TABLE II. Average Leucocyte Ratios.

Skin test	No.	Wright's PMN/lymph before	after	PMN % degran
+	(10)	1.56	1.39	33
		Difference –.17		
—	(8)	1.52	.98	38
		Difference –.54		

leucocytes accompanying phagocytosis has been described(7), the per cent degranulation was estimated from counts of Wright's stained blood smears made upon completion of the Warburg run. There is very little difference between positive skin reactors and negative skin reactors in this respect (Table II). Under the conditions described, no evidence of increased phagocytosis in positive skin reactors was obtained.

Comparable observations on a duplicate set of blood smears using the fluorochrome Acridine Orange according to the method of Bertalanffy(6), corroborated the Wright's stain observations with respect to leucocyte ratios. Degranulation of polymorphonuclear leucocytes could not be estimated because the granules do not take up the fluorochrome. No attachment of coccidioidin to either polymorphonuclear leucocytes or mononuclear cells was evident.

Summary. Results of Warburg studies on 22 whole blood samples from positive skin reactors and 16 whole blood samples from negative skin reactors indicate a transient small but statistically significant increase in oxygen consumption upon *in vitro* addition of Smith's Lot 64D4 coccidioidin.

Studies of blood leucocytes before and after addition of coccidioidin using both the fluorochrome Acridine Orange and Wright's staining technic show no differences attributable to the coccidioidin addition or negative or positive skin reaction.

Technical assistance of Mrs. E. C. Brodie, Mr. W. E. Elsesser, Mrs. L. N. Peck, and Mrs. I. B. Snow, is gratefully acknowledged.

1. Cohn, Z. A., Morse, S. I., *J. Exp. Med.*, 1960, v111, 667.

2. Sbarra, A. J., Karnovsky, M. L., *J. Biol. Chem.*, 1959, v234, 1355.

3. Krenis, L. J., Strauss, B., PROC. SOC. EXP. BIOL. AND MED., 1961, v107, 748.

4. Strauss, B., Stetson, C. A., *J. Exp. Med.*, 1960, v112, 654.

5. Rapaport, F. T., Lawrence, H. S., Millar, J. W., Pappagianis, D., Smith, C. E., *J. Immunol.*, 1960, v84, 358.

6. Bertalanffy, F. D., *CA Bull. Cancer Prog.*, 1960, v10, 118.

7. Hirsch, J. G., Cohn, Z. A., *J. Exp. Med.*, 1960, v112, 1005.

Received April 22, 1963. P.S.E.B.M., 1963, v113.

Action of Pentolinium on Cardiac Output and Other Hemodynamic Parameters in the Rat.* (28454)

RONALD VANDEN BRINK, RAMON ROSAS†, PEDRO BLAQUIER‡, and DAVID F. BOHR
(Introduced by J. M. Weller)
Department of Physiology, University of Michigan, Ann Arbor.

There have been various suggestions as to the mechanism by which ganglionic blocking agents lower blood pressure. Some investigators have found that cardiac output remains unchanged after ganglionic blockade and, therefore, attribute blood pressure reduction to reduced total peripheral resistance. Others have shown that cardiac output decreases markedly, with total peripheral resistance remaining unchanged or being increased(1). Of those who find a reduced cardiac output, some consider this to be a result of reduced venous return(2-5), others believe

* This work was supported by grant from Nat. Inst. of Health, U.S.P.H.S.

† Present address: Lab. de Fisiol., Esceula de Med., Univ. Catolica de Chile, Santiago.

‡ Present address: Centro de Invest. Cardiol. Med. School, Univ. of Buenos Aires, Arg.

there is a direct depression of cardiac function(6).

Species differences in reaction to ganglionic blocking agents have been demonstrated (2,8,9), and this may account for some of the lack of agreement in the literature. Because ganglionic blocking agents are used widely in cardio-vascular experiments involving the rat, more exact knowledge of the action of these drugs in this species is important. In the current study the relative action of pentolinium on cardiac output and total peripheral resistance in this species was determined. To our knowledge this problem has not been investigated previously in the rat.

A by-product of this study was the development of a technique by which cardiac output, total peripheral resistance, mean circulation time, central blood volume and total blood volume can be determined, simultaneously, for a rat, before and after ganglionic blockade. Measurement of these hemodynamic parameters in one animal, pre- and post-blockade, should be more meaningful than similar measurements in comparable, but not identical, animals.

Methods. Sprague-Dawley rats (160-290 g) were prepared by anesthetization (Na pentobarbital, 35 mg/kg, i.p.), heparinization (10 mg/kg, i.v.) and tracheotomy. The femoral vein was cannulated with 3 cm polyethylene tubing (i.d. 0.011", o.d. 0.024"), for injections. The femoral artery was cannulated with polyethylene tubing (i.d. 0.023", o.d. 0.038"); the length of the cannula was adjusted to arterial pressure (1 cm of tubing for each 10 mm Hg) to insure a constant sampling flow. Mean arterial pressure was measured by the same cannula, using a mercury manometer. The technique used for cardiac output determination was, essentially, the dilution method of Hamilton *et al.*(10), as modified by Sapirstein(11) for use in the rat. Details of the procedure are illustrated in Fig. 1. In preliminary experiments double cardiac output determinations were performed using simultaneously injected RISA (I^{131} labeled human serum albumin) and Rb^{86}. Cardiac output values obtained with Rb^{86} were slightly higher than values obtained with RISA; this is compatible with a partial diffusion of Rb^{86} and other ionic isotopes from the pulmonary circulation(12). RISA was, therefore, selected as the indicator to be used.

A standard reference solution was prepared for each shipment of RISA. Since RISA partially precipitates upon standing, solutions were well mixed before use. When a cardiac output determination was to be made, RISA (0.2 g in 0.2 ml, averaging 4 μc) was injected rapidly into the femoral vein and blood sampling was begun simultaneously. Approximately 20 samples were collected in successive depressions in a Plexiglass disc, revolving at the rate of 157 rpm 5.5 μl blood were transferred by pipette (Drummond "Microcaps," 10 μl) into a glass analyzer tube and gamma emissions were counted in a well scintillation counter.

Counts per minute were plotted on semilogarithmic paper and the descending limb of the curve extrapolated to the baseline, eliminating effects of recirculation. The area under the curve was used to calculate cardiac output (CO) using the modification of the Stewart-Hamilton formula described by McIntyre, *et al.*(13):

$$CO = \frac{\#\text{ samples/min} \times \text{wt of RISA inj} \times \text{dilution of std} \times \#\text{ counts/min in std}}{\text{Area curve} \times \text{wt of std RISA}}.$$

Area of curve is represented by the sum of the counts/min (cpm) for each sample point.

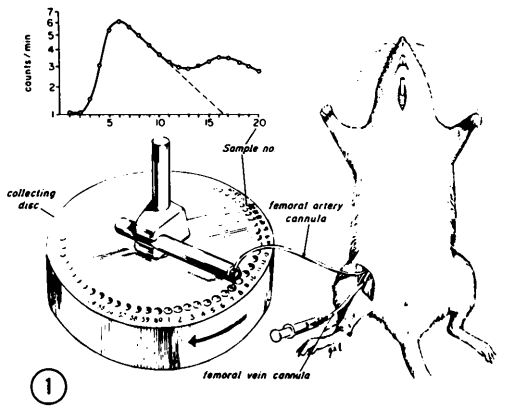
Using values determined for cardiac output and mean arterial pressure (MAP), total peripheral resistance (TPR) was calculated:

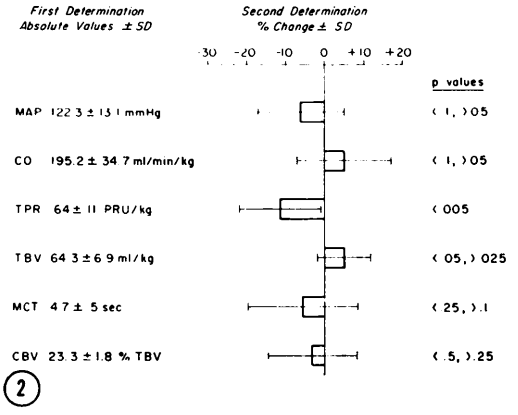
$$TPR = \frac{MAP}{CO/kg}.$$

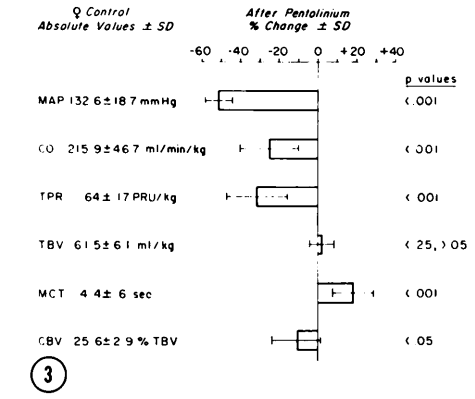
Three additional blood samples were obtained 10 minutes after the initial injection, for determination of total blood volume (TBV) (14):

$$TBV = \frac{\text{Wt RISA inj} \times \text{dilution of std} \times \text{cpm std}}{\text{Wt RISA (std)} \times \text{cpm sample}}.$$

Blood loss was determined by weighing the rat before and after bleeding. There was an average loss of 1.0 ml (approximately .6 ml







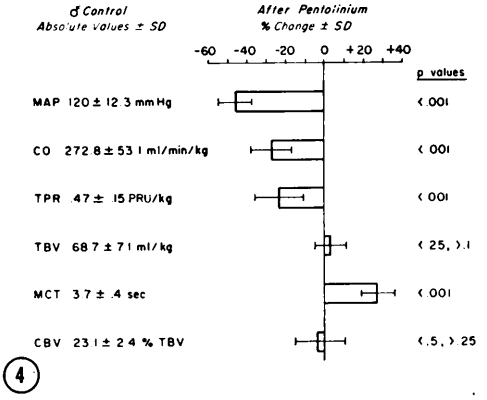


FIG. 1. Procedure for measuring cardiac output by RISA indicator dilution method. Upper part of figure illustrates a typical dilution curve as plotted on semi-logarithmic paper.

FIG. 2. Comparison of 2 consecutive sets of hemodynamic measurements in 11 normal rats: mean arterial pressure (MAP), cardiac output (CO), total peripheral resistance (TPR), total blood volume (TBV), mean circulation time (MCT) and central blood volume (CBV). Bars represent percent change between first and second determinations.

FIG. 3. Hemodynamic measurements in 14 female rats before and after pentolinium blockade. Bars represent percent change after blockade. Abbreviations as in Fig. 2.

FIG. 4. Hemodynamic measurements in 12 male rats before and after pentolinium blockade. Bars represent percent change after blockade. Abbreviations as in Fig. 2.

during CO determination); this was replaced by blood from a donor rat.

In the control series the MAP, CO and TBV were again determined 30 minutes after the blood transfusion. In the experimental series animals were given pentolinium bitartrate (5 mg/kg i.v.) after blood replacement. After the fall in arterial pressure due to the drug had reached its maximum, the series of measurements was repeated. Several blood samples were obtained just prior to injection of the second dose of RISA so that the radiation background of the blood was known.

From the data obtained, 2 additional parameters were calculated, using the following formulas(15):

$$\text{Mean circulation time (MCT)} = \frac{\Sigma\,(c \times t)}{\Sigma^{c}},$$

where c = counts/min at the time t, and t = time interval from time of injection to time at which concentration was measured.

This represents the average time required for a particle of indicator to pass from injection site to sampling site.

$$\text{Central blood volume (CBV)} = \frac{\text{MCT} \times \text{CO}}{60}.$$

This represents the volume of the vascular system between injection and collection points.

Results. Comparison of consecutive hemodynamic measurements in normal rats. Comparison of values obtained from 2 CO determinations on 11 female rats showed no statistically significant differences between first and second sets of determinations (Fig. 2). MAP decreased 6.2%, CO increased 4.9%, TPR decreased 11.0%, TBV increased 4.8%, MCT decreased 5.6% and CBV decreased 3.1%. Only the TPR measurements were significantly different. No reason for this apparent change is evident. It is of small magnitude when compared with changes occurring after ganglionic blockade.

These results indicate sufficient correlation between consecutive determinations in the normal condition to render meaningful any changes observed after introduction of a variable, such as ganglionic blockade, between determinations.

Hemodynamic effects of ganglionic blockade in the female rat. In 14 female rats (Fig. 3) the average fall in MAP after pentolinium blockade was 51.2%, CO decreased 24.9% and TPR decreased 31.2%. Apparently reduction in CO and TPR are of about equal importance in the acute lowering of arterial pressure by pentolinium. TBV increased 2.5% and CBV decreased 10.4%. MCT increased (18.6%) as would be expected when CO decreases and TBV remains constant.

Hemodynamic effects of ganglionic blockade in the male rat. In 12 male rats (Fig. 4) the average fall in MAP was 46.0%, CO decreased 27.6%, TPR decreased 23.8%, TBV increased 2.8%, MCT increased 26.8% and CBV decreased 3.3%. As in the female rat, reduction in CO and in TPR seem to be of equal importance in the acute lowering of blood pressure by pentolinium. TBV and CBV are not significantly changed and MCT is increased.

In this group of animals the CO of the male rat was significantly greater ($p < .001$) than that of the female. This finding was unexpected and remains unexplained.

Discussion. Values for CO (228.2 ml/kg) obtained using RISA as an indicator are in good agreement with those obtained by other techniques(16-19).

The value of 65 ml/kg for total blood volume of the rat is somewhat higher than that generally reported(20); the average of the latter is about 53 ml/kg, but there is much variation between series. The hematocrit of the arterial blood samples used to determine total blood volume may be higher than the total body hematocrit, resulting in a falsely high value for blood volume. The same error, however, would be present with most dilution techniques.

Central blood volume after pentolinium blockade was found to be lower in both male and female rats, but the change was significant ($p < 0.01$) only in females. Acute studies with ganglionic blockers in other species have shown significant reduction in central blood volume(7,21). It has been demonstrated that catheter sampling systems may produce errors in values obtained for mean circulation time and central blood volume(22). Such errors may have produced an increased standard deviation in our results and thus prevented them from showing statistical significance.

The mechanism by which blood pressure of the rat is reduced by pentolinium seems to be a combination of diminished peripheral resistance and reduced cardiac output. These two factors appear to play approximately equal parts, since each is reduced by approximately the same percentage. The reduction of cardiac output could be due either to a decrease in sympathetic support of the heart or to a failure in venous return; the observed decrease in central blood volume suggests that the latter is the more important in the rat. It is reasonable to assume that the fall in TPR is due to a decrease in sympathetic vasoconstrictor tone. There was no demonstrable difference in the mechanism of action of this drug between the male and female rat. These results represent the acute action of pentolinium blockade, *i.e.,* 10 to 15 minutes after intravenous administration, and do not necessarily represent the hemodynamic situation in the rat following chronic administration. It should be noted that the pentolinium blocked rat, which is so frequently used in the assay of pressor agents, especially angiotensin, is actually an animal with lower cardiac output and lower peripheral

resistance than the normal rat. These hemodynamic changes should be considered in interpreting results of experiments in which such animals are used.

Summary. A technique was developed by means of which determinations of cardiac output, total peripheral resistance, mean circulation time, total blood volume and central blood volume can be repeated in a given rat. The above hemodynamic parameters were determined before and after ganglionic blockade with pentolinium in both male and female rats. Blood pressure fell approximately 50% after blockade; the responsibility for the reduction being equally divided between fall in cardiac output and reduced total peripheral resistance. Total blood volume was not significantly changed and central blood volume decreased slightly. Mean circulation time was increased after blockade. There was no apparent difference between male and female rats in their response to blockade.

We are indebted to Dr. Ruth McVaugh for editing this manuscript and to Miss Paula Levy for technical assistance.

1. Aviado, D. M., *Circ. Res.*, 1960, v8, 304.
2. Combes, B., Preedy, J. R. K., Wheeler, H. O., Hays, R. M., Bradley, S. E., *J. Clin. Invest.*, 1957, v36, 860.
3. Freis, E. D., Rose, J. C., Partenope, E. A., Higgins, T. F., Kelly, R. T., Schnaper, H. W., Johnson, R. L., *ibid.*, 1953, v32, 1285.
4. Crosley, A. P., Jr., Brown, J. F., Tuchman, H., Crumpton, C. W., Huston, J. H., Rowe, G. G., *Circulation,* 1956, v14, 584.
5. Grob, D., Scarborough, W. R., Kattus, A. A., Jr., Langford, H. E., *ibid.*, 1953, v8, 352.
6. Beck, L., *ibid.*, 1958, v17, 798.
7. Smith, J. R., Hoobler, S. W., *ibid.*, 1956, v14, 1061.
8. Haas, E., Goldblatt, H., *Am. J. Physiol.*, 1959, v197, 1303.
9. Haas, E., Goldblatt, H., *ibid.*, 1959, v196, 763.
10. Hamilton, W. F., Moore, J. W., Kinsman, J. M., Spurling, R. G., *ibid.*, 1928, v85, 377.
11. Sapirstein, L. A., *ibid.*, 1958, v193, 161.
12. Yudilevich, D., *Circ. Res.*, 1961, v9, 925.
13. McIntyre, W. J., Pritchard, W. H., Eckstein, R. W., Friedell, M. L., *Circulation,* 1951, v4, 552.
14. Aust, J. B., Chon, S. N., Marvin, J. F., Brachney, E. L., Moore, G. E., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 514.
15. Doyle, J. T., Wilson, J. S., Lepine, C., Warren, J. V., *J. Lab. and Clin. Med.*, 1953, v41, 29.
16. Bullard, R. W., *Fed. Proc.*, 1956, v15, 28.
17. Beznak, M., *Circ. Res.*, 1958, v6, 207.
18. Ledinham, J. M., Cohen, R. D., *Clin. Sci.*, 1962, v22, 69.
19. Blood, F. R., Smith, D. L., D'Armour, F. E., *Am. J. Physiol.*, 1950, v163, 268.
20. Altman, P. L., in: *Blood and Other Body Fluids,* Ed., Dittmer, P. S., *Fed. Am. Soc. for Exp. Biol.*, Washington, D.C., 1961, p5.
21. Werko, L., Frisk, A. R., Wade, G., Eliasch, H., *Lancet,* 1951, v2, 470.
22. Lacy, W. W., Emanuel, R. W., Newman, E. V., *Circ. Res.*, 1957, v5, 568.

Received April 25, 1963. P.S.E.B.M., 1963, v113.

A Hog Cholera Virus-Fluorescent Antibody System. Its Potential Use in Study of Embryonic Infection. (28455)

ERNEST L. STAIR, MARVIN B. RHODES, JOHN M. AIKEN, NORMAN R. UNDERDAHL and GEORGE A. YOUNG*
Department of Veterinary Science, University of Nebraska, Lincoln

A new concept in virus-host relationships was introduced by Gregg(1) who concluded that rubella virus which attacked the mother in the first third of gestation could adversely affect the developing embryo. Although many later reports have appeared to support the general virus concept of Gregg, they are also based largely on circumstantial evidence. Experimental approaches to production of malformed fetuses in animals have failed to clarify basic mechanisms of damage to embryonic and fetal tissues. Rhodes(2) and

* Published with the approval of the director as paper No. 1375 Journal Series Nebraska Agri. Exp. Station.

Aided by a grant from the National Foundation.