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1. Theiler, M., personal communication, cited in: Casals, J., An. Microbiol. (Rio de J.), 1963, v11(A), 13.

2. Downs, W. G., Anderson, C. R., Spence, L., Aitken, T. H. G., Greenhall, A. H., Am. J. Trop. Med. and Hyg., 1963, v12, 640.

3. Parodi, A. S., Greenway, D. J., Rugiero, H. R., Rivero, E., Frigerio, M., de la Barrera, J. M., Mettler, N., Garzon, F., Boxaca, M., de Guerrero, L., Nora, N., El Día Médico, 1958, v30, 2300.

4. Pirosky, I., Zuccarini, J., Molinelli, E. A., Di Pietro, A., Barrera Oro, J. G., Martini, P., Orientación Médica, 1959, v8, 303.

5. Johnson, K. M., Wiebenga, N. H., Mackenzie, R. B., Kuns, M. L., Tauraso, N. M., Shelokov, A., Webb, P. A., Justines, G., Beye, H. K., Proc. Soc. Exp. Biol. and Med., 1965, v118, 113.

6. Mettler, N. E., Casals, J., Shope, R. E., Am. J. Trop. Med. and Hyg., 1963, v12, 647.

7. Causey, O. R., Causey, C. E., Maroja, O. M., Macedo, D. G., ibid., 1961, v10, 227.

8. Causey, O. R., Casals, J., Shope, R. E., Suchinda Udomsakdi, ibid., 1963, v12, 777.

9. Shope, R. E., Causey, O. R., Andrade, A. H. P., Theiler, M., ibid., 1964, v13, 723.

10. Herrmann, E. C., Jr., Engle, C., Proc. Soc. Exp. Biol. and Med., 1958, v98, 257.

11. Webb, P. A., Johnson, K. M., Mackenzie, R. B., Proc. Soc. Exp. Biol. and Med., 1966, in press.

12. Parodi, A. S., de la Barrera, J. M., Rugiero, H. R., Greenway, D. J., Yerga, M., Mettler, N., Boxaca, M., Frigerio, M. J., La Prensa Méd. Argentina, 1959, v46, 554.

13. Parodi, A. S., Rugiero, H. R., Greenway, D. J., Mettler, N., Martinez, A., Boxaca, M., de la Barrera, J. M., ibid., 1959, v46, 2242.

14. Marui, R. A., Ibarra Grasso, A., Rev. Soc. Entomol. Argentina, 1962, v23, 55.

15. Johnson, K. M., Mackenzie, R. B., Webb, P. A., Kuns, M. L., Science, 1965, v150, 1618.

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Influence of Diet and Heredity on the Serum Protein Components of The Rat. (31183)

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One of several criteria used in this laboratory to determine the nutritional response of the rat to different diets is the measurement of the concentration of the various serum protein fractions. Heredity is also known to be a major factor affecting the distribution and concentration of the blood proteins(1). With the increased application of the more definitive electrophoretic techniques, genetic variations in almost all the serum protein components have been established(2).

Marshall and Hildebrand(3) have shown that such measurements as body growth, food intake, urinary protein, organ weights, and body and liver composition are influenced by the strain of the rat as well as by the diet. The differences observed in liver and body composition among the 3 strains examined indicated that the response to diet must be related to differences in inherent metabolic characteristics, particularly the mechanism of lipid synthesis. The effect of level and kind of dietary fat on the serum protein components of one of these strains has been reported(4) and the component most affected was pre-albumin, a lipid-protein fraction migrating more rapidly than albumin. In this paper are reported the differences observed

in the concentration of the serum protein components of some of the animals of the 3 strains shown by Marshall and Hildebrand to differ in their metabolism.

Experimental. Male weanling rats, 21 to 23 days old, of the BHE, Holtzman, or Wistar strain(3) were housed individually in a room maintained at 77 to 82°F and 40 to 55% relative humidity. The animals were fed *ad libitum* either a stock diet in the form of pellets containing crude protein, not less than 25 g; crude fat, not less than 4 g; crude fiber, not more than 4 g; ash, not more than 10 g per 100 g of diet, or a semipurified diet containing 16 g casein, 8 g lactalbumin, 8 g hydrogenated vegetable oil (Crisco having approximately 10% linoleic acid), 10 g brewer's yeast, 4 g modified Osborne and Mendel salt mixture(5), 2 g cellulose flour and 52 g sucrose per 100 g of diet. All animals were given 10 g of fresh kale twice weekly. Animals fed the semipurified diet were also given one drop of vit A and D concentrate* twice weekly and 30 mg vit E in corn oil weekly. Water was available at all times. At 330 to 335 days of age the animals in a nonfasted condition were anesthetized with sodium amytal solution, the body cavity opened and blood removed by heart puncture.

Total protein and nonprotein nitrogen contents of the sera were determined by a micro-Kjeldahl method(6). Moving boundary electrophoretic analysis (AMINCO portable apparatus) of the individual serum protein components was performed on the blood serum diluted 1:5 with veronal buffer (pH 8.6, 0.1 μ) and dialyzed against the buffer overnight at 10°C. The concentrations and mobilities of individual components were calculated from ascending Schlieren patterns. The amounts of each component have been expressed in terms of percent of total serum protein (relative) and on the basis of grams of each component per 100 ml of serum (absolute). Significant differences at 5 and 1% levels of probability among strains and between diets were determined by the analysis of variance(7) and test differences for the measurements stated by the multiple range test(8). Nonprotein nitrogen values were transformed to logarithms because the variances were not homogeneous. This is in accordance with the report of Wootton(9) on the normal variations in blood constituents in which he observed that certain blood components as nonprotein nitrogen have a skewed distribution and statistical evaluation should be based on the logarithm rather than the normal value.

Results and discussion. The data showing the concentration of total serum protein and of individual serum protein components—pre-albumin (PA), albumin, alpha-1, alpha-2, beta and gamma globulin—are presented in Table I. Values reported are expressed as percent of total protein; absolute amounts may be calculated from the data for serum protein concentration. A combined value for albumin and alpha-1 globulin is reported because of the poor separation of these components in certain sera. The levels of probability for the significant differences in the measurements among the strains are shown in Table II.

Rats within strains fed either the commercial stock diet or the semipurified diet showed no significant differences in serum total protein, nonprotein nitrogen or the individual protein component concentrations. Comparison of the 3 strains fed either of these diets revealed no differences in electrophoretic mobilities of the individual protein components or in alpha-2 globulin concentration. When the semipurified diet was fed, a higher total protein value ($P<0.01$) was observed in sera of Wistar rats than in sera of BHE rats. When the stock diet was fed, nonprotein nitrogen concentration was higher ($P<0.05$) in sera of BHE rats than that of Wistar rats.

Pre-albumin was observed in almost all rats regardless of strain. Holtzman rats had a significantly higher concentration of PA in their sera than either the BHE or the Wistar rats. The presence of a pre-albumin fraction in the moving boundary electrophoretic patterns of serum from Sprague-Dawley rats (10), progenitors of the Holtzman strain, from the BHE rats(4), and from albino rats examined by Halliday and Kekwick(11) has

* Percomorph Oil, Mead Johnson Co., containing 60,000 units vit A and 8,500 units vit D/g; approximately 1,250 units vit A and 180 units vit D/drop.

been reported. Glover and Joo(12), Beaton *et al*(13), Wise *et al*(14), Neuhaus *et al*(15), and Escribano and Grabar(16) also have observed one or more pre-albumin components in rat sera analyzed by electrophoresis in stabilized media. Genetic studies on the serum proteins separated by starch gel electrophoresis have shown that the absence or presence of one, two or more PA components in mice(17), pigs(18), horses(19), and primates(20) is an inherited characteristic of the individual animals. The numbers of sera analyzed in the present study were not large enough to determine if the absence or presence of one or more pre-albumin components was genetically controlled.

TABLE I. Serum Protein Components of 3 Strains of Rats Fed Two Different Diets.

Strain	No. of rats: Total	No. of rats: With PA*	Total: Protein, g/100 ml	Total: NPN,† mg/100 ml	Concentration of the protein components, % of total: PA	Albumin & alpha-1 globulin	Alpha-2 globulin	Beta globulin	Gamma globulin
Stock diet									
BHE	12	11	5.9 ± .1 ‡	42 ± 5[a]§	6.4 ± 1.5[b]	70.0 ± 1.5[a]	7.6 ± .4	13.0 ± 1.2[b]	3.1 ± .6[b]
Wistar	8	7	6.3 ± .1 (7)‖	28 ± 5[b]	5.1 ± 1.2[b]	58.4 ± 2.6[b]	9.4 ± .8	19.5 ± .9[a]	7.6 ± 1.5[a]
Holtzman	3	3	6.0 ± .4 (5)¶	52 ± 18	16.4 ± .4[a]	65.7 ± 2.4[a]	8.6 ± 1.1	8.3 ± 1.2[c]	1.0 ± .5[b]
Semipurified diet									
BHE	11	7	5.8 ± .2[b]	35 ± 3	3.6 ± 1.0[c]	68.7 ± 2.0[a]	7.7 ± .5	15.6 ± .9[a]	4.5 ± .9[a]
Wistar	9	8	6.7 ± .2[a]	27 ± 5	7.4 ± 1.6[b]	61.4 ± 1.9[b]	8.8 ± .8	16.7 ± 1.4[a]	5.6 ± 1.3[a]
Holtzman	8	8	6.2 ± .2[ab](7)‖	26 ± 1	12.6 ± 1.0[a]	67.2 ± 2.0[ab]	7.7 ± .6	11.3 ± .8[b]	1.2 ± .5[b]

* Pre-albumin. † Nonprotein nitrogen. ‡ Mean ± S.E.

§ Significance of means determined by multiple range test(8). Within a set of means, any 2 means which do not have the same superscript are significantly different from each other.

‖ Insufficient serum from one rat to analyze for total protein and nonprotein nitrogen content.

¶ Five rats were included in the analysis of variance for total protein but sera from only 3 rats were analyzed by moving boundary electrophoresis.

The amount and incidence of pre-albumin in rat serum has been reported to be affected by diet, particularly by the kind and level of fat(4), and paper electrophoretic analysis has shown the presence of lipid material associated with this fraction. One of the PA components in rat sera(15) and in human sera(21) separated by gel electrophoresis has been identified as a protein-lipid complex. According to Glover and Joo(12) the pre-albumin fraction because of its small size and lability of its lipid components may play a significant part in the transport and uptake of lipids by tissues. If pre-albumin is an inherited variant in the blood of the rat, animals having this component, especially a high level, would have a different lipid transporting system from those animals without such a component. Thus the utilization of dietary fat or mobilization of fat from tissues may be affected by the amount of pre-albumin present in serum.

Lack of separation of albumin and alpha-1 globulin in some sera has made it impossible to discuss these components separately and differences may reflect a variation in either component. The relative concentrations of albumin plus alpha-1 globulin were significantly lower in Wistar than in BHE rats fed either diet. Comparison of the combined values for these components between the Holtzman and Wistar or BHE strains revealed no significant differences except for a higher relative amount in Holtzman rats than in Wistar rats fed the stock diet. The absolute amounts of albumin and alpha-1 globulin, however, were not significantly different among the strains.

The component most affected by the strain of rat studied was beta globulin. When the stock diet was fed, both the relative and absolute values for the 3 strains were significantly different from each other. In contrast, when the semipurified diet was fed, neither the relative nor absolute amounts of beta

TABLE II. Level of Probability for Significance of Differences Among Means Presented in Table I.

Strains	Total No. of rats	Concentration of the protein components, % of total: Pre-albumin — All rats	Pre-albumin — Rats with PA	Albumin and alpha-1 globulin	Beta globulin	Gamma globulin
		Stock diet				
BHE & Wistar	20			.01 (R)*	.01	.01 (R) .05 (A)
BHE & Holtzman	15	.01	.01		.01 (R) .05 (A)	
Wistar & Holtzman	11	.01	.01	.05 (R)	.01	.01
		Semipurified diet				
BHE & Wistar	20	.05 (A)		.05 (R)		
BHE & Holtzman	19	.01	.01		.05 (R)	.05 (R)
Wistar & Holtzman	17	.01 (R) .05 (A)	.05		.01	.01

* If neither (R) nor (A) follows the level of probability, values were significantly different for both relative (R) and absolute (A) concentrations.

globulin in the sera of Wistar and BHE rats differed significantly. The absolute and relative amounts of beta globulin were lower in the Holtzman than in Wistar rats fed this diet, but the difference in the absolute amounts in Holtzman and BHE sera was not significant.

Relative and absolute concentrations of gamma globulin were significantly lower in Holtzman sera than in Wistar sera regardless of diet. Although BHE rats tended to have a higher relative level of gamma globulin than Holtzman rats, the difference was significant only for rats fed the semipurified diet. Both relative and absolute gamma globulin contents of BHE rats were significantly lower than that of Wistar rats when the stock diet was fed but not when the semipurified diet was fed. Respiratory diseases were not uncommon in all 3 strains. The low level of gamma globulin in the Holtzman strain cannot be attributed to infection alone since Wistar and Holtzman rats were more susceptible to respiratory diseases than the BHE rats but kidney defects occurred more often in BHE and Holtzman rats than in Wistar rats(3). Variations in gamma globulin, therefore, seem to be due to heredity. The condition of hypogammaglobulinemia has been reported to be congenital or acquired(2).

Examination of the interaction of strain and diet in the analysis of variance revealed a significant interaction ($P<0.05$) for the absolute amount of pre-albumin and for the relative amount of beta globulin in the serum. Evaluation of the nutritional response of a group of rats to different diets based on the concentrations of both of these components must be considered with respect to the strain used.

Summary. 1. Wistar rats had a significantly higher amount of total serum protein than BHE rats fed the semipurified diet and a significantly lower relative amount of albumin and alpha-1 globulin than the BHE strain regardless of diet. 2. All 3 strains fed the stock diet varied significantly in their serum beta globulin concentration. BHE and Wistar rats fed the semipurified diet, however, had similar concentrations of beta globulin while Holtzman rats fed this diet had a significantly lower relative value than BHE or Wistar rats. 3. The Holtzman strain had the lowest level of serum gamma globulin, which was significantly different from the Wistar strain regardless of diet, but different only from the BHE strain when the semipurified diet was fed. 4. A significant interaction between strain and diet was found for both the serum pre-albumin and beta globulin fractions. 5. Comparison of rats within strains (BHE, Wistar, Holtzman) fed either a stock diet or a semipurified diet showed no significant differences in total serum protein, nonprotein nitrogen or in individual protein component concentrations. 6. No differences among

strains were observed in mobilities of individual serum protein fractions or amount of alpha-2 globulin.

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1. Bier, M., Electrophoresis: Theory, Methods and Applications, Academic Press Inc., New York, 1959, p391.
2. Putnam, F. W., The Plasma Proteins, Vol. II, Academic Press Inc., New York, 1960.
3. Marshall, M. W., Hildebrand, H. E., J. Nutrition, 1963, v79, 227.
4. Lakshmanan, F. L., Adams, M., ibid., 1965, v86, 337.
5. Osborne, T. B., Mendel, L. B., J. Biol. Chem., 1919, v37, 557.
6. Sher, I. H., Anal. Chem., 1955, v27, 831.
7. Dixon, W. J., Massey, F. J., Jr., Introduction to Statistical Analysis, McGraw-Hill Book Co., Inc., New York, 1951.
8. Duncan, D. B., Biometrics, 1955, v11, 1.
9. Wootton, I. D. P., Proc. Nutr. Soc., 1962, v21, 129.
10. Deutsch, H. F., Goodloe, M. B., J. Biol. Chem., 1945, v161, 1.
11. Halliday, R., Kekwick, R. A., Proc. Roy. Soc. London, 1957, v146, 431.
12. Glover, J., Joo, C. N., Biochem. J., 1963, v88, 65 P.
13. Beaton, G. H., Selby, A. E., Wright, A. M., J. Biol. Chem., 1961, v236, 2001.
14. Wise, R. W., Ballard, F. J., Ezekiel, E., Comp. Biochem. and Physiol., 1963, v9, 23.
15. Neuhaus, O. W., Havez, R., Biserte, G., Compt. Rend. Soc. Biol., 1962, v156, 1105.
16. Escribano, M. J., Grabar, P., Compt. Rend. Acad. Sci., 162, v255, 206.
17. Shreffler, D. C., Genetics, 1964, v49, 629.
18. Kristjansson, F. K., ibid., 1963, v48, 1059.
19. Ashton, G. C., Nature, 1958, v182, 1029.
20. Miller, J. B., ibid., 1963, v200, 810.
21. Uriel, J., Grabar, P., Bull. Soc. Chim. Biol., 1956, v38, 1253.

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Chemical Prevention of Radiation-Induced Leukemia in Mice.* (31184)

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Total body exposure of animals to ionizing radiation results in a spectrum of damage which is dose-related(1). The destructive effects have been mitigated by shielding of sensitive organs(2), and by pretreatment of exposed animals with chemical agents (3); 2 of the latter are *Salmonella typhosa* endotoxin(4) and 2-aminoethylisothiuronium bromide (AET)(5).

Leukemia virus persists as an occult infection among many strains of mice, and is very likely transmitted to progeny by congenital routes(6,7). Leukemic disease appears spontaneously in the majority of animals of 2 mouse strains (AKR and C58) when 4 to 12 months of age(8,9). In other mouse strains, development of significant levels of disease depends on early exposure to specific chemical agents or to ionizing radiations(10, 11,12). Total body exposures to a series of small doses of X-rays (150 R) are most effective in inducing leukemia in mice(13). By this regime the disease has been induced in germfree and in conventional mice of 6 strains: they are Swiss-Webster, ICR, CFW, Balb/C, C57 BL, and C3Hf(14). Lesions in the spontaneous and in the radiation-induced forms of leukemia are indistinguishable: enlarged thymus, and less frequently swollen lymph nodes, infiltration of visceral organs with large lymphoid cells, and increased numbers of lymphoid cells in the blood.

The mechanism of progressive leukemogenesis, spontaneous or X-ray induced, is as yet unknown. The homeostatic balance between host and virus is altered to the extent that pathology emerges. It may in some way be related to the immune mechanism of the

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