REVISTA ESPAÑOLA DE FISIOLOGIA, 39, 39-44. 1983

Intralipid and Free Plasmatic Tryptophan *in vitro*

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(Received on April 19, 1982)

J. M. PENA, C. AULESA, O. VIÑAS, J. BOSCH, M. FARRIOL and S. SCHWARTZ. *Intralipid and Free Plasmatic Tryptophan* in vitro. Rev esp. Fisiol., 39, 39-44. 1983.

In an attempt to investigate the role of the lipidic emulsion Intralipid in the development of metabolic encephalopathy in a patient showing high free tryptophan levels, the relationship between lipidic emulsion and free tryptophan was examined in *in vitro* experiments.

The addition of intralipid to normal serum produces an immediate increase in non-esterified fatty acids and a parallel rise in free tryptophan. Moreover, when serum with intralipid is incubated at 37° C, the lipases release new non-esterified fatty acids and the free tryptophan increases proportionally.

The non-esterified fatty acid content of intralipid was found to be 12 ± 2 mEq $\times 1^{-1}$. An inverse correlation was seen between free tryptophan and different serum albumin concentrations.

It is concluded that intralipid causes an increase in free tryptophan levels. It is known that *in vivo* free tryptophan modulates 5-hydroxytryptamine synthesis and thus may be considered a possible causal agent for encephalopathy.

Tryptophan, which in the blood is carried mainly by albumin, is at present considered a possible causal agent for metabolic encephalopathy. Free tryptophan enters the brain and influences 5-hydroxytryptamine turnover (9), while a competitive action is exerted by the branched chain amino acids (10).

In our hospital, a patient receiving parenteral nutrition with lipids developed metabolic coma. Laboratory determinations shoved low plasma branched chain amino acids and high free tryptophan values. After administration of the Fischer solution (7), the patient recovered from the coma and amino acid values normalized. Our observations in this case, together with the fact that an inverse Fischer relation is frequently seen during the first week after surgical intervention, prompted us to study the possible effect of lipidic emulsion on the free serum tryptophan.

CURZON *et al.* (2-4, 8) have clearly es-

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tablished that high non-esterified fatty acids (NEFA) values produce a rise in free tryptophan. The rise of cerebral tryptophan causes an increase in 5-hydroxytryptamine (5-HT) synthesis, since the limiting enzyme, tryptophan hydroxylase, is usually not saturated by tryptophan (6). In addition, ANDREW *et al.* (1) found increased concentrations of NEFA and triglyceride in newborns receiving intralipid solution.

CURZON *et al.* (3) reported that in *in vitro* studies the addition of NEFA at physiologic concentration to human and rat plasma caused a manifest rise of free tryptophan. All these findings point to a probable effect of intralipid emulsion on albumin tryptophan binding. The present study was designed to determinate the effect of intralipid on free plasmatic tryptophan.

The *in vitro* addition of intralipid to human serum always produced an increase of free tryptophan, which could be explained by the NEFA contained in intralipid and the NEFA resulting from serum lipase activity.

Materials and Methods

The lipidic emulsion used was 20 % intralipid (Vitrum Laboratories). Tryptophan of fluorimetric grade was obtained from Merck. Acyl CoA synthetase, acyl CoA dehydrogenase and other reagents for the enzymatic determination of NEFA were obtained from WAKO. The emulsionated olive oil for the lipase activity determination came from Perkin Elmer, Coleman Instruments Division. Centriglo Membrane Cones Type CF25 (more than 95 % retention for molecules with above 25.000 molecular weight) were from Amicon and precoated Thin Layer Chromatography silica gel plates from Merck. All other reagents were of analytical grade.

The human sera samples were pools of deep frozen normal sera obtained from 12-hour fasting patients. The intralipid 20 % was stored at room temperature.

The fluorimetric Denckla method (5) was used for tryptophan determination. Free tryptophan was determined in previously ultrafiltrated samples.

The free fatty acids were evaluated with two methods: First, a TLC was done on silica gel plates. The extractions of the lipidic emulsion and normal serum were done with chloroform/ethanol (2:1, v/v), the eluent was petrol ether/ethyl ether/acetic acid (90:10:1, v:v:v) and the plate developer was a 10 % (w:v) ammonium phosphomolybdate ethanolic solution.

The second method was the ACS-ACOD method (Wako Pure Chemical Industries, Ltd). After developing of the colour the samples, the blanks and standards were filtered with Amicon Membrane Cones (Type CF 25) to eliminate the turbidity produced by the emulsion.

The serum lipase was inactivated by 1-hour incubation at 57° C. No lipase activity could be demonstrated by the Zinterhofer method (11).

Results

The determination of free tryptophan in different sera showed an inverse correlation between free tryptophan and albumin levels. In all cases, addition of intralipid 20 produced a progressive rise of free tryptophan; this effect was strongly patent in a sample low in albumin and high in bilirubin (fig. 1).

Intralipid effect on tryptophan binding. Dose-Effect: The addition of intralipid 20 to a pool of normal sera produced an increase in free tryptophan. The percentage of this increase is linearly related to increasingly higher doses of intralipid

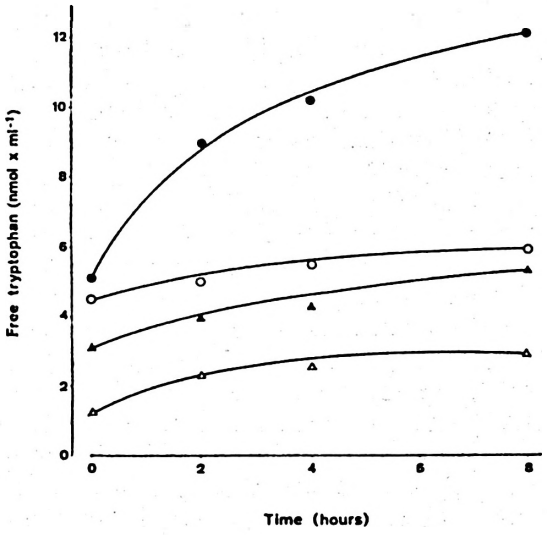

Fig. 1. *Free tryptophan, albumin and bilirubin relation in human sera.*
Effect of the addition of 50 μl intralipid/ml human sera in 4 pools. Free tryptophan levels are plotted versus incubation at 37° C. In all cases NEFA and total tryptophan were within normal range. Albumin (g × dl^{-1}) and bilirubin (mg × dl^{-1}) values for each pool: △ 4.2 and 0.9; ▲ 3.6 and 1.0; ○ 3.0 and 1.3; ● 2.7 and 5.0.

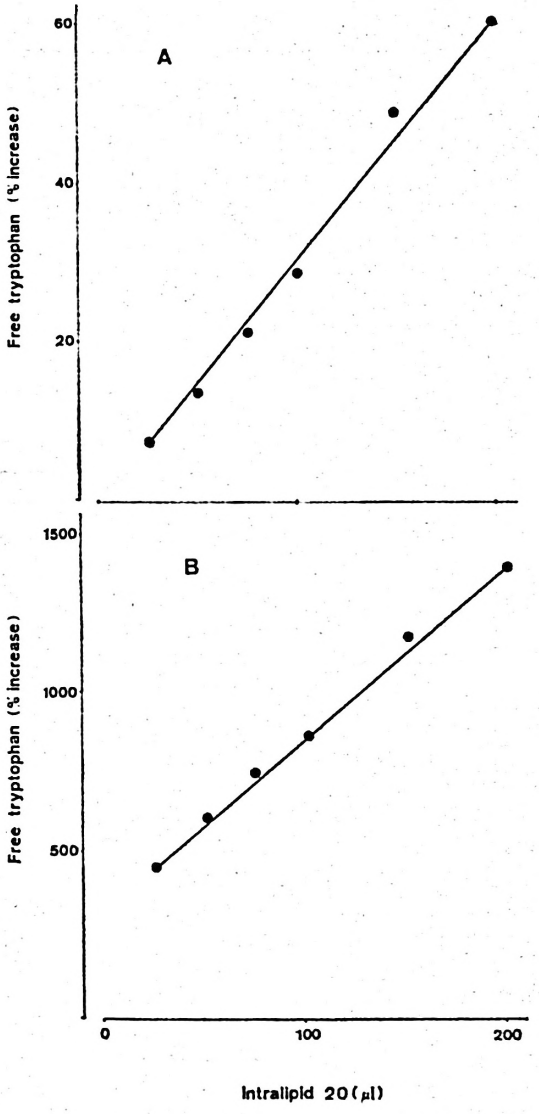

Fig. 2. *Dose-response to Intralipid addition in normal sera.*
Rise of free tryptophan is expressed as percentage of the increase from basal values. In 2A free tryptophan was measured immediately after lipid addition, in 2B after incubation at 37° C during 24 hours.

20 (fig. 2). Figure 2A shows the results obtained immediately after intralipid was added, while figure 2B shows that after 24-hour incubation at 37° C the effect was the same, but amplified 250 times. This demonstrates that the intralipid solution contains a compound which shifts the tryptophan-albumin binding immediately after the addition of intralipid (fig. 2A), while incubation at 37° C produces a substance which strongly amplifies this effect (fig. 2B).

Time-Course: The addition of intralipid 20 to normal serum (50 μl/ml), triggers a progressive release of free tryptophan from albumin (fig. 3). This time effect was already insinuated in figure 2. The findings expressed in figures 2 and 3 strongly suggest the possible presence of free fatty acids in the lipidic emulsion

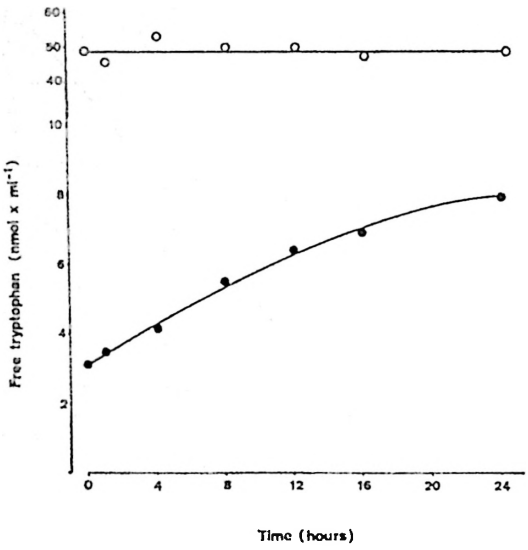

Fig. 3. *Time-course of the Intralipid effect after incubation at 37° C.*
Total tryptophan O and free tryptophan ●, assayed at indicated times.

as well as the probable metabolization of intralipid triglycerides and phospholipids by seric lipase, with the consequent interference between intralipid and the tryptophan-albumin binding.

Free fatty acids determination in intralipid 20. Classic thin layer chromatography was used to demonstrate the intralipid content of NEFA. The NEFA fraction of the intralipid emulsion was evident (fig. 4). Densitometric quantification gave 12.9 mEq $\times$ l^{-1}. Therefore, the addition of 50 μl intralipid per ml serum produces an increase of 0.6×10^{-3} mEq NEFA per ml serum.

Intralipid NEFA mediated effect on the free tryptophan level. The addition of 50 μl intralipid per ml of fresh sera pool resulted in an immediate rise in NEFA and free tryptophan (fig. 5); these values became still higher with time.

When intralipid was added to an aliquot from the same sera pool, but with previously inactivated lipase, a similar increase in NEFA and free tryptophan was seen. However, NEFA and free tryptophan values remained constant after the initial rise. Controls were performed simultaneously in sera lacking in intralipid, both with and without lipase inactivation. No immediate increase in values was seen in any of the controls, but a slight rise in NEFA and free tryptophan levels occurred subsequently in the control with active lipase, whilst values remained constant in the sample with inactivated lipase.

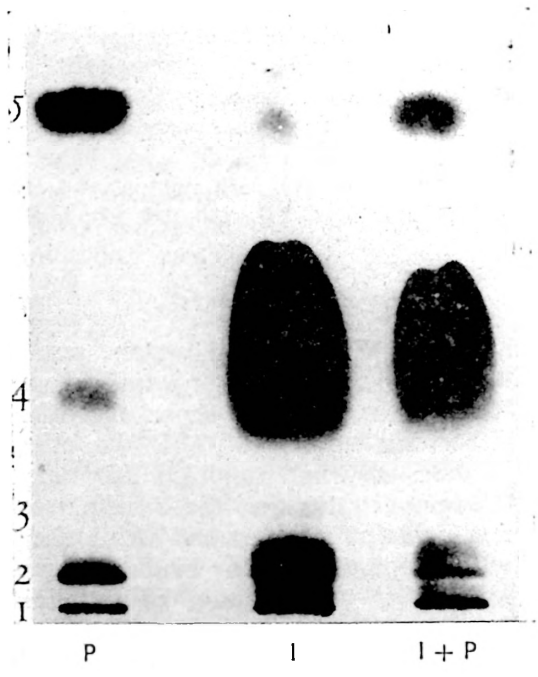

Fig. 4. *This layer chromatography of intralipid and human sera.*
Processed samples: Intralipid (I), human normal sera (P) intralipid/serum (1 : 1). Fractions: 1-mono and diglycerides, 2-free cholesterol, 3-NEFA, 4-triglycerides, 5-esterified cholesterol.

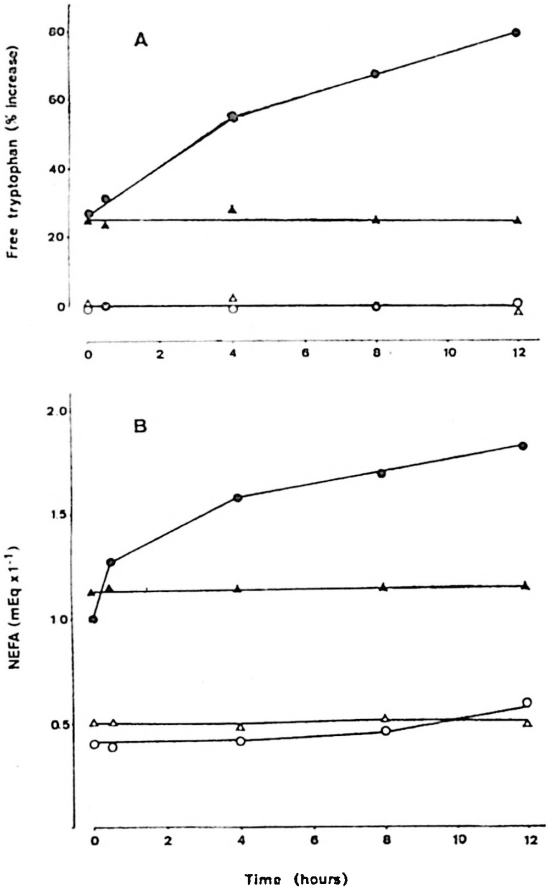

Fig. 5. *Free tryptophan and NEFA after intralipid addition to fresh and inactivated human sera.*
In A the percentual increase of free tryptophan is plotted versus time. In B, NEFA values versus time. After intralipid addition fresh ● and inactivated ▲ sera were incubated Controls without intralipid: fresh serum O and inactivated serum △.

Discussion

The present work, initially suggested by clinical data, was undertaken to study the possible effect of intralipid on the tryptophan-albumin binding. To simplify interpretation of the results, the study was done *in vitro*.

The results show that intralipid displaces tryptophan from the tryptophan-albumin binding, thereby causing an increase in free tryptophan concentration (fig. 2). The rise in free tryptophan immediately after intralipid addition suggests that intralipid contains NEFA. The NEFA content of intralipid was confirmed and calculated and is expressed in figure 4.

However, after incubation at 37° C, a progressive increase in free tryptophan is observed which could be explained by the serum lipase action on intralipid (figures 2B and 3). It has been reported that a parallel increase in NEFA and free tryptophan is produced by the injection of heparin, which releases tissue lipase into the plasma (9), and that a total NEFA concentration of 2.0 mEq $\times$ 1^{-1} led to 50 % of the total tryptophan being free.

The present study confirms both the NEFA content of intralipid and the progressive release of NEFA by serum lipase, as well as the displacement of tryptophan from albumin by an increase in NEFA. It might be expected that intralipid has a similar effect *in vivo* as *in vitro;* thus the administration of intralipid would suppose an immediate increase in NEFA, which would persist during lipidic perfusion. These high NEFA levels would produce a parallel rise of free tryptophan, considered as a possible causal agent for metabolic encephalopathy. We found a correlation between hypo-albuminemia and high free tryptophan levels (fig. 1), when hyper-bilirubinemia is also present, this effect is even more pronounced.

Based on the foregoing evidence, the potential risk of free tryptophan must be taken into account whenever a parenteral nutrition with lipids is considered.

Further clinical studies are being done to confirm the possible role of intralipid in the appearance of metabolic encephalopathy in patients receiving paranteral nutrition with lipids.

Resumen

Se estudia *in vitro* la relación de la emulsión «Intralipid» y el triptófano libre sérico.

La adición de Intralipid a un suero normal produce un aumento inmediato en los ácidos grasos no esterificados y una elevación paralela en el nivel de triptófano libre. Además, cuando se incuba suero con Intralipid a 37° C, las lipasas liberan nuevos ácidos grasos no esterificados, incrementando el triptófano libre proporcionalmente.

El contenido de ácidos grasos no esterificados del Intralipid es de 12 ± 2 mEq $\times l^{-1}$. También se observa una correlación inversa entre el triptófano libre y la concentración de albúmina en suero. Se concluye que el Intralipid causa un aumento en los niveles de triptófano libre.

Es conocido que el triptófano libre *in vivo* no modula la síntesis de 5-hidroxitriptamina, por lo que puede ser considerado como posible agente causal de encefalopatía.

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