Erythropoietin-Mediated Erythrocytosis in Rodents After Intrarenal Injection of Nickel Subsulfide

F. WILLIAM SUDDERMAN Jr., M.D., SIDNEY M. HOPFER, Ph.D., MARILYN C. REID, B.S., SAMUEL K. SHEN, Ph.D., AND CATHARINE B. KEVORKIAN, B.S.

Departments of Laboratory Medicine and Pharmacology, University of Connecticut School of Medicine, Farmington, Connecticut

Received March 22, 1982

Rats and guinea pigs developed pronounced erythrocytosis at one to four months after unilateral intrarenal (ir) injection of nickel subsulfide (Ni$_3$S$_2$). For example, at two months after ir administration of Ni$_3$S$_2$ (5 mg) to rats, blood hematocrit values averaged 70 ± 3 percent ($p < 0.001$ vs. 48 ± 2 in controls); at two months after ir administration of Ni$_3$S$_2$ (20 mg) to guinea pigs, blood hematocrit values averaged 67 ± 6 percent ($p < 0.001$ vs. 49 ± 1 percent in controls). Hamsters and gerbils did not develop erythrocytosis after ir injection of Ni$_3$S$_2$ (5 mg/animal). Administration of Ni$_3$S$_2$ to rats by intrasplenic injection did not increase blood hematocrit; splenectomy did not prevent erythrocytosis in rats that received ir injection of Ni$_3$S$_2$. Erythrocytosis in rats was completely blocked by excision of the Ni$_3$S$_2$-injected kidney but was unaffected by excision of the non-injected kidney. Partial inhibition of Ni$_3$S$_2$-induced erythrocytosis in rats occurred after simultaneous ir injection of Mn, Cu, or Al dusts, benzo(a)pyrene, or subcutaneous (sc) infusion of sodium diethylthiocarbamate. Erythrocytosis induced by ir injection of Ni$_3$S$_2$ was augmented by ir injection of Cr dust or intramuscular (im) administration of iron-dextran. Erythrocytosis occurred in rats after ir implantation of Ni$_3$S$_2$ within semi-permeable cellulose tubules, indicating that phagocytosis of Ni$_3$S$_2$ particles is unnecessary for erythropoietic stimulation. Erythropoietin (Ep) activity in rat serum increased sixfold at two weeks after ir injection of Ni$_3$S$_2$ ($p < 0.001$ vs. controls), but Ep activity in pooled extracts of Ni$_3$S$_2$-treated rat kidneys did not increase significantly. This study identifies several factors that influence erythropoietic stimulation by Ni$_3$S$_2$, and furnishes salient information concerning the pathogenesis of Ni$_3$S$_2$-induced erythrocytosis.

INTRODUCTION

In 1973, Jasmin [1] noted that rats develop polycythemia within one month after an intrarenal (ir) injection of nickel subsulfide (Ni$_3$S$_2$). Subsequent studies confirmed that blood hematocrit, hemoglobin concentration, and erythrocyte count are significantly increased from one week to approximately six months following ir injection of 5 or 10 mg of Ni$_3$S$_2$ [2–4]. Blood hematocrit values are greatest at six to 12 weeks; highest reticulocyte counts and maximum incorporation of $^{59}$Fe into erythrocytes occur at two to four weeks [5–10]. Pronounced erythroid hyperplasia is present in bone marrow of rats from two weeks to five months after ir injection of Ni$_3$S$_2$.

Sponsored by research grants from the National Institute of Environmental Health Sciences (ES-01337) and the U.S. Department of Energy (EV-01340).

Address reprint requests to: F. William Sunderman, Jr., M.D., University of Connecticut School of Medicine, 263 Farmington Avenue, Farmington, CT 06032

Copyright © 1982 by The Yale Journal of Biology and Medicine, Inc.

All rights of reproduction in any form reserved.
[3,7,9,10]. In rats that receive ir injection of 5 or 10 mg of NiS\textsubscript{2}, the circulating erythrocyte mass undergoes 2 to 2.5-fold enlargement; the plasma volume is slightly diminished, and the concentration of 2,3-diphosphoglycerate in erythrocytes remains normal [2,3,10]. When \textsuperscript{51}Cr-tagged erythrocytes from NiS\textsubscript{2}-treated rats are transfused into normal recipients, the circulating half-life of the labelled cells is prolonged, consistent with an increased proportion of juvenile erythrocytes [7]. NiS\textsubscript{2}-induced erythrocytosis is unaccompanied by leukocytosis or thrombocytopenia [3,10]. Erythrocytosis does not occur in rats after administration of NiS\textsubscript{2} by intramuscular (im), intravenous (iv), or intrahepatic injection, or after sustained intraperitoneal (ip) infusion of NiCl\textsubscript{2} [3,4,7,8,11].

Induction of erythrocytosis by ir administration seems to be peculiar to nickel compounds, inasmuch as ir injections of Au, Cd, Co, CoS, Cr, Cu, Fe, Mn, and Pb dusts do not stimulate erythrocytosis in rats [12,13]. The erythropoietic effects of 17 nickel compounds have been tested by ir administration to rats [13]. In order of decreasing potency, the following compounds cause significant increases of blood hematocrit: NiS\textsubscript{2} > NiO > NiSe > NiS > NiS\textsubscript{2} > NiSe\textsubscript{2} > NiFe\textsubscript{2} > Ni dust > NiAsS [13]. Equivalent doses of the following compounds do not affect blood hematocrit under the same conditions: amorphous NiS, NiTe, NiAs, Ni\textsubscript{11}As\textsubscript{9}, Ni\textsubscript{3}As\textsubscript{2}, NiSb, NiFe, and NiTiO\textsubscript{3} [13]. Stimulation of erythropoiesis in rats by ir injection of nickel compounds does not correlate with phagocytosis of the compounds by rat peritoneal macrophages in vitro [13,14], or dissolution of the compounds during incubation in rat serum or renal cytosol [13,15]. NiS\textsubscript{2}-induced erythrocytosis in rats is inhibited by simultaneous ir injection of Mn dust [16]. Until the present study, NiS\textsubscript{2} was known to induce erythrocytosis only in rats; ir injection of NiS\textsubscript{2} does not cause erythrocytosis in mice (BALBc strain) or squirrels [7].

Several reports have described the pathological lesions that develop in rats after ir injection of NiS\textsubscript{2} [3,7,9,17,18]. Inflammation and fibrosis occur along the needle tract in the injected kidney [3,7]; NiS\textsubscript{2}-particles are phagocytized by mononuclear cells and glomerular mesangial cells [9]; renal glomeruli become enlarged [9]; mesangial cells become hyperplastic [9]; and crystalline mitochondrial inclusions develop in renal tubular cells [17,18]. Carcinomas and sarcomas frequently become evident in the injected kidneys of rats at eight to 20 months after ir injection of NiS\textsubscript{2} [4,12,19]. Other pathological findings include splenomegaly, cardiomegaly, sialyl hyperplasia, and arteriosclerosis [9].

The following evidence suggests that NiS\textsubscript{2}-induced erythrocytosis is mediated by increased renal production of erythropoietin (Ep): (a) Ep activity is increased in rat serum at two to eight weeks after ir injection of NiS\textsubscript{2} [6,10,11]; (b) erythropoietin activity is increased in the light mitochondrial fraction from kidneys of NiS\textsubscript{2}-treated rats [10]; (c) NiS\textsubscript{2}-induced erythrocytosis is reversed by resection of the injected kidney [10,11]; (d) NiS\textsubscript{2}-induced erythrocytosis is suppressed by prolonged exposure to hyperoxia [10]; and (e) NiS\textsubscript{2}-induced erythrocytosis is prevented by ip infusion of anti-Ep antiserum [10].

The present paper describes ten experiments on NiS\textsubscript{2}-induced erythrocytosis that furnish salient information concerning species-specificity, route of administration, effects of nephrectomy and splenectomy, inhibition by chelating agents; antagonism by metal dusts, role of phagocytosis, effect of iron-dextran treatment, response to simultaneous ir injection of benzo(a)pyrene, and erythropoietin activity in renal cytosol.
MATERIALS AND METHODS

Test Materials

Nickel subsulphide (αNi₃S₂, median particle diameter < 2 μm) was donated by Stuart Warner, Ph.D., INCO Ltd., Toronto, Canada. The chemical and physical properties of the Ni₃S₂ sample were described previously [19,20]. Mn, Cu, Cr, and Al dusts (median particle diameters < 5 μm) were obtained from Alfa Inorganic Products Division, Ventron Corp., Danvers, MA. Purity of the metal dusts was > 99.9 percent, based upon the producer’s specifications. Benzo(a)pyrene (Mann Research Laboratories, Inc., New York, NY) yielded a single fluorescent spot after thin-layer chromatography, as previously described [21]. Iron-dextran solution [“Imferon,” Fe(OH)₃-dextran complex (50 mg Fe/ml), dissolved in NaCl solution (0.14 mol/liter)] was obtained from Richardson Merrill, Inc., Cincinnati, OH. d-Penicillamine (d-3-mercaptoproline hydrochloride) was obtained from Aldrich Chemical Co., Milwaukee, WI. Sodium diethyldithiocarbamate trihydrate (Sigma Chemical Co., St. Louis, MO) was recrystallized, as previously described [22]. Lyophilized sheep erythropoietin (“Stage 3 Preparation,” Connaught Laboratories, Ltd., Swiftwater, PA) was the standard for erythropoietin bioassays.

Experimental Animals

The animals included 596 male rats (Fischer-344 strain, mean body weight = 230 g, SD ± 44 g, Charles River Breeding Laboratories, Inc., Wilmington, MA); 120 virgin female mice (CF-1 strain, mean body weight = 27 g, SD ± 2 g, Charles River Breeding Laboratories, Inc.); 19 male guinea pigs (Hartley strain, mean body weight = 534 g, SD ± 18 g, Buckberg Laboratory Animals, Inc., Tomkins Cove, NY); 22 male hamsters (Syrian golden strain, mean body weight = 117 g, SD ± 13 g. Charles River Breeding Laboratories, Inc.); and 19 male Mongolian gerbils (mean body weight = 90 g, SD ± 13 g, bred in the University of Connecticut Vivarium, Farmington, CT). The animals were fed the appropriate Purina laboratory animal chows and water ad libitum. Gerbils received parenteral iron supplementation (Imferon, 2.5 mg Fe/gerbil, im) at biweekly intervals. Rats and guinea pigs were housed in stainless steel cages; mice, hamsters, and gerbils were housed in polypropylene cages with wood chip bedding.

Treatments

Anesthesia with diethylether was used for animal surgery. The kidney was exposed by a subcostal lumbar incision. For ir injection, the test substance [suspended in 0.1 to 0.25 ml of NaCl solution (0.14 mol/liter) or glycerol (50 percent solution in distilled water)] was injected into the kidney by a tuberculin syringe with No. 25 gauge needle. For nephrectomy, the ureter and renal vessels were ligated with silk sutures, and the kidney was excised with fine scissors. After ir injection or nephrectomy, the lumbar muscles were sutured with silk, and the skin incision was closed with surgical clips. For intrasplenic injection or splenectomy, similar procedures were used; the abdominal cavity was opened by a midline incision. In one experiment, osmotically driven pumps (“Alzet minipumps,” model 2001, infusion rate = 1 μl/hr, Alza Corp., Palo Alto, CA) were employed for sc infusion of chelating agents, as previously described [23]. The pumps were replaced at seven-day intervals during the 35 days of treatment. In another experiment, semipermeable
tubules ("Diaflo H10X50 Fibers," nominal molecular exclusion limit = 50,000 daltons, 0.5 mm internal diameter, 6 mm length, Amicon Corp., Lexington, MA) were implanted in rat kidneys. Each tubule was filled with Ni$_3$S$_2$ by the following procedure: (a) the empty tubule was weighed, (b) a tiny wad of cotton was inserted in one end, (c) the partially plugged end of the tubule was connected to suction via a blunt needle, (d) Ni$_3$S$_2$ powder was aspirated into the tubule from a weighing boat, (e) the cotton wad was removed, (f) the tubule was reweighed to determine the Ni$_3$S$_2$ content, and (g) both ends were sealed by application of forceps that had been heated in a flame. A No. 18 gauge needle was inserted into the rat kidney to a depth of 8 mm, with care to avoid the renal calyx. The needle was withdrawn, and the tubule was gently pushed into the renal parenchyma along the needle track.

Hematological Tests

Blood samples (~50 μl) were obtained from tails of rats and gerbils, from toepads of guinea pigs, and from retro-orbital venous plexuses of mice and hamsters. The blood samples were collected in heparinized capillary tubes; microhematocrit determinations were performed by the method of Strumia et al. [24]. Bioassays of erythropoietin (Ep) activity were performed upon serum and renal extracts from groups of 12 to 28 rats. The rats were exsanguinated by cardiocentesis, and 2 ml samples of serum from each rat were pooled. Each kidney was perfused in situ with 12 ml of cold phosphate-buffered NaCl solution (Na$_2$HPO$_4$-KH$_2$PO$_4$ buffer, pH 7.0, 10 mmol/liter; NaCl, 0.14 mol/liter, 4°C). The kidneys were homogenized in phosphate-buffered NaCl solution and centrifuged at 2300 x g for 30 minutes, as described by Fried et al. [25]. Each ml of renal extract corresponded to 0.33 g of kidney (wet weight). Each sample of pooled rat serum and pooled renal extract was apportioned into 12 tubes; the tubes were stored at -20°C; one tube was thawed immediately prior to each dosing in the Ep bioassay. Ep activity was measured in groups of four to six post-hypoxic polycythemic mice, as previously described [6]. Each mouse received 12 injections sc of 0.5 ml of pooled serum, renal extract, or Ep standard solution at six-hour intervals for three days (total dose = 6 ml per mouse). Ep activities of rat serum and renal extract were read from a six-point logit calibration plot of erythrocyte $^{59}$Fe-uptake in mice that received injections of sheep Ep standard solutions (8 to 500 IU/liter). The Ep detection limit was 12 IU/liter, defined as the concentration of Ep that yielded a response differing by its standard deviation multiplied by Student’s t value for $p = 0.05$ from the response for “zero” dose (NaCl vehicle solution) [6]. Null hypothesis for differences between observations in treated and control groups were tested by Student’s t test [26].

RESULTS

Species Susceptibility Experiment

Blood hematocrit was measured in groups of rats, hamsters, gerbils, and guinea pigs at one to four months after unilateral ir injection of Ni$_3$S$_2$ at dosages specified in Table 1. Control groups received ir injection of NaCl vehicle. No significant changes of blood hematocrit were observed in Ni$_3$S$_2$-treated hamsters and gerbils; significant increases of blood hematocrit were found in Ni$_3$S$_2$-treated rats and guinea pigs throughout the period of study. For example, at two months after ir injection of Ni$_3$S$_2$, the average increase of blood hematocrit in rats was 46 percent and that in guinea pigs was 38 percent, ($p < 0.001$ vs. corresponding values in controls).
**NI$_3$S$_2$-INDUCED ERYTHROCYTOSIS IN RODENTS**

TABLE 1

| Species      | Dose (ir) of Ni$_3$S$_2$ (mg/animal) | No. of Animals | Blood Hematocrit (%) after ir Injection (mean ± standard deviation) |
|--------------|-------------------------------------|----------------|-------------------------------------------------------------------|
|              |                                     |                | 1 mo                  | 2 mo                  | 3 mo                  | 4 mo                  |
| Rat          | 0*                                  | 11             | 50 ± 2               | 48 ± 2               | 52 ± 3               | 51 ± 2               |
|              | 5                                   | 11             | 64 ± 2$^{*}$         | 70 ± 3$^{*}$         | 72 ± 3$^{*}$         | 69 ± 4$^{*}$         |
| Hamster      | 0*                                  | 12             | 56 ± 2               | 52 ± 3               | 54 ± 1               | 54 ± 2               |
|              | 5                                   | 10             | 57 ± 2               | 58 ± 4               | 57 ± 3               | 53 ± 3               |
| Gerbil       | 0*                                  | 12             | 46 ± 4               | 50 ± 2               | 49 ± 2               | 48 ± 2               |
|              | 5                                   | 12             | 48 ± 2               | 50 ± 2               | 49 ± 2               | 48 ± 2               |
| Guinea pig   | 0*                                  | 11             | 46 ± 2               | 49 ± 1               | 48 ± 1               | 47 ± 2               |
|              | 20                                  | 8              | 62 ± 7$^{*}$         | 67 ± 6$^{*}$         | 63 ± 8$^{*}$         | 53 ± 6$^{*}$         |

*NaCl vehicle (0.14 mol/liter) was injected into the right kidney of controls (0.1 ml/rat, hamster, or gerbil; 0.25 ml/guinea pig).

*p < 0.05 vs. corresponding value in vehicle controls.

'p < 0.001 vs. corresponding value in vehicle controls.

**Injection Route Experiment**

Groups of rats were given intrasplenic or intrarenal injections of NaCl vehicle or Ni$_3$S$_2$ (10 mg/rat). As indicated in Table 2, blood hematocrit values were consistently increased at one to four months after ir injection of Ni$_3$S$_2$ ($p < 0.001$), whereas blood hematocrit values did not change significantly after intrasplenic administration of Ni$_3$S$_2$.

**Splenectomy Experiment**

Splenectomy or sham splenectomy (with exposure and palpation of the spleen) were performed in groups of rats at five days before or after ir injection of NaCl vehicle or Ni$_3$S$_2$ (5 mg/rat) (Table 3). Splenectomy had no effect upon blood hematocrit values in vehicle-treated controls. Splenectomy did not suppress the development of erythrocytosis in Ni$_3$S$_2$-treated rats.

**Nephrectomy Experiment**

Groups of rats were subjected to unilateral nephrectomy on the fourth day after injection of NaCl vehicle or Ni$_3$S$_2$ (5 mg/rat) into the right kidney (Table 4). Excit-
TABLE 3
Effect of Splenectomy on Ni$_3$S$_2$-Induced Erythrocytosis in Rats

| Treatment                | Dose (ir) of Ni$_3$S$_2$ (mg/rat) | No. of Rats | Blood Hematocrit (%) after ir Injection (mean ± standard deviation) |
|--------------------------|-----------------------------------|-------------|---------------------------------------------------------------|
|                          |                                   |             | 1 mo                              | 2 mo                              | 3 mo                              | 4 mo                              |
| Sham splenectomy$^a$     | 0                                 | 10          | 48 ± 2                           | 49 ± 1                           | 50 ± 2                           | 51 ± 2                           |
| Sham splenectomy$^a$     | 5                                 | 9           | 62 ± 6$^c$                       | 68 ± 5                            | 70 ± 6$^e$                       | 70 ± 8                           |
| Splenectomy$^b$          | 0                                 | 11          | 52 ± 2                           | 48 ± 2                           | 52 ± 3                           | 51 ± 2                           |
| Splenectomy$^b$          | 5                                 | 12          | 59 ± 4$^e$                       | 67 ± 5                           | 69 ± 6                           | 64 ± 8$^e$                       |
| Sham splenectomy$^c$     | 0                                 | 11          | 48 ± 2                           | 48 ± 2                           | 51 ± 2                           | 52 ± 3                           |
| Sham splenectomy$^c$     | 5                                 | 11          | 64 ± 2$^e$                       | 70 ± 3                           | 72 ± 3                           | 69 ± 4$^e$                       |
| Splenectomy$^d$          | 0                                 | 12          | 49 ± 2                           | 49 ± 2                           | 51 ± 3                           | 50 ± 1                           |
| Splenectomy$^d$          | 5                                 | 10          | 62 ± 4$^e$                       | 70 ± 6                            | 71 ± 7                            | 66 ± 6$^e$                       |

$^a$Sham splenectomy five days before the ir injection.
$^b$Splenectomy five days before the ir injection.
$^c$Sham splenectomy five days after the ir injection.
$^d$Splenectomy five days after the ir injection.
$^e$NaCl vehicle (0.14 mol/liter, 0.1 ml/rat) was injected into the right kidney of controls.
$^f$p < 0.001 vs. corresponding value in vehicle controls.

Sodium diethyldithiocarbamate and d-penicillamine were administered to rats by sustained sc infusions, according to the schedule in Table 5. The molar dosage of diethyldithiocarbamate was twice that of d-penicillamine, since the stoichiometric ratios of Ni[II]-bis-diethyldithiocarbamate and Ni[II]-penicillamine are 1:2 and 1:1, respectively. Diethyldithiocarbamate and d-penicillamine did not affect blood hematocrit values in rats that received ir injection of NaCl vehicle. Administration of diethyldithiocarbamate partially suppressed Ni$_3$S$_2$-induction of erythrocytosis, while d-penicillamine had no effect.

Chelation Experiment

TABLE 4
Effect of Nephrectomy on Ni$_3$S$_2$-Induced Erythrocytosis in Rats

| Nephrectomy$^a$ | Dose (ir) of Ni$_3$S$_2$ (mg/rat)$^b$ | No. of Rats | Blood Hematocrit (%) (mean ± std. deviation) |
|-----------------|--------------------------------------|-------------|------------------------------------------------|
|                 |                                      |             | 1 mo                              | 2 mo                              |
| None            | 0                                     | 10          | 52 ± 2                              | 53 ± 2                             |
| None            | 5                                     | 10          | 70 ± 3$^c$                          | 75 ± 3$^c$                         |
| Right           | 0                                     | 10          | 50 ± 2                              | 50 ± 1                             |
| Right           | 5                                     | 10          | 49 ± 1$^d$                          | 49 ± 2$^d$                         |
| Left            | 0                                     | 10          | 50 ± 2                              | 50 ± 1                             |
| Left            | 5                                     | 10          | 70 ± 5$^e$                          | 76 ± 3$^e$                         |

$^a$Nephrectomy four days after ir injection.
$^b$Injection of NaCl vehicle (0.14 mol/liter, 0.2 ml/rat) or Ni$_3$S$_2$ into the right kidney.
$^c$p < 0.001 vs. corresponding value in vehicle controls.
$^d$p < 0.001 vs. corresponding value in Ni$_3$S$_2$-treated controls.
Effect of Chelating Agents on Ni$_3$S$_2$-Induced Erythrocytosis in Rats

| Treatment   | Dose (ir) of Ni$_3$S$_2$ (mg/rat) | No. of Rats | Blood Hematocrit (%) after ir Injection (mean ± standard deviation) |
|-------------|-----------------------------------|-------------|---------------------------------------------------------------------|
|             |                                   |             | 1 mo | 2 mo | 3 mo | 4 mo |
| Buffer*     | 0*                                | 8           | 46 ± 3 | 51 ± 3 | 52 ± 3 | 54 ± 2 |
| Buffer*     | 1.2                               | 6           | 56 ± 5$^\dagger$ | 70 ± 4$^\ddagger$ | 71 ± 6$^\ddagger$ | 70 ± 6$^\ddagger$ |
| PEN         | 0*                                | 7           | 47 ± 2 | 48 ± 2 | 49 ± 3 | 50 ± 2 |
| PEN         | 1.2                               | 7           | 60 ± 4$^\dagger$ | 69 ± 5$^\ddagger$ | 75 ± 7$^\ddagger$ | 71 ± 11$^\ddagger$ |
| DDC         | 0*                                | 8           | 45 ± 2 | 51 ± 2 | 51 ± 3 | 52 ± 2 |
| DDC         | 1.2                               | 8           | 46 ± 2$^\dagger$ | 62 ± 6$^\ddagger$ | 62 ± 8$^\ddagger$ | 59 ± 6$^\ddagger$ |

*Phosphate buffer (Na$_2$HPO$_4$-KH$_2$PO$_4$, 0.1 mil/liter, pH 9) was administered to controls (24 μl/day) by an osmotic minipump implanted sc one day prior to the ir injection. The minipump was replaced at seven-day intervals; the sc infusion was maintained for 35 days.

$^\dagger$d-Penicillamine (0.9 mol/liter in phosphate buffer) was infused sc (22 μmol/day) for 35 days (total dose = 0.75 mmol/rat).

$^\ddagger$Sodium diethyldithiocarbamate (1.8 mol/liter in phosphate buffer) was infused sc (44 μmol/day) for 35 days (total dose = 1.5 mmol/rat).

$^\flat$NaCl vehicle (0.14 mol/liter, 0.2 ml/rat) was injected into the right kidney.

$^\dagger$ < 0.01 vs. corresponding value in controls that received phosphate buffer (sc) and NaCl vehicle (ir).

$^\ddagger$ < 0.001 vs. corresponding value in rats that received phosphate buffer (sc) and NaCl vehicle (ir).

$^\flat$ < 0.05 vs. corresponding value in rats that received phosphate buffer (sc) and Ni$_3$S$_2$ (ir).

$^\flat$ < 0.01 vs. corresponding value in rats that received phosphate buffer (sc) and Ni$_3$S$_2$ (ir).

$^\flat$ < 0.001 vs. corresponding value in rats that received phosphate buffer (sc) and Ni$_3$S$_2$ (ir).

**Metal Interactions Experiment**

Four metal dusts (Mn, Cr, Cu, and Al) were administered to groups of rats by ir injection (8 mg/rat), alone, or in combination with Ni$_3$S$_2$ (2.5 mg/rat). In the absence of Ni$_3$S$_2$, the four metal dusts had no significant effects upon blood hematocrit values (Table 6). Consistent with previous findings [16], induction of erythrocytosis at one and two months after ir injection of Ni$_3$S$_2$ was partially suppressed by admixture of Mn dust. Similar, but less marked, suppression of Ni$_3$S$_2$-induced erythrocytosis was also noted at one month after ir injection of Cu dust and Ni$_3$S$_2$, and at four months after ir injection of Al dust and Ni$_3$S$_2$. Enhancement of erythrocytosis was observed at two to four months after ir injection of Cr dust and Ni$_3$S$_2$, compared to that produced by Ni$_3$S$_2$, alone.

**Semi-Permeable Tubule Experiment**

Blood hematocrit values of rats were increased at one to four months after ir implantation of sealed cellulose tubules that contained 10 mg of Ni$_3$S$_2$ (Table 7). This observation indicates that phagocytosis of Ni$_3$S$_2$ particles is not essential for Ni$_3$S$_2$-induced erythrocytosis. Blood hematocrit values were also increased in rats at one to four months after ir implantation of Ni$_3$S$_2$-containing tubules that had been punctured 20 times with a needle. However, hematocrit values in these rats were not significantly higher than in rats that received ir implants of sealed Ni$_3$S$_2$-containing tubules.

**Effect of Iron-Dextran Treatment**

To ascertain whether augmentation of body iron stores might influence Ni$_3$S$_2$-induced erythrocytosis, iron-dextran complex ("Imferon") was administered to rats...
by nine biweekly im injections, commencing one week prior to ir injection of NaCl vehicle or Ni₃S₂, as specified in Table 8. In the absence of Ni₃S₂ treatment, Imferon administration had no significant effect upon blood hematocrit values. In contrast, rats that received im Imferon plus ir Ni₃S₂ had substantially higher hematocrit values than rats that received only Ni₃S₂.

**Benzo(a)pyrene Interaction Experiment**

Intrarenal injection of benzo(a)pyrene had no significant effect on blood hematocrit values in rats (Table 9). When a mixture of benzo(a)pyrene and Ni₃S₂ was administered ir to rats, benzo(a)pyrene partially inhibited Ni₃S₂-induced erythrocytosis throughout the period from one to four months after the injection.
TABLE 8
Effect of Imferon on Ni$_3$S$_2$-Induced Erythrocytosis in Rats

| Treatment  | No. of Rats | Blood Hematocrit (%) after ir Injection (mean ± standard deviation) |
|------------|-------------|-------------------------------------------------------------------|
| Vehiclea   | 0           | 50 ± 1 50 ± 2 51 ± 2 51 ± 2                                      |
| Vehicleb   | 10          | 65 ± 5a 71 ± 4a 71 ± 5a 67 ± 5a                                  |
| Imferona   | 0           | 50 ± 2 49 ± 2 50 ± 2 52 ± 2                                      |
| Imferonb   | 10          | 70 ± 6* 77 ± 5* 76 ± 4* 74 ± 5*                                  |

*NaCl vehicle (0.14 mol/liter, 0.1 ml/rat) was injected im one week before and 1, 3, 5, 7, 9, 11, 13, and 15 weeks after the ir injection.

*Imferon (iron-dextran, 5 mg Fe/rat) was injected im one week before and 1, 3, 5, 7, 9, 11, 13, and 15 weeks after the ir injection.

*NaCl vehicle (0.14 mol/liter, 0.2 ml/rat) was injected ir into the right kidney.

*p < 0.001 vs. corresponding value in controls that did not receive Ni$_3$S$_2$.

*p < 0.05 vs. corresponding value in rats that received Ni$_3$S$_2$ without Imferon.

*p < 0.02 vs. corresponding value in rats that received Ni$_3$S$_2$ without Imferon.

*p < 0.01 vs. corresponding value in rats that received Ni$_3$S$_2$ without Imferon.

Suppression of Ni$_3$S$_2$-induced erythrocytosis was not observed when benzo(a)pyrene was injected in the left kidney and Ni$_3$S$_2$ was injected in the right kidney.

Erythropoietin Bioassays

To determine whether increased Ep activity in serum of Ni$_3$S$_2$-treated rats is associated with increased Ep activity in kidney, Ep bioassays were performed upon pooled serums and renal extracts from rats killed at two weeks after ir injection of Ni$_3$S$_2$ or NaCl vehicle solution (Table 10, Experiment A). Consistent with previous findings [6,10,11], Ep activity was increased sixfold in serum of Ni$_3$S$_2$-treated rats (*p < 0.001 vs. controls). Ep activity in pooled extracts of right (injected) kidneys

TABLE 9
Blood Hematocrit of Rats after ir Injection of Benzo(a)pyrene (BP) and Ni$_3$S$_2$*

| Dose (ir) of BP (mg/rat) | Dose (ir) of Ni$_3$S$_2$ (mg/rat) | No. of Rats | Blood Hematocrit (%) after ir Injection (mean ± standard deviation) |
|--------------------------|----------------------------------|-------------|-------------------------------------------------------------------|
| 0a                       | 0                                | 15          | 48 ± 2 48 ± 2 49 ± 2 52 ± 2                                      |
| 2                        | 0                                | 15          | 49 ± 2 48 ± 1 48 ± 2 52 ± 2                                      |
| 0                        | 2                                | 16          | 66 ± 3 78 ± 6 78 ± 4 77 ± 6                                    |
| 2*                       | 2                                | 16          | 49 ± 2 61 ± 8 65 ± 9 68 ± 8                                    |
| 2*                       | 2                                | 16          | 63 ± 4 74 ± 5 74 ± 4 72 ± 6                                    |

*All rats received Imferon (iron-dextran, 5 mg Fe/rat, im) one week before and 1, 3, 5, 7, 9, and 11 weeks after the ir injection.

*Glycerol vehicle (50 percent, v/v; 0.2 ml/rat) was injected into the right kidney.

*Benzo(a)pyrene (2 mg/rat) and Ni$_3$S$_2$ (2 mg/rat) were administered as a single injection in 0.2 ml of glycerol vehicle into the right kidney.

*Benzo(a)pyrene (2 mg/rat, in 0.2 ml of glycerol vehicle) was injected into the left kidney, and Ni$_3$S$_2$ (2 mg/rat, in 0.2 ml of glycerol vehicle) was injected into the right kidney.

*p < 0.01 vs. corresponding value in controls that did not receive Ni$_3$S$_2$.

*p < 0.001 vs. corresponding value in controls that did not receive Ni$_3$S$_2$.

*p < 0.01 vs. corresponding value in rats that received Ni$_3$S$_2$ without benzo(a)pyrene.

*p < 0.001 vs. corresponding value in rats that received Ni$_3$S$_2$ without benzo(a)pyrene.
TABLE 10

| Exp. | Dose (ir) of Ni₃S₂ (mg/rat) | No. of Rats | Hematocrit (%), mean ± SD | Pooled Samples for Ep Assay* | No. of Assay Mice | RBC SC Fe Uptake (%)*, mean ± SD | EP Activity (IU/liter), mean ± SD |
|------|-------------------------|------------|-----------------------------|-----------------------------|-------------------|--------------------------------|---------------------------------|
| A    | 0                       | 24         | Serum 48 ± 1                | Right kidney 6              | 1.24 ± 0.26      | 12 ± 3                          | 14 ± 5                          |
|      | 5                       | 28         | Serum 56 ± 4*                | Left kidney 6              | 1.41 ± 0.27      | 13 ± 3                          |                                 |
|      |                         |            |                              |                            |                   |                                |                                 |
| B    | 0                       | 12         | Serum 48 ± 2                | Right kidney 5              | 1.55 ± 0.23      | 14 ± 3                          |                                 |
|      | 5                       | 12         | Serum 56 ± 4*                | Left kidney 5              | 2.87 ± 1.18      | 24 ± 8                          |                                 |

*Rats were killed two weeks after injection of NaCl vehicle (0.14 mol/liter, 0.2 ml/rat) or Ni₃S₂ in the right kidney.

*Pooled sera or renal extracts were administered to post-hypoxic polycythemic mice as 12 sc injections (0.5 ml/mouse at six-hour intervals during three days; total dose = 6 ml/mouse).

*SC-Fe-labelled ferrous citrate solution (2 μCi SC-Fe/mouse) was injected iv at eight hours after the last sc injection of serum or renal extract; SC-Fe-uptake into circulating erythrocytes was measured at 24 hours after the ferrous citrate injection.

*p < 0.01 vs. corresponding value for control rats.

*p < 0.001 vs. corresponding value for pooled serum from control rats.

from Ni₃S₂-treated rats did not differ from (a) Ep activity in extracts of right (injected) kidneys of NaCl-treated controls, or (b) Ep activity in left (non-injected) kidneys of Ni₃S₂-treated rats. Repetition of the Ep bioassays in pooled renal extracts from Ni₃S₂-treated and control rats (Table 10, Experiment B) also failed to disclose any significant variations in renal Ep activities.

DISCUSSION

Species Specificity

The observation that Ni₃S₂-induced erythrocytosis occurs in guinea pigs as well as in rats provides a new experimental animal for investigations of the erythropoietic effects of ir injection of Ni₃S₂. Species variations in susceptibility to Ni₃S₂-stimulation of erythropoiesis are clearly evident, since the present study of gerbils and a previous study of BALBc mice and squirrels [7] show that these species are refractory to Ni₃S₂-induction of erythrocytosis. In hamsters, blood hematocrit at two months after ir injection of Ni₃S₂ averaged 12 percent greater than in controls; this slight increase was not statistically significant. In future studies, we shall determine whether significant erythrocytosis develops in hamsters after ir injection of larger doses of Ni₃S₂.

Role of the Spleen

The possible involvement of the spleen in Ni₃S₂-stimulation of erythropoiesis was investigated, since splenomegaly and splenic erythroid hyperplasia occur in rats after ir injection of Ni₃S₂ [9], and since the spleen has been proposed as an extrarenal source of erythropoietin [27–29]. In the present study, intrasplenic injection of Ni₃S₂ did not affect blood hematocrit in rats, and splenectomy did not significantly influence the erythrocytosis that occurs after ir injection of Ni₃S₂. Therefore, the
spleen does not play an essential role in the pathogenesis of \( \text{Ni}_3\text{S}_2 \)-induced erythrocytosis. The lack of erythropoietic response to intrasplenic injection of \( \text{Ni}_3\text{S}_2 \) is consistent with the previously reported lack of erythropoietic response to intrahepatic injection of \( \text{Ni}_3\text{S}_2 \) in rats following partial heptectomy [7]. To date, ir injection is the only route of \( \text{Ni}_3\text{S}_2 \) administration that has been found to evoke erythrocytosis in rats.

**Nephrectomy**

McCully et al. [9] speculated that glomerular mesangial cells may be responsible for Ep production in \( \text{Ni}_3\text{S}_2 \)-treated rats, based upon the occurrence of glomerulomegaly and hyperplasia of mesangial cells in both kidneys of rats at one to 18 weeks after unilateral ir injection of \( \text{Ni}_3\text{S}_2 \). The present nephrectomy experiment shows that excision of the non-injected (left) kidney does not diminish the erythropoietic response, whereas excision of the injected (right) kidney completely prevents the response. The possible involvement of the contralateral kidney in the pathogenesis of \( \text{Ni}_3\text{S}_2 \)-induced erythrocytosis has not hitherto been investigated; consistent with previous findings [10,11], presence of the injected kidney appears to be essential for \( \text{Ni}_3\text{S}_2 \)-induction of erythrocytosis. Since mesangial cell hyperplasia occurs with equal intensity in both kidneys [9], the results of the nephrectomy experiment militate against the hypothesis that hyperplastic mesangial cells are responsible for Ep production in \( \text{Ni}_3\text{S}_2 \)-treated rats.

**Effects of Chelators**

Studies in rats indicate that diethylidithiocarbamate is superior to d-penicillamine for treatment of nickel carbonyl poisoning [22], but less effective than d-penicillamine as an antidote for nickel chloride poisoning [39]. \( \text{Ni}[\text{II}] \)-bis-diethylidithiocarbamate is lipophilic and \( \text{Ni}[\text{II}] \)-penicillamine is hydrophilic; these differences in chemical properties result in disparate effects of diethylidithiocarbamate and penicillamine on the tissue distribution of \( ^{64}\text{Ni}[\text{II}] \) [31]. The present study shows that ip infusion of diethylidithiocarbamate partially suppresses the erythropoietic response to ir injection of \( \text{Ni}_3\text{S}_2 \) in rats, whereas d-penicillamine has no inhibitory effect. The inhibitory action of diethylidithiocarbamate on \( \text{Ni}_3\text{S}_2 \)-induced erythrocytosis may be unrelated to its chelating affinity for nickel, since diethylidithiocarbamate also protects rats against the toxicity of carbon tetrachloride [32] and dimethylnitrosamine [33].

**Effects of Metal Dusts**

At one month after ir injection of Mn dust in combination with \( \text{Ni}_3\text{S}_2 \), Mn dust completely blocks \( \text{Ni}_3\text{S}_2 \)-induced erythrocytosis. The inhibitory effect of Mn dust on erythropoietic stimulation by \( \text{Ni}_3\text{S}_2 \) is less marked at two months; the inhibition is no longer significant at three months after the ir injection. Manganese has also been reported to antagonize the actions of nickel in other experimental systems; (a) Mn dust suppresses the carcinogenicity of \( \text{Ni}_3\text{S}_2 \) following im and ir administration to rats [19,20]; (b) Mn dust blocks *in vitro* morphological transformation of Syrian hamster embryo cells by \( \text{Ni}_3\text{S}_2 \) [34]; (c) Mn[II] protects organ cultures of mouse trachea from Ni[II] toxicity [35]; (d) administration sc of Mn[II] blocks Ni[II] induction of heme oxygenase activity in rat kidney [36,37]; (e) Mn[II] and Ni[II] compete for binding to identical sites on rabbit and human albumins [38]; and (f) Mn[II] and Ni[II] exert antagonistic effects upon action potentials of myocardial fibers in...
Erythropoietin that stimulates erythrocytosis in rats at one month and four months, respectively, after i.r. injection. The inhibitory effect of Cu dust is particularly interesting, since Cu[II] displaces Ni[II] from its binding site near the N-terminus of human albumin [41]. Enhanced erythrocytosis was noted in the present study at two to four months after combined i.r. injection of Cr dust and Ni3S2, compared to that produced by Ni3S2, alone. This observation is consistent with a recent report that Cr[III] potentiates the toxic effects of Ni[II] in cell cultures of murine fibroblasts [42].

**Semi-Permeable Tubules**

Stimulation of erythropoiesis was observed in the present study in rats that received i.r. implants of Ni3S2 within semipermeable tubules (nominal molecular exclusion limit = 50,000 daltons). The erythrocytosis was not significantly augmented when Ni3S2-containing tubules were punctured several times with a needle prior to i.r. implantation. In a pilot study that was reported previously [7], erythrocytosis did not develop in five rats that received i.r. implants of Ni3S2 within sealed tubules with larger pores (nominal molecular exclusion limit = 150,000 daltons). We attribute the discrepancy between the results of the pilot study and the present experiment to (a) improved implantation technique, with less peritubular hemorrhage, and (b) less obstruction of the smaller pores by fibrin. Ni3S2 gradually dissolves within the lumen of the cellulose tubules; Ni[II]-complexes with amino acids, peptides, and low molecular weight proteins slowly diffuse through the pores into the renal parenchyma [7,43]. The present experiment demonstrates that phagocytosis of Ni3S2 particles within the renal parenchyma is not essential for the induction of erythrocytosis.

**Effects of Imferon and Benzo(a)pyrene**

Supplementation of body iron stores by biweekly i.m. administration of iron-dextran (“Imferon”) augmented erythrocytosis in rats after i.r. injection of Ni3S2. This observation suggests that dietary intake of iron from Purina laboratory rat chow is insufficient to sustain the maximal rate of erythrocyte production in Ni3S2-treated rats. Reports of carcinogenic synergism between benzo(a)pyrene and nickel compounds [21,44,45] prompted us to administer benzo(a)pyrene and Ni3S2 to rats, singly and in combination, by i.r. injection. Instead of enhancing Ni3S2-induced erythrocytosis, benzo(a)pyrene partially inhibited the erythropoietic response. This finding provides a novel experimental system to investigate the metabolic interactions of benzo(a)pyrene and nickel compounds.

**Erythropoietin Bioassays**

Renal extracts, prepared by the method of Fried et al. [25], and pooled sera from Ni3S2-treated and control rats were assayed for Ep activity in post-hypoxic polycythemic mice. The bioassays demonstrated increased Ep activity in serum, but not in renal extracts of Ni3S2-treated rats. This finding is consistent with the concept that a renal erythropoietic factor (“erythropoietin”) is produced by the kidney and
acts in concert with a serum component to produce the active Ep molecule [46,47]. Meagher [10] detected increased erythropoietin activity in the light mitochondrial fraction from injected kidneys of \( \text{Ni}_3\text{S}_2 \)-treated rats. Attempts to confirm Meagher's observation and to investigate the mechanism whereby \( \text{Ni}_3\text{S}_2 \) stimulates renal erythropoietin activity are under way in our laboratory.

ACKNOWLEDGEMENTS

The authors are grateful to Leo Delaney and Bryan Stamm for skillful assistance.

REFERENCES

1. Jasmin G: Experimental production of polycythemia in rats with nickel sulfide. Clin Res 21:1068, 1973
2. Jasmin G, Solymoss B: Polycythemia induced in rats by intrarenal injection of nickel subsulfide, \( \text{Ni}_3\text{S}_2 \). Proc Soc Exp Biol Med 148:774–776, 1975
3. Morse EE, Lee T-Y, Reiss RF, et al: Dose-response and time-response study of erythropoietin in rats after intrarenal injection of nickel subsulfide. Ann Clin Lab Sci 7:17–24, 1977
4. Jasmin G, Solymoss B: The topical effects of nickel subsulfide on renal parenchyma. In Inorganic and Nutritional Aspects of Cancer. Edited by GN Schrauzer. New York, Plenum Press, 1978, pp 69–83
5. Hopfer SM, Sunderman FW Jr, Fredrickson TN, et al: Nickel-induced erythropoiesis: Efficacies of nickel compounds and susceptibilities of rat strains. Ann Clin Lab Sci 8:396–402, 1978
6. Hopfer SM, Sunderman FW Jr, Fredrickson TN, et al: Increased serum erythropoietin activity in rats following intrarenal injection of nickel subsulfide. Res Commun Chem Pathol Pharmacol 23:155–170, 1979
7. Hopfer SM, Sunderman FW Jr, Morse EE, et al: Effects of intrarenal injection of nickel subsulfide in rodents. Ann Clin Lab Sci 10:54–64, 1980
8. Oskarsson A, Reid MC, Sunderman FW Jr: Effects of cobalt chloride, nickel chloride, and nickel subsulfide upon erythropoiesis in rats. Ann Clin Lab Sci 11:165–172, 1981
9. McCully KS, Rinehimer LA, Gillies CG, et al: Erythropoiesis, glomerulomegaly, mesangial hyperplasia, sialyl hyperplasia, and arteriosclerosis induced in rats by nickel subsulfide. Virchows Arch Pathol Anat 394:207–220, 1982
10. Meagher RC: Mechanisms Underlying the Induction of Erythropoiesis in Rats by Intrarenal Implantation of Nickel Subsulfide. (Ph.D. thesis, New York University, 1979.) Ann Arbor, University Microfilms International, 1980, pp 1–123
11. Solymoss B, Jasmin G: Studies of the mechanism of polycythemia induced in rats by \( \text{Ni}_3\text{S}_2 \). Exp Hemat 6:43–47, 1978
12. Jasmin G, Riopelle JL: Renal carcinomas and erythropoiesis in rats following intrarenal injection of nickel subsulfide. Lab Invest 35:71–78, 1976
13. Sunderman FW Jr, Hopfer SM: Correlation between the carcinogenic activities of nickel compounds and their potencies for stimulating erythropoiesis in rats. In Biological Aspects of Trace Metals and Metal-Related Diseases. Edited by B Sarkar. New York, Raven Press, in press
14. Kuehn K, Fraser CB, Sunderman FW Jr: Phagocytosis of particulate nickel compounds by rat peritoneal macrophages in vitro. Carcinogenesis 3:331–336, 1982
15. Kuehn K, Sunderman FW Jr: Dissolution half-times of nickel compounds in water, rat serum, and renal cytosol. J Inorg Biochem, in press
16. Hopfer SM, Sunderman FW Jr: Manganese inhibition of nickel subsulfide induction of erythropoiesis in rats. Res Commun Pathol Pharmacol 19:337–345, 1978
17. Jasmin G: Ultrastructural patterns of Ni-induced crystalline inclusions in mitochondria of renal tubules. Exp Mol Pathol 29:199–210, 1978
18. Jasmin G, Bonneau R, Andre J: Etude par goniometrie des inclusion cristallines mitochondriales induites par le subsulfure de nickel chez le rat. Biol Cell 35:81–86, 1979
19. Sunderman FW Jr, Maenza RM, Hopfer SM, et al: Induction of renal cancers in rats by intrarenal injection of nickel subsulfide. J Envir Pathol Toxicol 2:1511–1527, 1979
20. Sunderman FW Jr, Kasprzak KS, Lau TJ, et al: Effects of manganese on carcinogenicity and metabolism of nickel subsulfide. Cancer Res 36:1790–1800, 1976
21. Maenza RM, Pradhan AM, Sunderman FW Jr: Rapid induction of sarcomas in rats by combination of nickel sulfide and 3,4-benzpyrene. Cancer Res 31:2067–2076, 1971
22. Baselt RC, Sunderman FW Jr, Mitchell J, et al: Comparisons of antidotal efficacy of sodium diethylthiocarbamate, d-penicillamine, and triethylenetetramine upon acute toxicity of nickel car- bonyl in rats. Res Commun Chem Pathol Pharmacol 18:666–688, 1977
23. Shen SK, Williams S, Onkelinx C, et al: Use of implanted minipumps to study the effects of chelating drugs on renal ⁴²Ni clearance in rats. Toxicol Appl Pharmacol 51:209–217, 1979
24. Strumia MM, Sample AB, Hart ED: An improved microhematocrit method. Amer J Clin Path 24:1016–1024, 1954
25. Fried W, Barone-Verelas J, Berman M: Detection of high erythropoietin titers in renal extracts of hypoxic rats. J Lab Clin Med 97: 82–86, 1981
26. Zar JH: Biostatistical Analysis. Englewood Cliffs, NJ, Prentice-Hall, Inc, 1975, pp 105–107
27. De Francis cis P, De Bella G, Cifaldi S: Spleen as a production site for erythropoietin. Science 150:1831–1833, 1965
28. Mirand EA, Murphy GP: Renal erythropoietic factor (REF) assays in anephric and intact baboons following varied erythropoietic stimuli. J Med 2:192–200, 1971
29. Kaplan SM, Rothmann SA, Gordon AS, et al: Extrarenal sites of erythropoietin production. Proc Soc Exp Biol Med 143:310–313, 1973
30. Horak E, Sunderman FW Jr, Sarkar B: Comparisons of antidotal efficacy of chelating drugs upon acute toxicity of Ni[III] in rats. Res Commun Chem Pathol Pharmacol 14:153–165, 1976
31. Oskarsson A, Tjvalve H: Effects of diethylthiocarbamate and penicillamine on the tissue distribution of ⁶³NiCl₂ in mice. Arch Toxicol 45:45–52, 1980
32. Popp JA, Shinozuka H, Farber E: The protective effects of diethylthiocarbamate and cyclohexi- mide on the multiple hepatic lesions induced by carbon tetrachloride in the rat. Toxicol Appl Pharmacol 45: 549–564, 1978
33. Abanobi SE, Popp JA, Chang SK, et al: Inhibition of dimethylnitrosamine-induced strand breaks in liver DNA and liver cell necrosis by diethylthiocarbamate. J Natl Cancer Inst 58:263–271, 1977
34. Sunderman FW Jr: Carcinogenicity and anticarcinogenicity of metal compounds. In Environmental Carcinogenesis. Edited by P Emmelot, E Kriek. Amsterdam, Elsevier/North Holland Biomedical Press, 1979, pp 165–192
35. Paulsen G, Jonsen J, Olsen I: Manganese-nickel interaction in a tracheal ring model system. Res Commun Chem Pathol Pharmacol 32:525–534, 1981
36. Drummond GS, Kappas A: Metal ion interactions in the control of heme oxygenase induction in liver and kidney. Biochem J 192:637–648, 1980
37. Yoshida T, Ishizawa S: Question on the biological significance of metal ion interactions in the con- trol of heme oxygenase induction. Biochem Intern 3:181–187, 1981
38. Nandedkar AKN, Morse CE, Friedberg F: Mn⁴⁺ binding by plasma proteins. Intern J Peptide Prot Res 5:279–281, 1973
39. Babskii EB, Donskikh EA: Opposing nature of the effect of manganese and nickel ions on the action potentials of myocardial fibers. Dokl Akad Nauk SSSR 207:1250–1258, 1972
40. Donskikh EA, Mukumov MR: Action of manganese ions on automatic activity of myocardial fibers induced by nickel ions. Bull Exp Biol Med USSR 78:971–974, 1974
41. Peters T Jr: Serum albumin: Recent progress in the understanding of its structure and biosynthesis. Clin Chem 23:5–12, 1977
42. Rossner P, Bencko V, Sram RJ: Combined action of chromium and nickel on mouse and hamster fibroblast lines. J Hygi Epidemiol Microbiol Immunol 25:252–258, 1981
43. Kasprzak KS, Sunderman FW Jr: Mechanisms of dissolution of nickel subsulfide in rat serum. Res Commun Chem Pathol Pharmacol 16:95–108, 1977
44. Costa M, Mollenhauer HH: Phagocytosis of nickel subsulfide particles during early stages of neoplastic transformation in tissue culture. Cancer Res 40:2688–2694, 1980
45. Rivedal E, Sanner T: Synergistic effect of morphological transformation of hamster embryo cells by nickel sulfate and benz(a)pyrene. Cancer Lett 8:203–208, 1980
46. Zanjani ED, Contrera JF, Gordon AS, et al: The renal erythropoietic factor (REF). III. Enzymatic role in erythropoietin production. Proc Soc Exp Biol Med 125:505–508, 1967
47. Gordon AS, Kaplan SM: Erythropoietin (REF). In Kidney Hormones. Edited by JW Fisher. London, Academic Press, 1977, pp 187–229