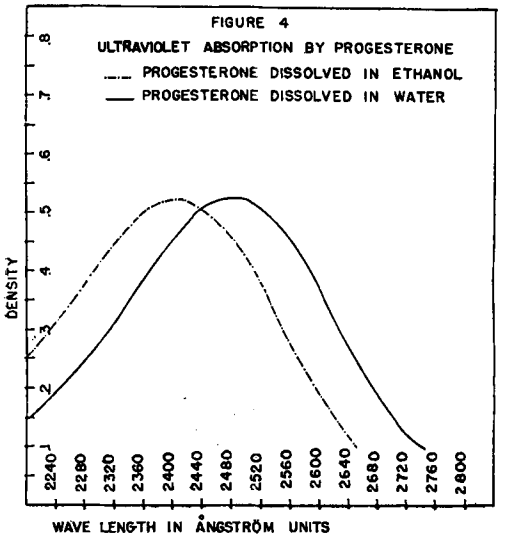
ture of 25° C until spectrophotometric readings were made at 24, 48, 72 and 168 hour intervals on the supernatant fluid. The solubility of progesterone thus obtained is presented in Fig. 2 and Fig. 3. It should be noted that equilibrium between the progesterone and its solvent was obtained at 72 hours and that the solution concentration remained constant to 168 hours. The slight irregularity in solubility noted in Fig. 2 is thought to represent supersaturation.



An incidental finding noted during these solubility studies was the shift of maximum ultraviolet absorption by progesterone from 2400 Å when dissolved in 95% ethanol to 2480 Å when dissolved in water. This shift occurred quite independently of the pH of the solution. Ultraviolet absorption curves are presented in Fig. 4 to illustrate this finding.

As will be noted in Fig. 2 and Fig. 3 the average solubility of progesterone in distilled water at room temperature for 72 hours was found to be 16.8 μg per ml and in 0.9% aqueous saline, 15.1 μg per ml, under identical conditions.

The solubility of progesterone in aqueous media as found in this experiment is at variance with that reported by Forbes and Hooker[1] of 6-9 μg per ml in saline. These results should not be construed as a marked disagreement since the smaller recorded solubility was based on bioassay primarily and the limited quantitative accuracy of bioassay is admitted.

Summary. The solubility of crystalline progesterone in aqueous media was determined spectrographically. Progesterone was found to have an average solubility at room temperature of 16.8 μg per ml in distilled water and 15.1 μg per ml in 0.9% aqueous saline.

[1] Forbes, T. R., and Hooker, C. W., *Science*, 1948, **107**, 151.

16884

Cell Proliferation Accelerating and Inhibiting Substances in Normal and Cancer Blood and Urine.*

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The rate of cell proliferation of bone marrow cells and of normal tissue cells cultured *in vitro* is accelerated by normal blood serum and inhibited by blood serum from cases of neoplastic disease, pernicious anemia, aplastic anemia and leukemia.[1] These pathological blood sera and normal blood sera counteract the effect of each other in a manner similar to the counteracting effect of xanthopterin and antixanthopterin[2] on cell proliferation *in*

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[1] Norris, E. R., and Majnarich, J. J., *Am. J. Physiol.*, 1948, **153**, 483.

[2] Norris, E. R., and Majnarich, J. J., *Am. J. Physiol.*, 1948, **152**, 652.

TABLE I.

The Effect of Fractions Obtained from Normal and Pathological Urine upon the Rate of Cell Proliferation in Bone Marrow Cultures *in vitro*. Time of incubation, 7 hours. The initial concentration of the bone marrow cell suspension was: red blood cells (RBC) 8400/cmm; nucleated cells (NC) 4760/cmm; reticulocytes 11/1000 RBC.

Supplement added	Final Concentration, RBC/cmm	RBC % Increase	Final conc., NC/cmm	NC % Increase	Retic. per 1000 RBC
No supplement	10960	28	7920	66	16
5 γ xanthopterin/ml	14300	70	10600	123	32
5 γ 7-MP*/ml	4400	—48	2960	—38	0
Normal Human Urine					
Original urine	13500	61	10500	121	30
Norit filtrate	10700	27	8440	77	30
NaOH eluate	8640	2	6520	37	12
NH_3-acetone eluate	15300	82	11800	148	36
Carcinoma of the Prostate					
Original urine	13800	64	10400	118	30
Norit filtrate	10600	26	6800	43	20
NaOH eluate	4850	—42	3040	—36	4
NH_3-acetone eluate	10600	26	8600	81	22
Broncheogenic Carcinoma					
Original urine	13500	61	9080	91	28
Norit filtrate	9700	15	6850	44	20
NaOH eluate	4680	—44	2280	—52	2
NH_3-acetone eluate	10800	29	9000	89	16
Gastric Carcinoma					
Original urine	15100	92	10100	112	30
Norit filtrate	8900	6	7320	54	16
NaOH eluate	4350	—48	2000	—58	6
NH_3-acetone eluate	11700	39	8250	74	16
Hyperrenal Carcinoma					
Original urine	13600	62	8700	83	30
Norit filtrate	9050	8	6080	28	20
NaOH eluate	4800	—43	1840	—61	2
NH_3-acetone eluate	12300	46	8750	84	16
Aplastic Anemia					
Original urine	11900	42	8680	82	22
Norit filtrate	10700	27	7000	47	14
NaOH eluate	4320	—49	2200	—54	2
NH_3-acetone eluate	10600	26	7520	81	24
Carcinoma of the Rectum					
Original urine	12880	53	10000	110	26
Norit filtrate	10440	24	8520	79	20
NaOH eluate	4520	—46	2640	—45	2
NH_3-acetone eluate	12300	46	8750	84	24

* 7-MP indicates 2-amino-4-hydroxy-7-methyl pteridine.

vitro. It was suggested[1] that all blood sera contained both stimulating and inhibiting substances in varying ratios, such that normal blood serum contained a predominance of substances which would accelerate normal cell proliferation *in vitro* and blood serum from certain diseases contained a predominance of substances which would inhibit cell proliferation of normal cell suspensions and accelerate proliferation of neoplastic tissue cells.

If both accelerating and inhibiting substances are present in biological fluids, they might be separated to demonstrate their presence. For this purpose normal and pathological blood serum and urine was fractioned by adsorption on norit and elution as decribed below.

The proteins of a given volume of blood serum were precipitated with an equal volume of acetone. After mixing and filtering the precipitated proteins, the filtrate and washings were evaporated to small volume under reduced pressure to remove the acetone. The concentrated solution was diluted with distilled water to the original volume of blood serum used. The deproteinated serum extracts and urines were treated in the same manner in adsorption and elution.

An aliquot, usually 10 ml of the blood serum extract prepared as indicated above or of urine was shaken with norit and filtered. The filtrate was saved for testing on cell proliferation.

The norit was eluted with normal NaOH and filtered. The NaOH filtrate and washings were neutralized and made up to the original volume of the blood serum or urine used. The NaOH eluate was saved for testing on cell proliferation.

After elution with NaOH, the norit was eluted with ammoniacal acetone containing equal parts of 0.4M ammonium hydroxide and acetone. The filtrate and washings were concentrated under reduced pressure to small volume to remove the acetone and ammonia. The concentrated solution was diluted to the original volume of the serum or urine used for adsorption and saved for testing on cell proliferation as the NH_3 acetone eluate.

The effect of the fractions obtained was tested on bone marrow cultures *in vitro* by the technique as previously described.[2] The fractions were made up to a volume such that aliquots could be used as supplements in the bone marrow cultures equivalent to 0.1 ml of the original serum or urine for comparison. The results are shown in Tables I, III and IV. Controls are given with no supplement, xanthopterin, 2 - amino - 4 - hydroxy-7-methyl pteridine (7-MP) and Vitamin B_{14}.[3] One x 10^{-7} γ per ml of Vitamin B_{14} accelerates the rate of cell proliferation as great or greater than 5 γ per ml of xanthopterin, or Vitamin B_{14} is at least 50 million times as effective in accelerating cell proliferation in a bone marrow culture *in vitro* as xanthopterin.

Table I gives the results for fractionation of normal and pathological urines. Although neoplastic blood serum contains a predominance of inhibiting substances, the urines from cases of neoplastic disease had a predominance of substances which accelerate the rate of cell proliferation. When 0.1 ml of urine, from the neoplastic cases tested, was added to 2 ml of bone marrow suspension, the rate of cell proliferation was similar to that obtained with 0.1 ml of normal urine. The NaOH eluate contained an excess of inhibiting substances. The NaOH eluate from normal urine produced a rate of cell proliferation less than that of the control, which contained no supplement, indicating the presence of an inhibiting substance. The NaOH eluate from pathological urines all inhibited cell proliferation strongly, and had an activity similar to that of 5 γ per ml of 2-amino-4-hydroxy-7-methyl pteridine.

Table II gives the effect of fractions from neoplastic urine and other factors on the rate of cell proliferation in a suspension of cells of Brown Pearce tumor. The tumor cells were cultured by the technique previously described.[4] The effect of the pteridines, normal blood serum, and pathological blood serum on cultures of cells of neoplastic tissue *in vitro* is opposite to the effect on suspensions of normal cells.[4] Folic acid (pteroyl glutamic acid) and teropterin (pteroyl triglutamic acid) had no effect. Xanthopterin, Vitamin B_{14} and normal blood serum inhibited cell proliferation. Two-amino-4-hydroxy-7-methyl pteridine and cancer blood serum accelerated the rate of cell proliferation. The urine of broncheogenic carcinoma which accelerated

TABLE II.

Effects of Fractions from Pathological Urine Compared with Those of Other Factors on Cell Proliferation of Cells of Brown Pearce Tumor *in vitro*. The initial concentration of cells in the suspension was 18700/cmm. Time of incubation, 6 hours.

Supplement added	Final conc. of cells	% increase in cells
No supplement	21000	12
10 γ/ml folic acid	21200	13
10 γ/ml teropterin	21600	15
5 γ/ml xanthopterin	10700	—43
10 γ/ml "	8080	—57
10^{-3} γ/ml Vit. B_{14}	10200	—45
0.1 ml normal blood serum	9950	—47
5 γ/ml 7-MP*	29000	55
10 γ/ml 7-MP*	31200	67
From Broncheogenic Carcinoma		
0.1 ml blood serum	29400	57
Original urine	13000	—30
Norit filtrate, from urine	25700	37
NaOH eluate, from urine	31000	66

* 7-MP indicates 2-amino-4-hydroxy-7-methyl pteridine.

[3] Norris, E. R., and Majnarich, J. J., *Science*, in press.

[4] Norris, E. R., and Majnarich, J. J., *Am. J. Physiol.*, 1948, **153**, 492.

TABLE III.

The Effect of Fractions Obtained from Normal Rat Serum upon the Rate of Cell Proliferation in Bone Marrow Cultures *in vitro*. The initial concentration of the bone marrow cell suspension used was: red blood cells (RBC) 6920/cmm; nucleated cells (NC) 4280/cmm; and reticulocytes 11/1000 RBC. Time of incubation, 5½ hours. All supplements were adjusted equivalent to 0.1 ml of the original serum.

Supplement added	Final Concentration, RBC/cmm	RBC % Increase	Final conc., NC/cmm	NC % Increase	Retic. per 1000 RBC
No supplement	7680	11	5360	25	18
5 γ Xanthopterin/ml	12800	85	8700	103	32
1 × 10-7 γ Vit. B_{14}/ml	14900	116	8400	96	34
1 × 10-6 γ " "	18000	160	11100	159	40
5 γ 7-MP*/ml	3820	—45	1960	—54	4
Normal Rat Blood Serum					
Original serum	14200	104	7500	75	30
Norit filtrate	8360	19	5100	19	18
NaOH eluate	4240	—39	4560	6	4
NH_3-acetone eluate	18800	173	12900	202	40

* 7-MP is used to indicate 2-amino-4-hydroxy-7-methyl pteridine.

the rate of cell proliferation of bone marrow cells *in vitro*, inhibited the proliferation of cancer cells of Brown Pearce tumor. The NaOH eluate from the cancer urine, which strongly inhibited cell proliferation in bone marrow cultures, strongly accelerated the cell proliferation in a suspension of cancer cells of Brown Pearce tumor. This showed that while in the original cancer urine there was a predominance of factors which accelerated normal cell proliferation and inhibited cancer cell proliferation a fraction was obtained as the NaOH eluate which had a predominance of substances which inhibited normal cell pro-

TABLE IV.

The Effect of Fractions Obtained from Normal and Pathological Human and Rabbit Blood Serum upon the Rate of Cell Proliferation in Bone Marrow Cultures *in vitro*. The initial concentration of the bone marrow cell suspension used was: red blood cells (RBC) 11,640/cmm; nucleated cells (NC) 4880/cmm; and reticulocytes 10/1000 RBC. All supplements added were adjusted equivalent to 0.1 ml of the original serum.

Supplement added	RBC % increase	NC % increase	Retic. per 1000 RBC
No supplement	12	24	20
5 γ xanthopterin/ml	29	77	30
5 γ 7-MP*/ml	—47	—53	4
1 × 10-7 γ Vitamin B_{14}/ml	60	108	40
Normal Human Blood Serum			
Original serum	26	75	36
Norit filtrate	9	30	20
NaOH eluate	—40	— 8	8
NH_3-acetone eluate	40	57	36
Serum from a Case of Broncheogenic Carcinoma			
Original serum	—35	—27	6
Norit filtrate	8	— 7	6
NaOH eluate	—79	—75	0
NH_3-acetone eluate	33	75	22
Normal Rabbit Blood Serum			
Original serum	34	81	32
Norit filtrate	7	36	18
NaOH eluate	— 33	6	8
NH_3-acetone eluate	58	118	40
Serum of Rabbit Brown Pearce Tumor Blood Serum			
Original serum	—32	—28	2
Norit filtrate	1	6	10
NaOH eluate	—70	—72	0
NH_3-acetone eluate	32	81	22

* 7-MP is used to indicate 2-amino-4-hydroxy-7-methyl pteridine.

liferation and accelerated cancer cell proliferation.

Table III gives the effect of fractions obtained from normal rat serum upon the rate of cell proliferation in bone marrow cultures *in vitro*. The normal rat serum accelerates the rate of cell proliferation. The NaOH eluate inhibits proliferation and the NH_3-acetone eluate accelerates the rate of cell proliferation more strongly than the original blood serum, when used in amounts equivalent to the original blood serum. While the fractionation was not quantitative and the substances which inhibit and accelerate the cell multiplication were not isolated, it shows definitely that both types of substances were present in the original blood serum.

TABLE V.

The Effect of Fractions from Normal Blood Serum upon the Rate of Cell Proliferation in Brown Pearce Tumor Cell Suspension, *in vitro*. Time of incubation was 7 hours. The initial cell concentration of the suspension was 9600/cmm.

Supplement added	Final conc. cells/cmm	% increase of cells
No supplement	11900	24
5 γ/ml xanthopterin	6200	—35
5 γ/ml 7-MP*	17300	80
1×10^{-6} γ/ml Vitamin B_{14}	4360	—54
1×10^{-7} γ/ml Vitamin B_{14}	7160	—25
Normal Human Blood Serum		
Original serum	6320	—34
Norit filtrate	13400	50
NaOH eluate	16000	67
NH_3-acetone eluate	7240	—24
Normal Rabbit Serum		
Original serum	7920	—12
Norit filtrate	11760	23
NaOH eluate	18600	94
NH_3-acetone eluate	6760	—29
Normal Rat Serum		
Original serum	7240	—24
Norit filtrate	13180	48
NaOH eluate	18920	97
NH_3-acetone eluate	4840	—50

* 7-MP indicates 2-amino-4-hydroxy-7-methyl pteridine.

Table IV gives the effect of fractions obtained from normal and pathological human and rabbit blood serum upon the rate of cell proliferation in bone marrow cultures ***in vitro***. The supplements of serum and fractions are all equivalent to 0.1 ml of the original blood serum in 2 ml of bone marrow suspension. Normal blood sera accelerated the rate of cell proliferation. Cancer blood sera inhibited cell proliferation. The NaOH eluates all inhibited proliferation and those from the cancer blood serums inhibited more strongly than the original serum. The NH_3-acetone eluates accelerated the rate of cell proliferations. While the Norit filtrates appeared to be somewhat inhibitory, nothing definite can be said about them at this time as the adsorption on Norit may not have been complete for all factors. The results show that there was present, in all urines and blood sera studied, two types of factors which influence the rate of cell proliferation, one type which accelerates the rate of proliferation and one type which inhibits cell proliferation.

Table V gives the effect of the fractions from normal human, rabbit and rat sera shown in Tables III and IV upon the rate of proliferation of Brown Pearce tumor cells ***in vitro***. The technique used was the same as that previously described.[4] The original serum, Norit filtrate and eluates were so adjusted in the experiment that each was equivalent to 0.1 ml of the original blood serum in 2 ml of tumor cell suspension. The results are opposite to those obtained on bone marrow culture, which indicates that the NaOH eluate contained factors which inhibited the normal cell proliferation and accelerated the rate of cancer cell proliferation; and the NH_3-acetone eluate contained factors which accelerated the rate of normal cell proliferation and inhibited cancer cell proliferation. The two types of factors were shown to be present in each of the original sera tested, and present in such a ratio that the net result of the original serum exhibited a predominance of factors which accelerated the rate of normal cell proliferation and inhibited cancer proliferation.

Conclusions. 1. Blood serum and urine contain two types of substances; the one which accelerates the rate of cell proliferation, and the other which inhibits the rate of cell proliferation.

2. Normal blood sera and normal and pathological urine accelerate the rate of cell proliferation in bone marrow cultures ***in vitro*** because of an excess of accelerating substances over inhibiting substances present.

3. Blood sera from neoplastic disease, pernicious anemia and leukemia inhibit cell pro-

liferation in bone marrow cultures because of an excess of inhibiting substances over accelerating substances present.

4. Individual urine and blood serum specimens have been shown by adsorption on Norit and elution with NaOH and ammoniacal acetone to have both inhibiting and accelerating substances present.

16885

The Effect of Methionine on Blood Coagulation.*

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In 1936 Sterner and Medes[1] reported that cysteine and methionine prolonged both the bleeding and coagulation times for several hours when administered orally or intravenously to human subjects. They further concluded that the effect was mainly on one factor of the coagulation system, namely, prothrombin. From the data reported by these investigators one could conclude that methionine might possibly be an effective and much safer anticoagulant for clinical use than either dicoumarol or heparin. Because of these possibilities the effects of methionine on blood coagulation and quantitative prothrombin levels were studied more fully in human subjects.

In these studies dl-methionine was used. The solutions (2.5 g of methionine per 100 cc) for intravenous administration were made isotonic by the addition of sodium chloride and the pH adjusted to near neutrality by the use of sodium dibasic phosphate. The prothrombin determinations were done by the method of Quick[2] and the coagulation time by the intravenous test tube method of Lee and White.[3] Control studies were also done. All studies were done over a 6 hour period with observations at the initial 30 minute period and then each hour for the 6 hour period.

Control Studies. The coagulation times and prothrombin levels were determined in 3 persons in a fasting state over a 6 hour period. Changes were minimal. These studies were repeated in 3 persons after a routine hospital breakfast. Slight changes were noted in that the coagulation time was decreased after a period of from 4 to 5 hours, the maximum being 4½ minutes, this being within the range of experimental error.

Oral Methionine. Methionine was given to 2 individuals in 3.0 g amounts. No effect on the coagulation time was noted. No prothrombin determinations were made.

Intravenous Methionine. The individuals with normal prothrombin levels. Amounts of 1.3 g were administered to 2 subjects and no significant changes were noted in either the coagulation times or the prothrombin levels. Methionine in 2.5 g doses was given to 8 subjects, one fasting and 7 on routine hospital diet. In no instance was the coagulation time increased or the prothrombin level altered. In 4 individuals, including the fasting subject, the coagulation time was decreased, the maximum being a 10 minute decrease in one individual. The curve was a moderate exaggeration of the one obtained in the control subjects who had breakfast. Methionine in 5.0 g doses was administered to 3 subjects. No significant changes were noted in either the coagulation times or the prothrombin levels.

Intravenous Methionine. Individuals with decreased prothrombin. 2.5 g were administered to an individual with cirrhosis of the liver and observations made over a 6 hour period. The initial prothrombin level was

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1 Sterner, J. H., and Medes, G., *Am. J. Physiol.*, 1936, **117**, 92.

2 Quick, A. J., *J.A.M.A.*, 1938, **110**, 1658.

3 Lee, R. I., and White, P. D., 1913, **145**, 495.