$$H_2S_2O_3 \rightleftarrows H_2SO_3 + S.$$

H_2SO_3 is further oxidized to H_2SO_4 by NO_2.

La Mer and co-workers assumed this phenomenon as a phase-transition from molecularly dispersed sulfur into droplets of supercooled λ-sulfur, but the present experiments seem to indicate that the separation of sulfur itself requires time, as is indicated by the slow building up of SO_3'' and the color reaction.

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A Simplified Recording Bubble Flow Meter[1]

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In the course of cross-transfusion experiments with rats, it became desirable to measure the rate of blood flow between the animals. The conditions of the experiment required an instrument that could fulfill the following requirements: (*a*) measure accurately flow rates between 0 and 15 ml/min; (*b*) impose the minimum resistance to flow, i.e., the minimum pressure drop; and (*c*) divert a minimum volume of blood from the system under investigation. In addition, simplicity of design and a method for objective recording were considered desirable.

The recent survey of flow meters by Green and others (*2*) critically evaluated the several methods that have been employed. Of the group, we considered the bubble flow meter as most nearly satisfying our requirements. The modification of this instrument by Selkurt (*3*) offers a method for recording that is fundamentally simple and accurate. The chief objections to instruments of this sort have to do with the relatively large volume of blood diverted to the meter (about 6 ml in the form described by Selkurt) and the cumbersome character of the instrument as a whole. The modifications to be described obviate these difficulties, and provide an instrument which is simple to build in any laboratory, but which nevertheless performs with remarkable accuracy.

Fig. 1 presents several drawings of the instrument.

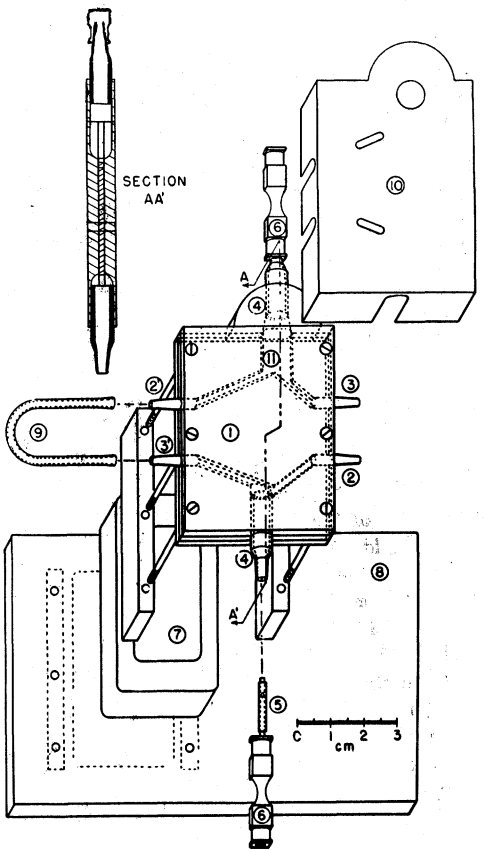

Fig. 1. Design of flow meter.

The numbers in the following description refer to the corresponding parts in the figure. The primary chamber (1) is laminated from 3 layers of lucite, the central layer being opaque and the 2 outer layers transparent. Fittings for the inflow (2) and outflow (3) tubes are turned from lucite and are cemented in place. Plastic male Luer connectors (4) are threaded into the block to provide the stability necessary for attachment of modified needles and syringes. Bubbles of desired size are injected through the lower connector via a Bunsen valve (5), with a drilled tuberculin syringe, as suggested by Bruner (*1*). The bubble trap (11) is emptied with any convenient-sized syringe through the upper Luer connector. Double female Luer connectors (6) are made from hypodermic needle hubs, and provide easy access to facilitate cleaning the system. The entire chamber is mounted directly in front of a General Electric, barrier-type photo emf cell (7), and the whole system mounted vertically as indicated on a suitable base (8). The two tubes (2′, 3′) are connected by a length of vinyl tubing (9) having an internal diameter of about 1.5 mm. The length of this tube may be varied to alter the range of the meter, while maintaining its inherent accuracy. In the model whose calibration curve

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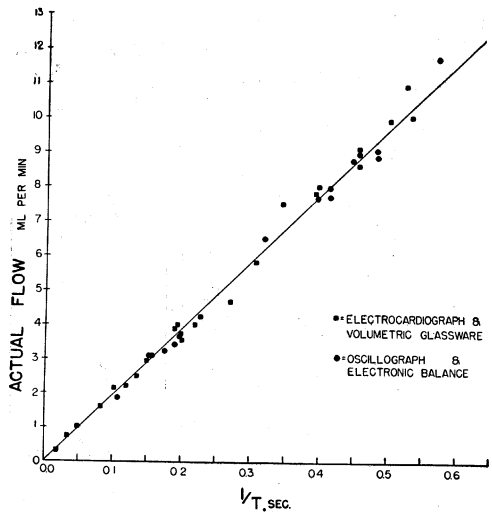

FIG. 2. Calibration curve for flow meter.

is given in Fig. 2, the length was 14 cm. Light is provided by a 6-v, 64-cp automobile headlight mounted directly in front of the chamber. To minimize stray illumination, a shield (10) placed between the light and the chamber masks the entire surface, except for 2 slits. Provision is also made for the observation of the bubble trap through this mask.

Since the blood is in contact only with smooth plastic surfaces, clotting is minimized; as a further precaution, however, the interior of the entire instrument is coated with "Dri-film."[2] In operation, blood from the animal enters the chamber through the lower inflow tube, passes on through the vinyl tubing, back past the bubble trap, and out to the animal again. The bubble is injected by the route described (5) and enters the flowing stream, thus breaking the continuous column of blood and permitting light to reach the photocell. The bubble then passes along through the vinyl tubing and again comes between the light source and the photocell, before it finally rises into the bubble trap. The output from the photocell is recorded directly on any suitable system, and the time between the 2 impulses is a function of the flow rate. We have found the conventional string galvanometer of the type used in a Cambridge electrocardiograph eminently suitable for recording the output of the photocell. The camera provided with such an instrument has a paper speed that allows time to be measured to within .02 sec.

The calibration curve (Fig. 2) was constructed by comparing true flow determined by direct measurement of the outflow and the apparent flow determined from bubble passage time. The reciprocal of bubble passage time, a direct function of apparent flow, is plotted against true flow rate to obviate the necessity for making a direct determination of the volume through which the bubble passes. However, calculations made from actual data for flow rate and for bubble passage time showed this volume to be approximately ⅓ ml, and the volume of blood in the entire instrument could be no more than double this volume. The linear relation obtained in the plot is an empirically determined one, and is not based on any assumptions concerning the volume involved in the flow meter.

Outflow rate was determined either directly by collection of the blood in a graduated cylinder over a measured period of time, or by means of a strain gauge balance. The latter instrument, to be described more fully elsewhere, employs a Statham-type dynamometer which is acted upon by one arm of a balance. The output of the dynamometer is amplified and finally recorded on an oscillograph. The relations in this system are all linear, so that the slope of the curve recorded is directly proportional to the change in weight on the balance pan. For use in calibrating the flow meter, the effluent blood is caught in a beaker on the balance, so that the rate of increase in weight, and hence the slope of the line are proportional to the rate of outflow. In addition to the greater sensitivity provided by this method of calibration, the output from the photocell of the flow meter may be recorded along with the flow curve. However, the data presented in Fig. 2 show no consistent difference between points obtained by the two methods. The data represent several different samples of dog and rabbit blood; the samples were heparinized or citrated in some cases and defibrinated in others. There are no significant differences in these data.

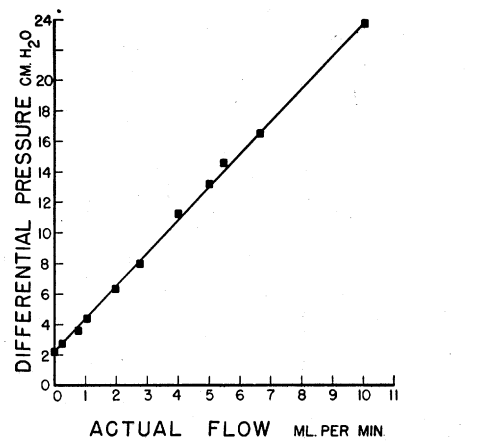

FIG. 3. Pressure fall across the meter as a function of flow rates.

Since the ratio of the volume of the bubble injected to the blood volume in the meter is so great, it was necessary to establish the fact that the injection of the bubble per se did not seriously alter the rate of flow. This was accomplished in two ways. First, the rate of flow was accurately determined over a period of time, during which no bubbles were introduced into the system. Then, holding all other conditions constant, a similar measurement was made with the injection of bubbles at frequent intervals. There was no significant difference in the measured flow rates. The second method has to do with the strain gauge type of calibration. There was no measurable change in the slope of the outflow curve

[2] Dri-film No. 9987, General Electric Company.

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.

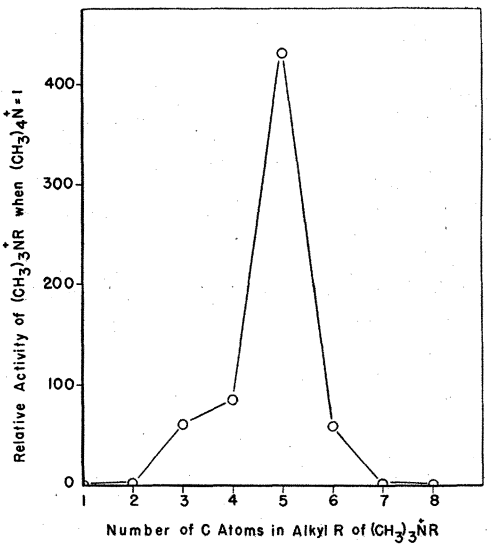

FIG. 1.

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-