ical pictures of regenerated fibers in the distal stump, but without substantiation of their number.

As in the former series, the diameters of the regenerated branches were subnormal, with only a few reaching the 8-9 micra class. This caliber deficit indicates that the fibers have been encroached upon at some proximal level by endoneural fibrosis, possibly at the points where the nylon cable was sewn through the stumps. It has been shown that the narrowing of the lumen of a fiber at any one level limits the caliber growth of the distal portion of the fiber.[3,4] While one must suspect that permanently subnormal calibers entail some deficiencies in function,[5] these have not been severe enough to become grossly discernible.[2]

Conclusions. Comparing these results with those of the earlier series involving gaps of about half the length, no evidence of a decline of the power of regeneration with the increase in the length of the gap can be detected. It may be reasonable to assume that the gap could be lengthened even more without becoming unbridgeable.

Summary. Gaps of from 22-32 mm in length in tibial nerves of monkeys were closed by nylon cables embedded in tubulated blood bridges.[1,2] The reinnervated muscles regained an average of 85% of their normal weight. The distal regenerated nerve fibers were approximately normal in number, though subnormal in size. There has been no reduction of regenerative power with increased length of the gap in this series as compared to a previous series with shorter gaps.

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Erythrophagocytosis in Bone Marrow Culture with Relation to Antagonism of Pteroylglutamic Acid.*

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A small series of cultures of bone marrow has been studied, as part of a larger investigation of the function of pteroylglutamic acid (PGA).[1] Through the addition of PGA-antagonists [(crude antagonist N67-A,[2-4] 'Methopterin' (a methyl pteroylglutamic acid of unidentified structure,[5]) and 'Aminopterin' (N-[-{[(2,4-diamino-6-pteridyl) methyl]-amino}-benzoyl]-glutamic acid][6]‡ it had been hoped to achieve *in vitro* a maturation arrest

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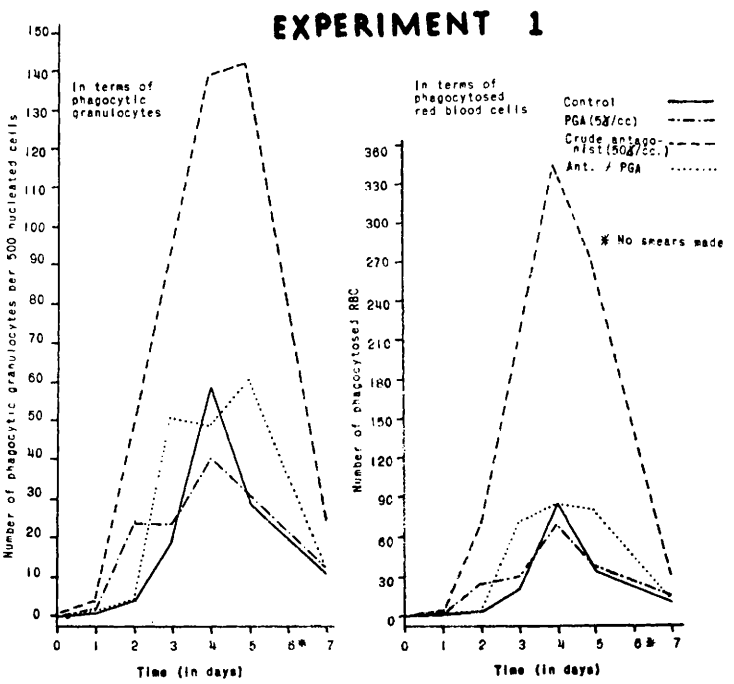

FIG. 1.
Erythrophagocytic effects of crude PGA-antagonist (N67-A) and its reversal by PGA.

in the erythroid series. Although this did not develop, extensive phagocytosis of erythrocytes, chiefly by granulocytes, was encountered, with a definite relationship to the presence of the PGA-antagonist. Because this phenomenon may have clinical implications, a summary of the results is being reported.

The culture method utilized was essentially that of Osgood and Brownlee.[7] Human umbilical cord serum in a concentration of 30-35% in a balanced salt solution was employed as the basic medium, in the manner described by these authors. Bone marrow cells were procured by the aspiration of 2 to 7 cc of marrow content from the sterna of patients without demonstrable hematologic disorders.

Observations in 2 experiments are shown in the accompanying graphs. A third experiment, involving the use of 'Aminopterin' (55 μg/cc), revealed, on the fifth and last day of the experiment, a count of 16 phagocytic granulocytes per 200 nucleated cells in the culture containing 'Aminopterin,' as opposed to 2 such cells in the control culture.

Except for an occasional eosinophil and several myelocytes, the phagocytic cells were either segmented or band-form polymorphonuclear neutrophils. Large macrophages, similar to those usually described, were found in all cultures after the first to third day, but these cells were only slightly phagocytic of erythrocytes, and were not included in the category of erythrophagocytic cells. (These macrophages have persisted in culture for more than 70 days). Only phagocytosed erythrocytes with distinct morphology were enumerated, thereby excluding cells in an advanced state of digestion. As many as 10 or 11 red cells have been counted in a single phagocyte. Occasionally, a normoblast was found phagocytosed.

From examination of the graphs, it will be noted that (a) the number of erythrophagocytic granulocytes in the culture containing a

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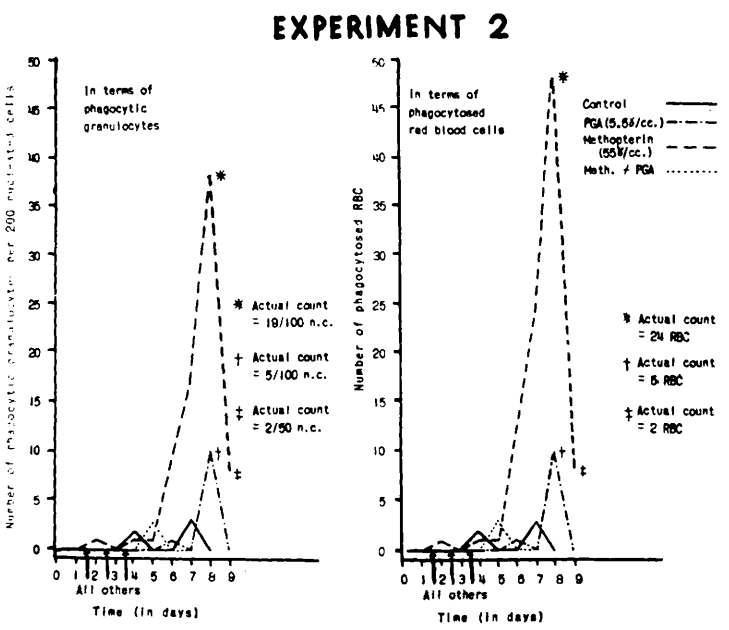

FIG. 2.

Erythrophagocytic effects of "Methopterin" and its reversal by PGA. Arrows indicate solid line representing all cultures other than those shown above this line.

PGA-antagonist became much greater than that of any other culture, (b) these cells, in the cultures containing antagonist, ingested more erythrocytes than those in the other cultures, and (c) these effects were prevented by the addition of PGA.

Daily leucocyte and differential counts of 200 nucleated cells revealed the relative and absolute numbers of segmented and band-form polymorphonuclear leucocytes in the PGA-antagonist cultures to be similar to, or often less than, those in the other cultures. Thus, the greater erythrophagocytosis prevailing in the antagonist cultures was not due to the presence of a greater number of potentially phagocytic cells.

These findings are of interest because of the occurrence of erythrophagocytosis in certain cases of marked anemias. Such phagocytosis has been observed in the peripheral blood in idiopathic aplastic anemia,[8] pernicious anemia,[9-11] acute hemolytic anemia of the Lederer type,[8,12] severe "secondary anemia,"[13] sickle-cell anemia (more pronounced in the liver, spleen, and lymph nodes),[8,14-17] erythroblastosis fetalis,[8,11,18] paroxysmal hemoglobinuria,[11] and in marked anemic states associated with leukemias,[11,19] parasitic infections (especially malaria),[11] and toxic or infectious states (especially subacute bacterial endocarditis).[11] Oroya fever, with a severe anemia resembling pernicious anemia, evidences marked erythrophagocytosis in the bone mar-

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row. spleen and liver.[20,21] The anemia of Hodgkin's disease has been ascribed to erythrophagocytosis (in lymph nodes, liver, lungs and bone marrow),[22] and the severe anemia of primary splenic panhematopenia has been attributed to excessive phagocytic function of the spleen.[23] Throughout the literature, macrophages have been the chief phagocytic cell reported. Only in patients with severe pernicious anemia,[9] and a severe "secondary anemia" (probably of a leukemia)[13] have granulocytes been the predominating phagocytic cell type.

In animals, toxic or infectious states have led to anemic conditions associated with erythrophagocytosis.[24] Most prominent of these is the bartonellosis of rats,[25] similar to Oroya fever in man. It is of interest that this condition is reported to be improved by treatment with PGA.[26] Other studies in man and rodents indicate an increased phagocytic function of granulocytes in anemia (including pernicious anemia), the increase in phagocytosis being roughly proportional to the severity of the anemia.[27]

The clinical and experimental data suggest, therefore, that erythrophagocytosis may be associated with a PGA-deficient state. Further investigation is required to ascertain if such a relationship exists.

Summary. Blood cell cultures grown in the presence of PGA-antagonists exhibited marked erythrophagocytosis by granulocytes when compared with cultures containing no antagonist or containing antagonist and PGA. It is suggested that PGA-deficiency might account for erythrophagocytosis observed in certain instances of anemia in humans and animals.

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16494

Electrokinetic Change in Human Erythrocytes During Adsorption and Elution of PR8 Influenza Virus.*

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Hirst[1,2] and McClelland and Hare[3] found that PR8 strain of influenza virus could be adsorbed by chicken erythrocytes with their accompanying agglutination. On standing it was also found that the virus was gradually eluted from the cells and that the cells could no longer adsorb subsequent additions of that

* Aided by grants from the National Foundation for Infantile Paralysis, Inc., and from the Commission on Influenza, Army Epidemiological Board, Office of Surgeon General, U. S. Army.

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