synaptic volleys, together with recording of the bioelectric activity of the ganglia following antidromic stimulation). It was found that the ganglion-blocking agents do not increase postactivating inhibition and have no influence on the conduction of excitation during antidromic stimulation. In contrast to the ganglion-blocking agents, procaine considerably impairs conduction of excitation in the postsynaptic neurones.

From the above data it appears that the inhibition of transmission of excitation in autonomic ganglia which occurs under the influence of ganglion-blocking drugs is related not only to the selective depression of the cholinoreceptores, but may also be the result of the influence exerted by these substances on the function of the presynaptic endings.

58 Enhanced Postganglionic Responses of Sympathetic Ganglia to Stimulating Agents Following Repetitive Preganglionic Stimulation. R. L. VOLLE (U.S.A.).

Asynchronous postganglionic discharges of the feline superior cervical ganglion, *in situ*, evoked by acetylcholine (ACh), carbamylcholine, and tetramethylammonium (TMA) were monitored with conventional electrophysiological techniques. Following repetitive preganglionic stimulation with supramaximal volleys at rates ranging from 20 to 100 c/s for periods of 10 sec to 15 min, the amplitudes and time–courses of the ganglionic responses to these agents were enhanced markedly. The duration of stimulation required to produce these changes appeared to be related inversely to the frequency of stimulation. Following repetitive stimulation, carbamylcholine, administered in doses which elicited postganglionic responses lasting 20–30 sec in previously unstimulated ganglia, evoked responses which last 3–5 min. Doses of ACh and carbamylcholine which produced just detectable postganglionic firing were reduced 8- to 16-fold by this procedure of conditioning. The threshold doses of TMA, however, were not reduced; suprathreshold doses were potentiated. The enhanced responses, once induced, persisted for the duration of the experiment (up to 5 hr after conditioning stimulation). Antidromic stimulation of the postganglionic trunks of normal and chronically denervated ganglia did not result in any significant alteration of the responses of the ganglia to these agents. Furthermore, ganglionic blockade by hexamethonium, infused into the arterial supply of the ganglia before and during repetitive stimulation in amounts which blocked completely the postganglionic responses to single preganglionic volleys, did not prevent the occurrence of enhanced responses to injected stimulating agents. It is suggested from these findings that: (1) conditioning with repetitive preganglionic volleys results in alterations in the presynaptic nerve terminals as reported previously by Larrabee *et al.*[1]; and (2) the action of ACh and carbamylcholine on the ganglion is due in part to stimulation of the nerve terminals.[2]

1. LARRABEE and BRONK (1947), *J. Neurophysiol.*, **10,** 139.
2. VOLLE and KOELLE (1960), *The Pharmacologist*, **2,** 88.

59 Classification of Ganglionic blocking Agents. J. M. VAN ROSSUM (Holland).

Transmission of impulses through the ganglionic synapse is to some extent similar to transmission through the neuromuscular junction. In both cases acetylcholine (ACh) is involved, which implies that drugs may act by: (a) inhibiting the synthesis of ACh; (b) affecting the release of ACh; (c) occupying ACh-receptors whilst having intrinsic activity and thus action as an ACh-mimetic; (d) merely occupying ACh-receptors and thus acting as a competitive antagonist; (e) occupying other receptors and so acting as a non-competitive antagonist.

When studying ganglionic blocking agents on neuro-muscular preparations evidence accumulates that some may act as competitive antagonists and others as non-competitive antagonists.

Ganglionic-stimulants and -blocking agents have therefore been investigated upon the isolated guinea-pig intestine under conditions that only ganglionic effects are measured. Dose–response curves were made of the stimulants using nicotine as a standard, whereas the blockers were studied as how they affect dose–response curves of nicotine.

At least three classes of ganglionic drugs occur: Class I: the mimetics as nicotine, DMPP, pyridine-3-methyl-yl-trimethylammonium; Class II: the lytics or competitive antagonists as hexamethonium, pentapyrrollidinium and azamethonium; Class III: the non-competitive ganglionic blocking agents as pentacyne, chlorisondamine. There exists a number of intermediate compounds, e.g. mecamylamine and pempedine, which are both competitive and non-competitive.

There exists a characteristic relationship between chemical structure and the class of drug.

60 The Effect of Various Amines on Transmission through the Superior Cervical Ganglion of the Cat. S. B. GERTNER and A. ROMANO (U.S.A.).

We have recently reported that guanethidine and bretylium blocked transmission when perfused through the superior cervical ganglion. This block which could be antagonized by the perfusion of epinephrine and norepinephrine, prompted a more thorough investigation of the effects of amines on ganglionic transmission. The actions of epinephrine, norepinephrine, isoproterenol, ephedrine, phenylephrine, tyramine, dopamine, tryptamine, 5-hydroxytryptamine and various other derivatives were tested on transmission. The stability of the

catecholamines during perfusion was greatly increased by the simultaneous use of ascorbic acid, which itself did nothing to transmission. In all the experiments there was never an indication of noticeable stimulation or facilitation of transmission. All of the catecholamines blocked transmission in doses varying from 50 γ/ml upwards. With lower concentrations no effect could be observed. Epinephrine and norepinephrine appeared to be equally effective in blocking transmission; dopamine was considerably less active. Of the drugs tested, tryptamine appeared to be the most potent, often causing considerable block of transmission when perfused in concentrations as low as 10 γ/ml. It was often possible to show a transient block when the drug was given as a single injection of 25 γ into the perfusion stream. Tyramine was somewhat less active. 5-Hydroxytryptamine showed very little if any effect on transmission although sometimes a slight potentiation was noticed. The blocking action of some of the amines was potentiated if given during the perfusion of a mono-amine-oxidase inhibitor.

61 The Mode of Action of Acetylcholine on the Adrenal Medulla. W. W. Douglas and R. P. Rubin (U.S.A.).

It is commonly accepted that the sympathetic nerves innervating the adrenal medulla are cholinergic, and that acetylcholine is the physiological stimulus which effects the release of catecholamines from the adrenal medullary cells. In experiments on perfused cat's adrenals, we have obtained evidence that the action of acetylcholine involves some Ca dependent process. Thus when the adrenals were perfused with Ca-free Locke's solution the amount of catecholamine liberated by acetylcholine fell to 5 per cent or less of the control value obtained in Locke's solution containing 2·2 mM Ca. Moreover, there was a quantitative relationship between the amount of catecholamine released by acetylcholine and the Ca content of the perfusion fluid: catecholamine output was about halved when the Ca concentration was lowered to 0·5 mM, and was increased by about half when the Ca concentration was raised to 8·8 mM. It was found that Ca itself (2·2 mM) caused secretion of catecholamine when introduced after a period of Ca free perfusion: excess Ca (to 35 mM) added during perfusion with Ca containing Locke had no such effect.

These findings, that catecholamine secretion in response to acetylcholine is related to the extracellular Ca concentration and that Ca itself (in appropriate conditions) causes catecholamine release, considered along with the known ability of acetylcholine to effect permeability changes at other post-synaptic sites, lead us to propose that acetylcholine causes catecholamine secretion by promoting an influx of Ca ions into the adrenal medullary cells.

62 The Influence of Ganglion-blocking Agents on Tissue Sulphydryl-Groups Content and Analysis of the Action of Thiol Substances on Impulse Transmission in Autonomic Ganglia. S. A. Mirzoyan (U.S.S.R.).

Some reactive groups of protein bodies undoubtedly play a responsible role in the primary chemical reactions of pharmacological agents with the receptors of effector organs. The present report concerns the interaction of gangliolytics with the tissue sulphydryl groups in structural homogenate. The objects for study have been the superior cervical ganglion and the ganglia of the vagal intestinal fibres in cats. The observations have shown that following the blocking of transmission in ganglionic synapses by hexamethonium, Gangleron or pentamine, a marked decreased in tissue sulphydryl content is observed. The experiments prove that the chemical interaction of gangliolytics with tissue reactive groups is not an accidental or side effect of the examined preparations. In experiments with nicotine it has been shown that the preparation, in doses which augment the sensitivity of ganglionic cells to electrical stimulation of presynaptic fibres and facilitate transmission in interneuronal synapses, causes an increase in tissue thiol groups content. In large doses, the blocking effect of nicotine on ganglionic transmission fully spreads over the sulphydryl groups content.

Titration of the homogenate in the presence of hexamethonium, Gangleron or pentamine, shows that the interaction of these preparations with the tissue sulphydryl groups takes place also in the non-structural homogenate, and under the action of hexamethonium, Gangleron or pentamine, a decrease is noted in sulphydryl groups content.

In perfo ion experiments an antagonism is observed between the sulphydryl groups and ganglion-blocking agents in their action on sympathetic ganglia and ganglia of the vagal intestinal fibres.

Thus, gangliolytics exhibit a capacity to decrease the thiol groups content and tissue sulphydryl groups have a definite significance in the chemical sensitivity of receptors towards ganglion-blocking agents.

63 Lipid Solubility as an Important Factor for the Penetration of Drugs into the Liver. H. Kurz (Germany).

The relative rate at which various foreign organic compounds of widely different chemical and physical properties enter the liver tissue was investigated in the isolated rabbit liver. The liver was perfused from the portal vein with a drug–saline solution at constant rate. The perfusate was collected in small fractions over a period up to 5 hr. The rate of disappearance of the drug was calculated from the decrease in concentration in the perfusate. Protein-binding was eliminated by calculation. The experiments were made at a temperature near 0°C to avoid metabolic interference