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# Transport of sulphate ion through intestine*

por

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It has been known for a long time that the sulphate ion is very little absorbed by the intestine and that this absorption is very slow. So slow, that an excess of sulphate ion in the digestive system gives aqueous retention to maintain the osmolarity.

It is an hydratable ion and takes on a large molecular volume. It is a strong electrolyte and has a double negative charge that inhibits its passage through a membrane of the same sign. Such a membrane is the intestine and hence, absorption by this path is difficult.

WILSON and STRAUS (10) in unpublished experiments, working with the intestine of the hamster, indicate that there is absorption of sulphate ion by the Jejunnum, Duodenum and the first part of the Ileum and furthermore that there is excretion by the latter against the concentration gradients.

The concentrations of sulphate ion in organic liquids and in the gastrointestinal tract are similar to these that are found in plasma (of 0.5 to 1 meq/l in the majority of animals). The passage of sulphate ion the intestinal liquids from the blood has been studied by EVERETT and SIMMONS (6). Normally a high sulphatemie leeds to a greater concentration of sulphate in the intestinal contents.

In our experiments, SANZ and ASTUDILLO (7) with radiosodium sulphate (S-35) have been able to prove that in the intestine of the animals studied is found the 1.6 of total sulphate ion in a few hours, the same as if it had been administered, orally or parenterally.

In our kinetic studies on the process of sulphoconjugation [ZUMEL, SANZ and ASTUDILLO (11)] in various organs we stated that this process is intense in the intestine and that the change of the S-35 activity in total sulphate in the intestine gave a curve with two biological periods, one of t= 7 hours, the other of t= 1.4 hours. Subsequently, ASTUDILLO, ESPLIGUERO and SANZ (2) published a work

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on intestinal absorption where they relate the process of sulphoconjugation with the absorption of sulphate by the intestine. DEYRUT (5) in studies *in vitro* indicates that the accumulation and transport of sulphate ion in the Ileum is not dependant on its own external contribution, and that these processes can be inhibited by 2-4 DNP, CN', and F' in the presence of sodium.

In this paper we have made a kinetic study of the movements of sulphate ion through different parts of the intestine, the same in absorption as in excretion, working *in vivo* and *in vitro*, and studying in addition the type of transport of this ion through this polymembrane.

## Methods

a) *Absorptions methods.* We carried out the *in vitro* experiments according to with DARLINGTON (4) and CRANE-WILSONS (3) methods.

As medium a Krebs's solution is labelled with $S^{35}O_4Na_2$ and also we added

TABLE I

*Comparative kinetic studies of activity in inorganic and organic sulphates-$S^{35}$ through the intestinal membrane.*

| INTESTINE | Inter. Hours | ABSORPTION | | EXCRETION | |
|---|---|---|---|---|---|
| | | Inor. sulph. c/m/mg. org. | Org. sulph. c/m/mg. org. | Inor. sulph. c/m/mg. org. | Org. sulph. c/m/mg. org. |
| DUODENUM | 1/2 | 121,1 | 0 | 31,5 | 0 |
| | 1 | 242,7 | 0 | 54,7 | 0 |
| | 1 and 1/2 | 331,4 | 0 | 66,2 | 0 |
| | 2 | 472,6 | 0 | 86,5 | 0 |
| | 3 | 750,3 | 30 | 78,7 | 23 |
| | 4 | 582,1 | 414 | 95,2 | 28 |
| JEJUNUM | 1/2 | 85,5 | 43 | 34,7 | 10 |
| | 1 and 1/2 | 109,6 | 55 | 56,5 | 21 |
| | 1 | 178,7 | 63 | 85,3 | 0 |
| | 2 | 238,9 | 64 | 101,6 | 9 |
| | 3 | 398,9 | 198 | 134,9 | 27 |
| | 4 | 473,3 | 205 | 151,4 | 25 |
| ILEUM | 1/2 | 98,3 | 48 | 21,2 | 4 |
| | 1 | 145,5 | 66 | 38,6 | 7 |
| | 1 and 1/2 | 205,0 | 122 | 70,0 | 28 |
| | 2 | 280,4 | 154 | 74,8 | 37 |
| | 3 | 461,9 | 214 | 104,6 | 21 |
| | 4 | 635,9 | 232 | 164,1 | 32 |
| COLON | 1/2 | 14,3 | 0 | 15,8 | 1 |
| | 1 | 22,8 | 0 | 28,0 | 14 |
| | 1 and 1/2 | 34,0 | 0 | 34,6 | 16 |
| | 2 | 43,9 | 0 | 51,3 | 28 |
| | 3 | 43,5 | 0 | 112,5 | 0 |
| | 4 | 53,6 | 18 | 117,3 | 31 |

$SO_4Na_2$ without $S^{35}$ in either one or both compartments to change the concentration gradients. Different parts of the rabbit intestine were used in the experiments: Duodenum, Jejunum, Ileum and Colon. It was taken as Duodenum the first 8 cm from the pylorus, as Jejunum the following 8 cm, and the same lengths taken of Ileum and Colon from well anatomically defined parts.

When we used the Crane-Wilson method, the intestine is placed in a normal or everted form and the samples are always collected from the inner compartment.

The *in vivo* experiments ware performed according to AKEDO'S method (1) ussing ileum of rabbit which had been anaesthetized with pentobarbital.

b) *Radioactive material and radiometric analysis* The $Na_2S^{35}O_4$ solution was added to the Ringer. The samples to be measured were precipitated as $Ba_4SO$ and dried before the determination of activity. Afterwards they were calcinated at 850° C and weighed to calculate the selfabsorption of the beta particles of $S^{35}$. The samples for organic sulphate determination were dialyzed and hidrolyzed before precipitation. The results of these analysis are expressed as c/m/mg of dry intestine.

c) *Kinetics*. The process is kinetically followed during four hours by taking samples at different times. In several cases we determine the transport coefficients according to SOLOMON equations (8) and in other cases we adjusted anali-

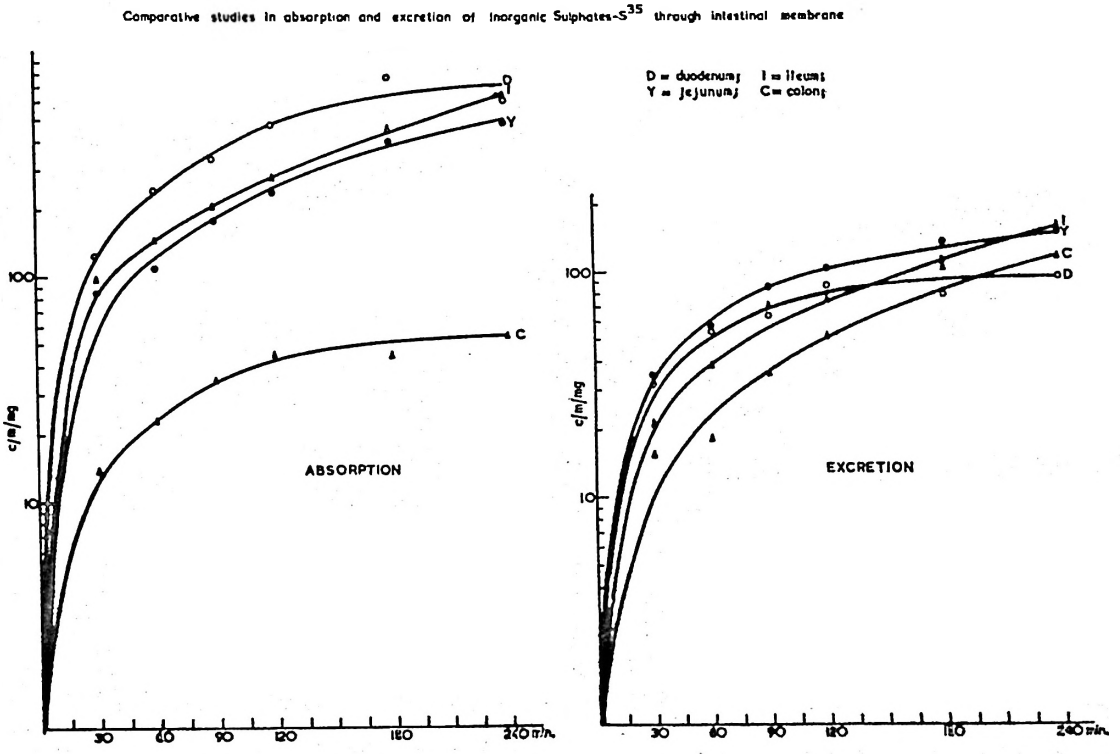


FIG. 1

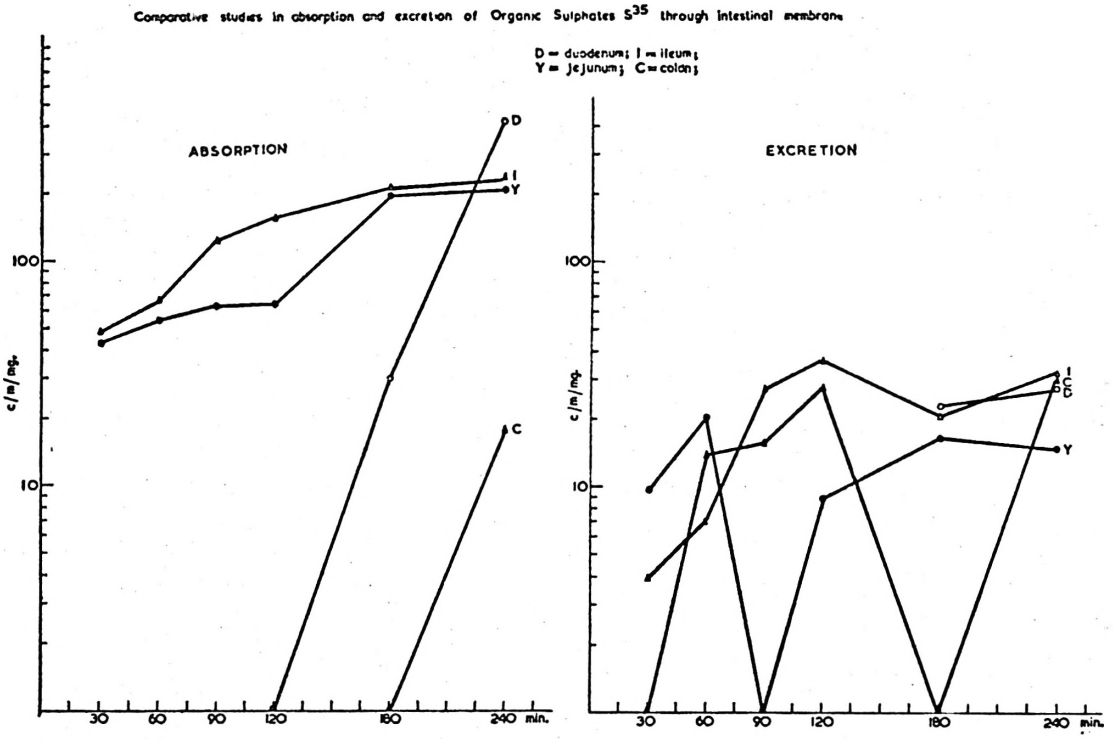


FIG. 2

tically to the experimental curves, the corresponding exponential equations and from them, calculated the time constants.

## Results

In Table I are summarized the results of the «in vitro» experiments. With these data Figs. 1 and 2 have been drown.

When the $S^{35}O_4Na_2$ is put in the mucosal compartment the activity of inorganic sulphates absorbed, follows this order: Duodenum-Ileum-Jejunum-Colon. That of organic sulphates is: Ileum-Jejunum-Duodenum-Colon.

When the S-35 is put in the serousal compartment the amount of inorganic sulphates excreted follows the order: Jejunum-Ileum-Duodenum-Colon, and the organic sulphates: Ileum-Colon-Duodenum-Jejunum.

We can see that in the Colon the excretion is greater than absorption and that the figures in Jejunum and Ileum are in general very similar.

If we consider the add of inorganic plus organic sulphates for both absorption and excretion we find the following order:

in absorption: Duodenum-Ileum-Jejunum-Colon.

and in excretion: Ileum-Jejunum-Colon-Duodenum.

If we consider now the ratio between organic and inorganic sulphates the order is:

| | | | | |
|---|---|---|---|---|
| in absorption | 0,70 | 0,43 | 0,36 | 0,34 |
| in excretion | 0,29 | 0,17 | 0,19 | 0,26 |

This figures seem to indicate that the zones where the sulphoconjugation is more intensive are Duodenum in absorp-

tion, and Duodenum and Colon in excretion.

The retention of sulphates by intestine is also different in Duodenum, Jejunum, Ileum and Colon. In absorption the Ileum and Jejunum retain the most activity and Duodenum in excretion (See Fig. 3).

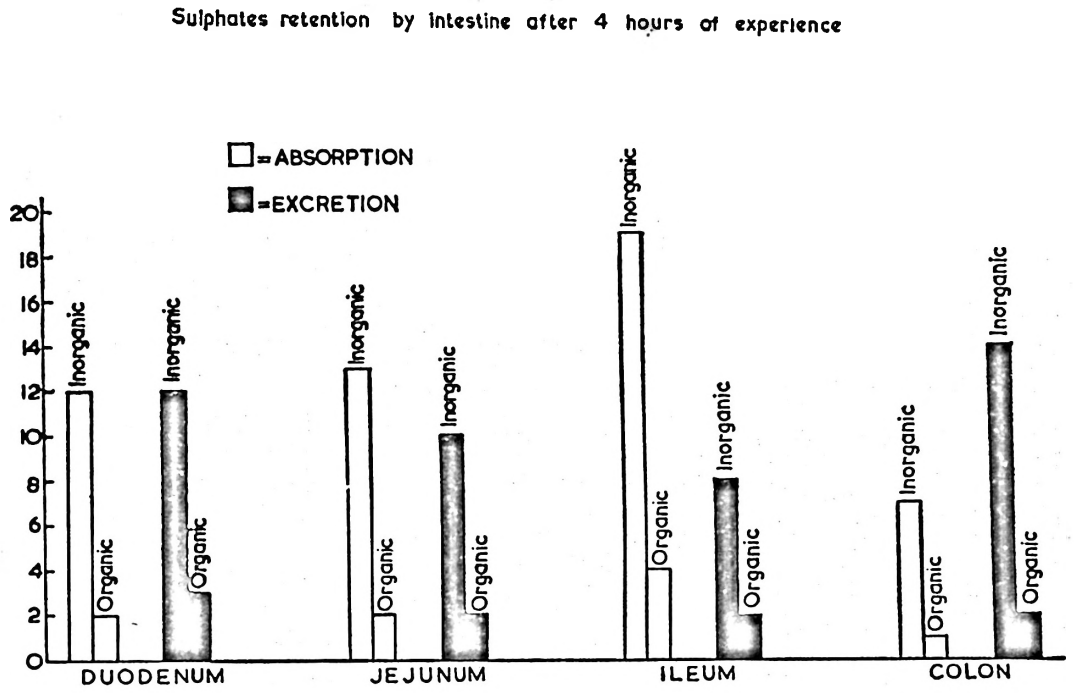


FIG. 3. Sulphates retention by intestine ofter 4 hours of experience.

*In the in vivo* experiments, see Table II, the intestine absorbs a great % of activity. The retention is 1.7 % in total sulphates of which 1.17 % are organic and only a 0.45 % are inorganic (Fig. 4 and 5).

TABLE II

*Radiactive Balance*

| | | |
|---|---|---|
| Initial Radiation . . . . . | 13.375.740 | c/m/90 ml/2000 gr. |
| Final Radiation . . . . . | 4.590.240 | c/m/ml. |
| Absorbed Radiation . . . . . | 8.785.500 | c/m/21 ml. |
| Radiation in extracellular volume . | 2.080.000 | 23,6 % |
| Retained Radiation by intestine . | 142.532 | 1,6 % |
| Intracellular Radiation . . . . . | 439.275 | 5,0 % |
| Controlled Radiation . . . . . | 2.661.807 | |
| Absorbed Radiation . . . . . | 8.785.500 | |
| Controlled Radiation . . . . . | 2.661.807 | 30,3 % |
| Eliminated Radiation . . . . . | 6.123.693 | 69,7 % |

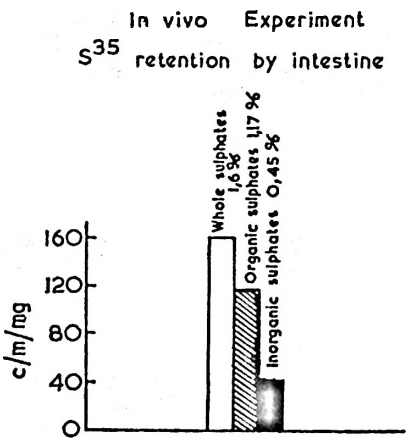


FIG. 4

The data of kinetic process, are summerized in the Table III and the curves correspond to the equation: $y = A\ (1 - e^{kt})$ whose time constants are for inorganic sulphates $K_1 = 0{,}40$, and for organic sulphates $K_0 = 1{,}45$.

TABLE III

*Inorganic and Organic Sulphates-$S^{35}$ in blood*

| Intervals min. | Inorganic sul. c/m/ml. | Organic sul. c/m/ml. |
|---|---|---|
| 30 | 3750 | 22 |
| 60 | 4633 | 50 |
| 90 | 4459 | 58 |
| 120 | 5396 | 55 |
| 150 | 5020 | 61 |

When we investigate the effect of several drugs on the intestinal absorption of sulphates, we can see that Hidrochlorothiazide and the Spyrinolactone do not modify the process, while that the 2-4 DNP and the F' modify it in opposite senses. (Fig. 6.)

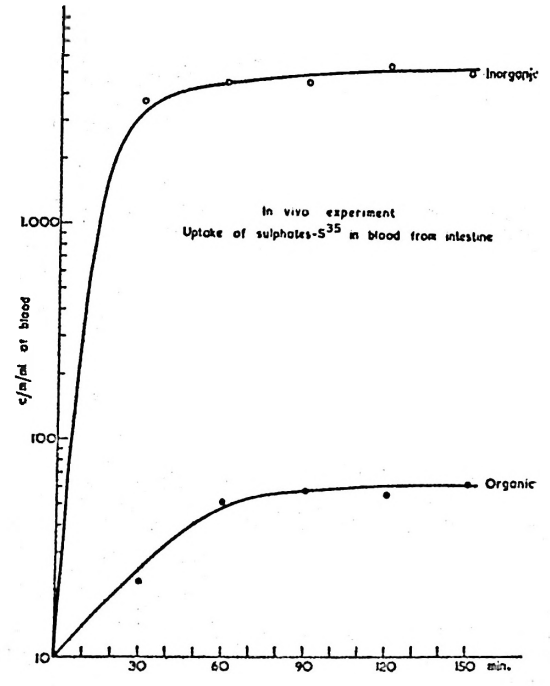


FIG. 5

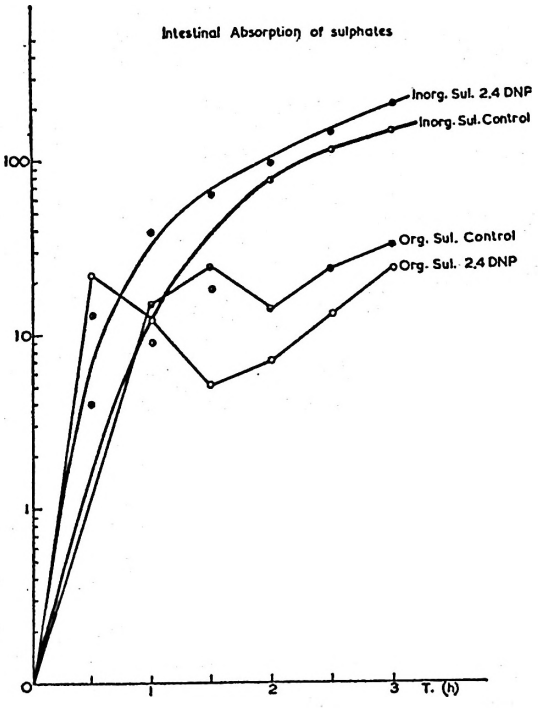


FIG. 6

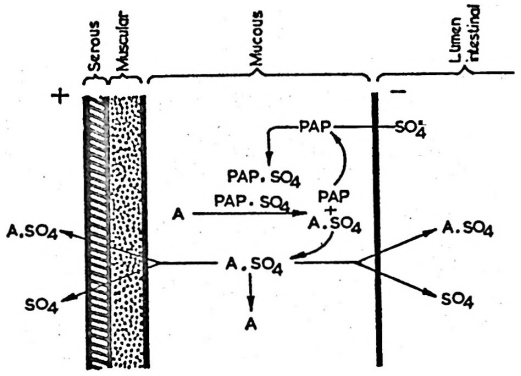


FIG. 7

## Discussion

The absorption and excretion of sulphates through the intestine is very little and slow. The intensity of values in absorption and excretion changes according to the different zones.

There is a process of the recrement of the sulphates and the activty of $S^{35}$ on the passage cis or trans is regulated by several factors.

The quantity of the organic sulphates in the trans step, in the intestinal wall permit us to think that the sulphoconjugations is a decisive factor in the sulphatic recrement.

The organic sulphates are weak electrolytes, they are in part ionizables, more lypo-solubles and hydrolizables, facts that dispose them for a better absorption by intestine.

When we have investigated the effect of several drugs which modify the membrane permeability, such as the Hidrochlorothiazide and some corticoids we have observed that they do not modify the absorption of sulphates; however, the inhibidor substances of the enzymatic process, as FNa and 2-4 DNP, modify this absorption at the concentrations we have used in these experiments (Fig. 6).

It is know that the sulphoconjugation is very intensive in the intestine and it seem be that the inorganic sulphate is the effective chemical form as substrate for the sulphoconjugation. In the intestine, there are divers polysaccharides acceptors of sulphate ion. (Fig. 7).

We could say that the absorption of sulphates can be in part arranged to the process of the sulphoconjugation for: 1) It realizes against of concentration gradients. 2) The activity/time curves representatives of kinetic process, became asintotic to the axis of the time, what indicates that the capacity of absorption has arrived to the saturation. 3) It can be modify by enzymatic inhibition.

## Resumen

Se ha estudiado el transporte del ion sulfato ($S^{35}$) a través de intestino de conejos y ratas (duodeno, yeyuno, ileon y colon ascendente) en las direcciones mucosal-serosal y serosal-mucosal, en experiencias «in vitro» e «in vivo». La cinética de absorción fue estudiada durante 2 y 3 horas y se tomaron muestras a intervalos de 15 minutos.

La intensidad del transporte se vio que seguía en ambas direcciones el siguiente orden: ileon-yeyuno-duodeno-colon.

En cada muestra se determinaron sulfatos totales, sulfatos inorgánicos y sulfoconjugados y el azufre total retenido por el intestino al final de la experiencia. Se calculó el influjo y el exoflujo y se deduce que el tipo de transporte es activo.

Se investigó el efecto de diuréticos, ADH y corticoides.

## Summary

The transport of $^{35}SO_4^{2-}$ was studied in rabbit and rat intestine (duodenum, jejunum, ileum, ascending colon), in the mucosal-serosal and serosal-mucosal directions, by Darlington and Wilson's method, «in vivo» and «in vitro».

The absorption kinetics were studied for 2-3 hours, taking samples at 15 min. intervals.

Transport intensity was seen in the order ileum-jejunum-duodenum-colon, in both directions.

Total sulphate, inorganic sulphate and sulphoconjugates were estimated in each sample, and the total sulphur still remaining in the intestine by the end of the experiment determined. Ionic influx and efflux were calculated. We conclude that an active transport mechanism is involved. Effects of diuretics, ADH, and corticoids were investigated.

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