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Direct evidence favouring the notion that erythropoietin alters iron transport across the isolated intestinal tract of the rat[1]

A. Gutnisky[2], E. Speziale, M.F. Gimeno[2] and A.L. Gimeno[2]

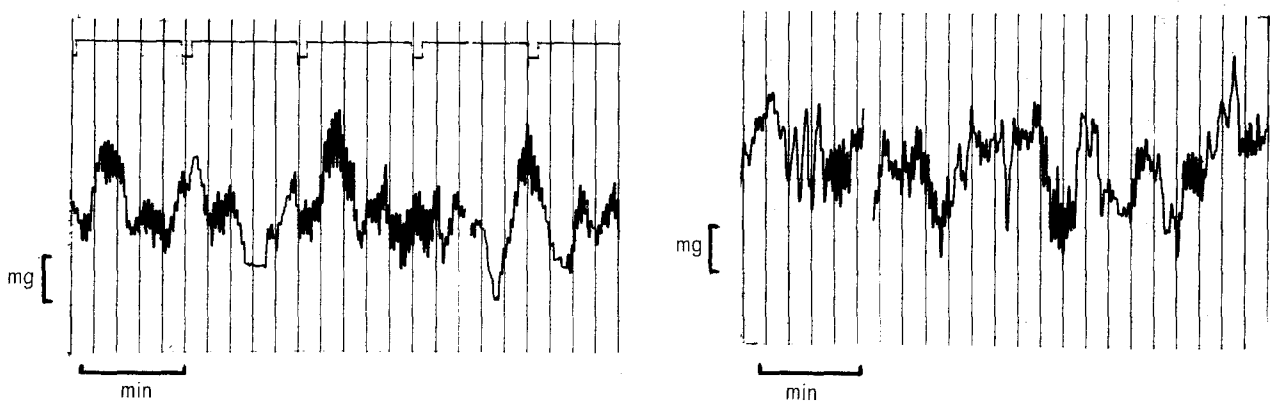
Centro de Estudios Farmacológicos y de Principios Naturales (CEFAPRIN), and Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Obligado 2490, Buenos Aires 1428 (Argentina), 2 August 1978

Summary. A simple and reproducible method, using the isolated not everted intestine of the rat, for the study of iron transport is presented. Erythropoietin (ESF) enhanced significantly the passage of ^{59}Fe across the intestine augmenting its movement at mucosal and serosal layers of the intestinal well.

A full understanding of the mechanisms subserving the control and regulation of intestinal iron absorption is still lacking. Numerous studies[3,4] have pointed out the relevance of several factors involved in the physiological process of iron absorption, namely: a) state of the intestinal mucosa; b) levels of iron at storage sites; c) quality of the ingested iron sources; d) chemical and pharmacological forms of iron preparations; e) concentration of transferrin and iron binding capacity; f) hormones, etc.

The prevailing concept holds that increased erythropoiesis enhances iron intestinal absorption[5] providing available mineral for hemoglobin synthesis. Furthermore tissue hypoxia increments erythropoietin (ESF = erythropoietin stimulating factor) which in turn regulates erythropoiesis[5,6]. However, the direct role, if any, of the ESF on the mechanisms of iron transport at intestinal level is not yet clearly documented. Therefore experiments were designed to ascertain the possible relevance of ESF on iron movement across the isolated rat intestine.

Methods. Male albino rats of the Wistar Strain, weighing 160±30 g, were used. After 24 h starvation, hemoglobin and hematocrit determinations were performed. Forthwith rats were sacrificed by cervical fracture; their intestinal tract removed and 6 cm of pilorous-jejunal segment was dissected in Petri dishes containing Krebs-Ringer-bicarbonate (KRB) solution kept at room temperature and gassed with a mixture of 95% O_2: 5% CO_2. The composition of the KRB medium has been reported elsewhere[8]. The piloric end was cannulated and firmly tied, whereas the jejunal one was also closed by a thread. Afterwards the preparations were transferred to a tissue chamber filled with 55 ml of KRB solution maintained at constant temperature (37 °C) and pH 7.4. One end of the isolated intestine was attached to a glass holder and the other to a force transducer coupled via en amplifier to a direct writing oscillograph. After a resting tension of 1000 mg was applied, the preparations were in a condition to be explored in terms of a) isometric developed tension and b) frequency of contractions. Following a period of stabilization, 1 µCi of ^{59}Fe (citrate salt) dissolved in 0.2 ml KRB and 0.2 ml of air, was introduced into the intestinal cavity via the catheter. Simultaneously erythropoietin (½ of the total concentration to be tested) was



Contractile activity of isolated intestine segments. Upper trace: Initial (postisolation) recording. Lower trace: Final (180 min) recording. Vertical and horizontal brackets: 100 mg and 1 min calibration, respectively.

In vitro effect of erythropoietin on iron transport by the intestinal tract of the rat

Experimental condition	Percent of ^{59}Fe uptake into the intestinal wall (per mg of dry intestine)*	Percent of ^{59}Fe uptake into the bath solution (per mg of dry intestine)*	Percent of ^{59}Fe uptake into the intestinal wall and the bath solution (per mg of dry intestine)*
Untreated controls	0.53±0.04 (19)	0.09±0.01 (19)	0.62±0.05 (19)
Erythropoietin (0.2 U/ml)	0.55±0.07 (7) NS	0.28±0.07 (7)**	0.83±0.10 (7) NS
Erythropoietin (1.8 U/ml)	0.55±0.05 (8) NS	0.24±0.07 (8)**	0.79±0.07 (8) NS
Erythropoietin (3.0 U/ml)	0.54±0.02 (7) NS	0.32±0.06 (7)**	0.86±0.06 (7)**
Erythropoietin (4.0 U/ml)	0.61±0.03 (13) NS	0.29±0.05 (13)**	0.90±0.05 (13)**

*Means ±SEM. Figures between parentheses are number of cases. NS, Not significantly different than controls. **Significantly different than controls ($p = 0.05$ or less).

delivered to the solution filling the tissue chamber (the other ½ was added at 90 min). 4 standards of the same ^{59}Fe citrate solution were then prepared to calculate the total radioactivity.

The preparations were followed during 180 min. In order to asses their functional conditions, records of contractile cycles were taken at regular intervals. Furthermore 0.2 ml of air was again introduced into the lumen at 60 and 120 min.

The ESF used was a crude extract (liophylized) obtained from urine of anemic patients due to hookworm. It was processed by the benzoic method[9,10]; its activity compared with the Standard B of the Biological Laboratory Standards of London and assayed with the post hypoxic mice method[11].

At the end of 180 min, the preparations were removed from the bath, untied and their lumen washed with KRB solution. Samples were collected in different fractions up to a point when all radioactivity had disappeared. At the same time, the radioactivity of aliquots of the KRB solution filling the bath was determined. Also, wet and dry weights of the whole tissue were examined. Forthwith the percent of ^{59}Fe in the intestinal wall, as well as the percent of ^{59}Fe in the KRB bath solution per mg of dry intestine were calculated. Results were compared with Student's t-test and their means considered significantly different if $p = 0.05$ or less. In some cases, tissue samples were obtained at different intervals and studied with conventional light microscopy techniques.

Results. The table summarizes the results obtained. As can be seen the uptake of ^{59}Fe into the intestinal wall of the control untreated group (exposed solely to 1 μCi of ^{59}Fe) was 0.53±0.04 per mg of tissue dry weight (mean±SEM; $n = 19$), whereas values in the KRB bathing solution were 0.09±0.01 per mg of dry intestine ($n = 19$). Delivering into the KRB solution, increasing concentrations of ESF elicited variable effects. None of them (0.2, 1.8, 3.0 or 4.0 units/ml) changed the control values of ^{59}Fe radioactivity in the intestinal wall. However counts were significantly higher in the KRB solution following 0.2, 1.8, 3.0 or 4.0 units/ml of ESF ($p < 0.05$, $p < 0.05$, $p < 0.01$ and $p < 0.005$, respectively). The total radioactivity (tissue plus KRB solution) was significantly greater after an exposure to 3.0 or 4.0 units/ml of ESF ($p = 0.02$ and $p < 0.005$, respectively) than in the control group.

It must be noticed that extracts from the urine of normal subjects failed to induce any of the previously described changes. The figure depicts records of the contractile activity of isolated intestine obtained at the beginning and at the end of experiments. As can be seen, there are no distinct functional differences during the whole experimental period.

Discussion. The isolated not everted intestinal preparation employed in the present study proved to be a simple method which preserves adequately histological and physiological characteristics of the tissue and provides reproductible results. Despite the fact that the thickness of the walls (1 mm or less) allows an appropiate oxygen diffusion and nutrition of the mucosa, extra care was taken by introducing a reduced amount of air into the lumen. Furthermore the procedure of isolation and mounting avoided superfluous handling which might unpair normal tissue conditions. In addition, the experiments were performed with intestinal segments subjected to an external resting tension. With all these precautions the tissue showed functional stability (as assessed by its unaltered contractions) and did not present light histological signs of deficient oxygenation.

Existing reports regarding a direct influence of erythropoietin in vivo on intestinal iron absorption are conflicting. Some favour the notion of a stimulating effect[12] while others are against it [13]. Such discrepancies might arise from the difficulties associated with the presence of many non-controllable variables.

ESF distinctly increases the passage of ^{59}Fe through the intestinal wall without a net final accumulation into its cells. Should a double control mechanisms exist, one at the level of mucosal lumen cells and the other at the serosal ones, the ESF appears to enhance both the influx and efflux the of ^{59}Fe. Although the normal circulating levels of ESF are unknown, the fact that the smallest concentration of the hormone employed was effective suggest the possibility of direct control of iron absorption even without the existence of an emergency condition such as that of iron deficiency, which is accompanied by higher levels of circulating ESF.

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