reagents which combine with the sulfhydryl groups of the sulfhydryl-containing enzymes would also bring about systolic standstill of the isolated frog heart. This assumption has been verified by the observations outlined below.

Use has been made in this investigation of the three types of sulfhydryl reagents used by Barron and Singer (*1, 4*) in the study of the role of sulfhydryl-containing enzymes in carbohydrate, fat, and protein metabolism and by Hellerman, Chinard, and Deitz (*2*) in their study of the reversible inactivation of urease. The isolated frog heart, mounted on a Straub-Fuhner cannula, has been subjected to the action of the following sulfhydryl reagents: (1) the oxidizing agents, porphyrindin and the sodium salt of *o*-iodosobenzoic acid; (2) an alkylating reagent, iodoacetamide; (3) mercaptide-forming compounds, the sodium salt of *p*-chloromercuric benzoic acid, and phenarsine oxide hydrochloride.

Porphyrindin in a concentration of 2×10^{-3} (7.5×10^{-3}M) causes an immediate systolic effect accompanied by a decrease in heart rate. Systolic standstill is brought about in a period of approximately 30 minutes. This effect is prevented when porphyrindin is dissolved in a solution of glutathione 6.5×10^{-3}M. Glutathione does not reverse the effect of porphyrindin once the systolic standstill has been achieved. Small concentrations of porphyrindin (up to 1×10^{-4}) cause temporary increase in the amplitude of contraction.

Ortho-iodosobenzoate in a concentration of 2×10^{-4} (8.3×10^{-4}M) causes systolic standstill in approximately 20 minutes, this effect usually being preceded by an increase in heart rate. A concentration of 1×10^{-4} to 0.5×10^{-4} causes a temporary increase in the amplitude of contraction.

Iodoacetamide in a concentration of 1×10^{-3} (5.3×10^{-3}M) brings about systolic standstill in a period of approximately 10 minutes. In one experiment a concentration of 1×10^{-4} caused no appreciable effect.

The study of the mercaptide-forming compounds presented more difficulties than that of the other two groups of reagents. Preliminary experiments show that systolic standstill is obtained with *p*-chloromercuric benzoate and with phenarsine oxide hydrochloride only under certain conditions.

Para-chloromercuric benzoate in concentrations of 1×10^{-5} to 1×10^{-4} causes a depressant effect which ends in diastolic arrest of the heart. Concentrations of 2×10^{-4} (5.6×10^{-4}M) produce a depressant effect of approximately one-minute duration followed by a gradually developing systolic effect, but before complete systolic effect is obtained, the heart stops beating. Complete systolic standstill can, however, be obtained when the heart is connected with an electrical circuit consisting of a platinum wire placed in the fluid of the Straub cannula and a cotton-wrapped copper wire, the cotton wick being placed in contact with the surface of the heart. The stimulating electrodes are connected through the output potentiometer of a condenser-discharge stimulator, thus closing the circuit of a voltaic cell. The reaction is obtained even when the stimulator is not operating.

Stoppage of the heart before achieving systolic standstill is also a troublesome matter when phenarsine oxide hydrochloride 1×10^{-3} (4.2×10^{-3}M) is applied. Complete systolic effect is obtained, however, when a clean, soft, copper wire, 0.025 inch thick, is placed in the fluid of the Straub cannula for a period of 40 to 60 minutes before applying the solution of the arsenical.

The interpretation of the results obtained with the mercaptide-forming compounds requires further investigation.

The preceding results show that certain sulfhydryl reagents cause systolic standstill of the frog heart. This investigation suggests the possibility of studying certain enzymatic reactions in the isolated frog heart, a living tissue.

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Reduction of Sympathetic Synaptic Transmission as an Index of Inhibition at Adrenergic Junctions in General[1]

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Physiological quantities of epinephrine have been shown to inhibit synaptic transmission in sympathetic ganglia (*7, 9*). Furthermore, drugs like ephedrine (*2, 3, 4*), which are dependent upon the presence of adrenergic fibers for the exercise of their major and typical actions, likewise produce inhibition at the synapses of sympathetic ganglia (*7, 10*). These findings agree in indicating that the synapses at which the inhibition in question takes place must be adrenergic and opposed to the cholinergic excitatory ones already known to function in sympathetic ganglia.

The action described therefore constitutes a means of studying sympathomimetic inhibition, e.g. by the amines, which has distinct advantages and avoids some of the drawbacks of methods hitherto available.

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The difficulties have been inherent in the use of smooth muscle as an indicator of drug action. Inhibition in such experiments has been followed by measuring the depression of pre-existing spontaneous activity. In the isolated preparation, the respective importance of myogenic and neurogenic factors in the mechanism of the spontaneous rhythmic and tonic activity of the muscle is uncertain and variable throughout each experiment as well as from one experiment to another. Consequently all the factors capable of modifying the pre-existing activity cannot be satisfactorily controlled. The multiplicity of factors responsible for motility in the isolated smooth muscle, and also the differing excitabilities of longitudinal and circular coats, is well illustrated in a recent analysis by Feldberg and Solandt (*5*). In experiments on the intact animal, on the other hand, the muscle is also subject to fluctuations in the number of sympathetic nerve impulses reaching it, as well as to regulation by the antagonistic parasympathetic innervation which may exercise at times an opposed, and at times a reciprocal, influence. The activity of neither nerve system is under the investigator's control and furthermore may even be an expression of drug action remote from the site under consideration or of compensatory reflexes initiated by the action of the amines anywhere in the body.

A further important factor is that some amines, e.g. ephedrine, tyramine, etc., may possess to a small but definitely complicating degree a direct (musculotropic (Tainter, *13*) exciting action on smooth muscle. This is most readily seen in the denervated preparation, but undoubtedly is present in the innervated tissue, where it obscures and reduces the apparent inhibition due to a true sympathotropic action. The interpretation of the smooth muscle record is thus complex and uncertain.

Barger and Dale (*1*) stated that "the accuracy with which inhibitor effects can be compared is, indeed, not sufficient to permit the assignment of exact ratios." Subsequent investigators have likewise suffered from the same handicap so that quantitative studies of sympathomimetic amines have for the most part been confined to pressor or excitatory actions while their inhibitory action, essential to the picture of sympathomimicity and therapeutically as important as the excitatory, has been comparatively neglected.

TABLE 1

ORDER OF INHIBITORY ACTIVITY IN DIFFERENT TISSUES*

Ganglion	Intestine		Bronchi
(Cat)	Isolated (Rabbit) (*6*)†	Intact (Cat) (*15*)	(Guinea pig (*14*) and dog (*12*)
1. Epinephrine	Epinephrine	Epinephrine	Epinephrine
2. Nor-epinephrine	.....	Nor-epinephrine	Nor-epinephrine
3. Epinine			
4. Di-OH-ephedrine	.....	.....	Di-OH-ephedrine
5. Di-OH-nor-ephedrine	Di-OH-nor-ephedrine Epinine and tyramine	Di-OH-nor-ephedrine Epinine	Di-OH-nor-ephedrine
6. Meta-OH-nor-ephed.	Meta-OH-nor-ephed. Synephrine	.....	
7. Para-OH-nor-ephed.	Para-OH-nor-ephed.	.....	.....
8. Neosynephrine	.....	Neosynephrine	Neosynephrine Epinine
9. Tyramine		.....	.....
10. Phenylethanolamine	Phenylethanolamine and nor-ephedrine	.....	.....
11. Synephrine		Synephrine	.....
12. Amphetamine	.....	.....	
13. Ephedrine	Ephedrine	.....	Ephedrine
14. Nor-ephedrine		.....	Meta-OH-nor-ephed. Amphetamine Nor-ephedrine

* With respect to the same isomer in cases of stereoisomerism.
† Data on large intestine are presented since it gave "more uniform and sensitive responses." The results on small intestine were similar. ..... indicates absence from the series.

The present method of measuring inhibition is extremely sensitive[2] and is free from the above disadvantages. It consists in maintaining synaptic transmission through a sympathetic ganglion, in this instance the superior cervical, at a uniform testing level by preganglionic shocks. Thus, constancy of the activity to be inhibited is insured, since it is initiated by stimulating with fixed shocks the preganglionic nerve which has been isolated from the cord by section. The height of the potential recorded from the postganglionic nerve by means of suitable amplifiers (*8*) serves as a direct index of synaptic activity, and thus its reduction, when drugs are introduced intravenously, is a measure of inhibition. The action

[2] The effect of 5 gamma of epinephrine injected intravenously in a 4-kg. cat can be easily detected.

so measured is strictly localized to the neuro-neuronal junction or ganglionic synapses and therefore cannot be obscured by musculotropic or other distant actions.

Although the various tissues innervated by sympathetic (adrenergic) inhibitory nerves have individual thresholds, together they constitute a group that typically is inhibited by average doses of epinephrine. Light would be shed both on the nature of the synaptic processes involved and on the applicability of the values obtained at the ganglionic synapses to other adrenergic neuro-effector junctions by a determination of the correspondence between values obtained for the same drugs at neuro-neuronal and at neuro-effector junctions. A final answer would depend upon the accumulation of considerably more extensive and diversified results, but a working answer suggests itself on comparing such data as are available. Table 1 presents, alongside the corresponding ganglionic data, results taken from the literature where experiments included a sufficient number of the amines in question to allow comparisons that begin to be significant. The four columns list in descending order, under the technique employed, the inhibitory potency of the amines tested. Taking into account the complicating factors already outlined, as well as species differences, a fair amount of agreement is apparent, particularly in that the most active and the least active compounds are in their approximately proper places. Four of the 14 compounds show frank discrepancies. These are epinine, tyramine, meta-OH-nor-ephedrine, and synephrine. In the case of epinine the isolated and intact intestine agree in placing it after cobefrine (di-OH-nor-ephedrine), whereas it precedes cobefrine in the ganglionic series. This difference, however, is no more marked, in fact less so, than that for the order of epinine in the two intestinal compared to the bronchial series. Tyramine shows a more serious difference but unfortunately is only represented in two series and so cannot be analyzed further. With meta-OH-nor-ephedrine, results in the ganglion and the isolated intestine agree well with each other but not with those in the bronchi. Finally, while the ganglionic synephrine value differs from that of the isolated intestine, it agrees well with that of the intact intestine. Even when there is noteworthy disagreement (4 out of 14) the agreement between the ganglionic and any of the other three series is no poorer than that among the non-ganglionic series themselves. Though each tissue is characterized by its own individual threshold, the comparative inhibitory activity of sympathomimetic amines at adrenergic neuro-effector and adrenergic ganglionic junctions appears to be, from such comparisons as are possible at present, of about the same order. Thus, in assaying the inhibitory action of amines, the ganglion, as much as one tissue can, may be taken as reasonably representative of adrenergic inhibitory junctions in general.

The comparisons favor the author's previously expressed contention that adrenergic (*11*) (and also cholinergic, *8*) processes at the neuro-neuronal junctions, represented by the synapses of sympathetic ganglia, and at neuro-effector junctions are essentially the same with only quantitative differences such as already exist between the various neuro-effector junctions themselves.

The point of view and method described have been found particularly useful in studying the relation between the structure and preponderance of inhibitory activity in sympathomimetic amines. Since both excitatory and inhibitory activities of the sympathomimetic amines are simultaneously influenced by the structure, correlation was attempted with both types of activity at once, as expressed by the ratio of inhibitory potency, measured in a reliable fashion such as described above, to excitatory potency. The correlation so obtained was attended with a greater degree of success than would otherwise have been possible. Moreover, this ratio, indicating the preponderance of inhibition or excitation, is exactly the knowledge desired for many therapeutic uses where the best drug would be the one most nearly manifesting a pure or exclusively inhibitory or excitatory action. A detailed presentation of the results is in preparation.

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