other pathogenic or non-pathogenic slow or fast growing mycobacteria show a wide range and variety of sensitivity to drugs[14,15], the drug sensitivity of these *M. scrofulaceum* type strains seems to reflect better the therapeutic effect of drugs in leprosy.

The proposed in vitro model is inexpensive and permits the in vitro testing of unlimited number of compounds in a short period of time. The drug sensitivity profile of these cultures seems to provide a genetic marker similar to the drug sensitivity of *M. leprae* in the diseased man[6-9,16]. The results obtained illustrate the possibility that potent therapeutic agents in the fight against leprosy might be sought among drugs which have a molecular mechanism of antibiotic action similar to rifampicin, interacting directly with DNA-directed RNA polymerase. Drugs affecting cell wall synthesis such as cycloserine, or interfering with protein synthesis similar to streptomycin, as well as other antituberculous agents, have practically no therapeutic effect in leprosy. Drugs with insignificant bactericidal effect, such as DDS and others, should be considered for second choice only and this mainly for economic reasons. An in vitro technique is necessary for rapid and large scale screening of drugs having efficiency similar to that of rifampicin. Compounds selected with the proposed in vitro techniques might then be subject to testing in the in vivo foot pad model.

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Species-dependent stereospecific serum protein binding of the oral anticoagulant drug phenprocoumon[1]

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Summary. 13 mammalian species are classified into 3 clearcut groups with respect to the stereospecific serum protein-binding of phenprocoumon: 2 groups showing opposed stereospecific binding characteristics and a 3rd group exhibiting no stereospecific binding. Structural differences in the albumin molecule account for these stereospecific differences in serum protein-binding.

It has been shown that optical isomers of drugs can interact in a stereoselective manner with mammalian serum proteins[2-5]. There is also evidence that the relative proportion of the binding capacity of serum proteins for enantiomers may vary from one species to another[6]. The order of binding strength of serum proteins for the enantiomers, however, was similar in all species (e.g. the enantiomer which showed the strongest binding in one species also did so in the others).

We now report that with respect to the stereospecific serum protein-binding of phenprocoumon mammalian species can be classified into 3 clearcut groups: 2 groups showing opposed stereospecific binding characteristics and a 3rd group exhibiting no stereospecific binding.

Material and methods. Blood samples were obtained from a peripheral vein (man, monkey, horse, goat, dog), carotid artery (cow, pig, sheep, cat, rabbit) or abdominal aorta (rat, mouse, guinea-pig). The freshly drawn blood was permitted to clot, centrifuged, and the serum was removed. Human and bovine albumin (dried, purified, electrophoretic purity of 100%) was purchased from Behring-Werke AG, Marburg/Lahn, BRD, and rat albumin (fraction V powder) from Sigma Chemie GmbH, Munich, BRD. The enantiomers of phenprocoumon (S(-)phenprocoumon $-113.8°$, 3.8% in methanol; R(+) phenprocoumon $+114.0°$, 3.4% in methanol) were gifts from Hoffmann-La Roche, Basel, Switzerland.

Protein binding was determined in undiluted serum or pure albumin solution (3.45 g albumin per 100 ml phosphate buffer, 0.15 M, pH 7.4) by equilibrium dialysis using custom made plexiglas cells of 1 ml separated by a cellophane dialysis membrane (Union Carbide Corp., Chicago, Ill.). Serum or albumin solution was dialysed for 18 h at 37 °C against phosphate buffer (0.15 M, pH 7.4) containing the desired drug concentration. Only fresh serum (stored no longer than 8 h at 4 °C) was used. Drug concentrations were measured fluorimetrically after extraction with n-heptane[7].

Results and discussion. The table shows the binding of R(+) and S(−)phenprocoumon to blood serum of 13 mammalian species at a total drug concentration of $3 \cdot 10^{-5}$ M. It is evident that the species under investigation can be arranged into 3 clearcut groups with respect to the stereospecific binding of phenprocoumon. Whereas in the serum of rat, mouse, guinea-pig and rabbit, the fraction of unbound S(−)phenprocoumon was markedly higher than that of R(+)phenprocoumon, the opposite was true in the serum of man, monkey, cat, dog, pig and horse. The serum of bovine, goat and sheep exhibited apparently the same

Relationship between the free fraction values of R(+) and S(−) phenprocoumon in the serum of 13 mammalian species

R<S			R>S			R=S	
	R(+)	S(−)		R(+)	S(−)	R(+)	S(−)
Rat	1.06±0.01	1.43±0.04	Human	1.07±0.03	0.72±0.03	Bovine 0.66±0.01	0.66±0.01
Mouse	9.57±0.16	11.07±0.37	Monkey	0.51±0.02	0.36±0.03	Goat 0.43±0.02	0.43±0.02
Guinea-pig	1.68±0.01	3.05±0.06	Cat	2.29±0.05	1.75±0.05	Sheep 0.65±0.03	0.67±0.03
Rabbit	1.87±0.09	2.43±0.10	Dog	2.83±0.08	1.44±0.05		
			Pig	1.41±0.04	0.97±0.03		
			Horse	4.33±0.17	3.04±0.07		

*Values are the mean ± SD of the free fraction × 100. 4–6 determinations were performed at a total drug concentration of 8.4 µg/ml.

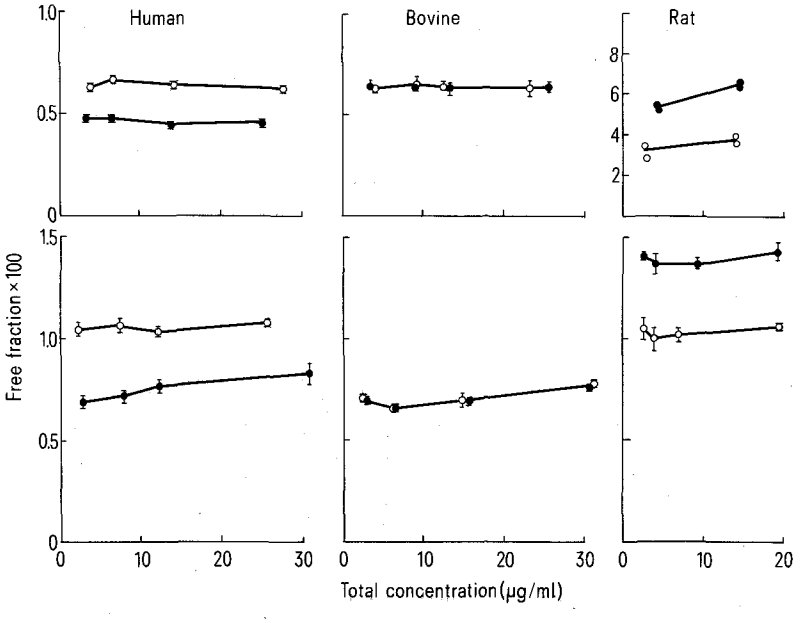
binding ability for both forms of phenprocoumon. The table also shows that there are considerable species differences in the degree of binding of serum proteins for phenprocoumon. This agrees with results obtained for the anticoagulant drug warfarin[8]. The stereospecific binding of phenprocoumon was investigated in greater detail using serum and albumin solutions of one representative species from each group (figure). This figure demonstrates that in the serum (lower part) of human, bovine and rat the free fraction of S(−) and R(+)phenprocoumon is almost independent of total drug concentration in a 20- to 30fold concentration range. Thus, stereospecific binding is also independent of total drug concentration in the designated concentration range. When the same experiment was performed using commercially available albumin from the 3 species, species-dependent stereospecific binding characteristics similar to those for blood serum were obtained (figure, upper part).

The results demonstrate intriguing species differences in the stereospecific protein binding of the enantiomers of the highly bound oral anticoagulant drug phenprocoumon. Until now, mammalian species can be classified into 3 groups with respect to the stereospecific binding of phenprocoumon: rat-like, bovine-like and human-like binding.

These stereospecific differences in serum protein binding are most likely related to certain structural differences in the albumin molecule, since a pure solution of human, bovine and rat albumin also exerts the same stereospecificity (figure). This could be demonstrated also for rat albumin, despite its much lower binding ability (figure). Differences in the preparation procedure of albumin (crystalline human and bovine albumin but fraction V rat albumin was used for the studies) probably account for this difference in binding ability.

Surprisingly, a species dependency, such as that shown for phenprocoumon, does not seem to exist for the structurally related drug warfarin. Although this drug also binds to rat and human serum in a stereospecific manner, the S(−)-form consistently shows a higher degree of binding[5]. This strongly supports the earlier finding that the mechanism of binding to human serum albumin is different for the warfarin and phenprocoumon enantiomers[9]. Our results are in accordance with studies on the pharmacokinetics of the enantiomers of phenprocoumon, in which stereospecific differences between man and rat in drug distribution were observed[7,10].

Despite the fact that the exact nature of the structural differences of the albumin molecule in the 3 groups of species responsible for the stereospecific binding is unknown, the results have several interesting implications. 1. They demonstrate that serum protein binding can be highly specific for certain drugs. 2. Stereospecific binding to 'silent' receptors (e.g. plasma proteins) does not always mimic the binding to the site of drug action, since in man and rat the S(−)enantiomer of phenprocoumon[7,10,11] and warfarin[12–16] is the more pharmacologically active component when compared with the R(+)enantiomer. 3. The easily measurable species-dependent stereospecific binding of phenprocoumon may be a useful guide in following the phylogenetic development of distinct structure(s) of the albumin molecule.



Free fraction values of S(−) (●) and R(+)-phenprocoumon (○) in serum (lower part) and albumin solution (upper part) of human, bovine and rat as a function of total drug concentration. Each symbol represents mean ± SD of 4–6 determinations. Human: serum was obtained repeatedly from 1 healthy male subject on 4 consecutive days, the total protein concentration was 7.2 g/100 ml and the albumin concentration was 4.5 g/100 ml. Bovine: serum was obtained from 1 cow, the total protein concentration was 7.8 g/100 ml and the albumin concentration was 4.0 g/100 ml. Rat: Serum taken from 12 male inbred Wistar-Lewis rats was pooled. The total protein concentration was 7.2 g/100 ml and the albumin concentration was 3.4 g/100 ml. Binding to albumin was studied at an albumin concentration of 3.45 g per 100 ml phosphate buffer.

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GABA-mimetic action of etomidate

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Summary. A comparison of antagonism by bicuculline or strychnine of the effects of GABA or etomidate on rat isolated superior cervical ganglia, frog isolated hemisected spinal cords and rat central neurones in vivo indicates that etomidate has GABA-mimetic actions.

Etomidate is a novel short acting, non-barbiturate hypnotic drug of high potency and low toxicity which is used in the induction of anaesthesia. Etomidate is of particular interest to the pharmacologist because it has 2 optical isomers, the (+)-form being considerably more potent than the (−)-form[2].

We have examined the effects of etomidate on rat neurones in vivo and also on the isolated superior cervical ganglion of the rat[3], the isolated hemisected spinal cord of the immature rat[4] and the isolated hemisected spinal cord of the frog[5]. In all preparations the effects of etomidate were depressed specifically by GABA antagonists, suggesting that this drug produces its central depressant action by mimicking the action of the inhibitory neurotransmitter.

Figure 1 shows the depolarizing effects of carbachol, GABA, etomidate and pentobarbitone recorded from the postganglionic nerve of the isolated superior cervical ganglion of the rat. Carbachol, GABA or (+) etomidate produced dose dependent depolarizations of the ganglion cells. Similar responses were recorded also from dorsal roots of the isolated immature rat spinal cord. Responses to (+) etomidate resembled those to pentobarbitone and were slower in onset and of longer duration than those to GABA. Responses produced by GABA, etomidate or pentobarbitone, but not carbachol, were antagonised by bicuculline. Responses to GABA or the 2 depressant drugs were unaffected by hexamethonium.

Figure 2 shows potentials recorded from dorsal and ventral roots of isolated frog spinal cords. GABA or (+) etomidate produced depolarization of primary afferent fibres and hyperpolarization of motoneurones. As with the superior cervical ganglion and rat spinal cord, the responses to etomidate were greatly prolonged in comparison to those of GABA. Bicuculline (100 μM) depressed responses to GABA or (+) etomidate and similar effects were observed with picrotoxin (50 μM). Strychnine has been shown to antagonize hyperpolarizations of frog motoneurones produced by β-alanine, taurine or glycine, but not those produced by GABA[6,7]. Figure 2, B shows that hyperpolarizations of frog motoneurones produced by (+) etomidate or GABA were unaffected by strychnine (1 μM), whereas responses to taurine were abolished. Each antagonist (bicuculline, picrotoxin or strychnine) was tested against reponses produced by etomidate and GABA or taurine on 2 preparations; complete recovery of frog spinal cord preparations from the antagonists was normally not sought since this took 12 h or longer.

Dose response plots for depolarization of frog primary afferent terminals produced by (+) or (−)etomidate and recorded from a dorsal root are shown in figure 2, C. The (+) isomer was 20 times more potent than the (−) isomer. Responses to the (−) isomer were antagonized also by bicuculline. These in vitro effects of (+) etomidate occur at concentrations similar to those measured in brain (5–30 μM) during behavioural and hypnotic effects[8].

(+) Etomidate, applied microiontophoretically to 67 glutamate excited neurones in the caudal medullary reticular formation (n. reticularis ventralis), produced depression of all of them. When the depressant actions of GABA, glycine and (+) etomidate were compared it was found that the mean currents necessary to produce the same level of depression were similar for applications of glycine or (+) etomidate whereas slightly higher currents were required for applications of GABA. On 13 neurones which were depressed by both GABA and (+) etomidate, iontophoretically applied bicuculline methobromide antagonized the action of GABA on all of these cells and that of (+) etomidate on 12 of them. An example of such an experiment is shown in figure 3. On 5 similar neurones strychnine was found to reverse the depressant action of glycine but not that of (+) etomidate.

Thus, both in vivo and in vitro experiments show that responses to etomidate are specifically antagonized by known GABA antagonists, which indicates that etomidate produces its central depressant effects by a GABA-mimetic action. Pentobarbitone has also been shown to have GABA-mimetic actions[9,10], and it is interesting that the relative GABA-mimetic potency of (+) etomidate and pentobarbitone, indicated in figure 2, approximates to the relative brain levels associated with the hypnotic effects of these 2 drugs[8,11].