served delayed hypersusceptibility to pentobarbital is the result of an increase in the sensitivity of the central neuronal systems responsible for the anesthetic effects of the drug. These results, of course, do not rule out other possible mechanisms of hypersensitivity, such as alterations in rate of permeation of the blood-brain barrier by pentobarbital.

Summary. Rats receiving a fixed anesthetic dose of pentobarbital developed hypersensitivity to the drug 28 days following tolerance induction. This delayed increase in sleeping time was non-significant in the male, but significant in the female animal ($P = 0.05$). Tolerant female rats showed an increase in liver weight (as % body weight) and an enhanced rate of disappearance of barbiturate from blood. Hypersensitive female rats failed to exhibit a significant change either in liver weight on in rate of decrease of barbiturate blood level compared to naive animals. Delayed hypersensitivity to pentobarbital in the female rat appears, therefore, to be unrelated to alternations in hepatic metabolizing enzyme activity.

1. Moir, W. M., J. Pharmacol. Exp. Therap., 1937, v59, 68.

2. Aston, R., ibid., 1965, v150, 253.

3. Conney, A. H., Burns, J. J., Adv. in Pharmacol., 1962, v1, 31.

4. Remmer, H., in Ciba Foundation Symposium on Enzymes and Drug Action, J. L. Mongar, A. V. S. de Reuck, eds., Little, Brown & Co., Boston, 1962, p276.

5. Williams, L. A., Zak, B., Clin. Chimica Acta, 1959, v4, 170.

6. Burn, J. H., Finney, D. J., Goodwin, L. G., Biological Standardization, Oxford Univ. Press, London, 1950.

Received October 4, 1965. P.S.E.B.M., 1966, v121.

Response of Heart Rate, Oxygen Consumption, and Arterial Blood Pressure to Graded Exercise in Dogs.* (30847)

DAVID E. DONALD AND DAVID FERGUSON

Mayo Clinic and Mayo Foundation, Section of Surgical Research, Rochester, Minn.

In man the relationship of heart rate to oxygen consumption during exercise is sufficiently direct that an increase in heart rate may be used as an objective measure of an increase in work load(1). It would be of value to know if the same direct relationship obtained in the dog since heart rate can be measured easily and without disturbing the animal. In addition, since there are few reports in the literature on the effect of graded exercise on arterial blood pressure in the dog, it was thought worthwhile to attempt this measurement in a reasonably large number of animals.

Methods. Dogs were trained to exercise on the treadmill until they were thoroughly accustomed to the procedure and would run on the moving platform without being attached by a leash to the apparatus. A complete exercise study consisted of a continuous run at grades of 0, 7, 14, 21, and 28% at 5.5 km/hr, for a period of 5 minutes at each work level. Measurements of heart rate, oxygen consumption, and aortic blood pressure were made during the period of exercise.

Heart rate was recorded at rest, throughout exercise, and during recovery by a direct-writing electrocardiograph coupled to an anterior-posterior lead.

Oxygen consumption was determined by collection and analysis of expired air. A permanent tracheostomy(2) was constructed to permit insertion into the trachea of a cuffed piece of Tygon tubing (⅜ to ½ inch I.D.) attached to a glass U tube bearing inlet and outlet valves (Collins J type valve). The U tube fitted snugly round the dog's neck and the unit was carried easily by the dog. The volume of air expired into a latex

* This investigation was supported in part by Research Grants H-6143 and H-5883 from Nat. Inst. Health, U.S.P.H.S.

collecting bag was measured in a spirometer, and the concentration of oxygen was measured by passing gas samples across 2 Clarke pO_2 electrodes situated in a thermostable cuvette. The pO_2 electrodes were calibrated, after each sample, with known concentrations of oxygen, and spot checks were made with the Haldane analyzer. Measurements of oxygen consumption were made at rest and through the last 2 minutes of each 5-minute exercise period; previous tests had shown that a stable state had been reached by this time. Oxygen uptake was measured over the full range of exercise on at least 2 occasions in each dog, the tests being separated by a period of 2 to 3 weeks.

Aortic blood pressure was recorded during exercise from catheters (1) previously implanted into the aorta or (2) on the day of the test, inserted (under local anesthesia) into a common carotid artery (enclosed in a previously constructed Van Lière loop) and thence advanced into the aorta. These methods were adopted since, in our hands, the insertion of catheters into the common carotid artery *via* a cutdown under local anesthesia on the day of or on the day prior to the test commonly resulted in the dog exhibiting an increased resting heart rate and a somewhat reduced capacity for exercise. The permanently implanted catheters were introduced into the aorta *via* either the left renal artery or the tenth or eleventh intercostal artery. The catheters were exteriorized on the dog's back and kept patent by daily flushing with heparin solution.

To record aortic blood pressure, the catheter was attached to a Statham P23Db strain gauge mounted on the dog's back, thus eliminating the need for hydrostatic corrections as the treadmill platform was raised and lowered and also reducing somewhat the artifact induced by movement of the catheter. The output of the strain gauge was electrically damped to indicate a mean pressure. The point of zero reference was taken as the junction of the upper and middle thirds of the anterior-posterior diameter of the chest at the level of the fifth rib with the dog standing horizontal on the treadmill. When a square wave of pressure (200 mm Hg) was forced on the assembly, 90% of the maximal response was reached in 1.5 seconds. An attempt was made to record aortic blood pressure over the full range of exercise on at least 2 occasions in each dog. On one occasion in each animal measurement of blood pressure was accompanied by simultaneous measurements of oxygen uptake. In examining the pressure records, particular attention was paid to the changes at the start of exercise and during the first 20 seconds after a change in work load.

Results. The relationship of heart rate to oxygen uptake in 23 tests in 8 dogs is shown in Fig. 1. The lowest values in each panel are those obtained in the resting state. In this group of dogs the mean resting heart rate was 89 beats per minute (SD, $\pm$ 14) and the mean resting O_2 uptake was 7.7 ml/kg/min STPD (SD, $\pm$ 2.7). On the average, the dogs were able to reach a maximal oxygen uptake of 85.2 ml/kg/min STPD (SD, $\pm$ 9.9), an 8- to 10-fold increase above the resting values. In the same dog the relationship of heart rate to oxygen uptake was closely reproducible from test to test and was linear over the lower one-half to two-thirds of the range. Likewise, when the average values from all of the dogs were examined, there was an increase in heart rate with increase in oxygen uptake; however, the linearity of the relationship was much less evident than in the individual animals.

In Fig. 2 are shown the values of mean aortic pressure measured at intervals of 6 seconds over the full range of exercise in 7 of the dogs. No records were obtained from one dog which refused to run properly on both test days. Commonly, fluctuations in blood pressure, often of 15 to 20 mm Hg, were observed when the dogs were lying at rest or standing quietly on the treadmill prior to exercise. This was particularly noticeable when the heart rate was between 50 and 70 beats per minute and the respiratory sinus arrhythmia normal for the quiescent dog was present. These fluctuations in blood pressure were noted to persist during the lighter grades of exercise. In general, blood pressure increased with increase in the severity of the work; blood pressure at the most severe grade

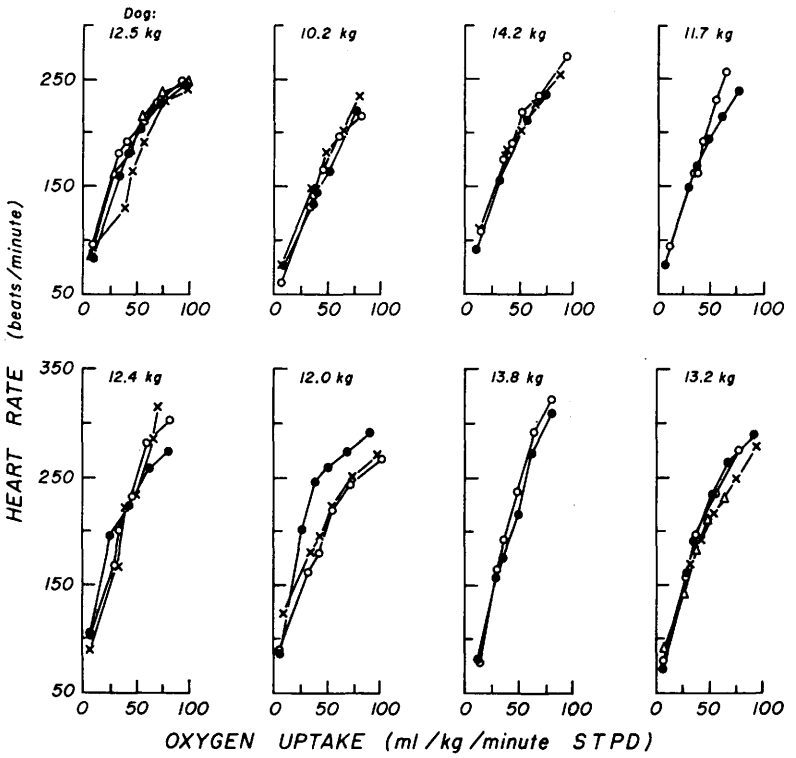

FIG. 1. Relationship of heart rate to oxygen uptake in 8 normal dogs running at 5.5 km/hr on a continually increasing gradient. Separate tests are indicated by different symbols.

of work was increased above the pre-exercise value by a mean of 30 mm Hg (range 10 to 50 mm Hg). However, the relationship of increase in arterial blood pressure to increase in work load was not as well defined or as constant as that of heart rate to oxygen consumption.

The considerable fluctuations in arterial blood pressure in the immediate pre-exercise period and during the lighter grades of exercise made it difficult to interpret the changes coincident with the onset of exercise. However, no pronounced or prolonged decrease in arterial pressure could be said to accompany the start of running. When the more severe grades of work were examined, it could definitely be said that no decrease in arterial pressure immediately followed an increase in work load.

Discussion. In man the close relationship between pulse frequency and circulatory capacity makes it possible to use pulse rate as an index in the determination of maximal working capacity(3). The present studies show a similar reproducible relationship between heart rate and oxygen uptake in well-trained dogs performing graded exercise. Thus, in any one animal the increase in heart rate may be used as an objective measure of the increase in the work performed, provided certain restrictions are met. The dog must be thoroughly accustomed to the procedure so that the effect on heart rate of fear and excitement is reduced to a minimum. The exercise must be of sufficient duration that a steady state is reached, since frequently there is a transitory overshoot in heart rate when exercise is started or when the work load is suddenly increased. The work load must be within the aerobic capacity of the animal. With these precautions the measurement of heart rate in a dog can provide quantitative evidence of a change in the work performed and also can be used to compare the work performed on separate occasions.

It has been proposed that, with the start of muscular work, the sudden vasodilatation in the working muscles would result in a

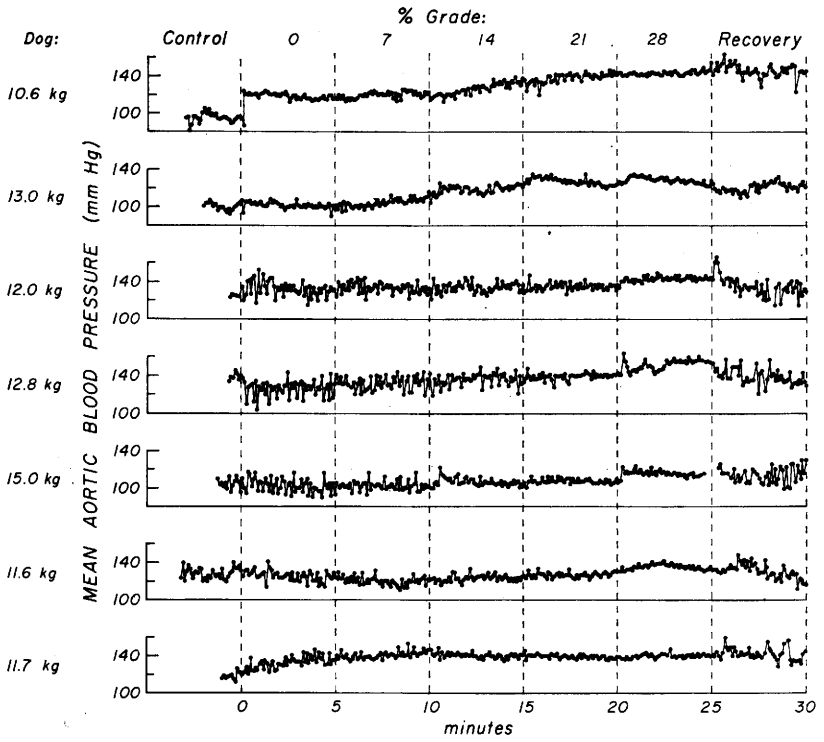

FIG. 2. Changes in mean aortic pressure in 7 dogs during control, exercise of increasing severity, and recovery periods. Pressure values are plotted at intervals of 6 seconds.

decrease in arterial pressure. This decrease in turn would activate the barosensitive receptors which would adjust the circulation to the new demands. From experiments in dogs, Warner and associates(4) have suggested that the regulation of cardiac output during exercise is accomplished by the arterial pressure receptors. Recently, Zitnik and colleagues(5) presented data from a study of leg exercise in supine man that showed a brief decrease in aortic pressure with the start of work. In a study of the changes in systemic arterial and central venous pressures during muscular work in man, Holmgren(6) noted that, at the start of exercise, there was an immediate oscillation in arterial pressure, consisting of a minor increase followed by a decrease; duration of the oscillation was about 15 seconds. This initial oscillation was seen only on transition from rest to exercise, was less marked with repetition, and was not observed when the work load was suddenly increased during continuous exercise. Holmgren thought that the arterial oscillation was a transient phenomenon and could not be taken to indicate that baroreceptive reflexes mainly govern the adjustments of the circulation during prolonged muscular work. Asmussen and Nielsen(7) and Eskildsen and coworkers(8) in studies in man and Skouby(9) in studies in dogs also did not observe an initial decrease in arterial pressure at the start of exercise.

Leusen and coworkers(10) studied the response to exercise in dogs in which the depressor nerves had been sectioned, the right carotid sinus had been denervated, and a balloon had been inflated in the left carotid sinus; they found the usual increase in cardiac output and blood pressure. From these studies, they questioned the role of the carotid sinus baroreceptors in reflex control of cardiac output during exercise. The studies in the dog reported here would be in accord with the findings of Holmgren and with the results of authors quoted subsequently. The data suggest that, in regard to the complex manner in which the circulation is adjusted to the demands of exercise, the role of the barosensitive receptors is still undefined.

Summary. In dogs performing graded exercise on a treadmill, there was a reproducible relationship between heart rate and oxygen consumption in the same dog. This relationship was linear up to moderately severe grades of work. Arterial blood pressure likewise increased with the severity of the exercise but the relationship was less direct. No decrease in arterial pressure was noted at the start of exercise or when the work load was suddenly increased during a continuous run.

1. Asmussen, E., Nielsen, M., Physiol. Rev., 1955, v35, 778.

2. Thilenius, O. G., Vial, C. B., J. Appl. Physiol., 1963, v18, 439.

3. Astrand, P. O., Rhyming, Irma, ibid., 1954, v7, 218.

4. Warner, H. R., Nicholes, K. K., Topham, W. S., The Physiologist, 1962, v5, 227.

5. Zitnik, R. S., Rodich, F. S., Marshall, H. W., Wood, E. H., Circulation Res., 1965, v17, 97.

6. Holmgren, Alf, Scandinav. J. Clin. & Lab. Invest., 1956, v8 (suppl. 24), 1.

7. Asmussen, Erling, Nielsen, Marius, Acta physiol. scand., 1951, v25 (suppl. 89), 5.

8. Eskildsen, P., Gotzsche, H., Hansen, A. T., Acta Cardiol., 1949, v4, 199.

9. Skouby, A. P., Acta physiol. scand., 1945, v10, 366.

10. Leusen, I., Demeester, G., Bouckaert, J. J., Arch. internat. physiol., 1956, v64, 564.

Received October 25, 1965. P.S.E.B.M., 1966, v121.

Effect of Interferon on RNA Synthesis in Sindbis Virus Infected Cells. (30848)

HILTON B. LEVY, LEROY F. SNELLBAKER AND SAMUEL BARON

U. S. Department of HEW, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Biology of Viruses, Bethesda, Md.

The step in viral replication that is the site of action of interferon has not been determined. In the case of some RNA viruses it has been demonstrated that viral RNA is not made in the presence of interferon(1,2). This effect on viral RNA synthesis may be the consequence of a still earlier action, as suggested by the observations of Levy(2). Interferon delayed the cutoff of cellular RNA synthesis normally induced in suspension cultures of L cells by infection with Mengo virus within ½ hour after infection. In these experiments, interferon reduced the yield of Mengo virus in a one-step growth cycle by 99.9%. If the effect of interferon on viral-induced cutoff of cellular RNA synthesis is a general one and not restricted to the Mengo virus L cell system, then interferon's action could be related to a very early step in virus replication. (It is, of course, true that such effect on RNA cutoff could be a reflection of a still earlier event.) We, therefore, have tested for the presence of a similar cutoff of RNA synthesis during Sindbis virus infection of chick embryo (CE) tissue culture.

Experimental. Materials and methods. RNA was determined by the method of Mejbaum(3), protein by the method of Lowry (4). Radioactivity was measured on Schleicher & Schull No. B-6 membrane filters in the Packard Tri-carb scintillation counter. Chicken interferon was prepared in allantoic fluid of embryonated eggs by infection with the WS strain of influenza virus, and mouse serum interferon by intravenous injection of mice with Newcastle disease virus, as previously described(5,6).

Interferon was assayed by a plaque reduction method, using vesicular stomatitis virus (VSV). One unit of interferon is defined as that amount which reduces by one-half the number of plaques of a challenge of approximately 50 pfu of VSV on CE cells(5).

The medium used was either Eagle's basal medium (BME) or BME modified for suspension culture, hereafter called "spinner" medium.

Results. Preliminary experiments were performed to determine if monolayers of CE cells infected with high multiplicity of Sind-