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New Technic for Study of Drug Actions on Bronchial Resistance in Isolated Lung.* (22236)

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In the estimation and evaluation of drug effects on specific organs and tissues, the use of isolated preparations perfused with a balanced salt solution has certain evident advantages. Drug and electrolyte concentration may be accurately and reproducibly controlled without the possible complications of metabolism, excretion, and binding by plasma proteins; temperature may be varied at will, and reflex and endocrine control is eliminated. Nevertheless, no completely satisfactory technic has been described for observation and measurement of drug actions on the bronchioles in an isolated lung. The object of this report is to describe an isolated lung preparation in which an attempt has been made to meet 4 basic requirements of *in vitro* technic: 1. Sensitivity to drugs should approach that shown in the intact animal. 2. Experimental conditions should simulate physiological conditions as closely as possible. 3. The method should not be encumbered with complicated apparatus and technic. 4. Results obtained should be easily interpreted and evaluated.

Most of the isolated lung preparations described in the literature have been designed for physiological studies, and technics used are rather elaborate(4-10). Usually the perfusion fluid was defibrinated or heparinised blood, which has the theoretical advantage of reducing oedema formation but introduces many new variables; it has been shown, for example, that substances may be liberated from blood which influence the tone of bronchioles(1,2). In cases where drugs were used, the sensitivity was 10-100 times lower than in the present method. Sollmon and von Oettingen(11) suggested perfusion of lungs with Locke's fluid through the bronchial tree. Although this method is very simple, it appears highly unphysiological and possesses very low sensitivity to drugs; moreover, the fluid must flow through the vascular system as well as the bronchioles, and flow rate will therefore depend on effects on the vasculature.

A large number of technics using intact animals have been described, some of which are based on principles similar to those used in the present method, *e.g.*,(12).

Method. General Principles. The object of this technic is to measure alterations in bronchial resistance in isolated lungs perfused through the pulmonary artery with a balanced salt solution. This is achieved by recording the pressure in a semiclosed system connected with the trachea while applying alternating pressure changes outside of the lungs. The recorded pressure changes will then be greatest when the bronchi and bronchioles are widely dilated, and smaller when the resistance to the passage of air between trachea and alveoli is increased. The apparatus is shown schematically in Fig. 1. It consists essentially of a warming chamber,

* The opinions or assertions contained herein are private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

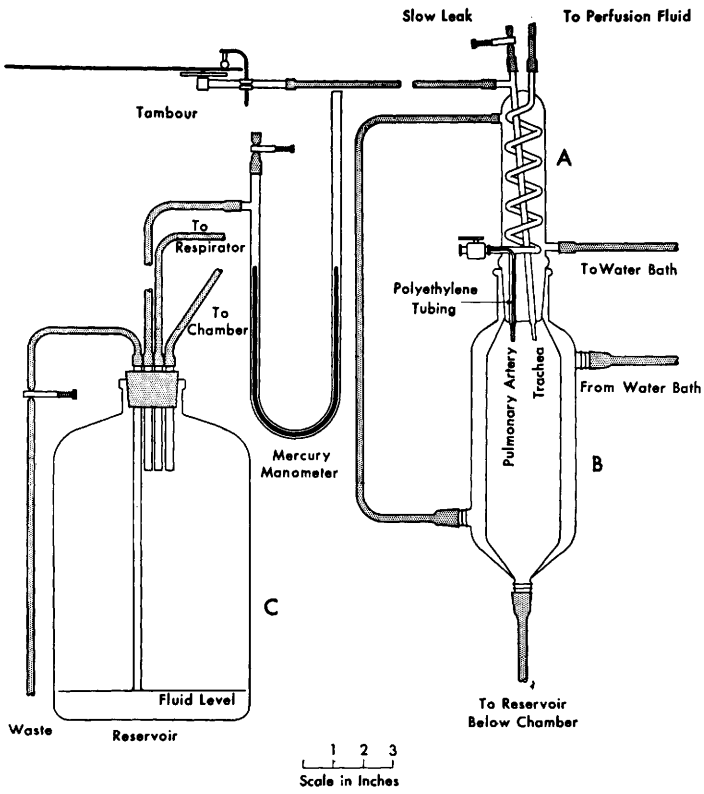

FIG. 1. Semi-schematic diagram of apparatus for lung perfusion. A is a warming chamber for tidal air and perfusion fluids; B, the lung chamber and C, the drainage reservoir, which is actually situated immediately beneath B.

A, in which the perfusion fluid and tidal air are warmed by water circulated from a thermostatic water bath at 38°C; a water jacketed lung chamber, B, in which the lungs are suspended by cannulae in the trachea and pulmonary artery from the appropriate outlets from A; and a drainage vessel C, which serves the dual purpose of collecting the fluid draining from the open pulmonary veins, and acting as a buffer between respiration pump and lung chamber to minimize alterations in applied pressure resulting from changes in volume of the lungs. A is connected with B by means of an airtight ground glass joint, and C is located immediately beneath B, to which it is attached with rubber tubing. *Perfusion* fluid is delivered to A from a Marriotte bottle, the air inlet of which is at the level of the pulmonary artery so that no positive perfusion pressure is used. Flow of fluid is maintained by pumping action of the applied air pressure changes, and is approximately 3-5 ml/min. A sidearm from the perfusion tube near the lower end of the warming vessel is plugged with a rubber stopper through which a hypodermic needle is inserted. On the inside this is connected to a polyethylene tube leading to the opening of the pulmonary artery, and on the outside a stopcock is attached. This serves to allow injections into the perfusion fluid to be made with minimum dilution and maximum speed of effect, while the stopcock prevents an air leak when the syringe is not in place. The dead space of the injection system is 0.2 ml and all injections were therefore washed in with 0.5 ml. The fluid used was a modified Krebs solution as follows: NaCl, $NaHCO_3$, KH_2PO_4, KCl, $MgSO_4 \cdot 7H_2O$, $CaCl_2 \cdot 2H_2O$, glucose, epinephrine in concentrations of 692, 210, 16.2, 35.4, 14.7, 18.7, 100.0 and .01 mg/100 ml, respectively. The glucose was added im-

mediately before use, and the solution was then equilibrated with 5% carbon dioxide in oxygen for at least 30 minutes. Epinephrine was added after oxygenation. The *tracheal cannula* is connected through the warming chamber with a Marey tambour recording on a kymograph. A sidearm bears a piece of rubber tubing with adjustable clamp, which during operation is kept almost closed. This slow leak serves to equalize the average pressure with atmospheric and improves the sensitivity to bronchoconstrictors. The *vessel C* is connected with the lung chamber B, respiration pump, mercury manometer and drainage tube. Total capacity of B and C is approximately 5 liters, and the respiration pump is set to move 600-800 ml of air in and out with each stroke. In a closed system the amplitude of the applied pressure change would therefore be 90-120 mm Hg. The drainage tube clamp is opened until this amplitude falls to 50-70 mm Hg, and this maintains the fluid level in C constant and at the same time equalizes the average pressure with atmospheric. Without the slow leaks on either side of the system to equalize the average pressure there was a tendency for the pressure to mount on one side and decline on the other, causing a progressive change in average volume of the lungs and in the sensitivity to drugs.

Procedure. Guinea pigs of both sexes weighed 450-550 g. The animal is given 400 units of heparin intramuscularly and 15 min. later is killed by a blow on the head. The carotid artery is immediately opened on one side, care being taken not to damage the trachea. A tracheal cannula is then inserted and artificial respiration started. The anterior wall of the thorax is opened and a polyethylene cannula tied into the common pulmonary artery through the right ventricle. The lungs are then dissected out with as little handling as possible and the heart removed. Artificial respiration is stopped and the 2 cannulae are attached to the appropriate outlets from the warming chamber A by means of adaptors. The water-jacketed vessel B is placed in position and the respiration pump started immediately. The useful life of this preparation is very dependent on the speed with which this procedure is carried out and degree of handling of the lungs. Five to 7 minutes were sufficient in our experiments.

Results. General Performance. When the preparation is first set up there ensues a progressive dilatation of the bronchioles, as evidenced by an increase in the size of the tracing, for 20-40 min. This process may be accelerated by small injections of epinephrine (2 γ), the effect of which tends to persist indefinitely (Fig. 2). During the first 60-90 min. of operation, the lungs gradually accumulate fluid until a steady state is reached, after which sensitivity to bronchoconstrictors remains approximately constant for the life of the preparation (6-8 hours). This accumulation of fluid does not interfere with movement of air, nor with response to drugs; on the contrary, the preparation does not become stable until it appears to be almost solid in the expired state.

In some preparations, however, fluid accumulated in the trachea and bronchi and persisted in spite of aspiration. This interfered grossly with movement of air and such preparations were useless for the study of drug actions. The causes of this phenomenon have not so far been determined with certainty, but certain precautions were found to reduce the incidence to a minimum and have been incorporated in the technic described. They are: 1. Addition of epinephrine to the perfusion fluid in a concentration of 1:10,000,000. In some experiments in which this was initially omitted, fluid accumulated in the trachea but recovery occurred when epinephrine was introduced and aspiration carried out. 2. Use of zero perfusion pressure. A wide range of perfusion pressures was tried, and in general the lower the pressure the less is the tendency to fluid accumulation in the trachea and bronchi. 3. Speed in dissection; avoidance of excessive handling of lungs and of inhalation of blood. Following a blow on the head, blood is readily aspirated, and rapid introduction of a tracheal cannula to avoid this is essential. 4. Premedication with heparin to prevent

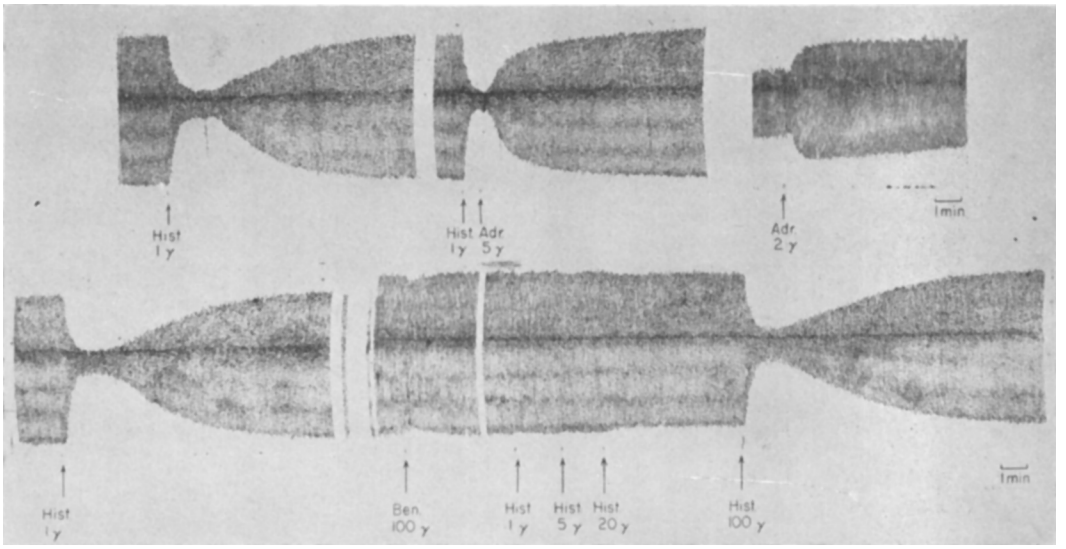

FIG. 2. Tracings showing effects of histamine, adrenaline and benadryl on the isolated lung. Tracings on top line were obtained from one preparation, those on bottom line from another. Effect of adrenaline alone is shown immediately after preparation was set up.

pulmonary emboli. 5. Positive pressure artificial respiration from the time spontaneous respiration stops until the preparation is finally set up. 6. Use of young animals. If the animal is too small, however, the tracing obtained is too small to work with; 450-500 g guinea pigs were found to be a suitable compromise.

Response to histamine. The injection of 1 γ histamine diphosphate produces a rapid reduction in amplitude of the tracing, indicating bronchoconstriction. The effect persists for a little over a minute and complete recovery ensues in 6-8 min. (Fig. 2, top line). Perfusion rate is almost unchanged, a 0-10% reduction in outflow being seen. This response is reproducible for several hours provided that the conditions are maintained constant. However, temporary mechanical disturbance of perfusion flow may cause a longer lasting change in sensitivity to histamine. It is therefore advisable for assay purposes to use a Marriotte bottle large enough to last for the entire experiment without refilling. An increase in sensitivity up to 10-fold may be achieved by omitting epinephrine from the perfusion fluid; however, there is then a greater tendency for fluid accumulation in the trachea, and recovery from histamine is slower. Sensitivity to histamine may be reduced very greatly by antihistamines. Fig. 2 (lower tracing) shows a sequence taken from an experiment in which a single injection of 100 γ of benadryl reduced the sensitivity to histamine by a factor of approximately 100.

Response to other agents. Acetylcholine produces a response closely resembling that of histamine, with slightly faster recovery, at

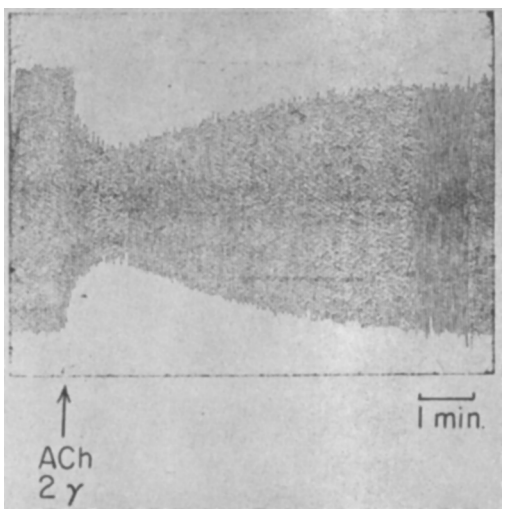

FIG. 3. Tracing showing effects of acetylcholine on isolated lung.

about 3 times the dose (Fig. 3). This response may be blocked by atropine. Morphine sulphate produces no bronchoconstriction in doses of up to 250 γ.

Bronchodilators have little or no action on the standard preparation, presumably because the bronchi are maintained maximally dilated by the epinephrine. Even epinephrine, in doses up to 5 γ, produces only a slight and very temporary effect. When superimposed on a typical response to histamine, however, epinephrine has a marked effect in accelerating recovery (Fig. 2); papaverine and theophylline also show some dilator activity in rather large doses (250 γ and 500 γ respectively). As mentioned earlier, epinephrine also produces a well marked effect on a preparation newly set up, and if epinephrine is omitted from the perfusion fluid, marked sensitivity is shown to bronchodilators.

Summary and Conclusions. A technic for the observation and measurement of the effects of drugs on bronchial resistance in an isolated perfused lung preparation is described. This preparation shows an absolute sensitivity to histamine comparable to commonly used biological assay objects, and shows a reproducibility adequate for assay purposes. Its simplicity makes it suitable also for teaching demonstrations of the effect of drugs on the bronchioles.

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Potentiation of Glycogenolytic Effect of Epinephrine by Desoxycorticosterone in Certain Skeletal Muscles.* (22237)

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The glycogenolytic effect of epinephrine on skeletal muscle (hind limb) is inhibited by cortisone acetate in normal rats(1), by adrenal cortical extract in adrenalectomized rats(2), and by growth hormone in hypophysectomized rats(3). On the other hand, adrenalectomy(2,4) or the injection of thyroxin(3,4) potentiates the glycogenolytic action of epinephrine and thyroidectomy depresses it(4). Those hormones which inhibit this effect of epinephrine also induce a storage of glycogen in these skeletal muscles (glycopexic effect)(1,2,3,5). While it is usually stated that desoxycorticosterone does not stimulate glycogen storage in muscle(5-8), there is no information on whether this mineral-corticoid influences epinephrine effects on carbohydrate metabolism in the intact animal. This report will show that desoxycorticosterone acetate (DOCA) potentiates the glycogenolytic action of epinephrine in certain skeletal muscles.

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Materials and methods. Adult female rats of the Long-Evans strain were selected for uniformity of age and weight within each ex-