through physical improvement of the O_2 supply, before the alternate mechanism of a more intimate interference as a reducing agent may be dismissed. The effect of epinephrine on photogenic glandular structures of the singing-fish, *Porichthys notatus,* is believed[7] to be directly on these light organs rather than through a nervous mechanism. Epinephrine decreases bacterial luminescence,[8] however, and Shoup and Kimler[9] have shown that 2,4-dinitrophenol also does not stimulate luminescence.

The following observations were made using an average of 10 ♂ fireflies for each agent. Conclusions are drawn from mean results of intraäbdominal injection of 0.01-0.02 cc of 1% solutions of the pure agents in 0.5% NaCl, except as noted. Hydrochlorides or sul-

TABLE I.
Effects of Certain Sympathicomimetic Amines on Bioluminescence in Fireflies.

Agent	Structure						Duration and intensity of induced glow	
							Duration, min.	Intensity
Phenethylamine	H	H	H	H	H	H	5	bright
Phenethanolamine	H	H	OH	H	H	H	15	"
							>120	moderate
Benzedrine	H	H	H	CH_3	H	H	5	"
Propadrine	H	H	OH	CH_3	H	H	10	"
l-Ephedrine	H	H	OH	CH_3	H	CH_3	15	"
							> 60	weak
d-Ψ-Ephedrine	H	H	OH	CH_3	H	CH_3	> 15	very weak
Neosynephrine, 0.25%	H	OH	OH	H	H	CH_3	15	bright
Tyramine	OH	H	H	H	H	H	15	"
							>360	weak
Hordenine	OH	H	H	H	CH_3	CH_3	120	bright
							>600	weak to moderate
Epinephrine, 0.1%	OH	OH	OH	H	H	CH_3	>360	bright

[7] Greene, C. W., and Greene, H. H., *Am. J. Physiol.*, 1924, **70**, 500.

[8] Taylor, G. W., *J. Cell. Comp. Physiol.*, 1932, **1**, 297.

[9] Shoup, C. S., and Kimler, A., *J. Cell. Comp. Physiol.*, 1934, **5**, 269.

fates of the bases were used and other agents were employed as noted, either as free acid or Na salt. Table I presents data for the sympathicomimetic amines studied.

Neither freshly prepared solutions of l-dihydroxyphenylalanine nor the same after 24 hours of eremacausis have any effect. Homogentisic and mandelic acids, and mescaline are also without effect. Resorcinol, pyrocatechol and pyrogallol produce a slow, spotty, very weak glow. A 1% suspension of tyrosine induces a weak glow in 3/10 fireflies and greatly stimulates flashing. Tyrosine partly dissolved in a large excess of $NaHCO_3$ causes a bright spotty glow for about an hour but a similar effect is produced by strong solutions of $NaHCO_3$ alone, presumably due to osmotic phenomena.

Yohimbine, ergotamine tartrate, piperidino-methylbenzodioxane and diethylamino-methylbenzodioxane have no effect alone but reduce the persistence of the bright glow after treatment with epinephrine to about 1 hour. Part of this action may be through adrenolytic effect but control injections of 0.5% NaCl also have an appreciable antagonistic effect, through dilution of the epinephrine. Cocaine has no effect alone and does not appear to potentiate the effect of 10^{-7} epinephrine, which produces a faint, spotty glow alone.

Atropine, hyoscyamine, homatropine, hyoscine, and trasentin either have no effect or produce a very weak glow. While none has any appreciable immediate effect, a persistent weak glow develops 30 minutes after injection of trasentin and less uniformly after atropine or hyoscyamine. The same latent effect is apparent several hours after the immediate effect of some of the weaker sympathicomimetic amines and may be related simply to general toxic action. A 1% emulsion of benzylbenzoate in 3% ethanol causes a very weak glow in about half the treated fireflies. Octin also causes a very weak glow. Papaverine produces a bright glow for 15 minutes followed by a persistent weak glow lasting more than 15 minutes.

Nicotine causes a weak to moderate glow in half the treated fireflies but is almost immediately lethal. Similar irregular effects on luminescence are produced with toxic concentrations of other agents which produce no effect in sublethal amounts, such as N_2O and ethylene,[10] and these can be considered as a common phenomenon postmortem. Fireflies exhibit an amazing tolerance towards lethal effects of other agents studied.

$NaNO_2$ causes a bright glow for 10 minutes. $BaCl_2$ produces a weak, spotty glow with much stimulation of flashing for 10 minutes. A commercial preparation of kallikrein, 7U/cc in 60% glycerol, pro-

[10] Emerson, G. A., *Anesthesia and Analgesia*, 1936, **15**, 134.

duces a bright glow becoming spotty in 1 hour but glycerol alone also produces this effect. 60% glucose causes a moderately bright glow for 30 minutes. Since 40% NaCl will produce the same intensity and duration of glow, it is more probable that glucose and glycerol act through osmotic effects than as reducing substances. The action of $NaHCO_3$ referred to above is also probably osmotic since weak buffer solutions of pH 2-13 have no effect. A weak, spotty glow follows injection of solutions of pH 1.

Injection of active amounts of O_2 dissolved in any of the solutions is improbable since 0.5% NaCl alone has no effect. Injection of air sometimes causes a weak spotty glow lasting less than 1 minute. Injection of O_2 causes a bright glow for 30 seconds followed by a very weak glow for 5 minutes. H_2O_2 produces a bright glow lasting less than 5 minutes and ammonium o-iodoxybenzoate has no effect but a momentary weak glow. These solutions contain more rapidly available O_2 than any of the other test solutions, and peroxide has a stimulant effect[11] on bacterial luminescence.

Effects of sympathicomimetic amines on luminescence of fireflies are analogous to pressor effects of these agents in higher forms of life, and the influence of phenolic and alcoholic hydroxyl groups and of substitution of the amino group is much the same in both instances. The extraordinary activity of epinephrine is related to the molecule as a whole and not to the influence of any single chemical group. Lack of significant effect of phenolic compounds not active as sympathicomimetics emphasizes the unimportance of catechol or similar groups alone. It is unlikely that the effect of epinephrine on fireflies is dependent on participation of the catechol group in the luminescence reaction. Whether the effect is directly on muscular tissue, on a receptive mechanism, or directly or remotely on nerves innervating the end-cells is not definable without a quantitative study of pharmacologic effects of antagonists and potentiating agents beyond the scope of the present study.

Summary. The action of epinephrine is not mediated through direct chemical stimulation of the luminescence reaction. Consideration of relative potencies of its congeners in evoking constant luminescence in fireflies suggests that the site of action is at the tracheal end-cells and that no nicotine-like effect is involved.

[11] Nakamura, H., *Acta Phytochim.* (Japan), 1939, **11**, 159.