carried out on groups of 10 pupae confined in copper mesh exposed to the different treatments in 100-ml capacity exposure chambers. After treatment, the pupae were kept in the culture room until adult emergence was observed. Insects were considered dead when adults failed to emerge. Loss in weight was determined by weighing each group of pupae before and after treatments.

Respiration rates, as expressed by carbon dioxide output (4 replicates), were determined on groups of 50 insects weighed and exposed to treatments in 50-ml test chambers. Daily carbon dioxide output was determined by stopping the gas flow for 2 h and measuring the gas composition.

The results on respiration (figure) show that, contrary to the typical U-shaped curve obtained at normal atmospheric conditions (21% oxygen)[10], respiration curves obtained at 3% oxygen or corresponding tension at low pressures (130 and 48 mm Hg) demonstrate a suppressed respiration rate. The 1% oxygen curves, on the other hand, showed the tendency of decreasing carbon dioxide output which leads to insect death. Results obtained at normal atmospheric pressure and those obtained at the corresponding low oxygen tensions, at the low atmospheric pressures tested, were very similar.

This similarity in the response of the treated insects is also apparent in the curves of weight loss and mortality. Accordingly, 100% mortality was obtained at 1% oxygen (normal pressure) as well as in the corresponding oxygen tension at 61 mm Hg. However, at 48 and 130 mm Hg and 3% oxygen, this effect on mortality was not obtained. Weight loss curves, on the other hand, did not indicate a critical upset of water balance in insects. It may be concluded that the effects on mortality and respiration of insects was due to the low oxygen tension only, at both normal and low atmospheric pressure.

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Effects of insulin on plasma fibrinogen levels in rats submitted to tissue injury or ACTH administration

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Summary. Insulin is necessary to produce an increase of plasma fibrinogen in rats submitted to tissue injury or ACTH administration. This increase is more significant when endogenous or exogenous excess of insulin is present, while in uninjured rats the absence or excess of insulin does not modify plasma fibrinogen.

It is known that tissue injury produces an increase in plasma fibrinogen levels in the rat[1,2]. Also during the tissue injury the liberation of ACTH is increased. The administration of ACTH increases the fibrinogen[3] due to an extra-adrenal mechanism[4] and to an increment of hepatic synthesis[5,6]. ACTH also increases the pancreatic synthesis and liberation of insulin[7-10]. As insulin contributes to the improvement of the nitrogeo balance after surgical injury[11], and is also important in regulating the activity of protein metabolizing enzimes[12], it was of interest to study the role of insulin on the increment of fibrinogen following tissue injury or the administration of ACTH.

Material and methods. 205 rats of both sexes, weighing between 140 and 200 g each, were used. The experiments were performed in 48-h fasted rats. Uninjured rats were used as control. Tissue injury was performed by laparotomy[13] wiuh careful manipulation of liver and visceras. The modifications of plasma fibrinogen by absence of endogenous insulin were studied in alloxan-diabetic rats. Aqueous alloxan (Sigma) in doses of 150 mg/kg was administered by i.p. injection. 48 h after the injection of alloxan or when the rats had finished the period of fasting, they were submitted to laparotomy or drug injection. Crystaline (Lilly) insulin was administrated in doses of 0.4 IU/100 g s.c., twice daily. Soluble ACTH (Elea), 1 IU twice daily, was given s.c. in 0.9% ClNa solution. Tolbutamide (150 mg/kg daily) was administrated s.c. in saline solution. 96 h after laparatomy or administration of drugs, blood was extracted over a mixture of ammonium oxalate and potassium oxalate in a 2:1 proportion. The methods used to extract the blood and to determine the plasma fibrinogen have been described elsewhere[14]. Glucose levels were determined by o-toluidine meuhod[15-17]. Rats were considered diabetic when plasma glucose was over 300 mg/100 ml. As the rats injected with alloxan presented a significant hemoconcentration (with a mean hematocrit of 53.4%), and control rats had a mean hematocrit of 44%, all the results were corrected according to normal values of hematocrit by Boas and Peterman equation[18]. Statistical treatment was made by the t-test. Significant differences were taken as $p < 0.05$.

Results. Solvent of drugs (distilled water or 0.9% ClNa solution) does not modify fibrinogen, compared with intact rats.

The results obtained in groups of rats submitted to tissue injury (laparotomy) and rats injected with alloxan or insulin are presented in table 1. Laparotomy produces an increase of fibrinogen compared with control. This increase was not observed in laparotomized rats injected with alloxan (Lap+A), in which fibrinogen levels are similar to those observed in controls. On the other hand, rats injected with alloxan or with insulin did not present variation of fibrinogen in comparison with control rats or rats injected only with solvent. On the contrary, the laparotomized rats injected with alloxan and insulin (Lap+A+I) increase the fibrinogen to levels similar to those observed in laparotomized uninjected rats. In laparotomized rats injected with

insulin (Lap+I), the fibrinogen increases significantly compared with tl e laparotomized uninjected groups ($p < 0.05$).
The results obtained in groups of rats with ACTH, alloxan or insulin administration, are presented in table 2. The administration of ACTH produces a significant increase of fibrinogen compared with control rats. This increment is not observed in rats injected with ACTH and alloxan (ACTH+A). On the contrary, in animals injected with ACTH, alloxan and insulin (ACTH+A+I) the levels of fibrinogen were similar to those observed in rats injected with ACTH only. Rats injected with insulin and ACTH presented an increment of fibrinogen which is significantly greater than that observed in the groups of rats injected only with ACTH ($p < 0.05$).
In order to know whether the increase of the endogenous insulin produces the same effect in fibrinogen as exogenous insulin administration, a group of rats injected with tolbutamide was studied. This is a drug that stimulates the liberation of insulin by the pancreas[19,29]. The results obtained in animals injected with tolbutamide, alloxan or ACTH are presented in table 3. In rats injected only with tolbutamide (Tol) and in alloxan-tolbutamide rats, the fibrinogen is not modified. On the contrary, tolbutamide produces in laparotomized or ACTH injected rats an increase of fibrinogen significantly greater than that observed in the rats laparotomized (table 1) or injected with ACTH but withouu tolbutamide administration (table 2).

Table 1. Effects of injury (laparotomy) on plasma fibrinogen levels in rats injected with alloxan or insulin

	Number	Fibrinogen (mg%)	p
Intact rats (control)	8	206.7 ± 6.6	
Injury 72 h	17	347.2 ±20.7	<0.001
Alloxan	8	198.5 ±14.4	
Injury+alloxan	16	177.9 ±16.4	
Insulin	20	190.06± 9.93	
Injury+alloxan+insulin	28	311.6 ±17.8	<0.001
Injury+insulin	13	414.0 ±20.14	<0.001
Distilled water	10	222.8 ±16.2	
ClNa 0.9%	10	228.4 ±19.4	

Mean ± SE. p, Degree of significance resulting from comparison of the other groups with control; p-values are shown only when the difference is significant.

Table 2. Effects of ACTH on plasma fibrinogen levels in rats injected with alloxan or insulin

	Number	Fibrinogen (mg%)	p
Intact rats (control)	8	206.7 ± 6.6	
ACTH	9	297.9 ±25.7	<0.01
ACTH+alloxan	9	219.58± 15.6	
Alloxan	8	198.5 ±14.4	
Insulin	20	190.06± 9.93	
ACTH+alloxan+insulin	10	304.5 ±26.5	<0.001
ACTH+insulin	10	416.9 ±36.6	<0.001
Distilled water	13	222.8 ±16.2	
ClNa 0.9%	10	228.4 ±19.4	

Means ± SE. p, Degree of significance resulting from comparison of the other groups with control; p-values are shown only when the difference is significant.

Table 3. Effects of tolbutamide on plasma fibrinogen levels in injured, alloxan or ACTH treated rats

	Number	Fibrinogen (mg%)	p
Intact rats (control)	8	206.6 ± 6.6	
Tolbutamide	9	236.32± 18.04	
Tolbutamide+injury	8	437.43± 13.0	<0.001
Tolbutamide+ACTH	10	478.37± 23.02	<0.001
Tolbutamide+alloxan	10	217.0 ±16.6	
Distilled water	10	222.8 ±16.2	
ClNa 0.9%	9	228.4 ±19.4	

Mean ± SE. p, Degree of significance resulting from comparison of the other groups with control; p-values are shown only when the difference is significant.

Discussion. The results obtained confirm that the injury or administration of ACTH produces a significant increase of plasma fibrinogen. As this is not found either in Lap+A nor in ACTH+A rats, but is found in Lap+I+A and ACTH+I+A rats, we think that the presence of insulin is necessary to produce the increase of fibrinogen in rats with injury or administration of ACTH. This is in agreement with the fact that insulin increases the activity of both polysomes[21] and DNA in the liver[22]. The synthesis of hepatic proteins is decreased in alloxan-diabetic rats[23,24]. But the plasma fibrinogen in 96-h alloxan-diabetic rats is not modified (table 1). It is known that the fibrinogen synthesis is only decreased during the first hours following alloxan administration and it is normalized within the following 18 h[25]. It is probable that this last fact was due to a feedback mechanism which has been described for fibrinogen[26].
Exogenous insulin (that in normal rats does not modify the fibrinogen), in Lap+I or ACTH+I rats produces a significant increment compared with the group of laparotomized untreated rats or with the group of rats injected with ACTH. On the other hand, in rats with increment of endogenous insulin (Tol), fibrinogen increases significantly in comparison with groups of laparotomized untreated animals. These results would indicate that in the presence of greater amounts of endogenous or exogenous insulin, the liver would synthesize more fibrinogen in response to injury or ACTH administration.
Fluidity of blood depends on the balance between the process of fibrinogenesis and fibrinolysis. In cases where there is no stress, endogenous or exogenous insulin does not modify fibrinogen, but in conditions of tissue injury, increase of fibrinogen would be capable of producing tromboembolic process in rats or human beings susceptible to it. It is possible to speculate that ACTH, and perhaps other substances liberated during tissue injury, must increase insulin secretion by the pancreas to produce an increase of fibrinogen synthesis by the liver. This mechanism would be common to other plasma proteins, such as albumin, in which the increases of its synthesis in the stress is annulled in alloxan-diabetic rats and restored by insulin therapy[13].

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

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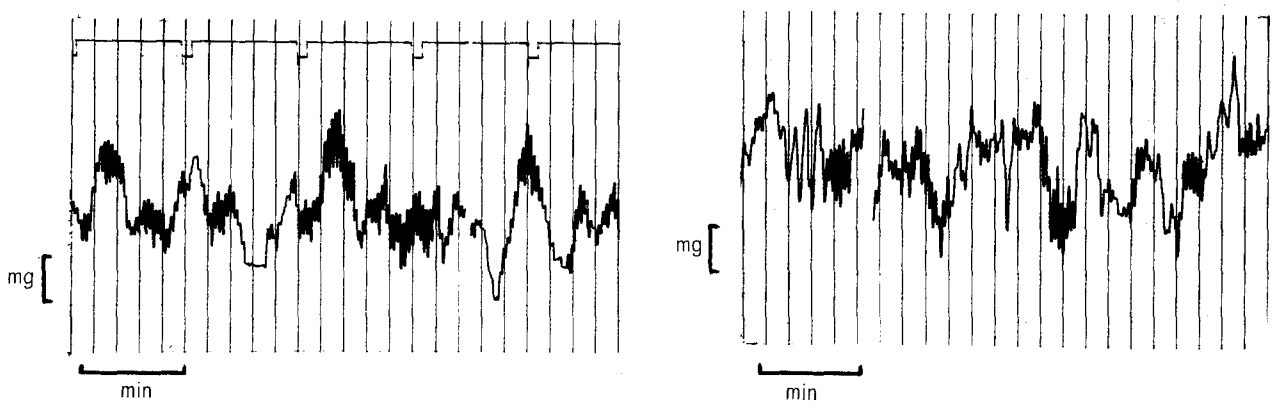
Summary. A simple and reproducible method, using the isolated not everted intestine of the rat, for the study of iron transport is presented. Erythropoietin (ESF) enhanced significantly the passage of ^{59}Fe across the intestine augmenting its movement at mucosal and serosal layers of the intestinal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