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Polycythemia Induced in Rats by Intrarenal Injection of Nickel Sulfide[1] Ni_3S_2 (38628)

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Current studies on nickel-induced carcinogenesis in rats have shown that intrarenal administration of nickel sulfide—unlike intramuscular injections—elicits a plethoric reaction a few weeks later (1), during which the ears and snout of the animals become purplish and swollen. These changes are somewhat similar to those produced by histamine releasers (2), but persist and only disappear when the treated kidney is extirpated or when a renal tumor develops destroying a large part of the organ. To study the nature of the plethoric condition and its possible relation to polycythemia, we investigated the effect of intrarenal nickel sulfide treatment upon erythropoiesis, by measuring hematocrit, hemoglobin, erythrocytes, the circulating erythrocyte mass and other parameters in rats.

Materials and Methods. Male and female Charles River CD rats (Canadian Breeding Farms and Laboratories, St Constant—Quebec), with an initial body wt of 120 g (110–130 g) and kept *ad libitum* on Purina Laboratory Chow and tap water, were used throughout these experiments. An aqueous suspension (0.5 ml) of 5 mg nickel sulfide (generously donated by J. P. W. Gilman, Ontario Veterinary College, Guelph, Ontario) was injected under light ether anesthesia, through a costo-lumbar incision, into each pole of the right kidney, approximately 3 mm below the surface. The controls were treated, under identical conditions, with 0.5 ml H_2O. Hematologic studies were performed 5 mo later when all the animals were exsanguinated under light ether anesthesia by aortic puncture. Blood coagulation was prevented by heparin.

Hematocrit was measured in capillary tubes after erythrocytes sedimentation (8000 *g*) for 10 min in a microhematocrit centrifuge (3). Hemoglobin was estimated spectrophotometrically in Drabkin's solution (4). After appropriate dilutions, erythrocyte values were obtained with an electronic Coulter counter. Plasma volume was determined by diluting J^{131}-albumin (Charles E. Frosst, Kirkland, Quebec), and the circulating erythrocyte mass by diluting Cr^{51}-labelled erythrocytes (Cr^{51} supplied by Charles E. Frosst) according to a standard technique (5). Radioactivity was quantitated in a Packard Tricarb scintillation counter. The 2,3-diphosphoglyceric acid (DPGA) level of erythrocytes was measured by an enzymatic method of the Sigma Chemical Company (6).

The results were statistically analyzed by Student's *t* test (7), and $P < 0.05$ values were considered to be significantly different.

Results. Nickel sulfide (5 mg), injected into each pole of the right kidney, induced a significant increase in hematocrit, hemoglobin and erythrocytes as well as in the circulating red cell mass (Table I). On the other hand, there was no change in plasma volume, indicating that these alterations were due to absolute polycythemia.

The hematocrit, hemoglobin and erythrocyte values were significantly enhanced by intracortical as well as intramedullar injections of nickel sulfide if compared to the controls (Table II).

The nickel salt did not alter the DPGA content of blood, calculated in relation to hemoglobin or hematocrit (Table III).

Discussion. Our findings (increased hematocrit, hemoglobin, erythrocyte and circulating erythrocyte mass values) indicate that the plethoric condition in rats given intrarenal injections of nickel sulfide is due to polycythemia. However, the mechanism of this action is not yet clarified and we do not know whether the nickel salt acts directly on bone marrow or induces polycythemia through augmented erythropoietin production.

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TABLE I. HEMOGRAM OF NICKEL SULFIDE-TREATED RATS.[a]

	Hematocrit (%)	Hemoglobin (g/100 ml)	Erythrocytes (10^6/mm^3)	Red cell mass (ml/100 g b.w.)	Plasma volume (ml/100 g b.w.)
Controls	44.9 ± 0.7[b] (8)[c]	15.5 ± 0.1 (8)	7.28 ± 0.06 (8)	2.39 ± 0.09 (6)	3.00 ± 0.23 (6)
Nickel sulfide-treated[a]	81.2 ± 1.4[d] (5)	26.8 ± 0.4[d] (5)	11.20 ± 0.14[d] (5)	5.79 ± 0.59[d] (6)	2.95 ± 0.08 (6)

[a] Each pole of the right kidney was injected with 5 mg of nickel sulfide.
[b] Means ± SE.
[c] Figures in parentheses denote number of rats.
[d] $P < 0.05$ vs controls.

TABLE II. HEMOGRAM 5 MO AFTER TWO (5 mg) INJECTIONS OF NICKEL SULFIDE INTO THE CORTICAL AND MEDULLARY REGIONS OF THE KIDNEY.

	Number of rats	Hematocrit (%)	Hemoglobin (g/100 ml)	Erythrocytes (10^6/mm^3)
Controls	8	44.9 ± 0.7[a]	15.5 ± 0.1	7.28 ± 0.06
Nickel sulfide in the renal cortex	5	71.8[b] ± 3.7	23.9[b] ± 1.0	10.70[b] ± 0.45
Nickel sulfide in the renal medulla	5	65.9[b] ± 3.5	21.9[b] ± 1.1	9.74[b] ± 0.42

[a] Means ± SE.
[b] $P < 0.05$ vs controls.

In man, primary polycythemia is ascribed to autonomic proliferation of erythropoietic and other hemopoietic elements (leading to erythrocytosis, leukocytosis and thrombocytosis) and is associated with an increased leukocyte alkaline phosphatase reaction, splenomegaly and other changes (8). In contrast, the development of secondary polycythemia is mediated through accelerated erythropoietin formation which, in turn, stimulates the differentiation of bone marrow stem cells into erythropoietic precursors (9). Erythropoietin is produced from a serum glycoprotein and, to a large extent (90%), under the influence of a renal erythropoietic factor (REF) (10). Since nephrectomy apparently reverses the plethoric condition, as mentioned in the introduction, intrarenal nickel sulfide administration presumably stimulates the REF and, consequently, erythropoietin production, thereby eliciting polycythemia.

In humans, hypoxia is the most frequent cause of enhanced erythropoietin formation. In the majority of cases it is induced by respiratory or circulatory insufficiency and, rarely by an increased affinity of hemoglobin for oxygen. The DPGA content of erythrocytes is altered under these conditions (11). The polycythemia induced by androgens is likewise associated with erythrocyte-DPGA changes (12). In our experiments, there were no alterations in the DPGA content of erythrocytes, indirectly suggesting that nickel sulfide did not elicit polycythemia by similar actions. On the other hand, the metal salt causes cellular lesions in the kidney (1) and possibly increases REF formation by evoking

TABLE III. DPGA CONTENT OF BLOOD IN UNTREATED AND NICKEL SULFIDE-TREATED RATS.[a]

	Number of animals	DPGA (μm) /g Hb	DPGA (μm) /% hematocrit
Controls	6	17.35 ± 1.76[b]	6.04 ± 0.60
Nickel sulfide-treated[a]	6	21.70 ± 2.08	7.15 ± 0.36

[a] Each pole of the right kidney was injected with 5 mg of nickel sulfide.
[b] Means ± SE.

local impairment of cellular respiratory processes. Nickel sulfide may even block the activity of a local REF inhibitor (13), creating a relative excess of REF. Regardless of the mechanism, the role of the kidney in the development of polycythemia is again suggested by the absence of a plethoric reaction after intramuscular administration of nickel sulfide.

Certain generally quite well differentiated renal tumors produce erythropoietin (14, 15) and, as mentioned earlier, nickel sulfide elicits kidney tumors. However, in our experiments, polycythemia clearly preceded the development of tumors, and when the latter infiltrated a large part of the renal parenchyma, the plethoric condition tended to subside. Furthermore, these neoplasms were highly undifferentiated.

Experiments are now in progress to clarify the mechanisms of this new model of polycythemia: the effects of nickel sulfide and other metal salts (injected into the kidney or elsewhere) on erythropoietin formation and other parameters (blood gas values, erythrocyte, leukocyte and thrombocyte production, leukocyte alkaline phosphatase reaction) are being studied in detail.

Summary. In rats, injection of nickel sulfide (5 mg) into each pole of one kidney, unlike intramuscular administration, elicits a plethoric condition a few weeks later. The resulting hematologic changes (increased hematocrit, hemoglobin, erythrocytes and circulating erythrocyte mass with normal plasma volume) indicate that the plethoric condition is due to polycythemia, which is not associated with alterations in the 2,3-diphosphoglyceric acid content of erythrocytes. Removal of the treated kidney, following the development of the polycythemia, as well as the tumor growth and expansion in the renal parenchyma, reverse the plethoric condition, suggesting that the erythropoietic changes derive from nickel-induced renal lesions. Further studies are required to elucidate the nature and mechanisms of the cellular alterations.

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