ON THE NATURE OF PARABIOSIS INTOXICATION: SHOCK AS THE PRECIPITATING CAUSE*

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Parabiosis has been widely employed to attempt elucidation of a number of problems of biological interest. Rats have been generally used and in them, although by no means confined thereto, there commonly occurs a condition of mutual systemic incompatibility usually termed parabiosis intoxication (1, 2). Estimates of the frequency vary widely, but an incidence of between 30 and 60 per cent of pairs is usually given (1–3). In those afflicted, one of the partners becomes anemic, with pale-colored paws, ears, and skin surfaces and often shows lipemia; while the co-twin develops polycythemia, and the skin areas are engorged with blood. These are often referred to as "white" and "red" rats respectively (1, 2). Various pathologic and physiologic derangements have been described in one or both of such partners. Certain of these (*e.g.* hypertension, arteritis, and renal lesions) seem to result from some characteristic of the parabiotic state although probably not specifically from incompatibility, since they may either occur in one of a normal pair of parabionts that do not demonstrate external signs of intoxication (4–6), or in the hyperemic member of pairs which do (7, 8). Other changes (*e.g.* splenomegaly, lymphoid hyperplasia, and erythrophagocytosis (3)), found in intoxicated pairs, may occur in either the anemic or the hyperemic animal and therefore are not clearly either causally or derivatively related to the respective hematologic states.

Various theories, none of them generally accepted, have been advanced to account for the syndrome. It would not be profitable to consider each of them in detail, but they include hemolysis of the cells of the anemic rat by the serum of its mate (9), cachexia and toxic effect (3), dehydration (10), nutritional disturbance (11), *Bartonella* infection (12), poor operative technique (13) and "stress" (14). The diversity of opinion has been emphasized in an excellent review on parabiosis (15). The most recent theory contends that following surgical union there are formed in one or both rats incomplete isohemagglutinin antibodies. These, it has been contended, lead to hemolysis and anemia in one rat, while such of its cells as reach the twin become agglutinin-coated and adhere together resulting in masses too large to renegotiate the capillary network joining the animals, and remain sequestered in the one circulation: these,

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together with that rat's own cells, produce polycythemia (2, 8). This hypothesis clearly fails to explain why the aggregates, if too large to pass through the capillaries joining the two, successfully traverse the various capillary beds of the animal to which they are confined, which, in the absence of evidence of thrombotic disease, they seemingly do quite successfully.

Various findings in this laboratory have not been entirely compatible with this view. Among the lines of evidence which, though not conclusively negating the immune theory suggested a possible alternative, were (*a*) the development of the condition in some pairs after months of harmonious partnership (16, 17), and (*b*) its presence in dead pairs which, as revealed by daily examination, had never manifested such changes during life. The latter finding suggested the possibility that one partner might die first and, because of differences in capillary pressure, be transfused by the survivor, thus leading to plethora in the recipient and anemia in the donor. Attempts to induce the condition experimentally in normal pairs with drugs proved to be futile. For if there was not an effective vascular connection between the two it was obviously impossible for both partners to participate in a hematologic response by treating only one of them; on the other hand if the vascular anastomosis was effective, then the agent entered the circulation of both partners and would not exert a qualitatively different action in each of them. A surgical method was finally adopted which permitted a state indistinguishable in its hematologic manifestations from spontaneous intoxication to be immediately induced in normal pairs.

Materials and Methods

The rats used in this study were of the Holtzman strain. Those parabiosed were paired for weight, litter, and sex and were joined surgically by the method of Bunster and Meyer (18). A number of such pairs are maintained at all times in our laboratory, and for the present study twenty-one normal pairs, three of them being males, were selected. They had been united when weighing between 65 and 100 gm.: and in parabiosis for between 11 and 41 days. Ten single animals of like age and weight served as controls. From each of the pairs blood samples were taken for a red cell count, hematocrit reading, and in most cases a hemoglobin determination. One of the rats—in eleven cases the left partner and in ten cases the right—was then subjected to severance of the spinal cord just caudad to the 7th vertebra. The operation was performed on the chosen animal while both were anesthetized with ether. The method was similar to that described by Frank (19) except that laminectomy was avoided. The wound was then closed by two or three stitches through the skin. Daily, and in some cases more frequent measurements of the blood components were made thereafter on both partners. The same procedure was carried out on the single controls prior to and following cord section.

RESULTS

Although the single controls actually followed temporally the studies on the pairs, the data obtained on them are given first in order to establish normal values. From Table I it will be observed that the values of hematocrit reading, red cell count, and hemoglobin were not changed appreciably in the 4 days following spinal section. Postoperatively all animals became poikilothermic and were paralyzed caudad to the level of transection. The paralysis affected

the urinary bladder, thus necessitating its frequent manual evacuation, and ultimately hemorrhagic cystitis of varying severity occurred. No particular attempt was made to prolong survival beyond the 3rd postoperative day, inasmuch as most of the pairs to be described succumbed within this period.

Transection performed on one of a pair of parabiotic rats had an effect on the blood composition of each in marked contrast to the hematologic stability characteristic of single rats. In each case the operated animal developed a progressive erythremia while the unoperated twin concurrently became increasingly anemic. These changes were reflected in an increased red cell count and hematocrit reading of the former and a decrease in the latter (Fig. 1). Hemoglobin determinations were not always made but when performed they paralleled the changes in count and hematocrit reading, values as high as 25.6 gm. per cent being encountered in plethoric animals and as low as 2.1 gm. per cent in anemic animals. Most unusual was the fact that in 17 cases the unoperated animal succumbed first. In 3 the transected animal died first, and in one case priority could not be established. In 5 of the 17 cases the surviving transected animal was separated surgically from the dead twin, and lived for 3 to 7 days thereafter. Survival of the intact animal following spinal transection of the twin ranged from less than 1 to 6 days with an average of 3.5 days. The plasma of these animals usually showed marked lipemia (Fig. 2), whereas that of the operated twin commonly evidenced mild hemolysis. The hematologic alterations were often detectable within hours of the operation (Table II). Animals developing anemia most rapidly had the shortest survival time, giving the impression that this was because of a well developed vascular anastomosis which allowed a rapid transfer of blood. On autopsy it was found that the intact anemic animals had viscera which were depleted of blood, whereas those of the transected partner were markedly engorged (Fig. 3).

TABLE I

The Effect of Spinal Transection on the Hemogram of the Rat

Data	Preoperative day	Postoperative day			
		1	2	3	4
No. of rats	10	10	10	8	2
Hemoglobin, *gm/100 cc.*	15.6 ± 0.29	16.3 ± 0.36	15.3 ± 0.44	16.3 ± 0.33	17.9 ± 0.23
Hematocrit count, *per cent*	45 ± 0.23	46 ± 0.88	44 ± 1.2	46 ± 1.3	47 ± 1.6
Erythrocytes, *millions/c.mm.*	8.41 ± 0.21	7.67 ± 0.27	7.54 ± 0.24	7.63 ± 0.28	7.69 ± 0.90

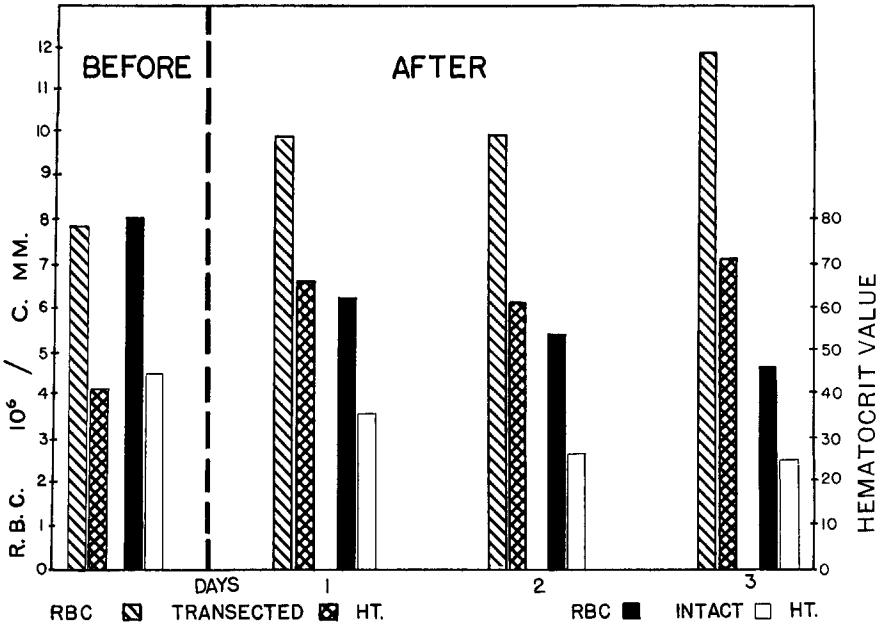

FIG. 1. RBC count and hematocrit changes in parabiotic rats before and after spinal transection of one of them. These are average values based upon 21 pairs prior to transection and upon 20, 15, and 10 pairs respectively on postoperative days 1, 2, and 3.

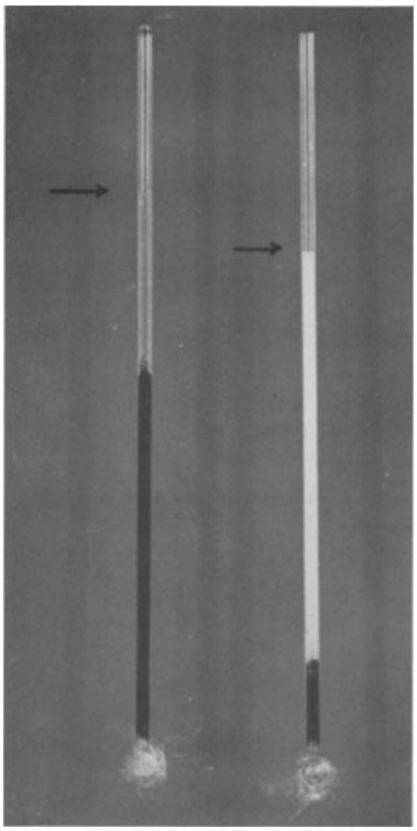

FIG. 2. Hematocrit findings in transected (left) and intact members of a parabiotic pair of rats taken 3 days postoperatively. Note marked disparity in red cell volume and the lipemia of the intact rat. The arrows indicate plasma levels.

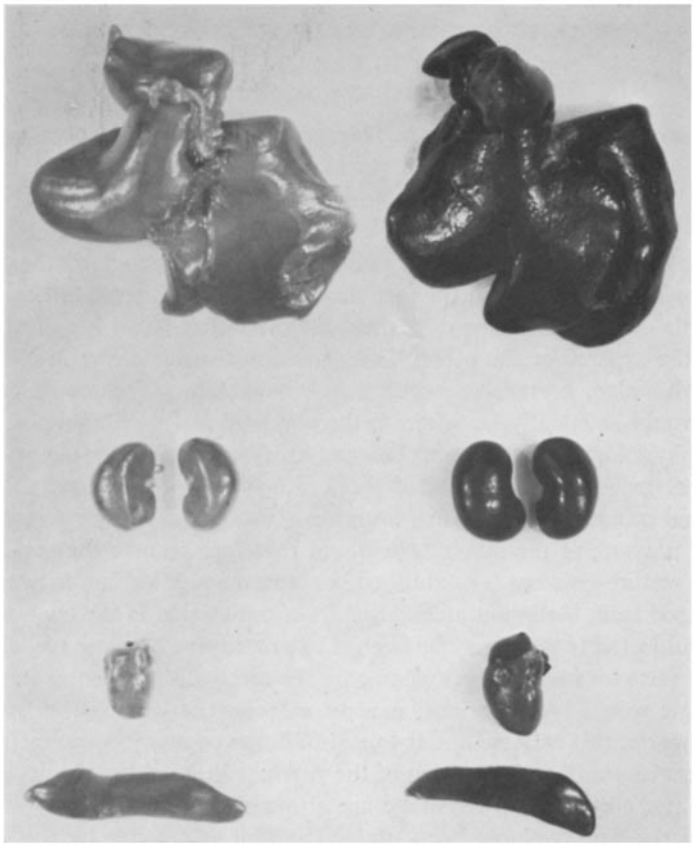

FIG. 3. Liver, kidneys, heart, and spleen from intact rat (left) and the partner with a spinal transection. Note pale color of the former due to blood depletion and the dark color of the latter due to engorgement.

TABLE II

Acute Changes in RBC Count and Hematocrit Value of Parabiotic Rats Following Unilateral Spinal Cord Transection

Time	State	Pair			
		1		2	
		RBC	Hematocrit	RBC	Hematocrit
		millions/ c.mm.	*per cent*	*millions/ c.mm.*	*per cent*
Preoperatively	Intact	5.02	42.3	8.04	44.1
	Intact	6.17	43.4	9.28	46.7
6 hrs. postoperatively	Transected	6.99	50.2	10.13	55.2
	Intact	3.99	33.4	8.84	34.8
24 hrs. postoperatively	Transected	8.33	68.4	10.73	63.6
	Intact	3.64	26.8	7.54	30.2

DISCUSSION

The experimental production in "normal" parabiotic rats of a syndrome which, like parabiotic intoxication (1, 2), consists of one of the partners developing anemia and often lipemia whereas the twin concurrently shows erythremia and hemolysis, suggests a new approach to an understanding of the etiology of the spontaneous condition. The rapid onset of the induced hematologic disturbance and the fact that both the erythremia of the one and the anemia of the other are progressive, indicates that blood is gained by one twin at the expense of the other. This should not result *per se* in changes of hematocrit value, erythrocyte count, or hemoglobin, inasmuch as cells and plasma would be equally transferred; the observed shifts are therefore attributed to physiological adjustments compensatory to the volume changes.

It is presumed that the state of shock which ensues in the parabiotic rat with spinal transection results in a lowering of its capillary blood pressure. The capillary pressure of the intact twin would therefore become the greater, and hence a pressure gradient is established between the pair leading to progressive loss of blood from the intact animal and its accumulation in the operated. The former, subjected to what may be likened to progressive hemorrhage, attempts to compensate for loss of blood volume by the mechanism which, if the animal were single would be efficacious, namely vasoconstriction. In the parabiotic state, however, this only enables it to maintain the capillary pressure at a level which permits continued perfusion of the partner. Presumably the erythrocyte reserves in the spleen and elsewhere are also mobilized during loss of the red cells, and the blood volume is maintained as well as possible by the addition of extracellular fluid to the plasma. This too being ineffective because of continued loss the animal succumbs. That such a sequence of events does occur is indicated both by the blood findings and by the marked contrast between the engorged spleen, liver, and kidneys of the transected plethoric rat, and the exsanguinated appearance of these same organs in the intact anemic partner (Fig. 3). Extracellular fluid added to the plasma would account for the observed decrease in erythrocyte count, hematocrit reading, and hemoglobin in the intact animal and may be responsible for the lipemia. It appears as if the transected animal gains blood from the twin, and attempts to keep its own blood volume under control both by the utilization of its storage facilities and by reducing the fluid component, which is more easily manipulated than the formed elements. This leads to increased cell count, hematocrit value, and hemoglobin. The lipemia which occurs in anemic rats is probably similar in nature to that which occurs in rabbits subjected to repeated hemorrhage (20), whereas the hemolysis in the erythremic partner likely reflects a compensatory destruction of the excess red cells or perhaps the action of autoantibodies. Fig. 4 represents schematically the concept of unequal transfusion.

Such a mechanism would account for the finding that many pairs which have never shown signs of intoxication during life do so when discovered dead.

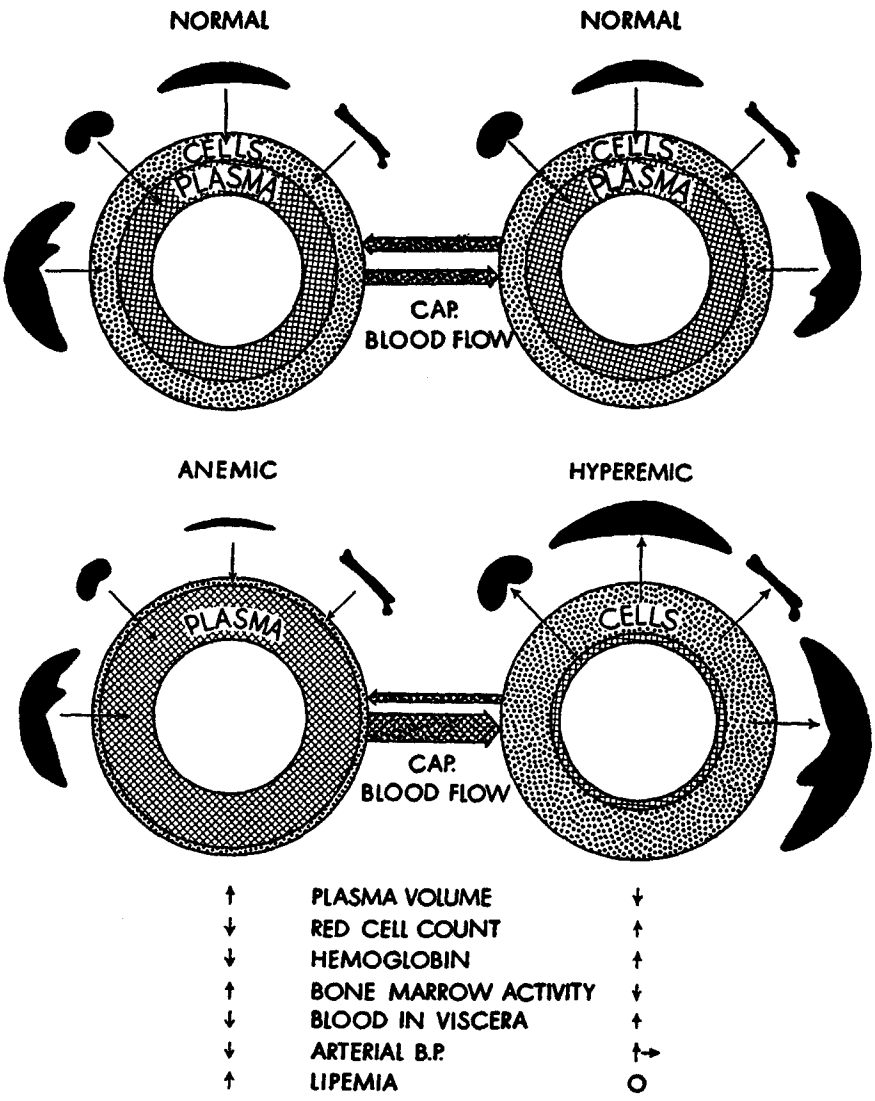

FIG. 4. The upper schema represents the interplay between the vascular compartments and certain organs in normal parabionts: the lower typifies what is believed to occur following shock in one or in intoxication. In the normals the arrows at the capillary bed between the two rats indicate an equal exchange of blood: those connecting the blood stream with the liver, kidney, spleen, and bone marrow indicate a normal equilibrium. In intoxication the disproportionate interchange of blood favors one rat. This together with compensatory changes leads to hemoconcentration and plethora while the twin develops hemodilution and anemia. In the anemic rat there is a depletion of erythrocytes from the liver, spleen, and kidney and increased erythropoietic activity of the bone marrow. The hyperemic rat stores red cells in the liver, spleen, and kidney, thus their engorgement, while erythremia supresses marrow erythropoiesis.

The one which succumbs first drains away the blood of the survivor, which then dies as a consequence of supplying it.

That the shock which follows spinal transection is responsible for the hematologic response is evidenced by the fact that in other experiments it has been possible to induce the typical visual anemia-hyperemia of parabiosis intoxication simply by killing one of a pair with ether. If the anemic member of an intoxicated pair or either member of a normal pair is thus killed and allowed to remain attached, then the surviving partner characteristically develops anemia. In examples of the first type the fatal anemia develops in a previously polycythemic rat whereas in the latter instance it develops in a hitherto normal animal (21). The point is further emphasized by the observation that the removal of elastic ligatures applied to both hind legs of one of a pair of parabionts produces anemia in the non-traumatized twin, although in this case it is only of moderate severity, probably because shock in the ligated animal is rapidly reversed and not fatal as in single animals similarly treated (22). It might be mentioned parenthetically that in pairs which do not have an effective vascular anastomosis, as determined visually by the union being reduced to a thin pedicle of skin, spinal transection of one, as might be anticipated, has no effect on blood composition of either.

It is not generally realized that, as has been indicated, after death the anemic partner usually becomes engorged with blood at the expense of its surviving but rapidly exsanguinated mate, which then succumbs in turn. Thus after death the gross appearance of the pair is the reverse of what it was during life in respect to anemia-hyperemia (21). The failure to appreciate this fact has doubtless been responsible for much of the confusion in the literature as to which of the partners is the smaller, which dies first, and which of them shows the various visceral alterations and lesions alleged to occur in parabiosis intoxication (1–3, 9, 14, 23, 24).

There seems little reason to support the contention that parabiosis intoxication results from an immune reaction, or that the anemia of one of the partners is due to a hemolytic process. The criteria used to identify hemolysis are often inadequate, and although some investigators have reported blood group differences in intoxicated pairs (2), others have consistently failed to do so (14, 25). The data indicate that the anemic rat is a victim which suffers death because of vascular attachment to a co-twin whose lowered capillary pressure causes it to accept more blood than it returns. The measurement of arterial blood pressure is of little use in evaluating this situation since it does not accurately reflect the concurrent pressures of the capillary anastomosis. Parabiotic pairs in which one of the partners has arterial hypertension, and in which its pressure may be twice that of the twin, show no such disproportionate transfer of blood (4, 5). This is probably because, as in human arterial hypertension (26), the capillary pressures are normal.

During the preparation of this paper there appeared a study the results of which are so basic to an understanding of the nature of parabiosis intoxication that it is deserving of detailed presentation (27). It also happens, according to the authors, to be the first case of its type reported in the medical literature.

Two identical twin girls were delivered after a normal full term pregnancy. Baby A weighed 2690 gm. and baby B weighed 1770 gm. The former was plethoric; the latter was anemic and had hepatosplenomegaly. The respective hemograms revealed: Hb 25.2 gm. per cent, RBC 7.47 million/c.mm., hematocrit value 87 per cent, total bilirubin 12.3 mg. per cent; and Hb 3.7 gm. per cent, RBC 1.85 million/c.mm., (hematocrit reading not given), and total bilirubin "normal." Both babies and the mother were found to be group A, CDE, MN. An indirect Coombs test with the mother's serum and each of the babies' cells was negative, as were indirect Coombs tests with the serum of each child and the cells of its twin. A direct Coombs test was also negative. It was the conclusion that no blood incompatibility existed between the infants or between either of them and the mother.

Examination of the placenta revealed one chorion separating two amniotic layers; a single ovum placenta divided into two unequal portions. Perfusion of an artery in each of the cords revealed arteriovenous shunts between the supposedly separate placental circulations substantiating a parabiotic circulatory system. From this and other studies it was concluded that there existed a relative obstruction to the return of venous blood to baby B, causing it to become anemic. The resulting back pressure extended to baby A's portion of the placenta through the parabiosed circulations and caused it to become plethoric. In brief, the hematologic findings were ascribed to the slow transfusion of one child by the other.

The authors were apparently unaware of the syndrome of parabiosis intoxication, but the case is clearly its clinical counterpart. The conclusion of the authors as to the fundamental disturbance is in entire agreement with the views herein expressed with respect to the experimental study, and reinforce the hypothesis that parabiosis intoxication is not an immune response but is due to unequal transfer of blood between the partners. It is possible that the cardiovascular-renal lesions which develop in one of a parabiotic pair of rats (4–8), are the result of a hypersensitivity reaction, but, as has been indicated earlier, these seem not to be related to the hematological disturbances.

It is surprising that although the syndrome has been known for almost 50 years (28) and has been the subject of intensive investigation, the problem has thus far eluded solution. Preoccupation with the idea of an allergic reaction presumed to result from an exchange between the pair of a substance highly antigenic to erythrocytes is evident from the literature (2, 7–9, 14, 23). This is probably derived from the plausibility of the concept in circumstances under which two animals, presumably genetically dissimilar, are joined by a common blood stream, rather than from any clear cut supportive evidence.

SUMMARY

Spinal transection of one of a parabiotic pair of rats is immediately followed by a state indistinguishable in visual and hematologic characteristics from spontaneous "parabiosis intoxication." The transected rat develops erythremia, hyperhemoglobinemia, and an increased hematocrit count; whereas the twin concurrently shows anemia characterized by a decrease in hemoglobin, erythrocyte count, and hematocrit reading, and often also lipemia.

These findings are ascribed to whole blood transfusion of one rat by the other followed by adjustments compensatory to the resulting distortion in the respective blood volumes. It is suggested that parabiosis intoxication is a manifestation of this same process and is not due, as has been contended, to an immune response.

BIBLIOGRAPHY

1. Finerty, J. C., and Panos, T. C., *Proc. Soc. Exp. Biol. and Med.*, 1951, **76,** 833.
2. Chute, R. M., and Sommers, S. C., *Blood*, 1952, **7,** 1005.
3. Herrmansdorfer, A., *Deutsch. Z. Chir.*, 1923, **178,** 289.
4. Hall, C. E., and Hall, O., *Arch. Path.*, 1951, **51,** 527.
5. Hall, C. E., and Hall, O., *Texas Rep. Biol. and Med.*, 1951, **9,** 714.
6. Turiaf, J., Zizine, L., and Sors, C., *Presse med.* 1954, **62,** 57.
7. Zeckwer, I., *Arch. Path.*, 1952, **54,** 84.
8. Sommers, S. C., Edwards, J. L., and Chute, R. N., *J. Lab. and Clin. Med.*, 1954, **44,** 531.
9. Mayeda, T., *Deutsch. Z. Chir.*, 1921, **167,** 295.
10. Morpurgo, B., *Verhandl. deutsch. path. Ges.*, 1925, **20,** 255.
11. Kojima, K., *Deutsch. Z. Chir.*, 1931, **232,** 578.
12. Sauerbruch, F., and Knake, E., *Klin. Woch.*, 1936, **15,** 884.
13. Perelman, L. R., and Kolpakow, I. V., *Vrach. delo.*, 1938, **20,** 871.
14. Diepenhorst, M. J., and de Vaal, O. M., *Acta physiol. et pharmacol. neerl.*, 1950, **1,** 342.
15. Finerty, J. C., *Physiol. Rev.*, 1952, **32,** 277.
16. Hall, C. E., and Hall, O., unpublished data.
17. Hoelscher, B., personal communication, 1954.
18. Bunster, E. and Meyer, R. K., *Anat. Rec.*, 1933, **57,** 339.
19. Frank, J. D., *Endocrinology*, 1940, **27,** 447.
20. Spitzer, J. J., *Fed. Proc.*, 1955, **14,** 143.
21. Hall, C. E., and Hall, O., *Am. J. Physiol.*, in press.
22. Hall, C. E. and Hall, O., *Proc. Soc., Exp. Biol. and Med.*, 1955, **90, 230.**
23. Moller-Christensen, E., *Acta Path. et Microbiol. Scand.*, 1932, **55,** 1939.
24. Niekau, B., and Duschl, L., *Deutsch. Z. Chir.*, 1925, **191,** 221.
25. Bichel, J., and Holm-Jensen, I., *Acta Physiol. Scand.*, 1949, **17,** 255.
26. Ellis, L. B. and Weiss, S., *J. Clin. Inv.*, 1929, **8,** 47.
27. Klingberg, W. G., Jones, B., Allen, W. M., and Dempsey, E., *Am. J. Dis. Child.*, 1955, **90,** 519.
28. Ranzi, E., and Ehrlich, H., *Z. Immunitätsforsch.*, 1909, **3,** 38.