by Hechter, that curves relating spreading to time are of 2 distinct types, one with rapid onset and S-shaped slope for the enzymatic spreading factor, the other typical for hyaluronate-inactive spreading factor with slowly ascending, progressively mounting slope. In addition, we noted that the area of ink spot retracts after 24 hours where inflammation and edema were instrumental in causing spreading. This type of curve was obtained repeatedly at various dosages of uterone and with several experiments done at different times by different observers. Uterone does not exert its spreading effect in dead skin, where inflammation and edema no longer occur but where enzymatic activity of injected hyaluronidase still is effective.

Summary. A spreading factor is contained in uterone which may be classified as a hyaluronate-inactive spreading factor exerting its effect through inflammation and edema. The nature of the component of uterone responsible for provoking inflammation remains to be determined and may well be a mucoprotein.

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Question of Purity of Erythropoietic Factor Concentrates.* (24457)

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There is now much evidence for a circulating erythropoietic factor and attempts have been made to concentrate it. Active material in plasma of rabbits made anemic with phenylhydrazine can be precipitated by ammonium sulfate and has been associated with a mucoprotein fraction(1,2,3). We prepared ethanol precipitates from anemic plasma according to Grant *et al.*(4)† and found them similar to concentrates made with ammonium sulfate. The stimulating factor found in urine of certain anemic patients is probably of the same nature as that found in anemic animals(5). This report compares certain properties and potencies of various active preparations made in 4 different ways.

Materials and methods. One preparation was from human urine(6),‡ the rest from plasma of anemic rabbits. These also were compared to a preparation made from plasma of normal rabbits. Starting material (PF) for some fractions was the filtrate obtained after boiling plasma at pH 5.5 for a few minutes(7). It contained only about 2% of plasma protein. We have shown that injections of unboiled plasma provoke immunological reactions which interfere with tests lasting 10 days or 2 weeks. After boiling there is no interference(3). The different preparations compared were: 1. PF from rabbits made anemic with phenylhydrazine was precipitated with 1.5 volumes of absolute ethanol. After 2 hours at 5°C the precipitate was removed in the centrifuge and an equal volume of absolute ethanol was added to supernatant. After 16 hours at 5°C it was centrifuged, the supernatant was poured off and the precipitate dried in a desiccator(4). About 25% of the protein and all erythropoietic activity of anemic PF was recovered in this fraction. In one case the yield was about 350 mg/l of

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TABLE I. Erythropoietic Activity of Various Preparations. Each assay was made with 6 mice or 4 rats, except where noted.

Preparation and source	Assay: Reticulocytes in normal mice after 3 doses — Dose	Reticulocytes, %	Assay: Fe^{59} uptake in fasted rats after 3 doses — Dose	Fe^{59}, %	Assay: Hemoglobin in normal rats after 12 doses — Dose	Hemoglobin, g % Day 1	Hemoglobin, g % Day 15
Saline	1.0 ml	2.1	2.0 ml	5.1	5.0 ml	14.7	14.2
PF anemic rabbit plasma	2.0 *†	9.0	2.0 *	32.0	5.6 *	14.3	17.3
1 *Idem*	.05 mg	4.0	.06 mg	9.7			
	.25	7.1	.24	22.3			
	2.0	11.0	.6	38.2			
2 "	.05	5.4	.5	14.2	2.16 mg	14.2	17.1
	.18	10.2					
3 "	.06	6.5	.93	21.5			
	.18	8.6					
4 Human urine	.1	4.8	1.0	25.6	2.5	14.5	16.9‡
	.5	8.5					
5 *Idem*			1.5	30.3			
6 Normal rabbit plasma	1.3	2.1	9.0	6.1			

* 1 ml PF contains 1.0-1.5 mg protein. † Reduced in vol. ‡ 2 rats only.

plasma. Nos. 2 and 3 were made from a fraction of anemic rabbit plasma, insoluble between 50-80% saturation of ammonium sulfate(2,3). 2. Material insoluble between 50-80% saturation of ammonium sulfate was boiled and a fraction soluble in 70% ammonium sulfate prepared as previously described(2,3). The yield was 100-130 mg/l of plasma. 3. Material insoluble between 50-80% saturation of ammonium sulfate was dialysed, boiled at pH 5.5 in 0.9% NaCl, the pH changed to 7.5, concentrated till protein was 1.4 mg/ml and precipitated with ethanol as in No. 1. The precipitate contained 25% of the protein and all activity of the filtrate after boiling. The yield was 180 mg/l of plasma. 4. *Urinary preparation.* This was the residue from ultrafiltration of urine of a patient with paroxysmal nocturnal hemoglobinuria.‡ 5. The same as 4, boiled 5 minutes. 6. Made the same as 1, but from plasma of normal rabbits. About 25% of the protein of boiled plasma was recovered. Yield about 300 mg/l of plasma. Preparations 1, 2 and 3 from anemic rabbit plasma and 6 from normal plasma, all of which had been boiled in the presence of all or much of the plasma proteins, showed the same main band in paper electrophoresis (pH 8.6, 0.05M veronal) with a mobility slower than albumin and faster than α_1-globulin. Preparations 4 and 5 from urine showed a main band between α_1- and α_2-globulin which after boiling was displaced to the origin. Activity of different fractions was tested by injecting them subcutaneously into mice or rats and by using the following assays: reticulocytes in normal mice after 3 daily injections(3), Fe^{59} uptake in fasted rats after 3 daily injections(8), and circulating hemoglobin concentration after 2 weeks of injections(7).

Results. Preparations 1, 2, 3, 4, and 5 all have activity by whichever method was used to test them. The effective dose was of the same order of magnitude for all 5 in mouse reticulocyte and rat Fe^{59} uptake assays (Table I).

In preparation 6 from normal rabbit plasma, we found no activity even at the high dose shown. This preparation can best be compared with 1, as they were made in the same way, yet 6 showed no activity at doses more than 20 or 30 times greater than 1.

Discussion. Isolation of such similar fractions of widely different effectiveness raises questions. The activity can be concentrated in a fraction containing highly soluble mucoproteins. It may be due to a special muco-

protein found only in anemic animals or to something not a mucoprotein. Because of the proteolytic destruction of activity(2,9) it is likely that it is of peptide nature or has such material as a necessary cofactor. Association with a different protein could account for the different electrophoretic behavior of the urinary material. Activity might be present in normal plasma but may not be separable by the methods effective with anemic plasma.

Summary. The bulk of erythropoietic activity of anemic plasma can be concentrated in a fraction which represents less than 0.5% of plasma proteins. Yet from the similarity in yield and electrophoretic behavior of the corresponding inactive fraction from normal plasma, it appears likely that the erythropoietic factor constitutes only a small portion of the present concentrates.

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Lipoprotein Lipase Response in Laennec's Cirrhosis.* (24458)

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Since the observation that heparin injected *in vivo* cleared alimentary lipemia in dogs(1), much work has been done in elucidating the mechanism of this phenomenon. It has subsequently been demonstrated that heparin-activated clearing factor is a plasma lipoprotein lipase which acts upon the triglyceride moiety of plasma lipoproteins to hydrolyze them to free fatty acid and glycerol(2,3). These studies have evoked increased efforts to relate concentration of this lipase to disorders of lipid metabolism(4), particularly atherosclerosis(4,5). In each instance, emphasis has been placed upon a *decreased* clearing factor or lipoprotein lipase response. Methods used in measuring lipoprotein lipase response or concentration have varied widely. Consequently, until recently, no attempt had been made to determine mean clearing factor response for a given "normal" population. A standardized *in vitro* method has been developed for determining clearing factor concentration and has been applied to an agewise study of a presumably "normal" population (6). Inasmuch as no significant agewise regression was found in clearing factor response, a mean for this population was established. In this study, it was incidentally observed that patients with primary diagnoses of chronic alcoholism and/or Laennec's cirrhosis demonstrated a significantly *increased* clearing factor response(7). In view of the observation that there is a lesser incidence of atherosclerosis in patients diagnosed as Laennec's cirrhosis, more extensive studies have been made on a larger group of subjects reported herewith. In a small group of patients with recent myocardial infarction (presumptive evidence of coronary atherosclerosis) evaluated in the original study, no significant difference in clearing factor response was observed when tested against the normal popu-

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