CARDIAC OUTPUT AND REGIONAL BLOOD FLOW IN CONSCIOUS RATS EXPOSED TO ACUTE HYPOXIA

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SUMMARY PAGE*

THE PROBLEM

To determine the effects of hypoxia on cardiac output and fractional distribution of regional blood flow in unrestrained and unanesthetized Sprague-Dawley rats.

FINDINGS

The animals were exposed 18-24 hours after cannulation to atmospheres of air and of 7% and 10% oxygen in nitrogen. Cardiac output as determined by thermodilution techniques was higher in the hypoxia environments. Fractional distribution of blood flow, determined by $Rb^{86}Cl$ and iodoantipyrine^{131} tracers, to the brain, heart, and adrenal was increased, while that to the kidney and spleen was decreased in reduced O_2 environment.

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*The experiments reported herein were conducted according to the principles enunciated in "Guide for Laboratory Animal Facilities and Care" prepared by the Committee on the Guide for Animal Resources, National Academy of Sciences-National Research Council.

INTRODUCTION

It is a widely accepted fact that acute hypoxia increases heart rate and cardiac output (1-5). It may be expected that various organs of the body participate differently in the redistribution of blood flow which follows such increases. Murray and Young (6), using a sine-wave electromagnetic flowmeter, found an increase in blood flow in the head, kidney, and hind limb of anesthetized dogs. No measurements were made of flow to the heart, adrenal, or other organs.

In the present study we applied the $Rb^{86}Cl$ and iodoantipyrine I^{131} indicator techniques (7) to determine the fractional distribution of blood flow in rats exposed to acute hypoxia. Because of the inability of Rb^{86} to pass the blood-brain barrier, I^{131} antipyrine was used to obtain data for the brain (8,9). In view of the known stressful effects of anesthesia and restraint, it is important to study the effects of hypoxic stress in unanesthetized and unrestrained animals.

PROCEDURE

Male Sprague-Dawley rats weighing 210-240 g were anesthetized by intraperitoneal injection of pentobarbital sodium (35 mg/kg). The right jugular vein was cannulated with polyethylene tubing (P.E. 10, Intramedic, Adams) inserted approximately 2.5 cm down into the precava and ligated in position. The free end of the tube was carried under the skin to the nape of the neck and brought to the surface through a small incision. Immediately behind the point of exit, a pack was sutured to the skin of the back to receive the jugular cannula.

The back pack was a lucite rod mounted on a sheet of plexiglass (1.8 cm x 2.8 cm) (Figure 1). This base had small holes on its perimeter through which the sutures passed to anchor it to the skin. When in place, the base of the pack was separated from the skin by a layer of plastic sponge. The lucite rod was drilled to contain a "Y"-shaped canal (Figure 1, inset). The single hole faced toward the head of the animal and had a blunt 27-gauge needle permanently secured in it to receive the jugular cannula. Extending from the opposite end, two lengths of P.E. 60 tubing were bonded to the other two holes. Through this system the isotopes, normal saline, and potassium acetate were delivered to the circulatory system. From the time of surgery until the injection of the indicator these tubes were filled with heparinized saline to prevent the formation of clots.

Electrocardiographic leads were implanted at the time of cannulation and carried under the skin and out by the base of the back pack along with the polyethylene tubes. These two polyethylene tubes and ECG leads were protected from gnawing by the rat by a flexible stainless steel wire coil. The ECG leads were made from fine wire coated with a biologically inert insulation and had a small stainless steel ring welded at the end, for suturing into position. Electrodes were placed subcutaneously at the posterior border of each axilla and at the twelfth ribs. The ECG was recorded on a Brush instrument (Model 200).

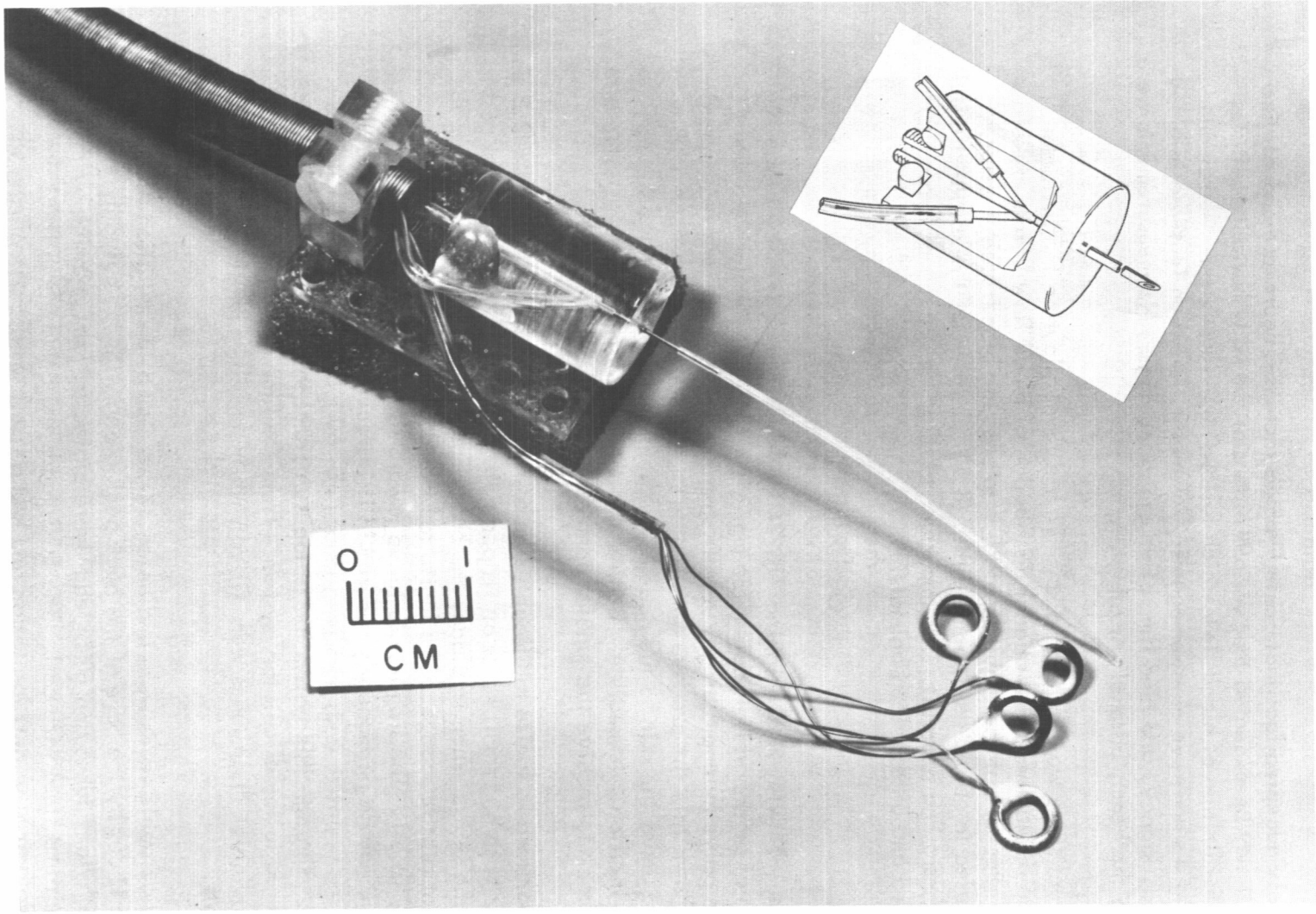

Figure 1

Back-pack for delivering solutions into chronically implanted juglar cannulae. Insert shows detail of the delivery tubes. Rings in foreground are ECG electrodes.

At the end of the operation the animals were injected intraperitoneally with 1 ml/100 g of body weight of a solution containing 0.5% terramycin in 5% glucose. After surgery the rats were placed in lucite metabolism cages (Figure 2). The wire coil carrying the leads and tubing extended out through the top of the cage and were suspended above by thread. This allowed limited movement but did not allow the rats to roll over and twist the tubing or leads. Water was supplied ad libitum, but the rats received no food between anesthesia and the end of the experiment. During the next 18-24 hours a stream of air passed through the cage at a rate sufficient to prevent a rise of environmental temperature.

The four groups of rats were then treated according to the following schedule: One group received 10% oxygen in nitrogen for two hours; a second group received 7% oxygen in nitrogen for two minutes; a third received 7% oxygen in nitrogen for two hours; and the fourth group as controls continued to breathe air. The gas concentration was determined by means of a Beckman oxygen analyzer (Model E2) at the outlet from the cage. Timing commenced when the composition of the exhaust gas from the cage was stable, which occurred after about one minute of flushing.

At the end of the appropriate gassing period a 5-10-μc dose of either $Rb^{86}Cl$ or antipyrine-I^{131} in 0.25 ml of normal saline solution was administered through the indwelling catheter by a calibrated Hamilton gas-tight syringe.* The line was flushed within ten seconds of the administration by 0.25 ml of normal saline and thirty seconds after the isotope dose by a lethal dose of 0.4 ml of potassium acetate (46 g/25 ml distilled water).

The electrocardiogram was monitored to assure that circulation ceased promptly. Circulatory arrest was indicated by the first disturbance of the regular ECG potentials. This occurred about one second after injection of the potassium acetate. Experiments in which carotid blood pressure was recorded showed that the first ECG change was accompanied by a fall of arterial pressure to 20 mm Hg and that any subsequent cardiac action potentials did not produce an arterial pulse.

Selected organs as well as the carcass (cut into six pieces) were placed in 1-1/2-inch deep paper cups and counted in a Nuclear-Chicago Tobor large sample counter. This detector has two opposed 3-inch sodium iodide crystals with a 2-inch separation to insure optimal geometry. The counts are summed and the fractional distribution of the isotope in each organ is determined. The summed counts of different organs and carcass were no less than 90 per cent of the dose injected.

The cardiac output of six unrestrained and conscious Sprague-Dawley rats (weighing 210-230 g) was determined by the thermodilution method (10). A P.E. 10 catheter was inserted through the jugular vein to the right atrium. A P.E. 10 thermistor-tipped

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*Hamilton Company, Box 307, Whittier, Calif. 90608

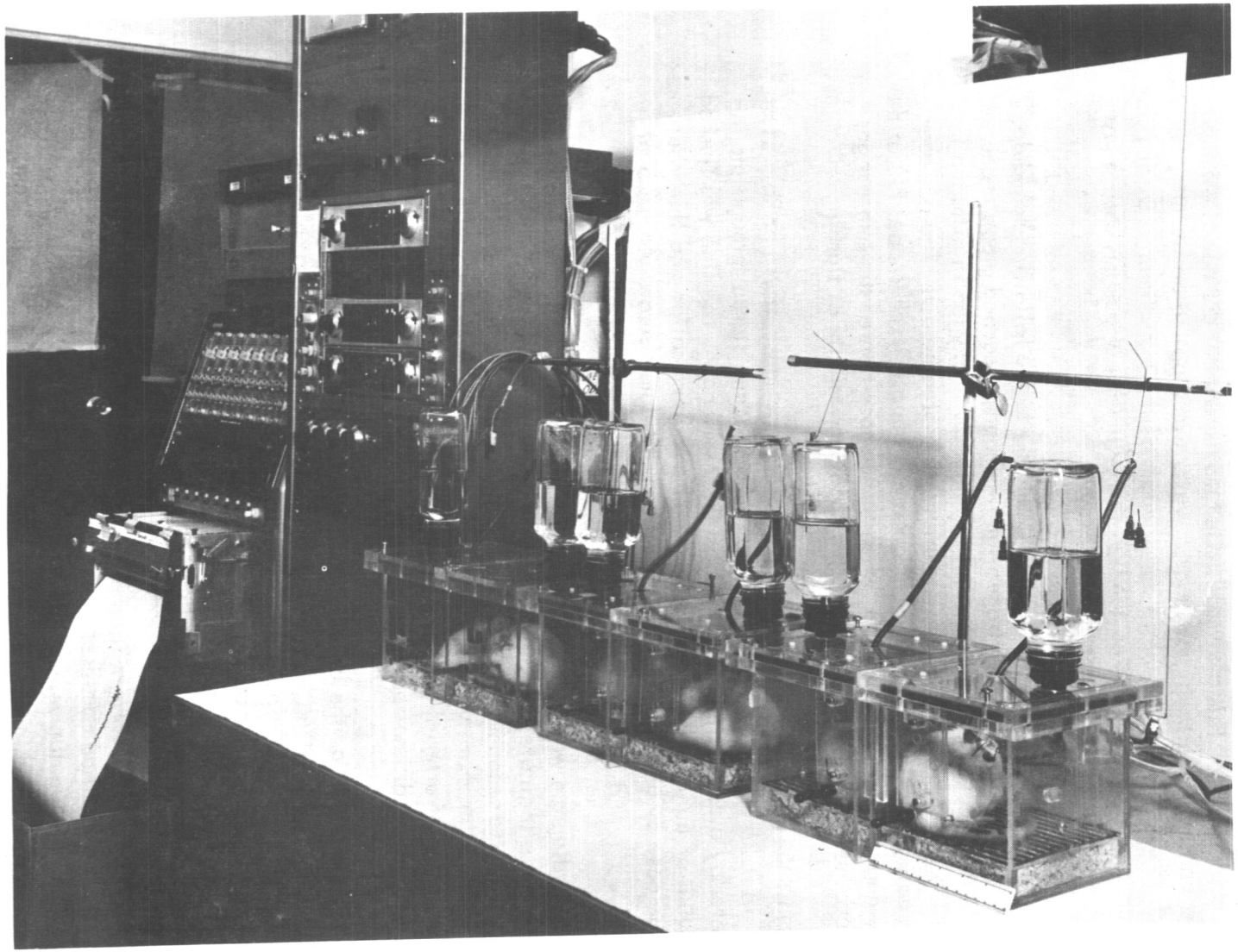


Figure 2

Metabolism cages for controlled oxygen atmospheres prepared for an experiment. Note dark spirals carrying tubing and leads.

radio-opaque tube guided by fluoroscopy was passed through the right carotid artery into the aorta just above the aortic valve. The lucite chamber, shown in Figure 3 (top) mounted on a sheet of plexiglass (1.8 cm x 2.8 cm), was sutured to the back of the animal. A thermistor inside the chamber indicated the temperature of the saline being injected. The saline chamber was surrounded with a second perforated lucite chamber. This latter chamber received a continuous flow of air from the hose line to prevent the rat's body heat from raising the temperature of the saline above 27° C. The anterior end of the saline chamber had a blunt 27-gauge needle permanently secured in it to receive the jugular cannula. A P.E. 60 tube was bonded to the rear end of the saline chamber, and its free end was connected to a 5-ml calibrated Hamilton gas-tight syringe. The syringe was mounted on a Hamilton PB 600 Repeating Dispenser and was driven by four solenoids (Figure 4). Thus, with a remote push button, the syringe injected 0.1 cc of saline.

In determining cardiac output, as in the technique described above, 18-24 hours were allowed for recovery after surgery. Thermodilution curves were obtained at five-minute intervals throughout the experiments. The area under the curve was determined by Gilford dye curve computer Model no. 104. Cardiac output was calculated by using the modified formula of Fegler (11).

RESULTS

REGIONAL DISTRIBUTION OF BLOOD FLOW

Table I shows the fractional distribution of isotope uptake in organs of unanesthetized rats subjected to room air, to 10% oxygen for a period of two hours, and to 7% oxygen for a period of two minutes or two hours. In this group of animals fractional uptake of organs was determined on the basis of total counts of $Rb^{86}Cl$ in the whole body $\left(\frac{\text{organ counts}}{\text{total body counts}} \times 100\right)$. This is related to the fraction of the cardiac output delivered to organ (7).

Table II shows fractional uptake by the brain in another four groups of animals that received iodoantipyrine (I^{131}) instead of Rb^{86}. These groups of animals were subjected to similar atmospheric regimens.

The results indicate that the carcass, liver, lung, and digestive tract respond slightly or not at all to varying respiratory oxygen content.

A 25 per cent decrease in uptake by the spleen was induced by 10% oxygen, and about a 50 per cent decrease was shown in the rats exposed to 7% oxygen either for two minutes or two hours.

Uptake by the kidney did not change significantly after two minutes' exposure of the animals to 7% oxygen. On the other hand, a decline of 19 per cent and 27 per cent was shown in the kidney blood flow of rats subjected to 10% and 7% oxygen, respectively, for two hours.

Table I

Fractional Distribution of Uptake of $Rb^{86}Cl$ by Various Organs in Rats Subjected to Hypoxia

Rb^{86}	No. of Rats	Adrenal Mean S.E.M.	Spleen Mean S.E.M.	Liver Mean S.E.M.	Digestive Track Mean S.E.M.	Kidney Mean S.E.M.	Heart Mean S.E.M.	Lung Mean S.E.M.	Carcass Mean S.E.M.
21% O_2 Control	6	0.13 ± .02	0.95 ± .07	11.46 ± .67	19.09 ± .55	16.72 ± 1.36	2.28 ± .07	3.60 ± .22	45.01 ±1.20
10% O_2 2 hr	13	0.11 ± .01	0.71** ± .05	12.11 ± .46	21.53 ± .71	13.48** ± .65	2.83* ± .19	3.40 ± .30	45.15 ± .91
7% O_2 2 hr	8	0.14 ± .03	0.47*** ± .05	12.04 ± .59	20.66 ± 1.09	12.11** ± 1.12	3.61*** ± .18	4.11 ± .49	46.11 ±1.00
7% O_2 2 min	7	0.18 ± .02	0.48*** ± .06	12.41 ± .58	17.37 ± 2.04	16.03 ± 1.15	3.75*** ± .28	3.99 ± .33	45.64 ±1.92

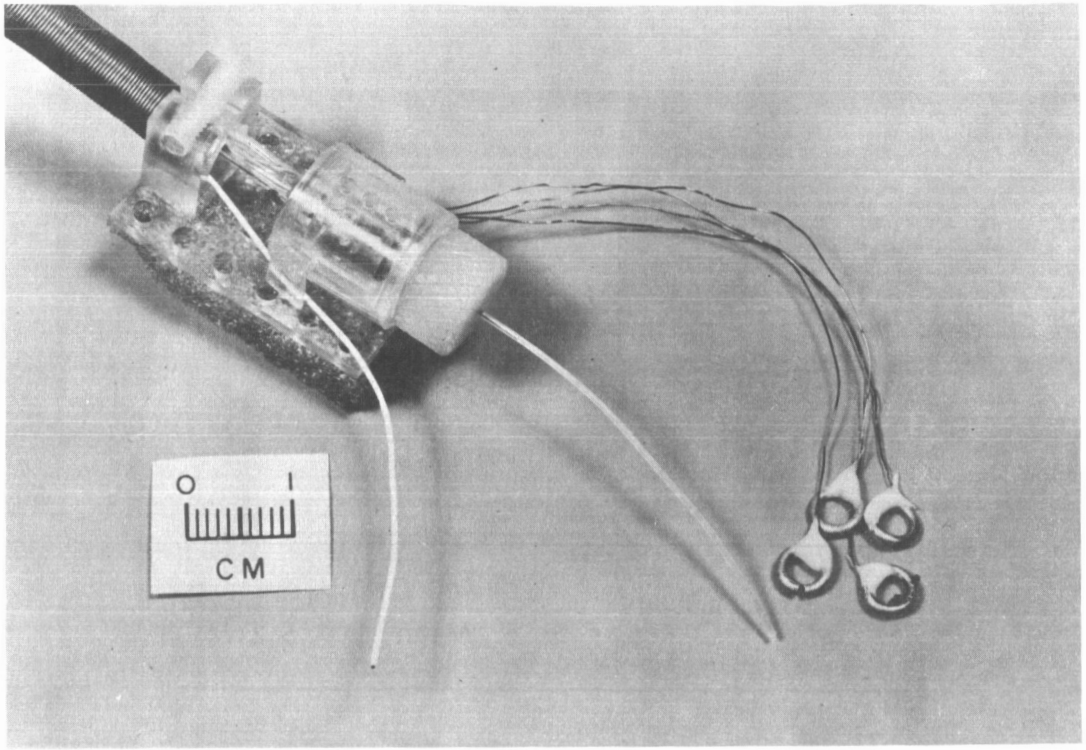
Significance of change from control:
* $P<0.1$
** $P<0.05$
*** $P<0.001$

Table II

Fractional Distribution of Uptake by Brain of ^{131}I Iodoantipyrene

^{131}I	No. of Rats	Brain Mean	S.E.M.
21% O_2 Control	8	2.05	± .21
10% O_2 2 hr	7	2.57*	± .21
7% O_2 2 hr	8	3.17**	± .38
7% O_2 2 Min	8	3.39**	± .55

Significance of change from control:
*P<0.10
**P<0.05



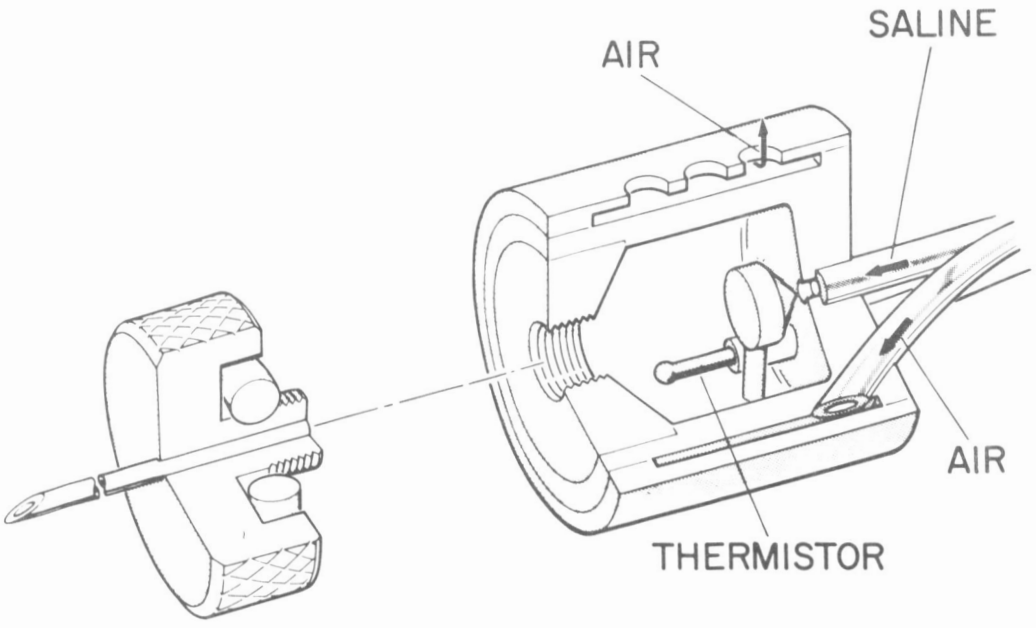


Figure 3

Thermodilution back-pack, Top: The radio-opaque carotid thermistor, the jugular cannula, and the four ECG leads. Bottom: An exploded view of the saline chamber.

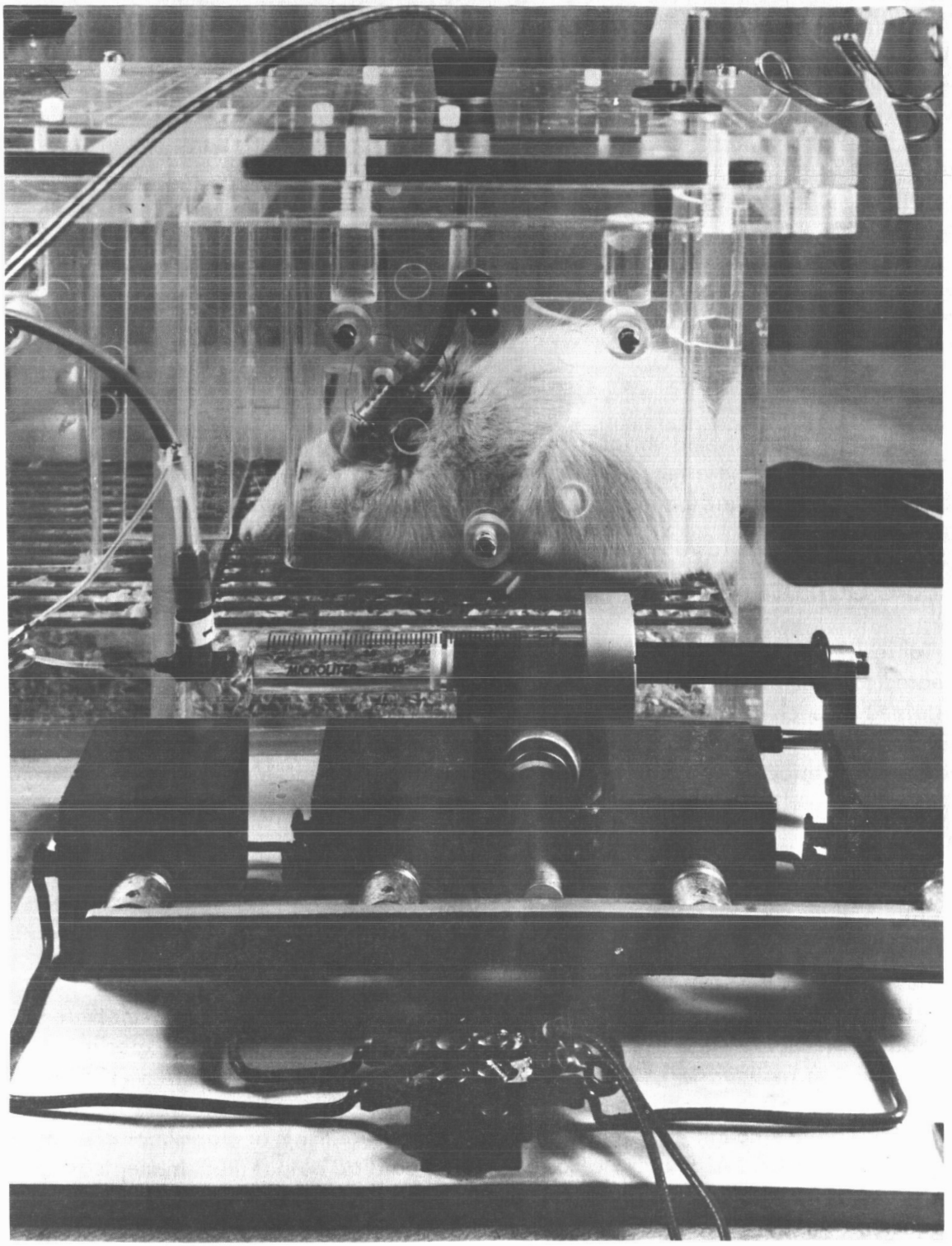


Figure 4

Hamilton repeating dispenser for injection of cooled saline for thermodilution measurements of cardiac output.

uptake by the adrenal glands was not significantly changed. However, the values suggest a possible increase in the adrenal fraction of rats subjected to 7% oxygen for two minutes.

Changes of myocardial uptake of rats under 10% oxygen were barely significant. However, rats subjected to 7% oxygen either for two hours or two minutes showed a more clearly significant and consistent increase (58% and 65%) in myocardial uptake.

There was an increase of 25 per cent in the uptake of antipyrine by the brain of rats under 10% oxygen and a 55 per cent and 65 per cent increase in those exposed to 7% oxygen for two hours or two minutes, respectively.

HEART RATE

Because of the extreme variability in the heart rate from minute to minute and the inconsistency of the effect of low oxygen on the heart rate in different rats, no positive conclusion could be made concerning the effect of low oxygen on the heart rate.

CARDIAC OUTPUT

Table III shows cardiac output of the relatively unrestrained and conscious rats subjected to room air, 10% oxygen, and 7% oxygen, respectively. All rats were maintained at each oxygen level for sixty minutes. Cardiac output was determined at five-minute intervals throughout the three successive hours of the control and experimental periods. For each rat the average of ten to twelve measurements in each period reveals a slight increase in cardiac output in hypoxia.

DISCUSSION

REGIONAL DISTRIBUTION OF BLOOD FLOW

The uptake of the isotopes used in our study is a useful measure of regional distribution of blood flow if the cardiac output is known and if the interval between injection of the isotope and time of sacrifice approaches zero. In these experiments this interval was reduced to half a minute. It has been shown by Sapirstein (12) that any delay between ten seconds and one minute is a sufficient approximation to zero time to give useful though not precise or absolute measurements of fractional distribution of blood flow. Although we do not present cardiac output measurements for any of the rats on which isotope uptake was measured, Table III indicates that our experimental conditions were without major effects on cardiac output. Therefore, our measurements of regional distribution of isotope uptake may be used with reservations as measurements of changes in the distribution of blood flow.

A marked decrease in apparent blood flow to the spleen in rats exposed to hypoxia may indicate contraction of spleen under that condition.

Table III

Mean Cardiac Output of Conscious and Unrestrained Rats Exposed to Successive Variations of Inspired Oxygen*

Rat	Determinations/hr	Cardiac output ml/min Room Air Mean	S.E.M.	10% O_2 Mean	S.E.M.	7% O_2 Mean	S.E.M.
1	10	74	± 1.3	79	± 1.1	80	± 1.6
2	11	70	± 1.2	74	± 1.1	72	± 1.2
3	12	69	± 1.1	75	± 1.2	76	± 1.2
4	12	68	± 1.0	76	± 1.1	74	± 1.1
5	10	60	± 1.0	67	± 1.1	65	± 1.2
6	10	66	± 1.2	73	± 1.1	72	± 1.0
Mean ± S.E.M.		68	± 1.9	74	± 1.6	73	± 2.0

*Each rat was subjected to each of the specified atmospheres for one hour during which 10-12 cardiac output determinations were made.

Two-hour exposure of animals either to 10 or 7% oxygen brought about a decrease in blood flow to the kidneys. These results are in accord with our recent findings in conscious greyhound dogs in a simulated altitude chamber (25,000 ft) showing about 30 per cent reduction in kidney blood flow as determined by the ultrasonic flowmeter (Nejad, unpublished data). The kidney is apparently affected by prolonged exposure but is not affected immediately.

An increase in blood flow to the adrenal glands of rats subjected to hypoxia might occur, since Marks, Battacharya, and Vernikos-Danellis (13) reported a significant increase in the weights of adrenal glands of rats exposed to 10% and 15% oxygen for twenty-four hours or longer. However, our results show no significant change in blood flow to those glands during short-term exposure to hypoxia stress. Goldman (14) using ether as a stressing agent for time periods of up to ten minutes also found no increase in blood flow to the adrenal glands. A change in blood flow to this organ may occur at some period beyond two hours.

The increase in the coronary blood flow and the decreases in the splenic and renal blood flows are consistent with the results of Fowler, Shabetai, and Holmes (15) who found an increase in adrenal medullary output in hypoxia.

The increase in myocardial and brain blood flow under hypoxia demonstrated in the present study has been shown by others (16-18). Blood flow in other organs examined here did not show consistent changes under hypoxic conditions.

CARDIAC OUTPUT

A slight increase was observed in the cardiac output of rats subjected to 10 and 7% oxygen in nitrogen. A variation in cardiac output of each rat was noted in successive determinations at each oxygen concentration. This variation may be due to acutal change of cardiac output at each determination rather than to experimental error. The large change in heart rate observed from moment to moment supports this concept. Cardiac output in hypobaric dogs is commonly associated with heart rate change (18). Moreover, the activity of these unrestrained rats varies from minute to minute, which may account for a variation in cardiac output at each determination.

CONCLUSIONS

The present study indicates that the redistribution of blood flow under hypoxic conditions is more important than an actual increase in cardiac output in supplying the vital organs with oxygen. The stress studied causes blood flow distribution changes which seem well adapted to the preservation of the organs whose functions are constantly needed at the expense of those whose functions may be temporarily suspended without evident harm.

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13. ABSTRACT

The effects of hypoxia on cardiac output and fractional distribution of regional blood flow were measured in unrestrained and unanesthetized Sprague-Dawley rats. Cardiac output was determined by thermodilution techniques, while blood distribution was determined by $Rb^{86}Cl$ and iodoantipyrine I^{131} tracers.

The animals were exposed 18-24 hours after cannulation to atmospheres of air and 7 and 10% oxygen in nitrogen. Cardiac output was higher in the hypoxic environments. Fractional distribution to brain, heart, and adrenal was increased, while that to the kidney and spleen was decreased in reduced O_2 environments.

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