therefore show that DNA synthesis can be inhibited in proliferating PHA-stimulated lymphocytes by short-term exposure *in vitro* to therapeutic concentrations of aspirin. Such inhibition would perforce be immunosuppressive in that depression of DNA synthesis would lead to reduction in proliferative response, and thus both ^{3}H-TdR incorporation and also mitosis would fall. This mechanism may explain the lowered growth rate *in vitro* of human embryonic cells caused by the aspirin metabolite, sodium salicylate (Paine & Nagington, 1971).

When DNA synthesis was inhibited by 5-fluorodeoxyuridine (Salzman, Pelegrino & Franceschini, 1966) and also when mitosis was reported to be affected directly by chloramphenicol (Pisciotta & DePrey, 1967; Nasjletti & Spencer, 1968), the degree and quality of morphological transformation of PHA-stimulated lymphocytes has been described as unchanged. Conversely, Caron (1967) expressed doubts about these results and found no blastoid cells when ^{3}H-TdR uptake was completely stopped *in vitro* by methotrexate. However, the degree of blastogenesis represents potential proliferation whereas incorporation of thymidine is a measure of active DNA synthesis and therefore of actual proliferation. These are two different phases of immunological response and may be affected independently by immuno-suppressive agents (Bradley & Elson, 1971). Failure to distinguish between the different phases of the proliferative response may lead to spurious results and may obscure the mode of action of some immunosuppressive and pharmacological agents.

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Enterohepatic circulation of sodium sulisatin* and its effects on glucose absorption in the rat

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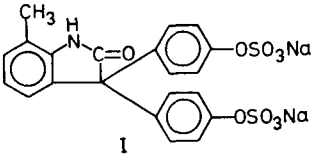
Synthetic laxatives with two phenolic groups either free or esterified with acetate or sulphate may undergo biliary elimination (Ferlemann & Vogt, 1965; Vogt, Schmidt & Dakhill, 1965; Fingl, 1975; Smith, 1966; Jauch, Hankwitz & others, 1975). We have studied the enterohepatic circulation of sodium sulisatin [disodium salt of sulphuric diester of 3,3-bis-(4-hydroxyphenyl)-7-methyl-2-indolinone, DAN-603, I], a new laxative whose activity and pharmacological properties have been reported recently (Garrido, Ibáñez & others, 1975; Moretó, Goñalons & others, 1976).



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The experiments were carried out with sodium [^{14}C]-sulisatin, labelled in the phenolic groups, with a specific activity of 0·9 μCi mg^{-1}. We first studied the metabolites present in portal blood plasma and bile of rats administered with sulisatin in the small intestine. Male albino Sprague-Dawley rats (8) (300–350 g), fasted for 24 h, were anaesthetized with urethane (1·25 g kg^{-1} subcutaneously). A midline abdominal incision was made and the common bile duct and the portal vein were cannulated. Sodium [^{14}C]sulisatin (11·1 mg kg^{-1} dissolved in water at a concentration of 1·11 mg ml^{-1}) was injected into the small intestine (4 rats) or into the large intestine (4 rats) isolated by double ligatures. The collection of bile and portal blood began immediately after the administration while preheated (37°) rat plasma was perfused through the external jugular vein, for 20 min. Examination of portal blood plasma by thin-layer chromatography on silica gel plates (G60 Merck), in several solvent systems, showed three spots corresponding in R_F value to the three standards (sulisatin, monophenolic derivative of sulisatin and the diphenolic derivative). 93·0 ± 0·5% (mean ± s.e.m.) of sulisatin, 4·5 ± 0·2% of the monophenolic derivative and 2·5 ± 0·4% of the diphenol were found in the portal plasma of rats administered in the small intestine and 84·9 ± 0·9% of sulisatin, 8·2 ± 0·8% of the monophenol and 6·9 ± 0·15% of the diphenol in rats that received sulisatin in the large intestine. The chromatographic analysis of the bile showed two spots, one corresponding to sulisatin (99·0–99·9%) and the other to the monophenolic derivative (0·1–1%). In all the chromatographic analyses spots were located by autoradiography (Curix RP 2, Afga-Gevaert). For quantification, the spots were scraped off, extracted with 80% ethanol (1 ml) and the radioactivity measured by liquid scintillation.

To establish whether sulisatin undergoes an enterohepatic circulation, bile was collected from 4 anaesthetized rats (300 g, fasted for 24 h), previously administered with sulisatin (1·11 mg kg^{-1}, 10 ml kg^{-1}), two in the small and two in the large intestine. The specific activity of bile (190·100 d min^{-1} ml^{-1}) was determined by liquid scintillation. This bile was placed in the small intestine (2) and in the large intestine (2) of anaesthetized rats (300 g, fasted for 24 h) at a dose of 10 ml kg^{-1}. After administration the bile duct was cannulated and bile was collected for 7 h. The specific activity of the bile excreted by the rats given bile in the small intestine was 6718 ± 177 d min^{-1} ml^{-1} while that of bile from rats given bile in the large intestine was 8216 ± 1064 d min^{-1} ml^{-1}.

In another experiment, 12 Sprague-Dawley male rats (300–360 g, fasted for 24 h) anaesthetized with urethane, received sodium [^{14}C]sulisatin, at the same dose as before, in the small or the large intestine, isolated by double ligatures. Rats were maintained at 37°. Two h after the administration the bile duct was cannulated and bile was collected. Portal blood was withdrawn by portal vein puncture. Systemic blood was obtained by cardiac puncture. After centrifugation, plasma samples (200 mg) were digested, using 0·6 ml of NCS (Amersham), and then counted by liquid scintillation. Bile (100 mg) was counted without additional manipulation. Quenching was corrected using the external standard channels ratio method.

Our results show that sulisatin may be mainly absorbed as such and undergoes excretion in the bile. The higher percentage of the mono and diphenols derived from sulisatin found in the portal blood plasma, when the drug is placed in the large intestine, as opposed to the small intestine, indicates that the bacterial microflora of this intestinal segment may play a role in the hydrolysis of the sulphate ester bonds, as occurs with sodium picosulphate [disodium salt of sulphuric diester of bis-(*p*-hydroxyphenyl)-2-pyridyl-methane, Jauch & others, 1975]. Reconjugation of the mono and diphenols might however occur in the intestine as happens with some other drugs. The absence of the diphenolic derivative and the low percentage of the monophenol found in the bile when compared with the percentages found in the portal blood plasma, would appear to indicate that in the liver the detoxification process involved is the reconjugation of the phenolic groups with sulphate, in contrast to bis-(*p*-hydroxyphenyl)-2-pyridylmethane which is excreted in the bile as glucuronide (Jauch & others, 1975). Moreover, sulisatin exhibits an enterohepatic circulation. The biliary excretion of sulisatin is of like nature in either small or large intestinal injection of bile containing the drug. The radioactivity levels of bile (B), portal plasma (PP) and systemic plasma (SP) showed that the B/PP ratio and B/SP ratio were similar regardless of whether the drug had been introduced into the small or into the large intestine (Table 1). The high B/SP ratio allows us to include sulisatin in Class B of Brauer's classification (Brauer, 1959). This comprises compounds whose bile/blood ratios are greater than 1, usually from 10 to 1000, and are therefore excreted in the bile by some kind of active secretory process.

As phenolphthalein and oxyphenisatin [3,3-bis-(4-hydroxyphenyl)-2-indolinone] may inhibit glucose absorption in the intestine (Hart & McColl, 1967) we have also studied the effect of sodium sulisatin, as well as sodium picosulphate and sodium sulphatin [disodium salt of the sulphuric diester of 3,3-bis-(4-hydroxy-

Table 1. *Bile/portal plasma (B/PP) and bile/systemic plasma (B/SP) ratio of the total radioactivity measured 2 h after the injection of sodium* [^{14}C]*sulisatin into the small or large intestine.* Results are expressed as mean ± s.e.m.

Injection site	n	B/PP ratio	B/SP ratio
Small intestine	6	112·0 ± 24·1	467·7 ± 83·1
Large intestine	6	111·8 ± 26·1	433·7 ± 74·2

phenyl)-2-indolinone], on the glucose absorption rate in the small intestine of the rat. Thirty four male Sprague-Dawley rats (350–400 g), fasted for 24 h, and anaesthetized with urethane (1·25 g kg^{-1} subcutaneously), were used.

The test was divided in five consecutive 30 min periods according to the Sols & Ponz technique (1946). In each period, 10 ml of warm saline solution containing glucose at a concentration of 10 mg ml^{-1} were placed in the small intestine segment (1st, 2nd, 4th & 5th period). In the third period, the drugs were added at a concentration of 0·5 mg ml^{-1}. Final glucose determination (glucose oxidase method, Werner, Rey & Wielinger, 1970), showed that only oxyphenisatin was capable of inhibiting the glucose absorption with a 48·1 ± 4·0% of inhibition (mean ± s.e.m.) while the other laxatives were devoid of any action. In addition we observed that after the withdrawal of oxyphenisatin and repeated washing of the intestine, the absorption of glucose in the two subsequent 30 min periods remained blocked to the same extent. The permanent inhibitory effect observed for oxyphenisatin is similar to the effect produced by coumarins on galactose absorption in chick and rat small intestine (Ruano, Bolufer & others, 1975).

In another set of experiments we studied whether the inhibition of the intestinal glucose transport could be attributed to one or both phenolic groups by comparing the activity of the monoester sulphate derivative of oxyphenisatin with its diphenol (oxyphenisatin) and with its diester sulphate (sulfatin). The experiments were carried out in rats (200–250 g), as described above, but with cannulation of the whole of the small intestine and using only two 30 min periods, the first as control and the second with the compound under study. Results, summarized in Table 2, indicate that a single free phenolic group had no inhibitory effect on glucose transport regardless of the three concentrations tested. The lack of inhibitory activity of the sulphuric ester derivatives is in accordance with the results of Bianchetti & Gianchetti (1972) who demonstrated that diphenolic laxatives inhibit tyrosine absorption in the small intestine of rats and guinea-pigs, while the corresponding sulphuric esters of the same compounds did not share this property.

Table 2. *Glucose absorption from the rat small intestine* in vivo. The values of glucose absorption are expressed as mean ± s.e.m. Oxyphenisatin significantly inhibits glucose absorption (*$P < 0.001$). Treatment with monoester and diester sulphate of oxyphenisatin does not affect glucose absorption.

Treatment concn (M)	n	Glucose absorbed (mg cm^{-1} in 30 min) 1st period glucose 0·5 mg ml^{-1}	2nd period glucose 0·5 mg ml^{-1} + compound
Oxyphenisatin			
10^{-3}	8	0·56 ± 0·03	0·22 ± 0·01*
$3{\cdot}10^{-3}$	6	0·60 ± 0·04	0·20 ± 0·01*
Monoester sulphate of oxyphenisatin			
10^{-3}	6	0·40 ± 0·05	0·40 ± 0·06
$3{\cdot}10^{-3}$	6	0·57 ± 0·02	0·55 ± 0·03
$6{\cdot}10^{-3}$	3	0·66 ± 0·05	0·63 ± 0·04
Diester sulphate of oxyphenisatin (sulfatin)			
$3{\cdot}10^{-3}$	6	0·70 ± 0·05	0·73 ± 0·02
$6{\cdot}10^{-3}$	6	0·64 ± 0·03	0·62 ± 0·03

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