associated with the injection or passage of the bubble through the meter.

The pressure loss across the instrument was determined with a Statham differential strain gauge manometer, whose output was measured by a Pfaltz & Bauer multiple mirror galvanometer. Inasmuch as the relations throughout this system are linear, there is a direct proportion between the pressure change and the galvanometer reading. Pressure loss was found to be linear over the range of flows used (Fig. 3). Because of the vertical position of the instrument, there is a gradient of about 2.2 cm water pressure between the inflow and outflow tubes, even with no flow through the system. This static drop, as well as the differences noted at the several flow rates, is not of sufficient magnitude to warrant redesign of the instrument but must be taken into account in studies which involve accurate pressure measurement.

Since the blood is in the measuring system for periods of time appreciably less than 30 sec, it was not considered necessary to make provisions for temperature control.

This modification of the optically recording bubble flow meter is simple to construct, it diverts smaller quantities of blood from the animals, it introduces only a minimal pressure drop in the system, and it is easily adapted to a wide range of flow rates by varying the length of the external tubing.

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Molecular Configuration and Biological Activity of Substances Resembling Acetylcholine[1]

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Articles appearing in SCIENCE during the past two years have discussed the structure-activity relations of the choline group of drugs and related compounds. In the first of these articles Pfeiffer (*5*) called attention to certain features common to a number of drugs having parasympathomimetic stimulant action. These were the presence of an ether oxygen adjacent to a carbonyl group, with a methyl substituted nitrogen distant by two saturated carbon atoms. He referred to these as "prosthetic groups" and considered them to be of particular significance in anchoring the drug molecule to cell receptors.

[1] The unpublished work reported here has been supported by a grant from the U. S. Public Health Service, and a report in greater detail will appear elsewhere.

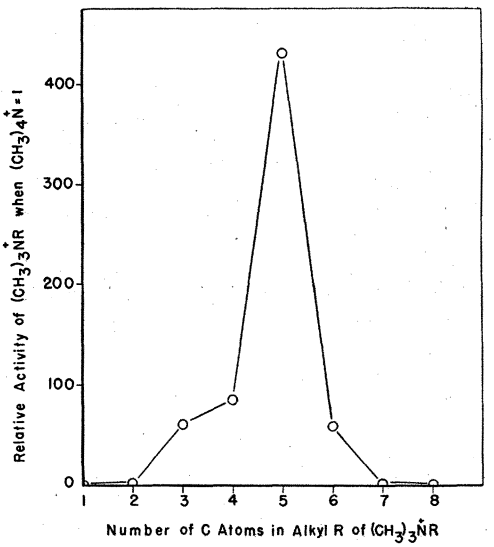

FIG. 1.

Pfeiffer also called attention to the presence of these groups in certain acetylcholine-blocking agents.

In a discussion of Pfeiffer's paper, Ing (*4*) pointed out that (1) not all molecules containing the three above-mentioned groups, disposed spatially as indicated, show parasympathomimetic stimulant actions, and (2) some molecules show such actions but do not contain all three groups. He suggested instead that the existing evidence favored the view that the preciseness of "fit" between the drug cation, as a whole, and some macromolecular structure in the cell determines the degree of activity that is observed. Opposing the views of Pfeiffer and of Ing, which imply a highly specific reaction between drug molecule and cellular constituents, is that of Barnes and Beutner (*2*), who believe that lipoid solubility and ionization of cholinergic drugs are sufficient to account for their pharmacological actions.

In a study of the fundamental mode of action of acetylcholine we have determined the relative activities of a considerable number of acetylcholine homologues and analogues, as well as simple quaternary ammonium ions, in depressing the spontaneous beat of the isolated heart of the mollusk *Venus mercenaria.* This preparation is extraordinarily sensitive to acetylcholine and is an especially favorable object for a quantitative comparison of the actions of compounds related to acetylcholine. It has been possible to test the effect of very slight alterations in size and spatial arrangement of groups within these molecules on their relative activities.

The action of acetylcholine on the *Venus* heart may be described as nicotinelike, and of a type such as is found at autonomic ganglia, for it is blocked by tetraethylammonium ions but not by curare alkaloids. At least two methyl groups on a quaternary nitrogen are required for a substance to have a significant acetylcholinelike, depressant action on the *Venus* heart. Quaternary ammonium ions with three alkyl groups other than methyl have a reversed or excitatory action and act as acetylcholine-blocking agents (*9*). This suggests that the di-

mensions of the cationic head are critical in determining the degree and kind of activity (*3*). Thus far we have found no compound related structurally to acetylcholine in which changes only in the long chain, or "tail," have resulted in a complete loss of acetylcholinelike action on the *Venus* heart. The results of some of these attempts have been reported previously (*3*). Any deviation from the acetoxyethyl group thus far tried, however, results in a decrease in activity. That the changes may be extensive and the activity yet remain high is seen when the relative activities of the alkyltrimethylammonium ion series are determined. Fig. 1 gives a comparison, on a molar basis, of the differences in depressant action of this quaternary ammonium ion series up to a carbon chain of 8 atoms in the odd alkyl group. The member with a 5-carbon atom chain, which has an over-all length approximately equal to that of the acetoxyethyl group of acetylcholine, is by far the most active member of the series in depressing the *Venus* heart. Compared with acetylcholine, the *n*-amyltrimethylammonium ion has been found to be about 1/70 as active. Earlier investigators (*1, 6*) had noted a similar peak of activity in this series when tested on a variety of preparations, the *n*-amyl member usually being most active or, when it was not, the difference between its activity and that of the *n*-butyl or *n*-hexyl was slight.

We have recently determined the relative activities of certain new acetylcholine analogues that are quaternary ammonium derivatives of pentanones and pentanols. We were interested in determining the effects of (1) substitution of methylene for the ether oxygen, (2) change in position of the carbonyl group, and (3) reduction of the ketone to hydroxy. The activities on the *Venus* heart of the pentanone derivatives are shown in Table 1. The values are expressed as approximately equiactive molar ratios, with the molar concentration of acetylcholine required for 50% decrease in amplitude of beat equal to one.

The substitution of a CH_2 group for the ether oxygen of acetylcholine reduces activity appreciably; nevertheless, the 4-ketoamyltrimethylammonium ion is the most active substance, next to acetylcholine, of the many structurally similar compounds that we have tested on the *Venus* heart. A shift of the ketone oxygen to the third carbon results in a further decrease in activity, which is more pronounced after a transfer to the second carbon of the 5-carbon chain. It would appear that the carbonyl group did represent an important point of attachment of drug molecule to receptor molecule, perhaps by hydrogen-bonding, and when the ketone oxygen is in position 4, the attractive force is maximal. The general pharmacology of the 4-ketoamyltrimethylammonium halide is being explored, and its activity relative to that of carbaminoylcholine (doryl), acetyl β-methylcholine (mecholyl), and acetylcholine (Ach) on different test preparations is given in Table 2.

Hydroxyamyltrimethylammonium halides were made with the hydroxy group in the 2, 3, and 4 positions. Compared with acetylcholine on the *Venus* heart, their activities were about 1/1500 as great. Little or no effect of change in position of the OH group has thus far been seen. The activity of the 4-hydroxyamyl- is less than 1/100 that of the 4-ketoamyltrimethylammonium ion. This difference appears too great to be accounted for on the basis of molecular configuration alone, and it seems probable that differences in rates of penetration are partly responsible for the dissimilarity in activities.

Among the substances that have been compared with acetylcholine on the *Venus* heart, to test the effect of changes in molecular configuration, are those of Table 3. Again, approximately equiactive molar ratios are given relative to a molar concentration of acetylcholine equal to one.

Although differences in general chemical properties doubtless are responsible for some of the differences in activity of the substances shown in Table 3, it seems

TABLE 1

		Equiactive molar ratios
Acetylcholine bromide	$[(CH_3)_3N^+CH_2CH_2O\overset{\overset{O}{\|}}{C}CH_3]Br^-$	1
4-Ketoamyltrimethylammonium chloride	$[(CH_3)_3N^+CH_2CH_2CH_2\overset{\overset{O}{\|}}{C}CH_3]Cl^-$	12
3-Ketoamyltrimethylammonium iodide	$[(CH_3)_3N^+CH_2CH_2\overset{\overset{O}{\|}}{C}CH_2CH_3]I^-$	160
2-Ketoamyltrimethylammonium iodide	$[(CH_3)_3N^+CH_2\overset{\overset{O}{\|}}{C}CH_2CH_2CH_3]I^-$	580

TABLE 2

	Before eserine	After eserine
Frog *rectus abdominis*	4-ketoamyl > doryl > Ach	Ach > 4-ketoamyl > doryl
Leech muscle	doryl > 4-ketoamyl > Ach	Ach > doryl > 4-ketoamyl
Frog heart	doryl > mecholyl > Ach > 4-ketoamyl	Ach > doryl > mecholyl > 4-ketoamyl
Venus heart	Ach > 4-ketoamyl > doryl > mecholyl	Ach > 4-ketoamyl > doryl > mecholyl

TABLE 3

		Equiactive molar ratios
Acetylcholine bromide	$[(CH_3)_3N^+CH_2CH_2OCCH_3]Br^-$ (O)	1
β-Carbomethoxyethyltrimethylammonium bromide	$[(CH_3)_3N^+CH_2CH_2COCH_3]Br^-$ (O)	15
Betaine ethyl ester chloride	$[(CH_3)N^+CH_2COCH_2CH_3]Cl^-$ (O)	660
4-Methylamyltrimethylammonium bromide	$[(CH_3)N^+CH_2CH_2CH_2CHCH_3]Br^-$ (CH_3)	1,370
4-Acetoxyamyltrimethylammonium iodide	$[(CH_3)N^+CH_2CH_2CH_2CHCH_3]I^-$ ($OCCH_3$ (O))	7,500

likely that the shifts in position of the carbonyl group and adjacent oxygen are, in part, responsible for the decreased activity of the β-carbomethoxyethyltrimethylammonium ion and the betaine ethyl ester.

This brief summary of results that we have obtained, using the isolated heart of *V. mercenaria* as a test object, supports the view of Pfeiffer that an ether oxygen and carbonyl group, spatially disposed as in acetylcholine, are of special significance in determining the activity of acetylcholine analogues. However, in agreement with Ing, we would emphasize the importance of exactness of "fit," or "dovetailing," between all parts of the drug molecule and structures in the receptor molecule. It has been suggested earlier (*7*) that acetylcholine may act as a coenzyme for an enzyme that plays a role in the regulation of membrane polarity and permeability. This further evidence of the significance of molecular configuration in the interaction of acetylcholine analogues and receptor substance is not inconsistent with the hypothesis.

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The Photochemical Action of Ultraviolet Light on the Absorption Spectra of Nucleic Acid and Related Substances[1]

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With the greater availability of materials capable of producing ionizing radiations, there has been an increase of interest in factors involved in the production of lethal effects in microorganisms and also of mutational changes in various types of living organisms. In view of the known cytochemical importance of nucleic acids, as well as their postulated significance, not only in the chromosomes but also in the cytoplasm, we have carried out experiments on the photochemical effect of ultraviolet radiation on nucleic acids and related substances, in the region of their major absorption, namely, about 260 mμ.

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[2] With the technical assistance of Marion Hyatt.

Previous reports of investigations on spectral changes in nucleic acids produced by ultraviolet radiations have been conflicting. Heyroth and Loofbourow (*2*), using a photographic colorimetric method, showed a reduction in the selective absorption of desoxyribonucleic acid (DNA), dichloromethyl pyrimidine, uracil, and adenine. Caspersson (*1*) noted similar effects upon DNA, adenine, guanine, and sodium urate and stated that the effect was least in the nucleic acid, whose "constitution seemed to stabilize the bonds." Hollaender (*3*) observed no change in the absorption spectrum of a .5% solution of sodium thymonucleate after irradiation for 83 hr. On the other hand, Uber and Verbrugge (*8*), irradiating a $12.5 \times 10^{-5} M$ solution of the pyrimidine component of thiamine for 2 hr, noted complete loss of selective absorption. More recently, Sinsheimer and Hastings (*7*) found under conditions of their experiment a 63% loss in the maximum absorption of uracil and uridine solution (6.2 μg/ml) following 16 hr of irradiation at pH 7.0, which was largely reversible by changing the pH to 1.0. Preliminary studies by them indicated similar reversibility for cytidylic acid. They stated, however, that thymine, cytosine, adenine, guanine, adenylic acid, and guanylic acid are not decomposed under the same conditions. Studying tobacco mosaic virus, Oster and McLaren (*6*) irradiated for 21 hr a .3% solution of