differs from that of beta-2M (Sell) and from the usual position of beta-2A(7), which has not been described in the guinea pig. Perhaps future work will show this line to represent another member of the known family of immune globulins(13). However, "extra gamma globulins," apparently unaffected by antigenic stimulation, have been found in chicken sera(14). The fact that free boundary electrophoresis showed little or no protein in the gamma globulin range of germfree guinea pigs fed diet L-445 might indicate that on this diet this fraction does not become evident or that it disappears in older animals.

Summary. The serum protein composition of germfree and conventional guinea pigs displays significant differences during young adult life (2-4 months) at which time the alpha, beta, and gamma globulins are less than those of the conventional animal. Immunoelectrophoretic analysis shows one component of the germfree gamma globulin range absent at this time.

In contrast to a diet containing only vegetable protein, which resulted in little beta and no detectable gamma globulin in the serum of adult germfree guinea pigs, a diet composed of complex vegetable and animal proteins stimulated the formation of larger amounts of globulins. This resulted in comparable or not significantly different alpha, beta, and gamma globulin fractions for year old germfree and conventional animals.

The authors acknowledge the technical assistance of Janine Bemelmans, Ann Hermans, and of Bernard A. Teah of the Germfree Animal Operations and his group for maintenance of the germfree animals.

1. Wostmann, B. S., *Ann. N. Y. Acad. Sci.,* 1959, v78, 255.

2. Newton, W. L., DeWitt, W. B., *J. Nutrition,* 1961, v75, 145.

3. Wostmann, B. S., *Ann. N. Y. Acad. Sci.,* 1961, v94, 272.

4. Phillips, B. P., Wolfe, P. A., Gordon, H. A., *ibid.,* 1959, v78, 183.

5. Wostmann, B. S., *ibid.,* 1959, v78, 175.

6. Friedman, H. S., *Clin. Chem. Acta,* 1961, v6, 775.

7. Grabar, P., Burtin, P., *Analyse Immuno-electrophoretique,* 1960, Masson et Cie Eds., Paris-Vie.

8. Ressler, N., Jacobson, S. D., *Clin. Chemica Acta,* 1957, v2, 372.

9. Korngold, L., *Ann. N. Y. Acad. Sci.,* 1957, v69, 681.

10. Benacerraf, B., Ovary, Z., Franklin, E., Block, K., *J. Exp. Med.,* 1963, v117, 937.

11. Wehmeyer, P., *Acta Path.,* 1954, v35, 54.

12. Yagi, Y., Macer, P., Pressman, D., *J. Immunol.,* 1962, v89, 736.

13. Heremans, J., *Les Globulines Seriques du Systeme Gamma,* 1960, Masson et Cie Ed., Paris-Vie.

14. Asofsky, R., Trnka, Z., Thorbecke, G. J., PROC. SOC. EXP. BIOL. AND MED., 1962, v111, 497.

Received March 6, 1964. P.S.E.B.M., 1964, v116.

Myocardial Proteins in Dogs of Various Ages.* (29408)

HARRY SOBEL, HERIBERTO THOMAS AND RICHARD MASSERMAN
Veterans Administration Hospital, Sepulveda, and St. Joseph Hospital, Burbank, Calif.

Recently, myocardial infarction was observed in dogs 6-8 years old which were maintained on a diet including thiouracil and cholesterol(1). Myocardial infarction in this species when fed an atherogenic regimen has not been previously reported, presumably because other investigators, obtaining animals at random from shelters, almost always used younger subjects. Accordingly, a systematic study has been undertaken to determine some of the age-dependent variables which could account for the increased susceptibility to myocardial damage(2,3). The present investigation concerns itself with myocardial protein fractions from dogs of different ages. Some age-dependent changes were observed.

Procedure. Male mongrel dogs of known ages were obtained from a reliable supplier.

* Supported by grant from U. S. Public Health Service.

TABLE I. Myocardial Protein Fractions in Dogs of 4 Age Groups.

Group*	I	II	III	IV
Wt (lb)	18	19	20	22
Heart wt (g)	59.1	71.5	72.4	87.9
Total protein†	128.11 ± 5.92‡	139.42 ± 7.70	138.35 ± 9.13	137.51 ± 6.43
Fraction I	22.39 ± 2.13	24.08 ± 3.61	25.12 ± 8.08	23.34 ± 2.56
" IA	3.87 ± .48	3.71 ± .66	4.74 ± 1.28	4.67 ± .77
" II	52.70 ± 5.01	54.08 ± 4.24	54.06 ± 5.98	56.55 ± 6.80
II_α	11.55 ± 1.51	10.89 ± .78	14.45§ ± 1.88	17.69[1] ± 2.51
II_β	31.00 ± 2.97	32.11 ± 2.73	28.47‖ ± 1.07	29.10 ± 3.36
II_γ	10.23 ± .96	11.25 ± 1.37	11.14¶ ± 1.29	9.76 ± 1.16
Myoglobin	2.31 ± .24	3.08[2] ± .15	3.45 ± .23	3.57[3] ± .18
Desoxyribose	.417± .021	.378 ± .031	.355 ± .029	.293[4]± .033
Ribose	2.78 ± .15	2.94 ± .17	2.90 ± .17	2.64 ± .21
R/D	6.66	7.77	8.16	9.04

* 9 dogs in each group.

† Each value represents the mean of findings with 9 dogs except in the case of total protein where there were only 8 values. All values expressed as mg/g of fresh tissue.

‡ Standard error of mean.

§ Represents only 8 values, a value of 25.78 deleted from calculation.

‖ " " " " " " " 128.68 " " " .

¶ " " " " " " " 27.14 " " " .

if included leads to Fraction II_α 17.13 ± 3.16, II_β 32.74 ± 5.29, II_γ 12.48 ± 1.75.

Difference statistically significant as compared to value of:

[1] Group I (t=2.10). [2] Group I (t=3.85). [3] Group II (t=2.13). [4] Group I (t=3.18).

An attempt was made to use dogs weighing 18-22 lb. However, this was not always possible; some younger dogs weighed less and some of the older dogs more.

The animals were heparinized and sacrificed with nembutal. The hearts were removed, washed of blood and perfused with saline through the coronary arteries until the organ was apparently blood free. They were then homogenized with a known volume of saline in a Virtis 45 homogenizer packed in ice. Aliquots were taken, frozen immediately and stored until used.

The procedure of Sobel *et al*(4), which was developed for the rat heart, was used for fractionation of the proteins. A thawed aliquot was ground with sand to initiate the procedure as described. Myoglobin was determined by the procedure of Reynafarje(5), desoxyribose and ribose according to the procedure of Schneider(6). Protein concentration was calculated by multiplying the nitrogen content, determined in a Technicon Autoanalyzer, by 6.25.

Results. For convenience the dogs were classified into 4 age categories as previously described(2): Group I, one year or less; group II, 2-4 years; group III, 5-8 years; and, group IV over 9 years. There were 9 dogs in each group.

The findings are shown in Table I. An attempt had been made to use dogs of equal weight throughout in order to avoid complications which might make interpretations of the findings difficult. Nevertheless, from the available material the average weight increased progressively from Group I through Group IV (18, 19, 20 and 22 lb respectively). The mean heart weights were also greater (59.1, 71.5, 72.4 and 87.9 g respectively). Inspection of the data revealed that the body weight-heart weight relationship was approximately linear on a log-log basis. This is a normal relationship(7) and did not suggest that the older dogs had hearts larger than expected on the basis of body weight. This possibility exists because hypertensive, renal and other cardiovascular diseases are seen in older dogs.

The amount of protein per gram of fresh heart remained constant in all age groups (approximately 128-139 mg/g) suggesting that there was no marked alteration in per cent of fat or water. It also suggests that the quantity of individual protein fractions may be documented on a weight basis.

Fraction I, which included cytoplasmic protein, was obtained as previously described for rat heart by extraction with sucrose-versene at pH 5.0.

Fraction IA was obtained by extraction with sucrose-versene at pH 7.4. The values for groups III and IV were greater than for groups I and II but the differences were not statistically significant (4.74 and 4.67 mg/g as compared to 3.87 and 3.71 mg/g).

Fraction II contained the contractile proteins. The 3 subfractions IIα, IIβ and IIγ had previously been identified with myosin, actomyosin and contractin respectively in the rat(4). The total fraction II was 52.70, 54.08, 54.06 and 56.55 mg/g respectively for the 4 groups. The values for subfraction IIα were 11.55, 10.89, 14.45† and 17.69 mg/g respectively. The difference between Group IV and Group I was statistically significant. The values for subfraction IIβ were relatively constant (31.00, 32.11, 28.47† and 29.00 mg/g respectively) and for subfraction IIγ they were 10.23, 11.25, 11.14† and 9.76 mg/g. The apparent decrease in value of subfraction IIγ was not statistically significant.

Myoglobin levels were lower than those reported by Reynafarje(5) for dogs (3.8-4.4 mg/g of heart) although the procedure of this investigator was used. The mean values obtained for Groups I-IV respectively were 2.31, 3.08, 3.45 and 3.57 mg/g of heart. The value for Group II was significantly greater than that of Group I while the value for Group IV was significantly greater than that of Group II.

There was a tendency for the mean desoxyribose values to decrease (.417, .378, .355 and .293 mg/g respectively). The difference between Group IV and Group I was statistically significant. The mean ribose values apparently remained constant with each age category (2.64-2.94 mg/g) and the ratio of ribose to desoxyribose accordingly increased with each group (6.66, 7.77, 8.16 and 9.04 respectively).

Discussion. It will be noticed that desoxyribose of the whole heart was relatively constant in all 4 groups (24.65, 27.03, 25.70 and 25.75 mg for Groups I-IV respectively). It is possible that the total number of cells in the hearts of all 4 groups was approximately the same and that the cells hypertrophied in order to accommodate the work demand required by the increase in weight of the total organism. This is consistent with the findings of Linzbach(8) who stated that all normal human hearts have essentially the same number of nuclei and fibers although the fibers become larger with growth. The concentration of the "myosin"-containing fraction was significantly greater in the oldest dogs while that of "actomyosin" remained constant and that of "contractin" may have decreased. The significance of these changes is not clear.

The data suggest that, although the question of cell loss with age has not been resolved, the compensatory mechanisms of the cells in the myocardium in old dogs permit growth so that heart weight is "normal" for body size. The concentration of protein fractions however is not entirely like that of younger animals. The significance of these differences and their relation to cardiac function remain to be determined.

Summary. Protein fractions of the hearts of dogs of different ages were examined and levels of desoxyribose, ribose and myoglobin were determined as well. There were 9 dogs in each of 4 groups: I, one year old or less; II, 2-4 years of age; III, 5-8 years of age; and, IV, 9 years or older. The desoxyribose concentration of the oldest group of dogs was 30% less than that of the youngest. Ribose concentration did not differ with age. Concentration of the fraction corresponding to myosin was significantly increased in the oldest group while the fraction corresponding to contractin showed a decrease that was not statistically significant, and that corresponding to actomyosin remained constant. Myoglobin concentration increased with each age group. The data suggest that cellular hypertrophy occurs with aging of the myocardium of dogs. Dogs in the oldest age group exhibit no impairment in ability to maintain a normal myocardial protein content although some age-dependent changes seem apparent.

† For qualification of this value see Table I.

The authors wish to express their gratitude to Riker Laboratories for a very generous supply of heparin.

1. Sobel, H., Mondon, C., Straus, H., *Circulation Res.*, 1962, v11, 971.
2. Sobel, H., Thomas, H. V., PROC. SOC. EXP. BIOL. AND MED., 1963, v112, 206.
3. Sobel, H., Thomas, H. V., Masserman, R., Parsa, K., *J. Gerontol.*, 1963, v18, 340.
4. Sobel, H., Myers, S. M., Cohen, F., *Exp. Med. and Surg.*, 1959, v17, 119.
5. Reynafarje, B., *J. Lab. Clin. Med.*, 1963, v61, 138.
6. Schneider, W. C., *Methods in Enzymology*, 1957, v3, Academic Press, New York.
7. Addis, T., Gray, H., *Growth*, 1950, v14, 49.
8. Linzbach, A. J., *Am. J. Cardiol.*, 1960, v5, 370.

Received March 9, 1964. P.S.E.B.M., 1964, v116.

Response of the Polycythemic Mouse and Polycythemic Guinea Pig To Human Erythropoietin.* (29409)

JOANNE H. JEPSON AND LOUIS LOWENSTEIN (Introduced by R. V. Christie)
Division of Hematology, Department of Medicine, Royal Victoria Hospital and University Clinic, McGill University, Montreal

The inconsistent erythropoietic response of guinea pigs to injections of erythropoietin extracts from other species has been described. Gordon(1) found an erythropoietic response in normal guinea pigs following injections of extracts of urine from a child with Thalassemia, but the guinea pigs did not respond to extracts from other sources which had been shown to stimulate erythropoiesis in rats. Guinea pigs have failed to respond to plasma from anemic ponies(2), erythropoietically active human urine extract(3), and erythropoietically active rabbit plasma filtrate(4). It has been reported, however, that erythroblasts in hanging drops of bone marrow cultures from guinea pigs or rats increase in number when serum or filtrates of boiled plasma from anemic patients or guinea pigs are added to the cultures(5). Recently, Schooley and Garcia(6) reported that an extract of human urine which produced an erythropoietic response in rats also stimulated erythropoiesis in hypertransfused guinea pigs.

Because of the paucity of information on the response of the guinea pig to erythropoietin from other species, the activity of an extract of urine from a patient with hypoplastic anemia was compared in polycythemic mice and polycythemic guinea pigs.

Materials and methods. 1. *Extraction of erythropoietically active human urine.* The human urine extract used in these experiments was obtained from a patient with hypoplastic anemia induced by chloramphenicol whose hemoglobin level was 5 g %. Urine extraction was performed by a modified method of Gordon(7). His procedure was followed up to the final step where, instead of lyophilization, 5 volumes of acetone at 0°C were added to each volume of the clear supernatant, and the mixture was allowed to stand overnight at 5°C. The precipitate which appeared was collected and washed with ether, evaporated to dryness *in vacuo* in the cold, and stored in a desiccator at 5°C until used. The final extract was light tan in color.

2. *Measurement of incorporation of Fe59 into erythrocytes of hypertransfused guinea pigs.* Inbred guinea pigs receiving a standard guinea pig laboratory diet with supplementary carrots and weighing 225 to 275 g were transfused intraperitoneally on 2 consecutive days with 5 ml of 80% suspension of washed homologous red cells in normal saline. Endogenous erythropoietin production was suppressed in these animals by the fifth day following the last transfusion. At this time, 50 mg of urine extract dissolved in 1 ml of normal saline were injected subcutaneously

* This investigation was supported in part by a Public Health Service Fellowship from Nat. Heart Inst.