nonspecific growth factor (NGF).

The inverse dose-response relationship noted in some experiments in which (McCoy) cells were used could possibly have resulted from the presence of an inhibitor as well as a stimulant substance in these extracts. This concept is consistent with the isolation of substances such as the growth promoting substance "promine" and the inhibitory substance "retine" reported by Hegyeli *et al*(13) and Szent-Gyorgyi *et al*(14). It is postulated that these substances are either identical with erythropoietin (NGF) or, more likely, they are members of a closely related family of nonspecific growth controlling substances. A reexamination of ESF (NGF) in this light could yield valuable information concerning control of cell division and tissue growth in normal and malignant tissue.

Summary. Cultures of 2 cell lines have been tested for their response to administration of erythropoietin (ESF). The ability of ESF to stimulate growth of cultures of human synovial membrane cells and human monocytic leukemia cells was demonstrated. ESF is ineffective in stimulating cell culture multiplication in very rapidly growing cultures. Suggestion was made that ESF be called "Nonspecific Growth Factor" (NGF).

The authors wish to acknowledge the excellent technical assistance of Misses Marilyn Guarino, Jesdon Haake and Rita Mortimeyer.

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Received October 24, 1963. P.S.E.B.M., 1964, v115.

Enhanced Efficacy of Plasma After Aging in Treatment of Tourniquet Shock.* (28998)

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In previously reported studies from this laboratory concerned with infusion therapy of rat tourniquet trauma, reconstituted lyophilized human plasma was shown to be the most effective infusion agent when compared with saline, dextran in saline and dextran in glucose-in-water(1). The initial choice of the human material was made because of the convenience of preparation of large quantities of sterile plasma using this commercially available material. When in practice it proved to be highly effective, its use was continued.

In later experiments, designed to investi-

* Supported by Dept. of Army, Office of Surgeon General, Defense Contract.

gate the properties of convalescent tourniquet serum, rat plasma was used in the therapy of rat tourniquet trauma; in these experiments it was established that the commercial human lyophilized preparation was more effective volume for volume than any of the rat plasmas which we were able to prepare. The reasons for this difference are still under investigation; some of the possibilities discussed were: 1) that the reconstituted human plasma had a protective effect universally present in all human plasma or 2) that commercial processing had in some way conferred a beneficial effect on it, or 3) that the aging of the blood prior to its lyophilization had in some way given it a protective effect not present at the time of its collection. In the experiments to be reported here comparison was made of the therapeutic properties in treatment of tourniquet shock in the rat of: 1) human plasma collected just prior to infusion (single donor and pooled), 2) aged pooled, human plasma at intervals up to 6 months after its collection and 3) commercial lyophilized human plasma.

Methods. Male albino rats of the Holtzman Farms, weighing between 200 and 270 g, were used. The details of the bilateral hind limb tourniquet trauma have been described(1). The application of tourniquets in this manner for 4½ hours has been found to be a consistently lethal trauma (*i.e.*, more than 96% of untreated control animals succumb within 48 hours).

One hour after tourniquet release infusion therapy was begun through a small polyethylene catheter inserted into the jugular vein. The delivery time of the infusion was 3 hours ± 9%; after completion, the vein was ligated and the neck wound closed. Three constant infusion pumps, each housing 12 syringes, were used.

By initiating fluid administration one hour after tourniquet release and continuing it for 3 hours, fluid therapy was given over the period of greatest fluid loss from the circulation into the traumatized extremity (as documented by Koletsky and Gustafson(2)). The volume of infusion in each instance was 3 ml per 100 g body weight. Survival was recorded at 48 hours after tourniquet release.

The experiments were divided into 2 groups as follows. In the first group of experiments simultaneous comparison was made of: 1) "fresh" (just collected) plasma from a single donor, 2) pooled plasma, shelf stored in the blood bank at room temperature for 6 months and 3) reconstituted, lyophilized plasma obtained commercially from the Hyland Laboratories. Each of the 3 solutions was infused into a group of 12 animals at a time (36 per day). In the second group of experiments, arrangements were made to prepare sterile fresh human plasma in our laboratory by passing it through a millipore filter sequence down to a pore size of 0.22 μ into a pre-sterilized bottle. A comparison of filtered and unfiltered reconstituted plasma showed no difference in therapeutic effectiveness. This procedure allowed for aging of a single large batch of human plasma, divided into aliquots for testing at various time intervals after collection. In each instance, bacteriological studies of the plasma before use showed it to be sterile. Simultaneous comparison of the following solutions were then made:

1) fresh pooled plasma (6 donors) *vs* fresh plasma from a single donor

2) 1-week-old pooled plasma *vs* lyophilized plasma

3) 5-week-old pooled plasma *vs* lyophilized plasma

4) 13-week-old pooled plasma *vs* lyophilized plasma

5) 14-week-old pooled plasma *vs* lyophilized plasma.

Throughout this sequence of experiments the protein concentration of solutions to be infused was determined by use of a T/S optical density meter. Discrepancies in protein concentration of solutions on any given day were equalized by dilution with 0.9% sodium chloride solution.

Results. It is apparent in Table I that pooled, shelf-stored, human plasma is as effective as commercial, human, lyophilized pooled plasma, which previously was found to be the most effective infusion agent, uti-

TABLE I. Comparison of Human Plasmas in Therapy of Tourniquet Trauma. (Volume equal to 3% body weight.)

Plasma infused	48 hr survival Lived	Died
Fresh (individual donor)	0	35
Shelf stored (pooled, 6 mo at room temperature)	24	11
Reconstituted, lyophilized (pooled, commercial)	26	8

TABLE II. Effect of Aging of Human Plasmas in Therapy of Tourniquet Trauma. (Volume equal to 3% body weight.)

	Plasma infused	48 hr survival Lived	Died
Exp 1	Fresh, pooled	0	18
	Fresh, single donor	0	17
" 2	Pooled, 1 wk old	1	17
	Lyophilized, reconstituted	16	0
" 3	Pooled, 5 wk old	8	9
	Lyophilized, reconstituted	11	7
" 4 & 5	Pooled, 13 and 14 wk old	22	14
	Lyophilized, reconstituted	22	13

lized in therapy of rat tourniquet shock. Fresh human, non-pooled plasma was completely ineffective in protection against this trauma, there being no survivors with the use of this material.

For each type of plasma, rat cells were crossmatched against donor plasma and showed clumping of cells. During one experiment one animal from each group was sacrificed to allow inspection of the serum for hemolysis. In the animals which received fresh and shelf-stored plasma hemolysis was present to a slight degree. In the animals infused with the reconstituted lyophilized plasma a "moderate degree" of hemolysis was present. It would thus seem that, although some hemolysis was present in all groups, this did not interfere with the beneficial therapeutic effect.

The results of the second group of experiments are summarized in Table II. The use of recently collected plasma, whether single donor or pooled, produced no survival after the standard tourniquet trauma, but as the pooled plasma aged, it gradually acquired the ability to protect, so that by 3 months after collection, it was as effective as the lyophilized plasma.

Discussion and summary. The data presented demonstrate that pooled or single donor human plasma immediately after collection exerts no beneficial effect in the therapy of rat tourniquet trauma, but that the aging of this material in some way converts it into a potent therapeutic solution. Whether this is due to neutralization of something harmful or to the conversion of an inactive material into something beneficial has not been determined. Investigations are underway to ascertain whether aged rat plasma is more effective than fresh rat plasma in treatment of this trauma. Sequential electrophoretic studies are also being made.

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Received October 28, 1963. P.S.E.B.M. 1964, v115.

Hydroxyproline Excretion in Urine of Patients with Muscular Dystrophy and Other Muscle Diseases.* (28999)

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In some forms of muscular disease, such as muscular dystrophy, the muscle fibers are replaced largely by connective tissue and fat. In muscular dystrophy occurring as an hereditary disease in mice, the muscles affected may have about 25% more collagen than the muscles of controls(1). In the advanced

* Aided by grants from Nat. Inst. of Arthritis & Metab. Dis., Nat. Inst. Health, and Muscular Dystrophy Assn. of America, Inc.