amine/galactosamine ratios, and we have measured N-sulfate hexosamine.

Three patients with Scheie's syndrome have been reported to excrete an excess of dermatan sulfate only in their urine(1), but determinations in another laboratory(9) on 2 of these patients have indicated the presence of heparan sulfate as well as dermatan sulfate. The two sisters in the present series excrete excessive quantities of both dermatan sulfate and heparan sulfate.

Summary. An assay for N-sulfate hexosamine has been adapted to the determination of the urinary excretion of N-sulfated glycosaminoglycans. Values were obtained for N-sulfate glycosaminoglycan concentrations in the urine of 56 pediatric outpatients without clinical signs of mucopolysaccharidosis. Abnormal levels of N-sulfate glycosaminoglycan were found in 6 cases of mucopolysaccharidosis.

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In vitro Porphyrin Synthesis by Iron Deficient Erythrocytes.* (32362)

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The synthesis of heme-containing compounds, hemoglobin, myoglobin and a variety of enzymes, is restricted in the iron deficient state(1). This is not solely the result of a lack of iron for incorporation into protoporphyrin to form heme. The associated anemia is characterized by erythrocytes with a low hemoglobin content. Despite an excess of free erythrocyte protoporphyrin in iron deficiency (2), when the hemoglobin deficit is considered, the total amount of porphyrin produced per erythrocyte is reduced. Adequate concentration of iron appears to be necessary for optimal heme biosynthesis(3,4). Erythrocytes from humans with clinical iron deficiency are as efficient *in vitro* as normal cells in augmenting the conversion of δ aminolevulinic acid to porphobilinogen. This implies normal activity of δ aminolevulenic acid dehydrase. Iron deficient cells, however, were found to be less adequate than normal as a source of necessary enzymes for the synthesis of uro and coproporphyrinogens from δ aminolevulinic acid(5).

In the experiments to be reported here the addition of iron to the *in vitro* incubation mixtures did not augment porphyrin synthesis by iron deficient erythrocytes. However, administration of iron salts to iron deficient patients greatly increased porphyrin synthesis from δ aminolevulinic acid by their erythrocytes *in vitro*.

Materials and methods. Using δ aminolevulinic acid as substrate, porphyrin production was measured *in vitro* by methods previously described(5). Coproporphyrin and protoporphyrin were determined by a modifi-

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TABLE I. *In vitro* Porphyrin Synthesis Using Erythrocytes from Iron Deficient Patients.

Patient	No iron added to incubation mixture				Iron added to incubation mixture					After iron treatment of anemia					
	Uro	Copro	Proto	Total	Uro	Copro	Proto	Total	Total % change	Uro	Copro	Proto	Total	Total % change	% of original value
1.	261	330	168	759	200	324	133	657	—13.4	638	470	146	1254	+ 65.2	165.2
2.	100	337	121	558	124	354	109	587	+ 5.1	480	1200	121	1801	+222.7	322.7
3.	24	335	198	557	0	364	189	553	— .7	220	352	200	772	+ 38.6	138.6
4.	29	78	139	246	21	92	156	269	+ 9.3	300	325	100	725	+194.3	294.3
									Avg %					Avg %	
Mean	103	270	157	530	86	283	147	517	+ .3	410	587	142	1138	+130.2	230.2

Uro = Uroporphyrins, Copro = Coproporphyrins, Proto = Protoporphyrins. Each represent net amount synthesized expressed as μg per 100 ml of erythrocytes.

cation of the procedure of Schwartz and Wikoff(6). Uroporphyrin was determined by the method of Dresel and Tooth(7). The hemolyzed erythrocytes of 4 patients with iron deficiency anemia served as the sources of required enzymes. The erythrocytes of these individuals were assayed before and approximately 4 weeks after administration of ferrous sulfate in therapeutic doses. Prior to iron therapy the cells were assayed with and without addition of ionic iron to the incubation mixtures. The incubation mixtures contained 2 ml of lysed erythrocyte suspension which included ghosts, 1 ml of maleic acid buffer at pH 7.2 and 0.2 ml of 0.1 M δ aminolevulinic acid. Ferrous ion, when it was used, was added as ferrous sulfate in a concentration of 2×10^{-5} M. Control tubes were identical except that they contained 0.2 ml of water in place of δ aminolevulinic acid. Incubations were allowed to proceed for 4 hours under aerobic conditions at 37°C, shaking in a water bath. Net synthesis of porphyrin was considered to be the difference in values between the experimental and control tubes. Porphyrin concentrations are expressed in micrograms per 100 ml of erythrocytes. Experiments were performed in duplicate and the results reported represent an average of the values for each measurement.

Results. As listed in Table I the total amount of porphyrin synthesized and the individual values for uroporphyrin, coproporphyrin and protoporphyrin were unchanged by addition of iron to the incubation mixtures. However, the erythrocytes from the same individuals 4 weeks after receiving iron therapy, were now much more active as stimulators of porphyrin synthesis. In the 4 patients the average increase in total porphyrin synthesized was +130.2% (of 230.2% of the original value). All of the increase was due to higher concentrations of uroporphyrin and coproporphyrin. In Table II are recorded the blood counts of the patients studied at the times porphyrin synthesis was measured. The hemoglobin values increased 2.8 g per cent on the average, the hematocrit 7% and the erythrocyte count about one half million cubic milliliter. The average mean corpuscular volume rose from 66.8 cubic

TABLE II. Blood Values of Patients at the Time Cells Were Studied.

	Before therapy				After 4 wk of iron therapy*			
Patient	Hb, g %	VPRC,† %	RBC $\times 10^6$	Retic, %	Hb, g %	VPRC, %	RBC $\times 10^6$	Retic, %
1	6.3	26	4.05	1.65	9.8	36	4.68	1.45
1	6.3	26	4.05	1.0	9.8	36	4.68	1.0
2	7.1	28	4.05	.6	8.4	30	4.88	2.3
3	6.0	24	3.35	.4	9.7	35	4.16	2.2
4	7.8	29	4.7	.6	9.7	36	4.6	.3
Mean	6.6	27	4.04	.65	9.4	34	4.56	1.45
Avg mean corpuscular volume			66.8 cu μ				74.1 cu μ	
Mean corpuscular hemoglobin concentration			24.4%				27.6%	

† VPRC = Volume of packed red cells.

micra to 74.1 cubic micra for the group and the mean corpuscular hemoglobin concentration rose from 24.4% to 27.6% (still in the microcytic hypochromic range).

Discussion. The details of the heme biosynthetic pathway involved in the conversion of porphobilinogen to the uroporphyrinogen isomers I and III are still obscure. At least 2 enzymes are concerned in this catalytic reaction, namely, porphobilinogen deaminase and uroporphyrin isomerase. The further conversion of uroporphyrinogens to the coproporphyrinogens I and III is influenced by uroporphyrinogen decarboxylase. All of these enzymes are cytoplasmic and persist to some degree in the mature erythrocyte. Coproporphyrinogen oxidase which expedites the conversion of copro III to protoporphyrin, and iron chelatase which enzymatically inserts iron into protoporphyrin to form heme are both associated with mitochondria.

The relative inadequacy of the iron deficient human erythrocyte as an enzyme source for *in vitro* synthesis of uroporphyrins and coproporphyrins(5) suggests that iron is concerned in heme synthesis as a step prior to its insertion into the protoporphyrin ring. Since the lifespan of iron deficient erythrocytes is markedly shortened(8), a youthful population of cells existed prior to iron administration. Therefore increased enzymic activity after therapy with iron cannot be attributed to the relative immaturity of the population of erythocytes tested. Iron may be involved by several possible mechanisms. For example, one or more of the enzymes, porphobilinogen deaminase, uroporphyrin isomerase and decarboxylase may require adequate concentrations of ionic iron as a cofactor for optimal function. The data presented make this explanation unlikely since the simple addition of ferrous ions did not augment porphyrin synthesis. These data are compatible with the alternative hypothesis that an adequate concentration of iron is necessary for enzyme induction within the intact cell. Enzyme induction generally implies protein synthesis, a process which no longer occurs in the mature erythrocyte. This favors the explanation that the increased activity resides in newly formed cells.

The post-treatment measurements were made at a time when the patients were still anemic and the average erythrocyte size and hemoglobin concentration were still low. It has been suggested(9,10) that iron may be transferred only to immature erythrocytes. Nevertheless the possibility remains that a small amount of iron may influence the mature cells sufficiently to activate latent capabilities. Therefore the available data suggest but do not establish with certainty that the augmented enzyme activity resides solely within erythrocytes newly formed under the stimulus of iron administration.

Conclusions. 1. The suboptimal support of porphyrin synthesis *in vitro*, found with iron deficient erythrocytes is not correctable by addition of inorganic ferrous ions *in vitro*. 2. Administration of iron to iron deficient

individuals restored the porphyrin synthetic capability of their erythrocytes.

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Effect of Litter Size Upon Milk Yield and Litter Weight Gains in Rats. (32363)

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In a series of studies from this laboratory, the effect of a number of hormones on milk secretion and removal has been reported. In these studies the litter size was reduced to 6. The milk yield was frequently increased significantly over the control groups; however, the mean litter weight in most experiments did not increase with increasing milk yield. In a study of this problem it was suggested that when milk secretion is intense the litters do not obtain all of the milk available due to the limited amount of oxytocin discharged and its short biological half-life. When 1 μ of oxytocin was injected at 6-hour intervals/day to enable the nursing young to obtain more of the available milk, the mean litter weight was increased 29 g, a highly significant increase of 13% (1).

In this and all previous studies, the number in the litter has been 6 pups. In an extension of the study to determine the relation between milk yield and litter growth, it seemed desirable to determine the effect of litter size. It was thought that such a study would contribute also to the problem of more complete milk removal when milk secretion was increased by one or more exogenous hormones.

Materials and methods. Lactating rats of the Sprague-Dawley-Rolfsmeyer strain were housed in individual cages, and fed Purina Lab Chow and water *ad libitum*. The animals were kept under standardized conditions of temperature and light (78 $\pm$ 1°F, 14 hours light and 10 hours darkness). On day 1 of lactation, the pups were adjusted in each litter from 2 to 12. The pups and mothers were weighed daily. On days 14, 16, 18 and 20 of lactation, milk yields were estimated from the increase of litter weights during a 30-minute nursing period following 10 hours of isolation from the mother. One USP unit of oxytocin‡ was injected subcutaneously into the dams immediately before nursing and a second unit after 15 minutes of nursing to aid in complete milk removal. On day 20 of lactation the dams were killed after nursing. Six posterior mammary glands were removed and DNA was determined by the Webb and Levy method (2). Ribonucleic acid (RNA) was determined by measuring the total nucleic acids (TNA) and obtaining RNA by the difference between TNA and

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