Iron Absorption by Bilaterally Nephrectomized Rats. (30239)

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The cause of anemia often observed in patients with chronic renal disease is not fully understood, but decreased erythropoiesis as a result of a lowering of circulating erythropoietin probably plays a very important role(1). The kidney is the only organ from which erythropoietin has been isolated with certainty(2). Destruction, removal or impairment of the functional capacity of the kidney or the site of production of this humoral agent may lead to a decrease in erythropoietin level and consequently erythropoiesis. The efficiency and absolute amount of iron absorption from the gastrointestinal tract is directly related to erythropoietic activity(3). Moreover, administration of erythropoietin also enhances iron absorption in experimental animals(4). If the kidneys are the major organs responsible for such regulatory mechanism, then iron absorption from the gastrointestinal tract should decrease following kidney removal in animals. In this paper, iron absorption by nephrectomized rats will be reported.

Experimental methods. Bilateral nephrectomy was performed on adult female rats, McCollum strain, sham operation on controls. Twenty-four hours after the operation, the experimental and control groups were each divided into 2 subgroups, one of which was maintained at normal atmospheric pressure, the other transferred to a chamber which was kept at about 50% atmospheric pressure. Twenty-four hours later, a solution of iron containing 20 μg of elemental iron in form of ferric chloride and 0.5 μc of Fe^{59} was administered *via* stomach tube. The animals were sacrificed 24 hours after iron feeding and radioactivity in the liver, kidneys, spleen and blood was measured in a well-type gamma scintillation counter.

In a second study the effect of decreased oxygen tension was studied by keeping animals in a small chamber provided with 8% oxygen and 92% nitrogen mixture. Four hours after adaptation, these animals were given a similar iron solution *via* stomach tube. Twelve hours later, they were sacrificed and the radioactivity in various tissues was measured. Comparable studies were also carried in hypophysectomized rats obtained from Charles River Breeding Co.

Results. The data in Table I show that under these experimental conditions nephrectomized animals absorbed less iron, as measured by the radioactivity in the blood, liver and spleen than sham-operated controls at both atmospheric and half-atmospheric pressure. At the lower pressure, significantly higher radioactivity was found in both nephrectomized animals and controls than at atmospheric pressure.

Similar results were obtained when comparisons were made between nephrectomized and sham-operated rats in air and under reduced oxygen tension at atmospheric pressure, as shown in Table II. Grossly, at least, parallel results (Table III) were obtained when the experiment involving reduced oxygen tension was repeated comparing hypophysectomized rats with controls. In both cases, iron absorption was apparently greater at lower oxygen tension, regardless of other treatment of the animals.

Discussion. There is much published evidence supporting the role of the kidneys on erythropoiesis. After bilateral nephrectomy, incorporation of Fe^{59} into red blood cells was markedly reduced(5), reticulocyte response to bleeding was lowered(6) and the increase of plasma iron turnover following administration of erythropoietin was abolished(7). The source of erythropoietin is believed to be kidney, not other tissues(8). Its production can be demonstrated in an *in vitro* system of perfused kidneys and can be increased by treatment with cobalt ion or by hypoxemia(9). Our finding of decrease in iron absorption following kidney removal again confirms the role of kidney in erythropoiesis.

TABLE I. Iron Absorption by Bilaterally Nephrectomized and Sham-Operated Rats Under Normal and Reduced Atmospheric Pressures.

Treatment	No. of rats	Radioactivity recovered (μg Fe) Blood (ml)	Liver	Spleen
Control N	6	.080 ± .011*	.542 ± .019	.066 ± .008
Nephrect N	5	.026 ± .003	.135 ± .007	.021 ± .005
Control R	6	.341 ± .015	.669 ± .127	.135 ± .021
Nephrect R	5	.097 ± .015	.527 ± .024	.047 ± .014

* Mean ± S.E. N = Normal atmospheric pressure. R = Reduced atmospheric pressure.

TABLE II. Iron Absorption by Bilaterally Nephrectomized and Sham-Operated Rats Under Reduced Oxygen Tension.

Treatment	No. of rats	Radioactivity recovered (μg Fe) Blood (ml)	Liver	Spleen
Control N	5	.060 ± .014	.476 ± .091	.054 ± .014
Nephrect N	7	.013 ± .003	.081 ± .019	.013 ± .004
Control G	3	.015 ± .011	.746 ± .132	.037 ± .006
Nephrect G	3	.057 ± .010	.503 ± .119	.029 ± .017

N = Normal atmospheric pressure. G = 8% oxygen and 92% nitrogen mixture.

TABLE III. Iron Absorption by Hypophysectomized Rats Under Reduced Oxygen Tension.

Treatment	No. of rats	Radioactivity recovered (μg Fe) Blood (ml)	Liver	Spleen	Kidneys
Control N	4	.026 ± .010	.140 ± .051	.061 ± .023	.027 ± .004
Control G	4	.399 ± .191	1.404 ± .269	.310 ± .017	.172 ± .055
Hypox N	3	.042 ± .013	.033 ± .009	.060 ± .032	.015 ± .003
Hypox G	3	.252 ± .010	.488 ± .049	.096 ± .013	.054 ± .005

N = Normal atmospheric pressure. G = 8% oxygen and 92% nitrogen mixture.

It is not clear whether the influence of kidney on iron absorption is an indirect effect related to the rate of erythropoiesis presumably mediated through the level of erythropoietin produced by the kidneys or it may also involve other mechanisms(10). It was reported that the effect of erythropoietin differing from hypoxia brought about no increase in iron absorption if the erythropoiesis was artificially suppressed(4). At least in man(11) and in rabbits(12) absence of kidneys will suppress erythropoiesis profoundly but will not completely abolish it nor the erythropoietic response to hypoxia. In our rats, bilateral nephrectomy resulted in a decrease in the efficiency of iron absorption but not its complete abolition. Furthermore, these animals can still respond to the reduced atmospheric pressure or oxygen tension and absorb more iron, presumably secondary to the increase of the rate of erythropoiesis under these conditions. Regardless of whether the influence of kidney on iron absorption is the direct effect or indirect effect secondary to the alterations in erythropoiesis, our data do seem to suggest that kidney is not the only organ influencing iron absorption.

The role of pituitary on erythropoiesis has been established and extensively reviewel(13, 14). Pituitary removal in rats can also decrease iron absorption(15). However, these hypophysectomized animals as shown in our data can still respond to reduced atmospheric pressure or oxygen tension and absorb more iron. In view of our failure to prepare hypophysectomized nephrectomized animals for iron absorption studies, it is not clear whether there is any interplay between pituitary and kidney on these processes or any compensating mechanism when one of them become defective.

Summary. Iron absorption as measured by tissue radioactivity is impaired in bilaterally nephrectomized rats. These animals still show

increased absorption, however, when subjected to reduced atmospheric pressure or oxygen tension, a situation very similar to that seen with hypophysectomized animals.

1. Kaye, M., J. Lab. and Clin. Med., 1958, v52, 83.
2. Osnes, S., Brit. Med. J., 1959, v2, 650.
3. Bothwell, T. H., Pirzio-Birli, G., Finch, C. A., J. Lab. Clin. Med., 1958, v51, 24.
4. Mendel, G. A., Blood, 1961, v18, 727.
5. Naets, J. P., J. Clin. Invest., 1960, v39, 102.
6. Reissmann, K. R., Nomura, T., Gunn, R. W., Brosius, F., Blood, 1960, v16, 1411.
7. Naets, J. P., Heuse, A. F., Nature, 1962, v195, 190.
8. Gordon, A. S., Physiol. Rev., 1959, v39, 1.
9. Fisher, J., Friederici, L., Acta Haemat., 1951, v66, 224.
10. Brown, E. B., Am. J. Clin. Nutr., 1963, v12, 205.
11. Nathan, D. G., Schupak, U., Stohlman, F., Merrill, J. P., J. Clin. Invest., 1964, v43, 2158.
12. Erslev, A. J., Acta Haemat., 1960, v23, 226.
13. Grant, W. C., Root, W. S., Physiol. Rev., 1952, v32, 449.
14. Gordon, A. S., Prog. Hormone Res., 1954, v10, 339.
15. Chow, B. F., Yeh, S. D. J., Eberspaecher, H., Endocrinol., 1963, v72, 871.

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Releasing Factor and Endogenous Vitamin B_{12}* (30240)

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The existence of a "releasing factor" capable of splitting vitamin B_{12} from its complex with intrinsic factor has been considered since the report by Ungley(1) who stated that a substance contained in the human small intestine could remove vit B_{12} from its complex with normal human gastric juice.

Cooper and Castle(2) found that rat intestinal extract contained "releasing factor" activity which split the bond between vit B_{12} and rat intrinsic factor and Doscherholmen(3) confirmed their findings. Herbert, Cooper, and Castle(4) questioned the physiologic importance of this vit B_{12} releasing factor when they were able to demonstrate releasing factor activity only in the proximal area of the rat's small intensine and not in the mid-ileum where vit B_{12} absorption takes place(5). It was later shown by Ellenbogen and Highley(6) that releasing factor activity was present in the mid-ileum as well as in the proximal area of the rat's small intestine.

A releasing factor was noted in various human organ extracts by Nyberg, Saarni, and Gräsbeck(7). Perlman, Guiffre, and Amrein (8) reported the displacement of radioactive vit B_{12} bound by mouse ascitic fluid or hog intrinsic factor by unbound non-radioactive vit B_{12}.

The experiments reported here were performed for the purpose of grossly identifying the substance(s) responsible for the release of vit B_{12} by rat intestinal extracts and extracts of various human organs. In a complex equilibrium system, endogenous vit B_{12} contained in rat intestinal extracts exchanges with intrinsic factor-bound radioactive vit B_{12}, as suggested by Donaldson and Katz (9). This mechanism appears to account for all the releasing activity exhibited by the mid-ileum and for a major portion, and perhaps all, contained in the proximal area, as well as the releasing effect shown by human organ extracts.

Materials and methods. Vitamin B_{12} labeled

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