that in turn releases ATP into plasma from blood cells or vessel walls. In either case the appearance of ATP in the plasma can account for the reduction in platelet adhesiveness.

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Analytical and pharmacokinetic studies on the optic isomers of oxazepam succinate half-ester

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PREVIOUS studies have shown an accumulation of oxazepam in the brain of mice, but not in rats after diazepam administration.[1] This accumulation is probably related to a longer half-life of oxazepam in mice than in rats.[2,3] However, since oxazepam is currently available as a racemate, it was of interest to investigate the toxic and the anticonvulsant effects of the two optic isomers prepared as succinate half-esters (see Fig. 1).

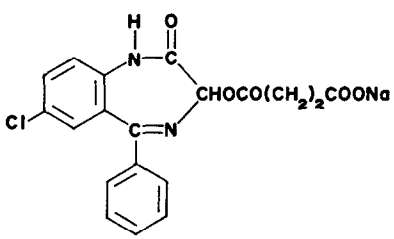

FIG. 1.

In addition the blood levels of oxazepam obtained after the administration of the two isomers or the racemic form of oxazepam succinate half-ester were determined.

Materials and methods

(1) *Animals.* Male Sprague–Dawley rats (body weight 200–250 g) and male albino Swiss mice (body weight 20 ± 2 g) fed *ad lib.* were used in all experiments.

(2) *Drug administration.* Oxazepam succinate half-ester (sodium salt) in the two optical isomeric forms (+), (−) and in the racemic form (±)* was administered both intravenously and orally, at a dose of 7·15 mg/kg (corresponding to 5 mg/kg of oxazepam) dissolved in a 0·006 M phosphate buffer at 1 pH 7·38. The LD_{50} was calculated in mice after i.v. administration, according to Lichtfield and Wilcoxon.[4] The anticonvulsant ED_{50} is calculated as the dose in mg/kg i.v. protecting 50 per cent of the mice from the mortality induced by metrazol (120 mg/kg i.p.). The preparation of blood extracts was made according to the method previously described.[5] Gas chromatograph Model G 1(Carlo Erba, Milan) equipped with a ^{63}Ni electron capture detector (voltage: 42V). The stationary phase was OV_{17} 3% on Gas Chrom Q (100–120 mesh) packed into a 2-m glass column (int. diam. 2 mm, ext.

* Kindly supplied by U. Ravizza, Muggiò, Milan.

diam. 4 mm). The flow rate of the carrier gas (nitrogen) was 33 ml/min and the column temperature was 245°. The electron capture detector sensitivity for oxazepam was 30 ng/ml of blood.

The oxazepam succinate half-ester as such is not measured in the employed method of oxazepam determination.

Results and discussion

Table 1 shows that the two optical isomeric forms of oxazepam succinate half-ester (sodium salt) exert a different anticonvulsant activity when given 30 min before metrazol. The ED_{50} of the (−) form is in fact about three times higher than that of the (+) form, while the ED_{50} of the racemic form (±) shows an intermediate value.

Results reported in Table 2 indicate that the LD_{50} of the two isomeric and racemic forms is comparable. Tables 3 and 4 summarize the levels of blood oxazepam obtained after the intravenous administration of the two isomers and the racemic form of oxazepam hemisuccinate half-ester (sodium salt). It is evident in both rats and mice that the (+) isomer, the most powerful anticonvulsant form, leads to the formation of higher or longer lasting levels of blood oxazepam than the (−) form. The racemic form shows somewhat an intermediate oxazepam blood level. However at certain times it is remarkable that the levels obtained with the racemic form are more close to the (−) than to the (+) form. It is evident, in agreement with previous findings[2] that the oxazepam formed from the succinate half-ester, independently from the isomeric form, shows a longer plasma half-life in mice than in rats.

TABLE 1. ANTIMETRAZOL ACTIVITY OF THE ISOMERIC FORMS OF OXAZEPAM SUCCINATE HALF-ESTER

Drug	Number of animals*	ED_{50} mg/kg i.v.†	Fiducial limits (95% confidence)
(+) form	60	0·770	(0·631–0·939)
(−) form	66	2·680	(2·061–3·484)
(±) form	60	1·290	(1·075–1·548)

* Swiss male mice (average weight 20 ± 2 g).
† Time between drug and metrazol (120 mg/kg i p.) was 30 min.

TABLE 2. TOXICITY OF THE ISOMERIC FORMS OF OXAZEPAM SUCCINATE HALF-ESTER

Drug	Number of animals*	LD_{50}(mg/kg i.p.)	Fiducial limits (95% confidence)
(+) form	72	498	(472–525)
(−) form	60	480	(421–547)
(±) form	60	490	(458–532)

* The LD_{50} was calculated 72 hr after drug administration to male Swiss mice (average weight 20 ± 2 g).

TABLE 3. RAT BLOOD LEVELS (μg/ml ± S.E.) OF OXAZEPAM AFTER i.v. ADMINISTRATION OF OXAZEPAM SUCCINATE HALF-ESTER (7·15 mg/kg)

Time after administration (min)	Administered drug form (+)	(−)	(±)
5	0·84 ± 0·03	0·24 ± 0·02	0·31 ± 0·03
30	0·31 ± 0·01	0·12 ± 0·01	0·14 ± 0·01
60	0·06 ± 0·007	0·08 ± 0·01	0·05 ± 0·005
180	< 0·03	< 0·03	< 0·03

TABLE 4. MOUSE BLOOD LEVELS (μg/ml ± S.E.) OF OXAZEPAM AFTER I.V. ADMINISTRATION OF OXAZEPAM SUCCINATE HALF-ESTER (7·15 mg/kg)

Time after administration (min)	Administered drug form (+)	(−)	(±)
5	1·15 ± 0·03	0·35 ± 0·02	0·40 ± 0·02
30	0·87 ± 0·02	0·18 ± 0·01	0·30 ± 0·01
60	0·60 ± 0·02	0·12 ± 0·008	0·24 ± 0·01
180	0·43 ± 0·015	0·08 ± 0·005	0·18 ± 0·02
300	0·34 ± 0·01	0·07 ± 0·004	0·12 ± 0·008

Data not reported here in details indicate that the difference between the two isomeric forms is not present when the oxazepam succinate half-esters are given by oral route to mice or to rats even if the animals were pretreated for 6 days with neomycin (1·5 g/kg) by oral route to minimize a possible hydrolytic activity of the intestinal flora.

The fact that there is a correlation between the anticonvulsant activity and the blood levels of oxazepam suggests that the succinate half-esters of oxazepam act by making available oxazepam. It remains to be established why the two optic isomers release different concentrations of oxazepam. As a working hypothesis it is suggested that a stereospecific esterase present in blood or in liver may be responsible for the difference observed.

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Effect of phenobarbitone on hepatic microsomal enzymes of the male rat*

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THERE have been contradictory reports of the effect of barbiturates on the activity of hepatic UDP-GT.[1–4]† This inconsistency may be due to the different species, substrates and enzymes preparations and incubations, as well as to the durations and doses of drug used. Small doses of phenobarbitone have been given to patients with hyperbilirubinaemia,[5] and the subsequent reduction of the plasma bilirubin levels may be due to an effect on UDP-GT. Doses in animals, however, have been much larger relative to body weight. In this paper we have compared in the male rat the effects of a dose

* A preliminary report of this work was presented at the meeting of the American association for the Study of the Liver, November, 1969.

† Abbreviations used: UDP-GT: UDP-glucuronate-glucuronyl transferase (EC 2.4.1.17).