Blood Proteins and Their Distribution in Rats with Parabiosis Intoxication. Determined by Paper Electrophoresis.* (23606)

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Parabiosis intoxication is a condition which develops in animals united surgically. In rats the haematologic manifestations are severe anemia in one of the partners and a marked polycythemia in the other(1-5). Recent evidence from this laboratory indicates that one of the partners develops a state of vascular collapse, and as a result, gains blood at the expense of the other(6,7). It is suggested that the former attempts to reduce blood volume by storing red cells in the available reservoirs and diuresing water; the latter endeavors to increase blood volume by hemodilution with extravascular fluids. If this is true then polycythemic partners should show hyperproteinemia and anemic partners hypoproteinemia. Although anemic partners often show ascites, hydrothorax and subcutaneous edema, which might be construed as indicative of hypoproteinemia, direct data are not available.

It was the purpose of this study to determine the level of blood protein and the distribution of the various serum protein components in rats with parabiotic intoxication, in the belief that an understanding of the mechanisms underlying this syndrome might suggest an explanation for the high incidence of hypertensive cardiovascular disease(8-10), arthritis(11), and neoplasia(12,13) encountered in parabiotic rats.

Materials and methods. The rats used were from our colony, the forebears of which were of the Holtzman strain. The animals were maintained throughout the experiment in air-conditioned quarters and received tap water and Purina Laboratory Chow *ad lib.* The technic of parabiosis was as previously described(8), and was carried out on animals paired for sex and body weight (60-90 g) before union. The series consisted of 23 pairs, 20 female-female and 3 male-male pairs of comparable weight; and 8 single male and female controls. Hematocrit, hemoglobin and red-cell counts were determined by routine methods and when diagnosis of parabiosis intoxication was confirmed by these findings, the pairs were anesthetized with ether. Blood was drawn from the abdominal aorta of each of the partners and controls for analysis of the proteins. After withdrawing blood, the partners were killed with ether, carefully separated along the suture line and weighed. Controls were also weighed after withdrawal

TABLE I. Characteristics of the Blood in Single Rats Contrasted with Those of Rats with Parabiosis Intoxication.

Data	Single controls	Parabiotic pairs: Anemic twins	Parabiotic pairs: Polycythemic twins
Body wt, g—Initial	75 ± 2 *	85 ± 3	85 ± 2
Final	103 ± 2	85 ± 8	115 ± 7
Hematocrit, %	45 ± 1.4	20 ± 1.4	66 ± 1.9
Hemoglobin, g/100 cc	13 ± .3	6.5 ± .5	20.1 ± .5
Erythrocytes, millions/mm³	6.72 ± .23	3.28 ± .25	10.67 ± .43
Plasma protein, g/100 cc	6.10 ± .21	4.35 ± .70	7.96 ± .24
Serum proteins, % albumin	38.0 ± 2.1	27.3 ± 1.4	26.6 ± 1.7
α_1 globulin	13.0 ± 1.3	16.9 ± 1.1	16.6 ± .9
α_2 "	7.4 ± .6	16.5 ± 1.0	17.2 ± 1.6
β "	31.1 ± 3.0	31.0 ± 1.8	30.5 ± 1.9
γ "	10.6 ± 1.3	8.3 ± 1.0	10.3 ± .9

* ± S.E. of the mean.

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Single Control, Normal

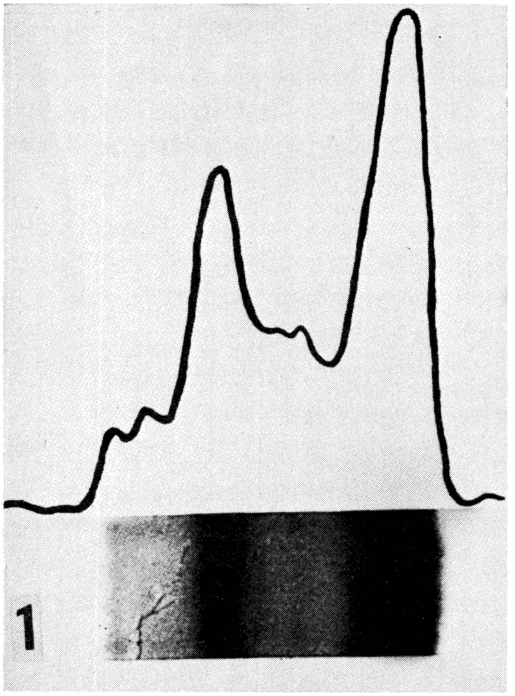

FIG. 1. Electrophoretic separation of serum proteins from a control rat. Plasma proteins 6.25 g/100 ml.

of blood. Plasma proteins were estimated by the method suggested by Lowry and Hunter (14), using plasma samples collected from a heparinized capillary tube after centrifugation. Blood samples were centrifuged and the plasma removed, allowed to clot, and 0.01 ml of undiluted serum was removed, subjected to paper electrophoresis and the protein components estimated by means of an Analytrol recording scanner and integrator.

Results. The results are given in Table I. It will be noted that the hematocrit, hemoglobin and red cell counts of the control rats were within the limits established as normal for the species, and that a severe state of anemia and polycythemia respectively existed in the partners with intoxication. Plasma protein concentration was found to average 6.10 g/100 cc for the controls, in contrast with 4.85 and 7.96 g/100 cc respectively for anemic and polycythemic animals. Serum electrophoretic patterns of anemic and polycythemic animals as compared to controls, both showed a decline in the percentage composition of albumin, and a concomitant increase in the percentage of α_2 globulin.

Rat 1368—Left, Anemic

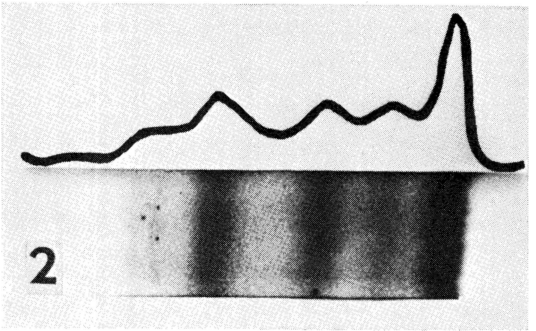

FIG. 2. Electrophoretic pattern of serum proteins from the anemic partner of a pair, #1368, with parabiosis intoxication. Plasma proteins, 1.75 g/100 ml.

Rat 1368—Right, Polycythemic

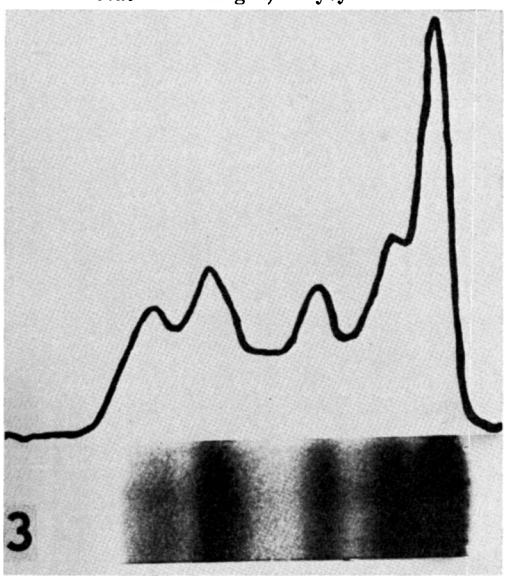

FIG. 3. Electrophoretic pattern of serum proteins from the polycythemic partner of pair #1368. Plasma proteins, 9.25 g/100 ml.

Discussion. The control values of both total plasma protein(15) and its percentage composition(16,17) were in general conformity with the figures reported for the rat. β globulin accounted for a greater percentage of the total than others have reported for the species but the significance of this is obscure in view of the known variability of serum protein electrophoretic patterns with age and strain(16,17). The animals utilized in this

study were very young, and such are known to have a proportionately greater serum β globulin level than older animals(17).

It is evident that the plasma protein concentration is augmented by about 30% in anemic animals and diminished by a comparable amount in their polycythemic partners. These findings are confirmatory of the inferences drawn as to their probable distribution from the physiologic derangements known to characterize animals with parabiosis intoxication(6). Furthermore, the fact that the changes in protein concentration are of comparable magnitude but opposite in direction from the normal values, is much easier to reconcile with the thesis that the fundamental disturbance is haemodynamic in nature, than with any of the proposed alternatives.

Despite the apparent differences in blood protein concentration in the partners, the percentage which each of the constituent proteins contributed towards the total was the same in anemic animals as in polycythemic. However, compared to normal values both partners demonstrated a decline in the percentage of albumin and a concomitant increase in that of the α_2-globulin. Such a pattern in man is said to be characteristic of conditions in which there occurs extensive and fairly severe tissue inflammation and destruction(18), which may well be the case in parabiosis intoxication. Statistical analysis revealed that the other serum protein partitions were normal in both polycythemic and anemic partners.

Normal parabiotic rats are said to show a diminished percentage of serum albumin and an augmentation of β globulins as compared with single controls, although total plasma protein concentration is unaffected(19). These data indicate that when intoxication supervenes there is an apparent change in total protein concentration favoring the polycythemic animal at the expense of its partner, and that in both the percentage of serum α_2-globulin is increased, and that of the albumin decreased.

Summary. Rats which have developed parabiosis intoxication show, in addition to the other hematologic aberrations which characterize the condition, changes in the blood proteins. Anemic partners have a low plasma protein level and polycythemic animals an elevated plasma protein titre. The partitions which albumin and the respective globulins contribute toward the total serum proteins average the same in both anemic and polycythemic twins, although as compared to control levels there is a diminished serum albumin and an elevated α_2-globulin component in each.

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