ference by prolactin release *in vivo* and furnishes a homogeneously primed mammary gland *in vitro*. A large stock of donor rats are required, however, to supply animals in metestrus.

In this test, growth hormone gave a positive response in concentrations 100 times higher than does prolactin. This is not surprising since growth hormone and prolactin are both lactogenic, apparently due to a common core of amino acids (like MSH and ACTH), and thus their lactogenic effect can be differentiated only quantitatively.

Summary. The mid-part of the inguinal mammary gland of non-primed virgin, metestric albino rats, weighing 270 ± 10 g, is sensitive to 0.001 I.U./ml of prolactin when cultivated in enriched synthetic medium M-199 for a period of 2 days. This method may replace the standard crop-gland method for prolactin assay and other routine tests for prolactin, because of its reproducibility, high accuracy and specificity. Its quantitative aspects are being worked out.

We wish to thank Dr. A. Danon who advised us during the first steps of this study. Aldosterone was generously supplied by Prof. M. J. Bein of CIBA, Basle. The anterior pituitary tropins tested were obtained from the Endocrinology Study Section of the NIH, Bethesda, Md., by courtesy of Mr. M. M. Graff.

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Simulated Aortic or Pulmonary Stenosis in the Rat.* (32092)

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Research concerning growth failure and other problems of cardiovascular disease has depended mainly upon clinical observations or the use of the dog as an experimental animal. The latter is expensive, particularly if an inbred strain is used and if the survival period is long; also, surgery in this species requires sterile procedures. In view of these considerations our immediate goal was to devise an experimental model of congenital cardiovascular lesions in the rat, which can be used in large numbers, at little expense, and without sterile technique. The lesions selected were aortic and pulmonary stenosis simulated by a banding procedure previously reported in abstract(1). Many investigators have banded, or otherwise narrowed, such vessels. The earlier procedures, based almost entirely on dogs or rarely on pups(2) were

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reviewed by Clatworthy *et al*(3). Smaller species, namely chicks(4) guinea pigs(5,6) and rats(7-9) have also been used; except in the first instance these were adult animals. Since growth is one of our interests we wished to use very young animals; the present report then concerns the effects of banding intrathoracic vessels of weanling rats.

Materials and methods. Bands were made from 22-gauge (0.025″ o.d.) tantalum wire†; this was wound under tension around a core of appropriate diameter to form a helix which, when cut (with a jeweler's saw) parallel to its axis, yielded a number of rings. The saw cut reduced the internal diameter to 0.75 or 1.0 mm for use on the pulmonary artery or aorta, respectively; these dimensions caused slight constriction at the time of application. The rings were smoothed with fine sandpaper under a dissecting microscope and opened slightly to allow placement around the vessel.

The animals were weanling male albino rats of the Wistar strain which at the time of operation weighed 40 ± 8 g. Litter mates were randomly assigned as controls, shams, or animals for aortic or pulmonary banding.

Anesthesia was induced with ether. The trachea was exposed by a midline incision and an endotracheal tube inserted for artificial respiration. Anesthesia was maintained by 20 ml of ether and ethanol (1:1) in a one-quart jar interposed in the air supply tube. The chest was opened at the fourth interspace and the exposed lung packed off with moistened cotton. The connective tissue was penetrated with an aneurysm hook and the vessel could then be readily slipped through the narrow gap left in the ring; the ends were approximated with a hemostat under a dissecting microscope. The lung was re-expanded and the chest closed with three (3-0 silk) sutures around the adjacent ribs; the last was pulled tight at the height of inspiration. No chest tube was necessary. The skin was closed with a continuous suture of the same material. The time from induction of anesthesia to cessation of artificial respiration was less than 30 minutes. Benzathine penicillin G (15,000 units) was given intramuscularly

† Available from Fansteel Metallurgical Co., North Chicago, Ill.

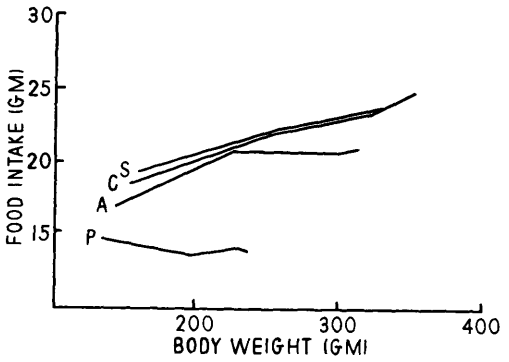

FIG. 1. Regression of mean daily food intake upon body weight in rats with banded aorta or pulmonary artery, and their controls. S, sham-operated; C, control; A, aorta banded; P, pulmonary artery banded.

twice, once at operation and again one month later, to all rats. Sham-operated animals were subjected to the same procedure except that the tantalum ring was not applied. Water and food were allowed *ad libitum*, the latter in the form of ground Purina Laboratory Chow. During the survival period (67 days) food intake was recorded in 46 randomly selected animals (Fig. 1). Body weight of all animals was recorded twice weekly, beginning when all animals had been operated upon.

All animals were killed with ether and the following statistics gathered: body weight, length (tip of snout to anus), and density (body weight/unit volume as determined by displacement of water), length and weight of the dried femur, wet and dry weight of the heart, and wet weight of the major divisions of the heart, separated as by Keen(10). The dissection was done under a stereoscopic microscope. The atria were first removed. The right ventricle was then separated by holding the scissors against the septum and cutting around the margin. The papillary muscles were left attached to the free part of the wall. The free portion of the left ventricular wall was separated in a similar manner. Each part was then blotted and weighed.

The band was removed from the vessel and this region was preserved for histological study. Stains used were H & E and Verhoeff-Van Gieson stain for elastic tissue(11).

Results. Of 141 rats, 113 were operated upon; of these 82% survived the surgery and, of the latter, half survived until the end

TABLE I. Statistical Analysis of Food Intake.

A. Analysis of Covariance				
Source	d.f.	Mean Square	F	P
Sham-operated	93			
Control	81			
Aorta banded	57			
Pulmonary artery banded	61			
Within	292	7.358983		
Regression Coeff.	3	47.786967	6.494	.001
Common	295	7.770115		
Adj. Means	3	407.93589	52.501	<.001
Total	298			
B. Completely Randomized Design (Analysis of final point on each curve)				
Source	d.f.	Mean Square	F	P
Total	42			
Treatments	3	261.11401	34.928	<.001
Within	39	7.4758049		

of the experiment. Cause of death postoperatively included rupture of the banded vessel, hemorrhages in the lung, and infections.

At autopsy the chests of the animals which had been operated upon were relatively free of adhesions. Fluid was found in the chest cavity of the animals with pulmonary bands, and the livers of these animals appeared mottled and deeply colored. No ascites was observed.

The metal band was covered with fibrous tissue; when this was incised, the band could easily be removed. At the junction of the two ends of the band, where erosion was prone to occur, histological sections revealed loss of elastic tissue (and, rarely, calcification) and thickening of the intima.

No differences were observed between controls and sham-operated animals in any respect.

Food intake per unit body weight is shown in Fig. 1 and the statistical analysis of same in Table I. The analysis indicated no difference between shams and controls but a highly significant difference in the food intake of animals with either vessel banded. Duncan's multiple range test, applied to the data contributing to the final point on each curve, indicated that the controls and shams did not differ at the 5% level, but that the animals with banded aorta differed from the shams at the 5% level and those with banded pulmonary artery, at the 1% level. Also, those with banded aorta or pulmonary artery differed from each other at the 1% level.

The growth curves of the 4 groups are shown in Fig. 2. Banding either the aorta or

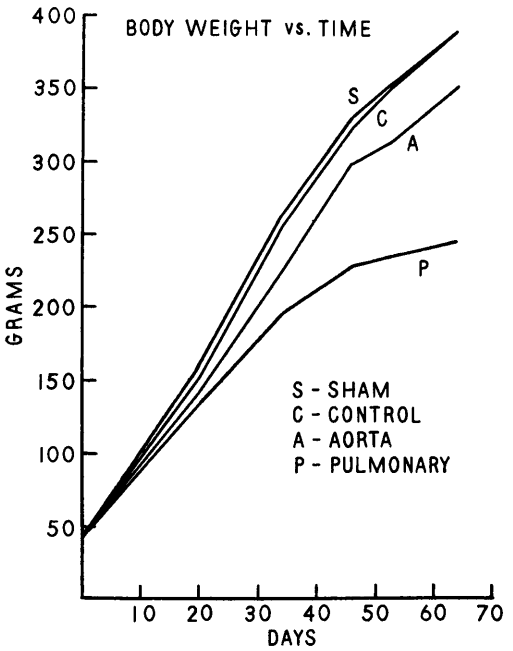

FIG. 2. Regression of mean body weight upon time in rats with banded aorta or pulmonary artery, and their controls. S, sham-operated; C, control; A, aorta banded; P, pulmonary artery banded.

the pulmonary artery reduced the body weight. Body length (Table II) was reduced significantly only by banding the pulmonary artery and body weight was reduced much more in this group than in those with banded aorta.

Femur weight was reduced in both experimental groups (Table II) but the p value relating to the arithmetical reduction in femur length in animals with banded aorta was

TABLE II. Criteria Relating to Growth and Body Composition.

	N	Body weight, final (g)	P	Body length, final (cm)	P
C	23	388 ± 42		23.9 ± .9	
S	25	388 ± 45		23.9 ± .7	
A	16	350 ± 51	<.02	23.6 ± 1.0	<.4
P	13	244 ± 32	<.001	21.6 ± .7	<.001
		Body density	P	Femur length* (cm)	P
C		1.04 ± .02		3.41 ± .01	
S		1.04 ± .01		3.41 ± .01	
A		1.03 ± .02	<.6	3.32 ± .01	<.1
P		1.08 ± .03	<.001	3.09 ± .01	<.001
		Femur weight (gm)	P		
C		.576 ± .004		C = Control	
S		.581 ± .003		S = Sham-operated	
A		.515 ± .004	.01	A = Aorta banded	
P		.408 ± .002	.001	P = Pulmonary banded	

* Measured by vernier calipers from the tibial articular surface of the medial condyle to the most remote point on the head of the femur.

<0.1, compared to <0.001 in those with banded pulmonary artery. Any change in femur weight was reflected in a very similar percentage change in body weight, and similarly in the case of femur and body length. Body density was increased only in the group with banded pulmonary artery.

The ratios of the weight of the heart, and various of its parts, to body weight are given in Table III. Banding in either location caused hypertrophy, whether referred to wet or dry weight. All parts were hypertrophied, to varying degree, by both banding procedures with the exception of the interventricular septum in animals with banded pulmonary artery. Banding the aorta caused a greater relative increase in the weight of the left ventricle and banding the pulmonary artery, a greater increase in the right.

Discussion. The bands at the time of application caused only slight constriction but were intended to produce a relatively greater constriction with time since the vessel could not grow at the region of application.

Banding the pulmonary artery caused more severe stunting than did banding the aorta. Rings smaller than 1.0 mm on the aorta promptly killed the animals. Since the band on the aorta could not be further reduced without killing the animal it would seem that initially the embarrassment to the organism was at least equal to that caused by banding the pulmonary artery. Since the stunting, changes in bone, etc., were greater in the "pulmonary" group some additional factor must have operated. The fluid found in the chest of these animals, as it is in patients with right ventricular failure(12), and the abnormal appearance of the liver suggest congestive heart failure and this may have contributed to quantitative differences. We can not exclude the possibility of heart failure in the group with banded aorta but if such obtained it would seem to be of lesser degree.

The data concerning food consumption show that banding the pulmonary artery, or, to a lesser degree, banding the aorta, altered the relation between food intake and body weight; the intake, which is low in absolute terms, failed to increase appropriately with increasing weight. Growth failure then was associated with reduced food intake, but this in turn must have been a consequence of the banding procedure or its resultant effects, such as heart failure, since no effect was seen in the sham-operated animals.

With regard to the specific changes in various groups one may first note that banding the aorta was associated with a significant decrease in body weight, but not length (and also weight, but not length, of femur) and yet density was unchaanged; presumably both lean body mass and fat were reduced. Density was increased in the animals with banded pulmonary artery in spite of accumulation of edema fluid in at least one area—the

TABLE III. Heart Weight, Whole and Fractional, as a Ratio of Body Weight (mg/g).

	Whole heart (dry)	Whole heart (wet)	Right ventricle (wet)
C	.60 ± .04	3.03 ± .46	.51 ± .05
S	.58 ± .04	2.97 ± .17	.52 ± .07
A	.99 ± .16†	5.01 ± .94*	.69 ± .15*
P	1.03 ± .13†	5.12 ± .60*	1.24 ± .21*
	Left ventricle (wet)	Inter-ventricular septum	
C	1.45 ± .12	.51 ± .06	
S	1.43 ± .18	.50 ± .06	
A	2.37 ± .27*	.82 ± .14*	
P	2.00 ± .25*	.58 ± .10‡	

* $p < .001$ † $p < .01$ ‡ $p < .05$

C, Control; S, Sham-operated; A, Aorta banded; P, Pulmonary artery banded.

thorax; presumably a disproportionate loss of fat occurred in these animals.

The chests of the operated and sham-operated animals at autopsy were relatively free of adhesions. It seems feasible to us to remove the bands from living animals; therefore our method may be useful for various studies before, during, and after vascular obstruction.

In view of the choice of experimental animal the method may prove especially useful for studies of endocrine function, cardiac hypertrophy, metabolic balance, etc., in which the expense of a larger animal might be prohibitive.

Summary. We have demonstrated the feasibility of banding intrathoracic vessels in the weanling rat, with survival of an acceptable proportion of the animals for 9 weeks. The vascular obstruction resulted in reduced food intake, growth failure, cardiac hypertrophy, and other changes indicative of cardiac failure.

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Volume Distribution and Separation of Normal Human Leucocytes.* (32093)

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The volume distribution of leucocytes of normal adults has been measured with a modified Coulter counter described below. This distribution curve has been investigated with the electronic cell separator described by Fulwyler(1). Cells from the separated fractions were identified and counted using leucocyte differential staining procedures. These methods can be extended to separation of different classes of white blood cells for metabolic and other studies in both health and disease.

Materials and methods. The electronic equipment for determining cell volume distribution uses a modified Coulter counter sample stand,† a specially designed aperture 75 μ diameter and 250 μ long, an aperture current supply set normally at 100 μamp, a transistorized low-input impedance amplifier‡ (integrating time 5 μsec, differentiating time 50 μsec, maximum gain 10^4), and an RIDL Model 34-12 400-channel analyzer§ with input modified to accept the relatively slow pulses from the Coulter aperture. The purpose of the low-input impedance is to make the pulse amplitude insensitive to diluent conductivity. The analyzer modification consists of increasing the input RC coupling time constants and duration of the linear gate which admits the pulse to the analog-digital converter, to accommodate the longer pulses (25-

* This work was performed under the auspices of U. S. Atomic Energy Commission.

† Coulter Electronic Sales Co., Hialeah, Fla.

‡ Designed at Los Alamos Scientific Laboratory; details available on request.

§ Radiation Instrument Development Laboratory, Melrose Park, Ill.