PHYSIOLOGICAL TECHNIQUES IN PHARMACOLOGY[1]

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Useful techniques are usually rapidly applied and become well-known; therefore, with a few exceptions, the scope of this review was limited to publications which appeared in 1961 and the first half of 1962. Of the many novel techniques published, selection for review was limited to those which measured functions of whole animals and tissues, with special emphasis upon those applied to unanesthetized animals. It is hoped that the survey will suggest to the reader new approaches to his own research.

Techniques Applied to Unanesthetized Animals

Although there are specific exceptions, in clinical practice the useful action of a drug is desired for at least several hours and the drugs are given to conscious patients. Since anesthetics may obscure or alter drug actions, testing in unanesthetized animals is often to be preferred. However, not only must pain be avoided, but also the effect of restraint itself should be considered. Dogs can be trained to accept experimental conditions calmly, but rats are more often severely restrained [for methods, see Davies & Grice (1); Cotlove (2)]. For example, restraint alone more than doubled corticosterone secretion in rats and caused a marked increase in acid concentration of the gastric juice [Knigge, Penrod & Schlinder (3); Brodie, Marshall & Moreno (4)].

Cannulation techniques.—Permanently implanted cannulas in blood vessels are necessary for injections and drawing samples. Polyethylene and polyvinyl tubes are most commonly used, but Teflon and Silastic (silicone rubber) appear superior for chronic use [Barr & Soila (5); Stewart & Sanislow (6)]. Teflon has the disadvantage of being too stiff, but Silastic tubing is soft and flexible. Silastic tubing has recently been made 0.025 in o.d., comparable to the smallest polyethylene and polyvinyl tubes (distributed by Becton, Dickinson Co., Rutherford, N. J. as Vivosil).

Permanent cannulas may readily be put into the right ventricle of dogs or right atrium of monkeys for chronic intravenous injections without fluoroscopic control by monitoring the position of catheter tip by pressure changes [Clark, Schuster & Brady (7); Rudolph & Paul (8); Weitzman *et al*, (9)]. In addition, the coronary artery of dogs was chronically cannulated by Barger *et al.* (10), and the coronary sinus by Galla *et al.* (11). The latter workers noted that in addition to use of heparin, intraluminal clotting could be temporarily corrected by fibrinolysin (Actase) injections.

Several methods have been proposed for chronic venous cannulation of rats [Popovic & Popovic (12); Slusher & Browning (13); Weeks (14)]. In all of

[1] The survey of literature pertaining to this review was concluded in July 1962.

these methods one end of a small polyethylene cannula was threaded down the jugular vein either into the superior vena cava or right heart. The exit for the tube was on the back of the neck, a location a rat would not disturb, although rats housed together quickly chew each other's tubes. The method of Weeks (14) can more readily be used repeatedly for injections and can be expected to remain functional without attention for much longer times (3 or 4 months compared to 3 or 4 weeks). However, a special cannula must be constructed. Rappaport *et al.* (15) have described a mechanical aid for cannulation of small mesenteric veins for continuous portal perfusion in rats. If their tube were brought out to the back of the rat's neck instead of through the abdominal incision, a restraining cage should not be necessary.

The aorta has likewise been chronically cannulated by Popovic & Popovic (12) via the carotid artery and by Weeks & Jones (16) directly into the abdominal aorta using a modified method of Still & Whitcomb (17). Arterial pressure and blood samples may readily be obtained.

Automatic injections as reinforcing agents.—Cook *et al.* (18) showed that intravenously administered epinephrine or acetylcholine could serve as conditioned stimuli in a conditioned-avoidance experiment in dogs. In monkeys, Clark, Schuster & Brady (7) showed that saline, injected upon a lever press, served as reinforcing stimulus when drinking water was not available. Weeks (14), and Epstein & Teitelbaum (19) described techniques for chronic injections in freely moving rats. Weeks studied experimental morphine addiction in which rats pressed a lever for automatic intravenous injections of morphine. Epstein & Teitelbaum (20) studied self-feeding experiments using rats with chronic gastric cannulations [Epstein (21)]. The mechanical devices for administering fluids to the freely moving rats were very different. Epstein & Teitelbaum (19) used a lightweight plastic swivel screwed permanently to the skull, while Weeks (14) fitted the rat whith a small saddle connected to a swivel above the cage by means of sprocket chain. Neither have evaluated whether these devices led to restraint-induced stress. Yanagita & Deneau (22) developed an ingenious device whereby freely moving monkeys may be given automatic intravenous injections and applied the technique to the study of experimental morphine addiction.

In addition to use in operant behavioral experiments, chronic techniques have many other applications. Thus, hormones may be infused continuously at rates comparable to those secreted physiologically, drug tolerance induced, drug metabolites collected continuously, and effects of continuous medication evaluated (due to the rapid metabolism of drugs by rats, hourly injections may be more comparable to four times daily in a dog or man).

Telemetry.—The impetus of aerospace research and electronic ultraminiaturization has led to practical telemetry of many physiological functions. Such techniques are nearly ideal for studying changes in physiological functions under practically normal conditions. These devices usually consist of a battery-powered oscillator whose basic frequency is modulated by altering a resistance, capacitance or inductance.

During this review period, "endoradiosondes" to be swallowed were described for gastrointestinal pressures [Farrar, Berkley & Zworykin (23); Jacobson & Nordberg (24)], deep body temperature [Fox, Goldsmith & Wolff (25)], and pH [Noeller (26); Storer *et al.* (27)]. All these devices, because of their necessarily small size, are limited by battery life and short range. Farrar, Berkley & Zworykin's (23) device did not have the battery problem since it was externally energized from a coil worn by the subject. This technique might well be applied to other self-contained telemetering devices if problems of relative position of the capsule antenna and energizing antenna can be solved.

A combination of pH and motility (pressure) endoradiosondes should be very useful in evaluating anti-ulcer drugs. The pH capsule of Storer *et al.* (27) is too large for practical use. Noeller's capsule is about the size of an average medicinal capsule (26).

More versatility is possible using dogs with surgically implanted sensing devices and exteriorized lead wires. The transmitter can be carried readily by the dog. Hawthorne & Harvey (28) described the telemetering for 150 feet of ventricular circumference changes of an unanesthetized dog using a mercury-in-rubber strain gauge. Their transmitter, even without microminiaturing techniques, weighed only 260 g, and utilized commercial pickup and amplification equipment.

Drugs on the brain.—Direct action of drugs on the brain after intravenous administration may be obscured by peripheral actions or failure to penetrate the blood-brain barrier. Consequently, intraventricular injections have been given through chronically implanted cannulas. This method may effectively reach deeper brain structures, but action on the cortex would be questionable. Kobayashi (29) developed a simple technique for permanent implantation of a polyethylene cannula in the arachnoid space over the cerebral cortex of dogs. Marked differences were seen in response to certain drugs between cortical and intraventricular injections.

For study of drug action on discrete areas of the brain, solids (solutions diffuse too rapidly) were implanted stereotactically under anesthesia [Davidson and co-workers (30 to 32)]. Grossman (33) described results obtained by introducing solids into the hypothalamus of unanesthetized animals through previously placed metal cannulas but did not describe their construction. Stein & Seifter (34) confirmed some of Grossman's findings and published directions for making and installing the cannula. In view of circumstantial evidence that gold thioglucose-induced obesity in mice and estrogen-induced sexual behavior in cats may be due to selective affinity for these substances of specific neurones in the hypothalamus, this technique should have many applications [Debons *et al.* (35); Michael (36)].

Cardiovascular Techniques

Blood flowmeters.—Until recently, cardiovascular measurements have centered about pressure, but only by simultaneous measurement of flow can

resistance be evaluated. Ideally, a flowmeter should not require opening of the vessel and be capable of implantation for chronic use on unrestrained animals. Reasonably non-technical reviews of the various types of flowmeters have been given by Wetterer (37), and by Jochim (38). At present, the most practical is the electromagnetic type which measures the potential induced when blood, acting as a moving conductor, flows between the poles of a magnet. All have the technical problem of separating the minute induced potential from stray potentials arising indirectly from the current energizing the magnet. The original instruments [and the commercially available models (38)] energize the magnet by either a sine-wave or square-wave pulse. Both are serviceable instruments, the latter has less background electrical interference and the former is more suitable for small-sized probes [Jochim (38); Olmsted (39)]. Westersten, Herrold & Assali (40) designed a flowmeter using a gated sine-wave; Yanof (41), one using a trapezoidal-wave; and Olmsted & Aldrich (39, 42) one using a new principle dependent upon phase detection instead of induced potential.

All electromagnetic meters share the problem of obtaining an accurate electrical zero reference after implantation of probes unless blood flow is actually arrested. A flowmeter around the root of the aorta may use the reading during diastole as zero. If the implantable arterial occlusion cuff designed by Jacobson & McAllister (43) were made of silicone rubber (Silastic), which is apparently inert in tissue, this problem might be solved.

Two flowmeters using ultrasonic waves were described by Franklin and co-workers (44, 45). One used a transit time of pulses of sound, the other the Doppler frequency shift when sound passed through the moving blood. The probes were much simpler and cheaper than electromagnetic probes.

Cardiac output, indicator dilution.—Dye dilution methods have become standard for measuring cardiac output, and Hansen & Pace (46) developed a completely automatic apparatus for use with unanesthetized animals. Considerable variations were observed at times, but this is probably to be expected. Concerning blood pressure and cardiac output recorded continuously, Olmsted (47) said, "When a conscious animal is lying quietly, irregular changes, which disappear under anesthesia, are continuously present in the recordings, some equivalent in magnitude to those from injected vasoactive substances."

It is possible that dye dilution curves may be obtained without removal of blood. Enson *et al.* (48) described a "reflection oximeter," two bundles of flexible glass fibers (light tubes) passed through a catheter with their tips in the blood stream. One bundle was lighted, the other measured light reflected from the blood. The ratio of intensity at two wave lengths was linearly related to the concentration of indocyanine green up to 10 to 12 mg/1.

Hirche & Lochner (49) were able to measure coronary blood flow in anesthetized closed-chest dogs by the dye dilution method after coronary artery and sinus catheterization under fluoroscopic control. The technique

seemed difficult, and several arbitrary correction factors were applied to their quantitative calculations.

The thermal dilution method for cardiac output uses cold saline instead of dye and the dilution curve is described by temperature changes of arterial blood [Fegler (50)]. Although Goodyer *et al.* (51) confirmed its accuracy, the method has not been widely used. Evonuk, Imig, Greenfield & Eckstein (52) again carefully evaluated the technique in anesthetized dogs. Using room temperature saline, results were practically identical with the dye dilution method and reproducibility was comparable. Injection into the right atrium and temperature recording from the aortic arch was most accurate [Mohammed, Imig, Greenfield & Eckstein (53)]. Richardson *et al.* (54) described an adaptation of the method to anesthetized rats. The simplicity of the method, with no withdrawal of blood or distortion of curves by sampling systems, recommends it for more widespread use [Hosie (55)].

Computers have been developed commercially for calculation of the area under dye dilution curves. These are necessarily complex instruments because of recirculation of dye before the curve is complete. Wessel *et al.* (56) designed a simple computer for the area of a thermal dilution curve, since there is no significant recirculation.

Cardiovascular reflexes.—Cardiovascular reflexes are frequently studied in acute anesthetized preparations using mechanical devices to control certain components of the circulation. Thus, pressure stabilizers maintain arterial pressure constant in the face of changes in cardiac output, peripheral resistance or in both. Beck (57) developed a stabilizer which deserves more widespread use. Since it operates on the Mariotte bottle principle, using compressed air or oxygen, the pressure is not influenced by the level of the blood in the reservoir. Settling of blood is avoided by constant bubbling of gas in the reservoir. The stabilizer is suitable for either arterial or venous pressures.

Pressor baroreceptor reflexes are readily elicited by clamping the carotid arteries, but eliciting depressor reflexes has not been so simple. Holland & Maxwell (58) perfused blood from the common carotid arteries into the femoral artery of a dog at a constant rate using a Sigmamotor pump. Changes in vessel tone in the leg were reflected by changes in perfusion pressure. Then, using a second Sigmamotor pump, blood from the severed proximal end of the femoral artery was used to perfuse the carotid sinuses and head through the common carotids. A shunt around the pump permitted perfusion at normal pressures. Reflex vasodilatation was induced by closing the shunt and pumping blood at a pressure above systemic arterial.

Drug-induced changes in vasomotor tone may arise either from direct action on vessels or mediated reflexly through the nervous system. Beck (59), and Laverty (60) independently introduced a technique to separate these actions in a single animal preparation. A limb was perfused at a constant rate as above, except that blood was delayed in reaching the limb by passing it through a coil of tubing before reaching the pump. Reflex changes in the re-

sistance of the perfused limb could be observed before injected drug arrived.

Local tissue blood flow.—At times it is sufficient to know only whether there are qualitative changes in blood flow. Modifications of the thermoelectric blood flow technique of Gibbs (61) were developed for the cerebral cortex and the hypothalamus in chronic experiments on unanesthetized cats [Betz & Hensel (62, 63); Kanzow and co-workers (64 to 66)]. Basically, this method consisted in placing two thermocouples in the tissue, one of which was warmed. The degree to which the second thermocouple was warmed varied inversely with tissue blood flow. Blood flow changes were correlated with the electroencephalograph and blood pressure in sleep and even while a cat caught and ate a mouse! Systemic blood pressure and local flow did not always change together.

Miscellaneous Techniques

Gastrointestinal motility.—Experimental studies directly measuring intestinal motility on unanesthetized animals commonly use trained dogs with Thiry-Vella loops. Although innervation and blood supply are intact, such loops lack the stimulation associated with intestinal contents. Two devices were developed which can be permanently implanted for months on the serosal surface of dog intestines to record movement without leading to adhesions or fibrosis. Louckes *et al.* (67) used an "inductograph," two small electromagnetic coils, one of which induced a current in the other, the induced potential depending upon their distance apart. These were used primarily for evaluating pyloric sphincter activity. A strain gauge transducer was imbedded in silicone rubber (Silastic) and stitched directly to the intestine of dogs by Jacoby, Bass & Bennett (68). Depending on the orientation of the transducer, circular and longitudinal activity could be recorded independently.

Ulcers.—Hanson & Brodie (69) showed that clinically effective antiulcer drugs would protect rats against restraint-induced ulcers. This method has the advantage over other methods in that drugs need be given but once and no surgical or chemical interference is required. Kuroyanagi & Necheles (70) described preparation of Mann-Williamson rats. Although not a simple operation, rats are much cheaper and easier to maintain in a small space than dogs.

Isolated muscle.—Isolated arterial tissue is commonly used to study vasoactive substances, but in the animal it is the arteriole which is responsible for actions observed. Bohr, Goulet & Taquini, Jr. (71) achieved the ultimate in studying isolated muscles when they measured tension developed by helical strips cut from arterioles of the mesenteric, pulmonary, renal, and cerebral beds. They compared reactivity to various vasoactive substances.

Gaddum & Szerb (72) introduced goldfish intestine to pharmacology for a sensitive assay for substance P. They also described a simple modification of the microbath (0.05 ml capacity) of Gaddum & Stephenson (73). The technique and assay were an attempt, with inconclusive results, to detect

substance P and other substances in the brain by extracellular tissue perfusion, using a "push-pull cannula" [Gaddum (74)], a modification of the subcutaneous perfusion technique of Fox & Hilton (75).

Renal and electrolytes.—Cotlove (2) described a method for renal clearance measurements in restrained unanesthetized rats. Infusions and drawing of blood samples might well be carried out using some of the cannulation techniques described earlier to lessen the stress on the rats. Williamson, Skulan & Shideman (76) have adapted the stop-flow technique to rats. Portnoy *et al.* (77) describe a much-improved glass electrode for continuously monitoring blood sodium and potassium concentrations.

Anesthesia.—When it is necessary to anesthetize small animals (mice to rabbits) for only about a minute, a can or jar containing carbon dioxide from a cylinder or dry ice is quick and safe [Hyde (78); Stone *et al.* (79)].

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