Bull., 1956, v22, 319.

6. Reeve, E. B., Gregerson, M. I., Allen, T. H., Sear, H., *Am. J. Physiol.*, 1953, v175, 195.

7. Hope, A., Verel, D., *Clin. Sci.*, 1955, v14, 501.

8. Chaplin, H., Jr., Mollison, P. L., Vetter, H., *J. Clin. Invest.*, 1953, v32, 1309.

9. Gibson, J. C., 2nd, Seligman, A. M., Peacock, W. C., Aub, J. C., Fine, J., Evans, R. D., *ibid.*, 1946, v25, 848.

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Production of Experimental Cardiac Hypertrophy in Rats by Reduced Atmospheric Pressure and Elevated Environmental Temperature.* (23858)

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In our investigations on proteins of the heart a need arose for production of graded cardiac hypertrophy in the rat. It was also necessary that the cardiac changes be related entirely to increase in work load. Use of an aorta or renal artery tie yielded erratic results. The possible occurrence of secondary cardiac pathology due to renal necrosis made these technics undesirable. Hypertrophy has been reported frequently following exposure to discontinuous hypoxia. Van Liere and Feder (1) observed 24% increase in relative heart weight following exposure of rats to atmospheric pressure of 303 mm Hg, 4 hours a day for 41 days. This technic seemed to offer many advantages and it was employed along with elevated environmental temperature. It was possible by this means to produce graded hypertrophy of any desired degree. For example a 50% increase in heart weight occurred after approximately 12 days of treatment.

Methods. Rats of the Addis-Slonaker strain maintained on stock diet were used. The weight range was 135-165 g. They were daily exposed (including weekends) for 4 hours to 37°C ± 1°C and an elevation of 25,000 ± 1,000 ft. in an evacuation apparatus.‡ When heart weights were to be obtained, the rat was killed on the morning following last exposure and the heart was freed of the great vessels and both atria. The chambers were cut and blood removed as completely as possible by tapping on filter paper. Growth did not occur during period of treatment; the animals either gained or lost a few grams over the starting weight. If an occasional rat lost more than 6 g, it was discarded.

Results. The findings were documented as regression of log of relative heart weight (R) on accumulated exposure time (T) (Fig. 1). R is the ratio of observed heart weight/predicted heart weight. For predicted heart weight the equation of Addis and Gray(2) was used. This had been derived for animals of this strain. R for untreated animals is approximately .96 rather than 1.00 since the Addis-Gray equation is based upon the intact heart. Since a sex difference was not observed, the data of males and females were pooled.

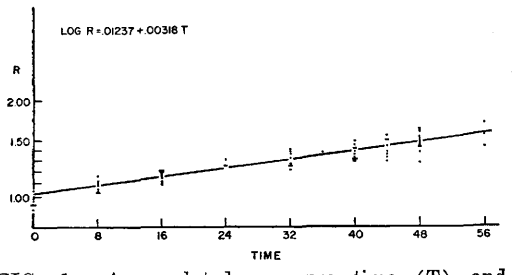

FIG. 1. Accumulated exposure time (T) and heart wt observed/heart wt expected (R). T = time in hr. n = 75. Correlation coef. = .83. z transformation yielded $\frac{z}{\sigma_z} = 10$. t = 12.7. V = .00144.

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It may be seen that a 50% increase in heart weight occurs after approximately 12 days of treatment. Van Liere and Feder obtained an increase in heart weight of only 24% after 41 days of treatment employing reduced atmospheric pressure alone.

Summary. Graded cardiac hypertrophy has been produced in the albino rat following exposure to 37°C ± 1°C and 25,000 ± 1,000 ft. for 4 hours a day. After 12 days of treatment a 50% increase in heart weight is produced by this means.

1. Van Liere, E. J., Feder, E. J., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 676.

2. Addis, T., Gray, H., *Growth,* 1950, v14, 49.

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Purification of Human Leukocytes.* (23859)

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Attracted by availability of human white blood cells (WBC) as compared to other cellular elements of body, numerous investigators have utilized a variety of methods for leukocyte isolation. Most technics for separating white cells from whole blood have emphasized leukocyte yields. However, as more exacting measurements are required, it becomes increasingly important to obtain preparations which are free of other formed elements. In these situations purity rather than yield must be the criterion for evaluating the merit of an isolation procedure. Of many technics we examined, the most promising for obtaining high white cell purity were fibrinogen sedimentation method of Buckley, Powell, and Gibson (1) as modified by Skoog and Beck(2) and the phytohemagglutinin method of Li and Osgood(3). The present report summarizes our experience with these 2 methods and introduces several modifications to facilitate evaluation and to increase purity of white cells.

Methods. Phytohemagglutinin prepared from McCaslan Pole Bean (*Phaseolus vulgaris*) by the method of Boyd(4)† was used and found to be a panagglutinin. The bean extract, at its optimal concentration for leukocyte isolation, has a titer of 1/256 with fresh erythrocytes of varying blood group and Rh types. Venous blood is collected with a few drops of potassium oxalate solution serving as anticoagulant. All syringes, pipettes, and other glassware are siliconed. The bean extract (0.3 ml) is added to 10 ml blood in a 15 x 100 mm test tube at room temperature. The contents are mixed by inversion, and the glass surface above blood is cleaned with cotton swab. The tubes are centrifuged (International Size 1, Type C centrifuge, Head No. 246 or International Clinical Centrifuge, Model CL) at 50 g for 4 min. The supernatant is removed and centrifuged at 100 g for 10 min. to sediment the leukocytes. The WBC are resuspended in 5-10 ml of phosphate buffer. This buffer is prepared from 2 g sodium acetate, 0.003 g ascorbic acid, and 1.42 ml 85% phosphoric acid; the mixture is diluted to one liter after adjusting to pH 7.45 with NaOH. (Tullis(5) pointed out the advantages of acetate and ascorbate.) The cell suspension is centrifuged at 90 g for 7 min., and the final button is resuspended in the same phosphate buffer. With experience, this procedure from time of collection to preparation of final phosphate suspension should require 45-60 min. WBC counts are prepared immediately using Toisson's solution as diluting fluid. (Toisson's solution is composed of 20 ml glycerin, 160 ml distilled water, 8 g sodium sulfate, 1 g calcium chloride, and 0.025 g methyl violet.) Slides for differential leukocyte counts are prepared by adding a

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