support this concept. In cases of malignant proliferation, the amount of excreted 5-ribosyluracil increases. The same is true after therapy that causes nucleic acid destruction (X-ray, Cytoxan). The latter results are consistent with similar data (17) on increased urinary 5-ribosyluracil excretion in rats following irradiation of the animals. We could not find patients with untreated, primary gout to confirm the reported(1) increase in the excretion of this nucleoside. But it may be worth noting that in one case of a patient with gout, complicated by other diseases of uncertain diagnosis, a single 2-mg dose of colchicine caused the amount of excreted 5-ribosyluracil to drop from 163 to 140 mg per 24 hours. This alkaloid, known for its inhibition of mitosis, and used in the therapy of gout, may therefore account for the "normal" values of excreted 5-ribosyluracil found by us in gout. The results of the study of excretion of 5-ribosyluracil in pathological conditions, as reported, give some indication of the potential value of such studies.

Summary. A method described for estimation and isolation of 5-ribosyluracil in 24-hour specimens of normal human urine (7) has been used to study its excretion in patients suspected of abnormal RNA turnover. In accord with the previously demonstrated lack of catabolism of 5-ribosyluracil in man(7), there is support for the view that the amount of excreted 5-ribosyluracil may be indicative of the turnover of transfer RNA, intimately associated with protein biosynthesis. With the use of this method, several as yet unidentified components have been isolated from human urine and partially characterized.

1. Adler, M., Gutman, A. B., Science, 1959, v130, 862.
2. Adams, W. S., Davis, F., Nakatani, M., Am. J. Med., 1960, v28, 726.
3. Eisen, A. Z., Weissman, S., Karon, M., J. Lab. & Clin. Med., 1962, v59, 620.
4. Weissman, S., Eisen, A. Z., Karon, M., ibid., 1962, v59, 852.
5. Weisman, S., Eisen, A. Z., Lewis, M., Karon, M., ibid., 1962, v60, 40.
6. Dlugajczyk, A., Eiler, J. J., Fed. Proc., 1963, v22, 470.
7. ———, Nature, in press.
8. Markham, R., Smith, J. D., Biochem. J., 1949, v45, 294.
9. Dlugajczyk, A., Ph.D. Thesis, Univ. of California, San Francisco, 1963.
10. Chang, M. L. W., Johnson, B. C., J. Biol. Chem., 1961, v236, 2096.
11. Fink, K., Adams, W. S., Pfleiderer, W., ibid., 1964, v239, 4250.
12. Dunn, D. B., Biochim. Biophys. Acta, 1959, v34, 286.
13. Osawa, S., Otaka, E., ibid., 1959, v36, 549.
14. Zamir, A., Holley, R. W., Marquisee, M., J. Biol. Chem., 1965, v240, 1267.
15. Holley, R. W., Everett, G. A., Madison, J. T., Zamir, A., ibid., 1965, v240, 2122.
16. Ofengand, J., Schaefer, H., Senderowitz, J., Chu, L., Pacific Slope Biochemical Conference, August 1965.
17. Drahovsky, D., Winkler, A., Skoda, J., Nature, 1964, v201, 411.

Received June 13, 1966. P.S.E.B.M., 1966, v123.

Inhibition of the Stem-Cell Action of Erythropoietin by Estradiol.* (31513)

JOANNE H. JEPSON AND LOUIS LOWENSTEIN (Introduced by J. C. Beck)
Division of Haematology, and McGill University Medical Clinic, Royal Victoria Hospital, Montreal, Quebec, Canada

Previous observations have revealed that hematological values of females of various species, after the attainment of sexual maturity, are lower than those of males, and a comprehensive analysis of the data has shown these differences are due to the pres-

* This investigation was supported by a special USPHS Fellowship 2-F3-HE-18, 509-02 from Nat. Heart Inst. and by grant MBA-1664 from Medical Research Council of Canada.

ence or absence of estrogens and androgens (1). It has also been shown that estrogen can depress the incorporation of Fe^{59} into erythrocytes and appears to antagonize the effect of erythropoietin(2), although the exact mode of action of estrogen has not been clearly established. Despite the high levels of estrogens present during pregnancy in the mouse, the erythropoietic activity of pregnant mouse plasma is high during the latter half of pregnancy and is not completely abolished by exposing pregnant mice to hyperoxic environment.† This suggests that there is a complex interplay of various hormones controlling erythropoiesis.

During our investigations of the effect of various hormones on erythropoiesis during pregnancy and lactation, the following experiments were done in an attempt to elucidate the effect of estrogens on erythropoiesis and their relation to the production and action of erythropoietin(3).

Materials and methods. The hypoxia-induced polycythemic mouse assay employed has been described(4). CF-1 female mice weighing 22-25 g were used. Mice with hematocrits less than 55% were discarded. A commercial preparation of estradiol valerate (Squibb-Delestrogen 10 mg/ml) in sesame oil was used. This preparation was diluted with sesame oil for the required dose.

Results and discussion. Following the return of polycythemic mice to a normal oxygen environment, by the fourth posthypoxic day, all erythroid precursors have disappeared and the effect of an erythropoietic stimulus on the stem cell can be measured. The site of inhibition of erythropoiesis by any material can then be determined by altering the time of injection of such a substance after the initiation of erythropoiesis by stimulation of endogenous erythropoietin secretion.

Female polycythemic mice, therefore, were reexposed to 20 hours of a 10% O_2 environment between the fourth and fifth day following their initial removal from hypoxia. They were divided into groups, each composed of a minimum of 5 mice, and injected with 0.4 μg of estradiol valerate in .05 ml sesame oil in the following schedule: Group 1 was injected 4 hours prior to their exposure, and Group 2 immediately upon removal from exposure to 20 hours of hypoxia; Groups 3-5 were injected at 24, 48, and 72 hours, respectively, after the end of exposure to hypoxia; Group 6 was exposed to hypoxia after injection of .05 ml of sesame oil. Fifty-six hours after the end of the 20 hours of hypoxia, 0.5 μc of $Fe^{59}Cl_3$ was injected into the tail vein and the percent incorporation of Fe^{59} into erythrocytes was measured 72 hours later (4).

† Jepson, J. H. and Lowenstein, L. unpublished.

TABLE I. Seventy-Two Hour Incorporation of Fe^{59} into Erythrocytes of Polycythemic Mice (♀) Following Injection of Estradiol Valerate at Various Intervals Before and After Exposure to Hypoxia.

Treatment	No. of mice	% Fe^{59} RBC ± S.E.
Sesame oil	5	.41 ± .07
Estradiol, .2 μg × 2	5	.25 ± .04
20 hr hypoxia:		
+ sesame oil	5	15.64 ±1.56
+ .4 μg estradiol (4 hr prior)	5	5.76 ± .82
+ .4 μg estradiol (on removal)	5	5.61 ±1.95
+ estradiol (24 hr after hypoxia)	5	16.40 ±1.79
+ estradiol (48 hr after hypoxia)	5	14.98 ± .62
+ estradiol (72 hr after hypoxia *and* 6 hr after Fe^{59} given I.V.)	5	15.58 ±1.47

Those groups which received estradiol either prior to or immediately following exposure to hypoxia (Table I), showed marked inhibition of erythropoietic activity, but when the estradiol was injected at 24 to 72 hours after the end of the hypoxic stimulus, no inhibition was observed. This suggested that estradiol could be acting during the stage of stem-cell differentiation and erythropoietin utilization and that it was not acting on the later differentiation of erythroid precursors.

To determine whether injection of estradiol impaired the production of erythropoietin or blocked the utilization of erythropoietin by the stem cell, plasma was collected, pooled, and stored until used, as previously described(4), from female mice who had been injected with 0.1 ml of sesame oil and exposed to 16 hours of hypoxia (10% O_2), and from female mice which had been injected with 0.5 μg of es-

TABLE II. Seventy-Two Hour Incorporation of Fe^{59} into Erythrocytes of Polycythemic (♀) Mice Following Injection of Plasma Collected from ♀ Mice Exposed to Hypoxia or Exposed to Hypoxia and Injected with Estradiol Valerate.

Treatment	Normal plasma	16 hr hypoxic plasma	16 hr hypoxic plasma (+ .5 μg estradiol)	24 hr hypoxic plasma	72 hr hypoxic plasma	72 hr hypoxic plasma (+ estradiol 5 μg)
% Fe^{59} RBC (mean ± S.E.)	.77 ± .03	5.05 ± .49	4.97 ± .3	7.28 ± .48	2.86 ± .11	15.94 ± .92
No. of mice	(3)	(5)	(4)	(5)	(5)	(5)

tradiol valerate 9 hours prior to their exposure to 16 hours of hypoxia. Plasma was also collected from female mice who were exposed to 72 hours of continuous hypoxia and received 0.1 ml sesame oil, daily, and from mice which, in addition to 72 hours of continuous hypoxia, received 3 injections of 5 μg of estradiol valerate in 0.1 ml sesame oil, daily, starting 24 hours prior to their exposure to hypoxia. This plasma was then tested for erythropoietic activity in polycythemic mice by injecting 1.0 ml of plasma on the fourth and fifth posthypoxic days. 0.5 μc of $Fe^{59}Cl_3$ was injected into the tail vein of each mouse 56 hours later and the percent incorporation of Fe^{59} into erythrocytes determined at 72 hours as described previously(4). Results are shown in Table II. The secretion of erythropoietin induced by exposure to 16 hours of hypoxia, was not inhibited by the estradiol at this dose level. Plasma from mice exposed to 72 hours of hypoxia alone had decreased their erythropoietin titer, presumably due to utilization of erythropoietin by the bone marrow(5), while the plasma of mice concurrently injected with 5 μg of estradiol daily, had a markedly increased plasma erythropoietin titer, strongly suggesting that the utilization of erythropoietin by the bone marrow was blocked by the estradiol. The increased plasma erythropoietic activity in the presence of active erythropoiesis observed during pregnancy in the mouse(3,4) may be due to the partial inhibition of the stem-cell action of erythropoietin by the increased endogenous estrogens present during pregnancy. It is known, however, that increasing erythropoietin can overcome the inhibitory effect of estrogens in rodents(2). Since estrogens are known to stimulate protein binding of thyroid hormone and adrenal steroids(6,7), they may also affect the protein binding of erythropoietin, resulting in increased titers during pregnancy and a more efficient regulation of the release of this hormone to the tissues.

Injection of very large doses, 250 μg to 1 mg, of estradiol cyclopentylpropionate has recently been reported to prevent the production of erythropoietin in mice, rats, and tamarins(8). This is approximately 1,000 to 4,000 times the daily endogenous production of estradiol in the mouse. This difference in results, therefore, can be attributed to the dose of estradiol employed. In our experiments, the doses used were much lower, in the range of 2 to 20 times that of daily endogenous estrogen production. It is well known that the action of estrogens differs according to the dose employed(9).

Summary. Injection of estradiol into polycythemic mice stimulated to produce endogenous erythropoietin inhibited the incorporation of Fe^{59} into their erythrocytes when it was injected during the stage of stem-cell differentiation, but not during the latter stages of erythroid cell development. In doses up to 20 times that of daily endogenous estrogen production, secretion of erythropoietin was not inhibited and utilization of erythropoietin by the bone marrow appeared to be blocked.

1. Grant, W. C., Root, W. S., Physiol. Rev., 1952, v32, 449.

2. Dukes, P., Goldwasser, E., Endocrinology, 1961, v69, 21.

3. Jepson, J. H., Lowenstein, L., Clin. Res., 1966, v14, 318.

4. ———, Blood, 1964, v24, 726.

5. Stohlman, F., Brecher, G., Proc. Soc. Exp. Biol. and Med., 1959, v100, 40.

6. Dowling, J. J., Freinkel, N., Inghar, S. H., J. Clin. Invest., 1960, v39, 111.

7. Wallace, G. Z., Carter, A. C., ibid., 1960, v39, 601.

8. Mirand, E. A., Gordon, A. S., Endocrinology, 1966, v78, 325.
9. Zarrow, M. X., Yochim, J. M., McCarthy, J. L., Exp. Endocrinol., Academic Press, N. Y., 1964,

Received June 17, 1966. P.S.E.B.M., 1966, v123.

Chemical Studies of Paolin II, an Antiviral Substance from Oysters. (31514)

B. PRESCOTT, C. P. LI, G. CALDES, AND E. C. MARTINO
U.S. Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, National Institute of Allergy and Infectious Diseases, and Division of Biologics Standards, Bethesda, Md.

The antimicrobial activity of substances named "paolins" isolated from marine animals has been described(1-7). The "paolins," active *in vitro* as well as *in vivo* are perhaps a new class of substances concerned with defensive mechanisms of animals against invading microorganisms including viruses(6, 8). These substances seem to be normal constituents of water or acetic extracts of all mollusc species thus far studied. This report concerns further chemical purification and characterization of an antiviral substance (paolin II) isolated from oysters.

Material and methods. Fresh frozen oysters bought at a local market were weighed and then homogenized with 1 volume of 50% acetic acid in a Waring blender for 5 minutes. The homogenate was allowed to extract overnight at room temperature, after which it was adjusted to pH 5.0 with NaOH. The extract was recovered by centrifuging the homogenate for 30 minutes at 2000 $\times$ *g*. The clear supernate was dialyzed against distilled water for 4 days in the cold room with 2 changes of water daily. The extract was then filtered through a K-5 clarifying pad (Hercules Filter Corp., New York) and lyophilized. The methods for assay of antiviral activity *in vitro* and *in vivo* have been described(5).

Ethanol separation: The procedure for the ethanol precipitation is shown in Fig. 1. Briefly, the lyophilized acetic extract from oysters was dissolved in distilled water in a 10% solution, precipitated with one-half volume of cold 95% ethanol and the mixture held at 4°C overnight. The precipitate which formed was collected in a refrigerated centrifuge, dissolved in 4 times the volume of cold distilled water and then lyophilized. This material constituted the one-half volume ethanol precipitate designated (A). Three subsequent precipitates formed with increasing amounts of ethanol to the supernatant (1, 2 and 4 vol), treated similarly as A. The precipitates which resulted from this treatment were designated B, C and D, respectively, while the evaporated supernatant of D represented fraction E. The most active antiviral agent was concentrated into a single fraction (D). The yield of active material was small—usually 0.5 to 1% of the acetic acid product.

Paper chromatography: The active material was analyzed for amino acid content by means of descending paper chromatography using the procedure of Wolfe(9) substituting the ninhydrin reagent of Barrollier(10) for that of Wolfe. Carbohydrates were identified according to the method of Colombo *et al*(11) using a 2.5% aniline hydrogen phthalate as a spray.

Purine and pyrimidine analysis: Purines and pyrimidines were determined by heating a sample of material in N-HCl in a boiling water bath for 1 hour. The hydrolysate was applied to Whatman No. 1 filter paper for descending paper chromatography(12).

Ultracentrifugal analysis: Sedimentation studies were performed at 20°C in a Spinco Model E ultracentrifuge at 60,000 r.p.m. for 65 minutes.

Results. Chemical properties of fraction D: The antiviral product is a white powder, water soluble, non-dialyzable, thermostable at a