# Rat Heart-Lung Preparation: Standardization of Work Performance; Lung Weights

*By* NEIL SOLOMON AND GEORGE SAYERS

**The preparation of an isolated rat heart-lung is described in detail. This isolated system exhibits a high degree of uniformity of work performance as measured by left ventricular work index (LVWI) (proportional to the product of cardiac output and mean arterial blood pressure). The procedures described are of considerable importance to studies of the tissue-level activity of corticosteroids.**

**Le preparation de un isolato de corde e pulmon del ratto es describite in detalio. Iste isolate systema exhibi un alte grado de uniformitate in le effective capacitate de travalio, mesurate per le nidice de travalio sinistro-ventricular (que es proportional al producto de rendimento cardiac con tension medie de sanguine arterial). Le proceduras describite es de considerabile importantia pro studios del activitate de corticosteroides al nivello tissutal.**

EXPERIMENTAL CONDITIONS have been developed for the operation of a rat heart-lung which exhibits a high degree of uniformity of work performance as measured by left ventricular work index (LVWI) (proportional to the product of cardiac output and mean arterial blood pressure). The secretion of the adrenal cortex has been demonstrated to influence the work capacity and wet-lung weight of this isolated preparation. The observations are of interest in connection with the well established fact that adrenocortical insufficiency is associated with a hypodynamic state of the cardiovascular system.[1-3]

## METHODS

### *Apparatus*

The isolated rat heart-lung preparation is fundamentally similar to that described by Knowlton and Starling for the dog.[4] An important difference is that the rat heart-lung is an open system and the lungs were not ventilated and remained collapsed during the course of an experiment; blood was oxygenated by passage over a modified Gibbon screen oxygenator,* a device which consists of a 2.5 cm. x 15 cm. x 30 cm. rectangular lucite container in which a stainless steel wire screen hangs in an atmosphere of 95 per cent oxygen, 5 per cent carbon dioxide, and collected at the bottom of the oxygenator which served as the "venous reservoir." A series of preliminary observations demonstrated that with positive pressure respiration the development of pulmonary edema limited the survival of the preparation. Extrapulmonary oxygenation prolonged the survival time significantly and promoted stability of the preparation. Concentrations of arterial and venous oxygen,

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*Aided by a research grant (A–331–C4) from the United States Public Health Service and by a research grant (DA–49–007–MD–990) from the Department of the Army.*

*The authors are indebted for technical assistance to the following High School Research Assistants: Bill Ginsberg, George Nadzam, Bill Sayers, Richard Shaw, and Peter Sindell.*

*One of the authors (N. S.) is a Fellow of the Arthritis and Rheumatism Foundation.*

*This modified Gibbon screen oxygenator was made available to us by Dr. Richard Eckstein.

determined at the mid-point of a typical experiment, were 15 and 12 volumes per cent, respectively.

A polyethylene catheter (1.2 mm. outside diameter) was inserted into the right carotid artery. Blood was conducted through the cathether and connecting tubing to a T-tube, one limb of which was attached to a capillary mercury manometer. The T-tube was succeeded in the circuit by a stopcock which prevented all outflow. Peripheral resistance was adjusted by a modified screw clamp. Blood then entered a bubble flowmeter which drained into the oxygenator.

Blood from the venous reservoir was led through a coil of polyethylene tubing immersed in a thermostatically controlled water bath. A stopcock was provided to prevent all inflow. The circuit was completed by a polyethylene catheter (2.4 mm. outside diameter) inserted into the thoracic inferior vena cava. Right atrial pressure was recorded from a saline manometer connected to the right atrium through the cannulated right superior vena cava (polyethylene cannula = 2.4 mm. outside diameter). Figure 1 shows a diagramatic scheme of the isolated heart-lung, and figure 2 is a photograph of the preparation. The legend to figure 1 supplies additional information concerning the length and diameter of tubing and of cannulas used in the circuit.

## *Animals*

For all experimental procedures white male rats weighing 320 to 370 grams were obtained from the Holtzman Farms and acclimatized for at least one week prior to their use as donor animals, prior to adrenalectomy or prior to sham adrenalectomy. Bilateral adrenalectomy was performed, via the dorsal approach, two weeks before isolation of the heart-lung; all animals were housed in a constant temperature room and fed Purina Laboratory Chow ad libitum. Adrenalectomized animals were maintained postoperatively on 0.9 per cent sodium chloride solution; sham-adrenalectomized animals drank tap water. Completion of adrenalectomy was checked by inspection of the suprarenal area. The anesthesia for the surgical procedures was sodium pentobarbital (40 mg./Kg.) administered intraperitoneally as a 1 per cent aqueous solution.

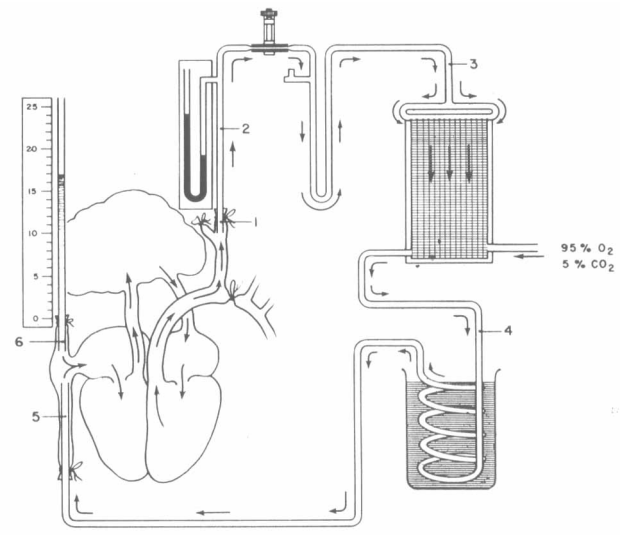


**Fig. 1.—Diagrammatic scheme of rat heart-lung preparation.**

Numbers on drawing correspond to the following dimensions of tubing: (1) Right common carotid artery cannula, polyethylene tubing; 2.0 cm. length, inner diameter (ID) = 0.8 mm., outer diameter (OD) = 1.2 mm. (2) Infusion tubing from right common carotid artery to bubble flowmeter; 58.0 cm. length, ID = 3.0 mm., OD = 4.5 mm. (3) Infusion tubing from bubble flowmeter to screen oxygenator; 16.0 cm. length, ID = 3.0 mm., OD = 4.5 mm. (4) Infusion tubing from screen oxygenator to inferior vena cava catheter; 90 cm. length, ID = 3.0 mm., OD = 4.5 mm. (5) Inferior vena cava catheter; 3.5 cm. length, ID = 1.7 mm., OD = 2.4 mm. (6) Superior vena cava catheter for right atrial pressure; 6.0 cm. length, ID = 1.7 mm., OD = 2.4 mm.

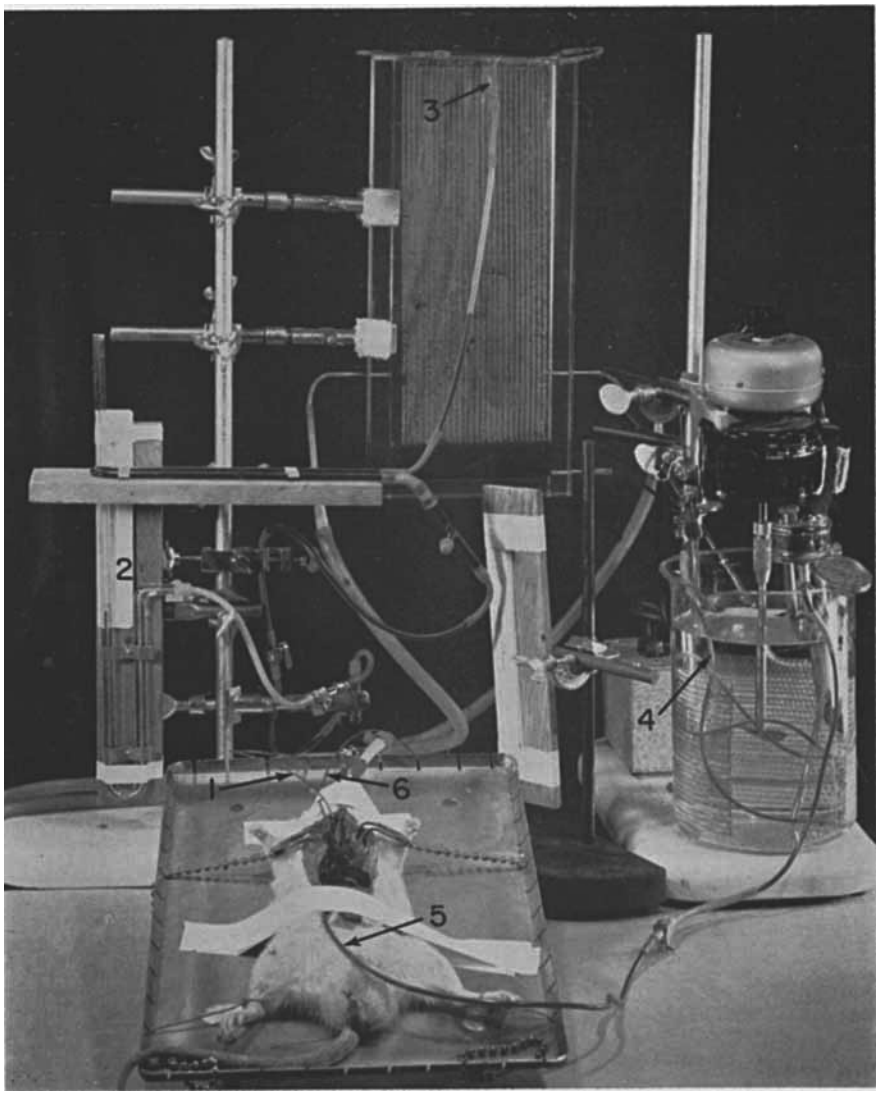


**Fig. 2.—Photograph of an isolated rat heart-lung preparation. Numbers on photograph correspond to numbers on figure 1 drawing.**

*Donor blood.*—Donor perfusion blood was collected through a number 20 gauge needle which was inserted into the abdominal aorta at the bifurcation of the iliac arteries of a heparinized animal which had been acclimatized for one week. The blood was used immediately or stored for less than two hours at 4 C. while other manipulations were in progress.

Depending upon the experiment, 50 or 100 ml. of donor blood was perfused through the isolated heart-lung. When steroids were added to the preparation, the steroid-ethanol solution was pipetted into a 100 ml. beaker and dried under vacuum. The perfusion blood was then added to the beaker, stirred gently, and transferred to the heart-lung preparation. The tubing, the screen oxygenator, the stopcocks, the hypodermic needles and all glassware were siliconized.

*Heart-lung preparation.*—The venous reservoir was charged with either 50 or 100 ml. of

fresh heparinized blood obtained from anesthetized donor animals. An anesthetized experimental animal was secured supine to the animal board. Tracheotomy was performed and artificial respiration started and maintained at a rate of 40 per minute.

A mid-line incision was carried from the symphysis pubis through the manubrium, and the thoracic walls were retracted; with blunt dissection the thymus was exposed. After thymectomy and with a single ligature, the left superior vena cava, the left common carotid and both subclavian arteries were securely ligated. Two loose ligatures were placed under the right common carotid artery high in the neck. Loose ligatures were placed about the thoracic vena cava, the arch of the aorta just distal to the origin of the brachiocephalic artery, and the azygos vein at the level of the fourth rib. Heparin sodium (1.5 mg.) was injected into the left renal vein. The distal ligature under the right common carotid artery and the ligature under the azygos vein were tied. A small artery clamp was placed around the brachiocephalic artery. A 1.2 mm. (outside diameter) catheter was inserted into the right common carotid artery, advanced into the brachiocephalic artery and secured with a ligature. The right superior vena cava was cannulated and the proximal portion of the cannula advanced to the right atrium. The distal portion was connected to a saline manometer for right atrial pressure measurements. Then, in rapid succession (1) a catheter was inserted into the inferior vena cava at the level of the diaphragm, (2) the aortic arch was ligated between the right common carotid and the left carotid artery, (3) the inferior vena cava catheter was secured with a ligature, (4) stopcocks on both inflow and outflow tubing were opened, and (5) positive pressure respiration was terminated.

The procedure must be performed in a relatively uniform manner, in less than ten minutes and with minimal hemorrhage. If surgery was protracted or almost perfect hemostasis not achieved, the preparation was discarded. An initial period, less than one minute, of cardiac arrhythmia and unstable arterial pressure was succeeded by a stable period of variable length. During the stable period, preselected mean arterial pressure was maintained by adjusting the peripheral resistance clamp; cardiac output, mean aterial blood pressure, ECG.,* temperature of venous blood, right atrial pressure were periodically recorded or adjusted. At the end of an experiment wet and dry heart† and lung‡ weights were determined. Heart and lung were dried by desiccating 24 hours over calcium chloride in a 100 C. oven.

*Sequence of experiments.*—The effects of seasonal variation and endemic infections were minimized by performing the experiments in a rotation among the groups to be compared. For example, in an experiment designed to determine the influence of corticosterone ($\triangle^4$-pregnene-11$\beta$, 21-diol-3, 20-dione) in a concentration of 2 $\mu$g. per 100 ml., on the left ventricular work index (LVWI) of the heart-lung from an intact rat perfused with blood from adrenalectomized rats (intact-adrenalectomized) the following sequence was replicated until there were nine preparations in each group: (1) heart-lung from intact rat perfused with blood from intact rats (intact-intact); (2) intact-adrenalectomized; (3) intact-adrenalectomized to which corticosterone was added in a concentration of 2 $\mu$g. per 100 ml.; and (4) intact-adrenalectomized to which tetrahydrocortisol (pregnane-3$\alpha$, 11$\beta$, 17$\alpha$, 21-tetrol-20-one) was added in a concentration of 2 $\mu$g. per 100 ml. In certain instances the sequence was modified as the experiment progressed. In some cases, additional groups were introduced into the sequence; in other cases, other groups were deleted from the sequence. For this reason the "No. preparations" in table 1 varies among the groups.

## *Calculation of Left Ventricular Work Index (LVWI)*

Left ventricular work index is proportional to the sum of the cardiac outputs (C.O.) for each interval times mean arterial pressure, and calculated from the equation: $LVWI = \Sigma (C.O._t)$ (Pressure) (K), in which $C.O._t$ is the cardiac output for a given interval

*Heart rate was calculated from ECG recording.

†Wet-heart weights are not presented because of the large statistical variation. Dry heart weights were not significantly different for the various Groups in table 1.

‡Dry-lung weights were not significantly different for the various Groups in table 1.

**Table 1.—*LVWI and Corresponding Lung Weights of Isolated Heart-Lung**

| Group | Steroid added | Conc. μg. per 100 ml. | Average LVWI (±S.E.) gram-meters per 100 mg. heart | No. preparations | Wet-lung weights (±S.E.) Grams |
|---|---|---|---|---|---|
| 1. Intact-intact† | — | — | 951 (±99) | 12 | 2.07 (±.01) |
| 2. Intact-adrenx‡ | — | — | 491 (±44) | 12 | 3.42 (±.10) |
| 3. Intact-adrenx | B§ | 2 | 892 (±101) | 12 | 2.65 (±.04) |
| 4. Intact-adrenx | B | 10 | 674 (±51) | 9 | 3.04 (±.04) |
| 5. Intact-adrenx | B | 50 | 303 (±32) | 7 | 3.44 (±.05) |
| 6. Intact-adrenx | B | 500 | 116 (±15) | 7 | 3.84 (±.11) |
| 7. Intact-adrenx | THF‖ | 2 | 482 (±42) | 9 | 3.42 (±.08) |
| 8. Intact-adrenx | THF | 10 | 496 (±46) | 7 | 3.32 (±.15) |
| 9. Intact-adrenx | THF | 50 | 271 (±22) | 7 | 3.50 (±.10) |
| 10. Intact-adrenx | THF | 500 | 295 (±25) | 7 | 3.60 (±.13) |

Mean arterial blood pressure, 110 mm. Hg; temperature, 32 C.

†Intact-intact = heart-lung preparation from intact rat perfused with blood from intact rats.

‡Intact-adrenx = heart-lung preparation from intact rat perfused with blood from adrenalectomized rats.

§B = Kendall's compound B, corticosterone ($\triangle^4$-pregnene-11$\beta$,21-diol-3,20-dione).

‖THF = Tetrahydrocortisol (pregnane-3$\alpha$, 11$\beta$, 17$\alpha$, 21-tetraol-20-one).

t; $\Sigma$ ($C.O._t$) is the sum of the cardiac output for the intervals, and represents the cardiac output from the start of the experiment until the preparation was unable to expel blood at the preselected level of mean arterial blood pressure (MABP); MABP* is constant for a given experiment and is expressed in mm. Hg; K, a conversion factor equal to 0.0136, is used to express LVWI as gram-meters of work. LVWI may be expressed as gram-meters per 100 mg. dry heart. (See legend to figure 3 for calculation of LVWI). LVWI does not include that fraction of left ventricular work required to perfuse the coronary arteries or that fraction of left ventricular work required to accelerate the blood in its exit from the left ventricle.† For these reasons the evaluation of the performance of the left ventricular is designated an *index*.

## Results

The results of a number of experiments performed to determine the effect of corticosterone on LVWI and on lung weight are presented in table 1. MABP was 110 mm. Hg and the temperature of the perfusion blood was maintained at 32 C. It is evident from the data that heart-lung preparations from an intact rat perfused with blood from adrenalectomized rats (intact-adrenalectomized) showed a decrease in LVWI, and an increase in wet-lung weight when compared to the intact-intact preparation (cf. groups 1 and 2). It is of further interest to note that the addition of corticosterone to the intact-adrenalectomized preparation in a concentration of only 2 μg. per 100 ml. increased LVWI and decreased lung weight toward intact-intact values (cf. groups 1, 2 and 3). At higher concentrations of corticosterone (10 and 50 μg. per 100 ml.), LVWI for the intact-adrenalectomized preparation decreased and lung weight increased when compared with the intact-adrenalectomized prepara-

*If the resistance clamp was adjusted for a lower mean arterial blood pressure, the preparation would expel blood for an additional period.

†The fraction of left ventricular work required to accelerate the blood in its exit from the left ventricle is less than 0.5 per cent of the total LVWI.

tion to which only 2 μg. corticosterone was added (cf. groups 3 vs. 4 and 5); the addition of corticosterone to the intact-adrenalectomized preparation in a concentration of 500 μg. per 100 ml. had an inhibitory effect on LVWI, and increased lung weight even when compared with the intact-adrenalectomized preparation (cf. groups 2 and 6).

Tetrahydrocortisol added to the intact-adrenalectomized preparation in a concentration of 2 and 10 μg. per 100 ml. had no effect on LVWI, and no effect on lung weight when compared to the intact-adrenalectomized preparation (cf. groups 2 vs. 7 and 8); tetrahydrocortisol added in a concentration of 50 or 500 μg. per 100 ml. exhibited an inhibitory effect on LVWI without showing a significant influence on lung weight when compared to the intact-adrenalectomized preparation (cf. group 2 vs. 9 and 10).

LVWI does not reveal certain characteristics of the rat heart-lung. For that reason, cardiac output against time has been plotted in figure 3 for four typical preparations: intact-intact, intact-adrenalectomized, intact-adrenalectomized plus 2 μg. corticosterone, and intact-adrenalectomized plus 2 μg. tetrahydrocortisol. Initial cardiac output was approximately the same for each preparation (9.5 to 10.0 ml. per minute) and the decrease in LVWI of the intact-adrenalectomized and the intact-adrenalectomized plus 2 μg. tetrahydrocortisol must be ascribed to a decrease in *duration* of function when compared to the intact-intact and the intact-adrenalectomized plus 2 μg. corticosterone. It is of further interest to note that the addition of corticosterone in a concentration of only 2 μg. per 100 ml. prolonged the running time of the intact-adrenalectomized preparation. On the other hand, the addition of the same concentration of tetrahydrocortisol had no effect.

## Discussion

Technics for the preparation of a rat heart-lung have been described by Cruikshank and Kosterlitz,[5] by Malinow et al.,[6] by Begović and Stern,[7] and by Bubnoff et al.[8] For the purpose of their experiments it was not necessary to precisely standardize the rat heart-lung. However, the present study was designed to determine whether an isolated system, namely, the rat heart-lung preparation would respond to the corticosteroids and to establish conditions for a high degree of reproducibility of the function of this isolated system. A degree of standardization[9] has been developed to a point where application to bio-assay of cardiotonic or cardiac inhibitory substances appears feasible.

Drugs, particularly those used in the treatment of hypertension, may have a direct depressant action on the myocardium.[10] The standardized rat heart-lung preparation appears to offer an opportunity to exclude such agents from consideration in the rational approach to treatment of cardiovascular disorders in man.

The following factors which influenced the work performance of the rat heart-lung were standardized:

*Screen oxygenator.*—Early attempts to standardize the rat heart-lung preparation were materially improved when a screen oxygenator was introduced into the circuit. Rat lungs subjected to positive pressure respiration

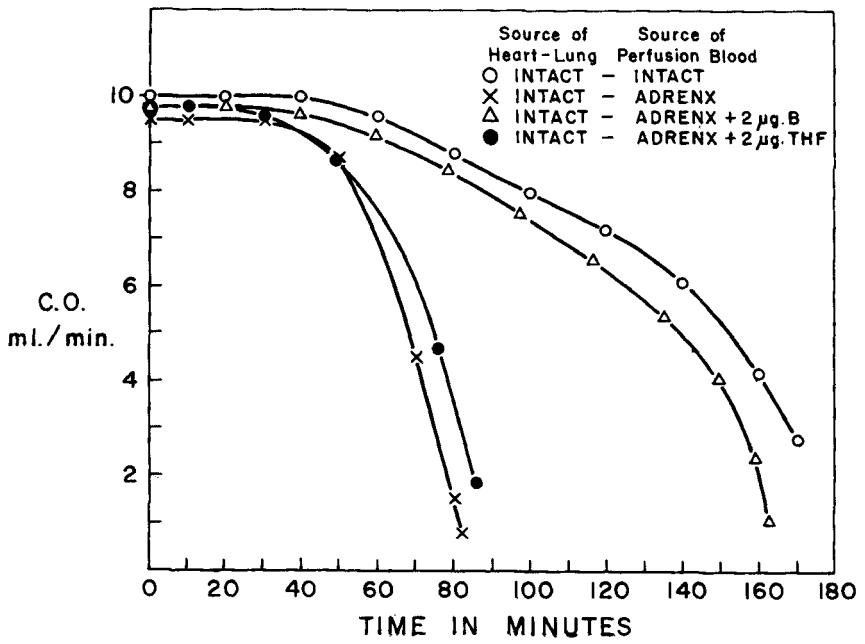


**Fig. 3.—Cardiac output plotted against time for four typical experiments taken from table 1 (groups 1–3 and 7). Mean arterial blood pressure, 110 mm. Hg; temperature of perfusion blood, 32 C. Area under each curve is related by a constant to LVWI and typical calculation taken from group 1 follows:**

| Time (min.) | C.O. (ml./min.) | Average C.O. (ml./min.)* | C.O. (ml.) |
|---|---|---|---|
| 0 | 10 | | |
| 20 | 10 | 10 | 200 |
| 40 | 10 | 10 | 200 |
| 60 | 9.8 | 9.9 | 198 |
| 80 | 9.6 | 9.7 | 194 |
| 100 | 8.4 | 9.0 | 180 |
| 120 | 7.8 | 8.1 | 162 |
| 140 | 6.8 | 7.3 | 146 |
| 160 | 4.6 | 5.7 | 114 |
| 170 | 3.0 | 3.8 | 38 |
| 171 | 0 | | $\Sigma$ (C.O.$_t$) = 1432 ml. |
| 172 | 2.8† | | |

LVWI = $\Sigma$ (C.O.$_t$) (MABP) (K)
= (1432) (110) (0.0136)
= 2142 gram-meters‡

*Average cardiac output is obtained by averaging two successive cardiac output readings.

†Despite maximum tension on the resistance clamp, the mean arterial blood pressure could not be maintained at the preselected level of 110 mm. Hg. Reduction in resistance for a pressure of 80 mm. Hg resulted in continued performance.

‡Dry heart weighed 230 mg. Total LVWI may be expressed as gram-meters per 100 mg. dry heart. In this case LVWI, 2141 gram-meters, times 100/230 = 931 gram-meters per 100 mg. dry heart.

with open chest appear to be particularly prone to develop pulmonary edema.

*Surgical skill.*—The surgical manipulations must be completed with minimum trauma to the heart and with minimum loss of blood. LVWI increases as the operator becomes more skillful. The surgical time required for preparing an isolated heart-lung (from the tracheotomy until completion of surgery) takes a trained operator about 10 minutes.

*The rats.*—No data are available on the influence of the sex, age or dietary history of the rats on LVWI. The present series has been restricted to the type of rats described under Animals.

A series of eleven heart-lung preparations, from rats kept at a relatively high and fluctuating temperature (26.5 to 35 C.) for two weeks, exhibited LVWI significantly less than heart-lung preparations from a similar group of rats kept at an environmental temperature of 25.5 ± 0.6 C. Respiratory infection as judged by sniffles, by rales, and more definitively by hemorrhagic consolidation of the exposed lungs, was associated with a markedly reduced performance of the heart-lung.

*Perfusion blood: volume, "aging."*—The reservoir was charged with 50 ml. or 100 ml. of blood from donor rats. For all experiments in table 1, 50 ml. of donor blood was used. The impression has been gained that the larger volume of blood is associated with increased LVWI. More definitive studies are needed to establish this point. The donor blood was collected from donor rats (at a rate of 2 ml. per minute) and employed immediately after collection or within 2 hours of such. Preliminary studies suggest that storage of perfusion blood overnight at 4 C. significantly reduces the LVWI.

*Toxins.*—An interesting incidental finding is the fact that ethanol (0.08 Gm. per 100 ml.) added to perfusion blood significantly reduces LVWI. For this reason steroids are now introduced into the perfusion blood as described in Methods.

It was of interest to note that corticosterone, a steroid which is biologically active in the whole animal, when added in physiologic concentration[11] to the intact-adrenalectomized preparation, increased LVWI[12] and decreased wet-lung weight, whereas the addition of the same concentration of tetrahydrocortisol, a steroid which is biologically inactive in the whole animal, had no apparent effect on LVWI or on wet-lung weight when compared to the intact-adrenalectomized preparation to which no steroid was added. The mode of action of corticosterone in increasing the LVWI and decreasing wet-lung weight of the heart-lung preparation remains obscure. Actually, the site of action cannot be definitely localized to the myocardium or lung on the basis of the present study. It is possible that corticosterone exerts its effect in whole or in part by way of the lung (data with reference to wet-lung weight are compatible with this possibility). For example, increased resistance to flow through the pulmonary capillaries is a reasonable possibility. However, preliminary studies with an isolated heart preparation (perfusion of the left atrium; pulmonary vessels out of the circuit) suggest that corticosterone has a direct action on the myocardium. This action of corticosterone on increasing

the left ventricular work index can be accomplished in the isolated rat heart perfused with whole blood or with 0.9 per cent sodium chloride solution. This latter action of corticosterone on the isolated rat heart, in the absence of whole blood, clearly shows a direct action of corticosterone on the myocardium.


## Summary

Experimental conditions have been established for the operation of a rat heart-lung preparation which results in a high degree of uniformity of work performance as measured by the left ventricular work index (LVWI) (proportional to the product of cardiac output and mean arterial blood pressure). The effect of steroids on this isolated system have been described.


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