nitude with *in vitro* BT output rates reported for American bullfrog adrenals(2). Because of low secretory rates and difficulties in obtaining sufficient quantities of tissue, qualitative analysis of steroids produced *in vitro* by interrenals of *Raja erinacea* was not attempted in this preliminary investigation. However, characteristic reactions with BT and UV provide strong presumptive evidence that the secretory products measured were α ketol-Δ^4-3 ketosteroids. Phillips(7) has shown definitively that cortisol is the predominant plasma corticoid of the female skate (*Raja eglanteria*).

Summary. The effect of mammalian ACTH on *in vitro* secretion of skate (Raja erinacea) interrenals was investigated directly. Interrenal weights also were compared between males and nonovulating and ovulating females. ACTH increased interrenal output of BT and UV measurable substances in both sexes. Preincubation output rates in the absence of ACTH were substantially greater than those during subsequent incubation. A strong but not perfect correlation was observed between body and interrenal weights in both sexes. F-test analysis for statistical significance of differences between means of covariance-corrected interrenal weights showed that those of males were significantly less than those of either combined or ovulating females but not different from those of nonovulating females. Ovulating females had significantly heavier interrenals than nonovulating females.

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Erythropoietic Stimulating Factor (ESF) as a Stimulant of Tumor Growth.* (27542)

FLOYD E. LEADERS, ROBERT L. DIXON, JAMES W. OSBORNE AND J. P. LONG
Departments of Pharmacology and Radiation Research, College of Medicine, State University of Iowa, Iowa City

Partial hepatectomy (PH) of rats is known to be followed by regeneration of the liver(1,2), and increased mitotic rate in the cornea. Increased compensatory hypertrophy of the kidneys is also observed after unilateral nephrectomy and PH(3). Evidence that this growth stimulating effect is due to a bloodborne agent is demonstrated by the ability of PH in one member of a parabiotic pair of rats to stimulate mitotic activity in the liver of the intact parabiont(4). Partial hepatectomy also results in a decreased rate of liver regeneration in these animals when compared with nonparabiont, PH controls(5).

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When injected into normal recipients, serum from hepatectomized but not from normal rats induces mitosis in livers of these animals (6). This serum from PH rats, when administered to other animals in which livers were regenerating following PH, increased mitotic activity over that observed in animals which were only PH.

It has been reported that rats and mice bearing malignant tumors demonstrated an increase in liver mitotic activity(7). Injections of tumors or saline extracts of tumors also resulted in an increase in liver mitotic index. Conversely, PH itself(8) or injections of serum from PH rats(9) increased hepatoma and sarcoma in mice.

Clinical reports appearing recently have demonstrated the association of polycythemia with several different types of cancer. These types include carcinoma, fibroyoma and hemangioblastoma(10,11,12). An erythropoietic stimulating factor was found to be present in the fluid from the cystic cerebellar hemangioblastoma(12). Remission of the polycythemia occurred following surgical removal of the fibroyoma and the renal carcinoma. McLaughlin reported(13) that periodic injection of 2 ml of pooled plasma from patients with various forms of cancer increased the rate of hepatic regeneration significantly over the rate following administration of blood from patients without cancer.

There is much evidence to support the existence of an erythropoietic stimulating factor or factors (ESF) or erythropoietin(14,15,16). The possibility that the erythropoietic stimulating factor (ESF) could be related to PH and tumor growth was suggested by the reports mentioned above. Therefore, the hypothesis which was tested is that increased tumor growth, liver regeneration and erythropoiesis may be related to liberation of a nonspecific "growth factor" into the circulation and this "growth factor" may be identical with the substance or one of the substances referred to as erythropoietin or ESF.

Materials and methods. Two hundred and twenty-one Holtzman rats weighing between 200 and 250 g were used. Some animals were partially hepatectomized. Others received injections of ESF and $CoCl_2$. Tumors were implanted into all animals.

ESF activity was measured using red cell Fe^{59} uptake. This method has been reported previously(17). Fe^{59} was injected intraperitoneally as ferrous citrate approximately 15 minutes before tumor implantation. Each experimental group was divided into 2 subgroups, one of which was used for Fe^{59} uptake studies. Animals were weighed and etherized 24 and 48 hours after injection of Fe^{59}. Five-tenths-ml samples of blood were obtained by cardiac puncture. The samples and standards were counted using a Nuclear-Chicago well type DS5-5 scintillation counter and a 132A analyzer-computer. Hematocrit readings were recorded and per cent Fe^{59} uptake was calculated using the method of Hennessey and Huff(18).

Novikoff hepatomas (19) were injected subcutaneously into rats and removed surgically 10 to 17 days later. They were weighed and the values expressed as gross weight and per cent of total body weight. Average tumor weights were calculated separately for the sub-groups receiving Fe^{59} treatment and those which did not. These values did not differ from each other significantly within any given test group. The two parts of each treatment group were then pooled.

Four experimental methods were used in this study. They are as follows: A. *Erythropoietin prepared in this laboratory.* This material was prepared by a modification of the method described by Borsook(15) using plasma from rabbits made anemic by phenylhydrazine injection. The plasma was deproteinized and the precipitate was removed by centrifuging at 9000 $\times$ gravity for 15 to 20 minutes instead of by filtration. Supernatant and washings were combined, and the volume reduced by lyophylization to $\frac{1}{3}$ the original plasma volume using a VirTis Freezemobile (VirTis, Inc.). Rats received subcutaneously (S.C.) 2 ml (full dose) and 1 ml (half dose) of this extract daily for 3 successive days prior to Fe^{59} injection and tumor implantation. B. *Erythropoietin from U.S. Public Health Service.* Erythropoietin (ESF) was obtained from the Armour Pharmaceutical Co. through U.S. Public Health Service. This

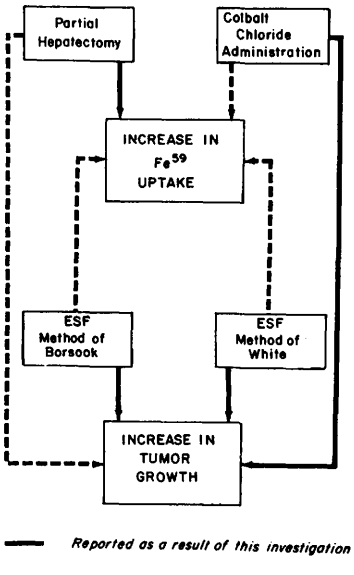

FIG. 1. Relationship between results first reported in this investigation and responses previously reported and confirmed by this work.

ESF was prepared from sheep plasma by the method described by White *et al.*(16). One unit of ESF is equivalent to 5 μm of cobalt chloride using the fasted rat assay(20). ESF in doses of 25, 50, and 100 units/kg were each administered S.C. in equally divided doses 24 and 48 hours prior to Fe^{59} administration and tumor implantation. C. *Cobalt chloride.* Animals which received cobalt chloride ($CoCl_2 \cdot 6H_2O$) received 250 μm or 500 μm S.C. in equally divided doses 24 and 48 hours prior to Fe^{59} injection and tumor implantation. D. *Partial hepatectomy.* Partial hepatectomies were performed using the technics of Higgins and Anderson(20). Fe^{59} injection and tumor implantation were done 24 hours after partial hepatectomy.

These four experimental designs were used to evaluate the influence of partial hepatectomy and administration of ESF and cobalt chloride on tumor growth and Fe^{59} uptake. The "student t" test(21) was used throughout this work to evaluate significance of difference between values. A p value of less than 0.05 was regarded as significant. The relationship between reports in the literature and the data presented here is summarized in Fig. 1.

Results. Each of the 4 procedures used has been previously reported to effect either ESF production or tumor growth. The response of both of these parameters, tested simultaneously in the same animals, is reported here. The effect of partial hepatectomy, erythropoietin (ESF) prepared by the methods described by Borsook and White, and administration of cobalt chloride on tumor weight and ESF activity (as measured by per cent Fe^{59} uptake) are illustrated in Fig. 2.

In all instances, values for the greatest tumor weight in grams, tumor as percent of body weight and percent uptake of Fe^{59} by red blood cells elicited by all 4 treatments differed significantly from the values recorded for control preparations.

Discussion. Partial hepatectomy, already known to enhance tumor growth(8), has been shown to produce increased levels of ESF as measured by the Fe^{59} uptake method. This finding adds support to the suggestion that ESF may be involved in tumor growth.

Responses to erythropoietin (ESF) prepared by either method confirmed the reports of increased Fe^{59} uptake(15,16) and suggested the presence of ESF in these extracts. In both experiments tumor growth was enhanced significantly. This could be due to the action of the ESF *per se,* an artifact produced by a substance formed during isolation, or a response to some other material present in the extract. However, the use of ESF preparations from 2 different species, concentrated by 2 different methods, to produce similar responses would tend to favor a direct action of ESF. To further limit the possibility that the tumor growth stimulant factor was merely an impurity or artificially produced substance, it appeared desirable to stimulate endogenous ESF production in the test animals. Cobalt chloride has long been known to stimulate erythropoiesis. It has recently been shown to do this by increasing the production of ESF(22). This relationship was confirmed.

We have concluded that ESF extracts produced by a modified method of Borsook or the method of White and procedures which increase endogenous ESF production (as meas-

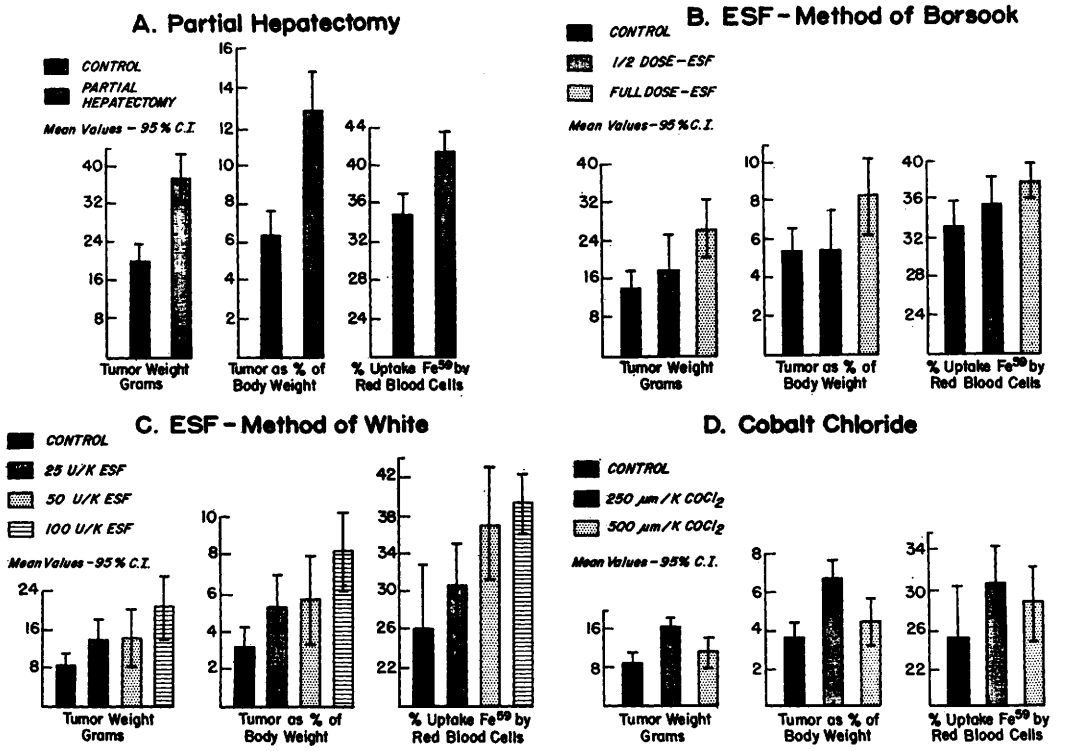

FIG. 2. A. Effects of partial hepatectomy of rats on Novikoff tumor weights and uptake of Fe^{59} by red blood cells. B. Effects of erythropoietin (ESF) prepared by method of Borsook on Novikoff tumor weights and uptake of Fe^{59} by red blood cells. C. Effects of erythropoietin (ESF) prepared by method of White on Novikoff tumor weights and uptake of Fe^{59} by red blood cells. D. Effects of cobalt chloride on Novikoff tumor weights and uptake of Fe^{59} by red blood cells.

Values are mean values of 15 to 21 responses per point. Values for the greatest tumor wt in g tumor as % of body wt and % uptake of Fe^{59} by red blood cells elicited by all 4 treatments differed significantly from values recorded for control preparations.

ured by Fe^{59} uptake) are capable of stimulating Novikoff hepatoma growth in rats. The apparent interrelation between tumor growth, ESF production, and erythropoietic activity opens many areas to investigation.

Erythropoietin (ESF) may have properties other than erythropoietic stimulating action. If subsequent work demonstrates the ability of this material to stimulate growth in tissue other than Novikoff hepatomas, then perhaps a name such as "nonspecific growth factor" or NGF might be more appropriate.

The possibility must be considered, but remains to be established, that a cause-effect relationship exists between blood levels of ESF and tumor growth. A detailed study of tumor patients and associated ESF blood levels would help determine whether such a relationship exists.

Summary. Four experimental procedures have been tested for ability to increase ESF concentrations (as measured by Fe^{59} uptake) and increase tumor growth. It was concluded that ESF extracts prepared by a modified method of Borsook or the method of White and procedures which increase endogenous ESF production (partial hepatectomy and cobalt chloride administration) are capable of stimulating Novikoff hepatoma growth in rats.

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Enzymes of *Neisseria gonorrhoeae* and Other *Neisseria*. (27543)

HENRY TAUBER AND HAROLD RUSSELL

Venereal Disease Research Laboratory, Communicable Disease Center, Public Health Service, Atlanta, Ga.

This study deals with enzyme systems of *Neisseria* thus far not described for these species of bacteria. Tonhazy and Pelczar(1), however, have reported an interesting manometric study concerning oxygen uptake by an isolate of *Neisseria gonorrhoeae* in the presence of citric acid cycle compounds. The present investigation was done to aid in classification and identification of *Neisseria*. The Thunberg technic, using color-forming 2,3,5-triphenyltetrazolium chloride (TPT) as the electron acceptor, has been employed. Included are a lactic dehydrogenase (LDH), a reduced diphosphopyridine nucleotide (DPNH) oxidase system, an alcohol dehydrogenase, an aerobic cysteine oxidase (CyO), an aerobic desulfhydrase (DSH), and an acetate oxidase system. The DPNH oxidase and alcohol dehydrogenase appear to be increased in penicillin resistant *N. gonorrhoeae* isolates.

Materials and methods. The culturing procedure has been described(2). The bacterial cells were washed twice with 4 volumes of sterile 0.85% NaCl and once with sterile distilled water. A portion of washed cells was lyophilized and the remainder was stored frozen at −20°C. For most experiments, bacteria were suspended in distilled water and dispersed in a glass homogenizer. Nitrogen was determined by micro-Kjeldahl procedure. When the formazan method was employed, enzyme activities were expressed as μg of formazan per 100 μg of cell nitrogen. Preparation of a formazan calibration curve has been described(3).

Determination of reduced diphosphopyridine nucleotide oxidase. Fifty mg of wet bacteria were suspended in 10 ml of distilled water and dispersed in a glass homogenizer. In Thunberg tubes were placed 1-ml portions of the material, 1 ml of 0.1 M phosphate buffer of pH 7.5, KCN (1×10^{-2} M) and TPT (2×10^{-3} M). In the side arms of the tubes was placed 0.25 ml of a solution containing DPNH (9×10^{-4} M) and cysteine (4×10^{-3} M) of pH 7.5. Compounds were calculated per final volume of 3 ml. Tubes were