The binding of salicylate to plasma proteins in different species

SIR,—In man, salicylate is extensively bound to serum proteins, and the extent of binding decreases with increasing salicylate concentration. Davison & Smith (1961), using an equilibrium dialysis technique, found that human albumin showed a greater affinity for salicylate at low concentrations than bovine albumin. Potter & Guy (1964) employed a dextran-gel filtration system and reported that dog plasma bound much less salicylate than human plasma. These results suggested that there may be some difference in the affinities between salicylate and plasma proteins in different species. We have therefore investigated the binding curves of salicylate and plasma from man, baboon, rhesus monkey, horse, dog, rabbit, guinea-pig, rat, mouse, turkey and the toad.

Blood samples were collected in lithium heparin bottles, centrifuged at 2000 *g* for 30 min and the separated plasma either used immediately or stored at −20°. Sodium salicylate was added to 0·5 ml aliquots of the plasma samples to give final concentrations up to 2 mM and the mixtures allowed to equilibrate at room temperature for at least 60 min. The protein-bound and free salicylate fractions (see Potter & Guy, 1964) were separated by passing 0·1 ml of each sample through a Sephadex G25 (fine beaded form) dextran gel and subsequently eluting with 2·8 and 5·0 ml quantities of 0·1M potassium phosphate buffer, pH 7·4. The salicylate in the separated fractions was determined with an Aminco-Bowman spectrophotofluorometer using an activating wavelength of 310 mμ and detecting fluorescence at 420 mμ, both values being the maxima for salicylate with the particular instrument employed.

The results showed that the various species differed in the extent to which salicylate was bound by their plasma proteins. The species could be separated into two main groups. The first, comprising baboon, horse, dog, rat, mouse, turkey and toad exhibited a low protein-binding capacity for the drug (Fig. 1). The second group (rhesus monkey, rabbit and guinea-pig) resembled man in

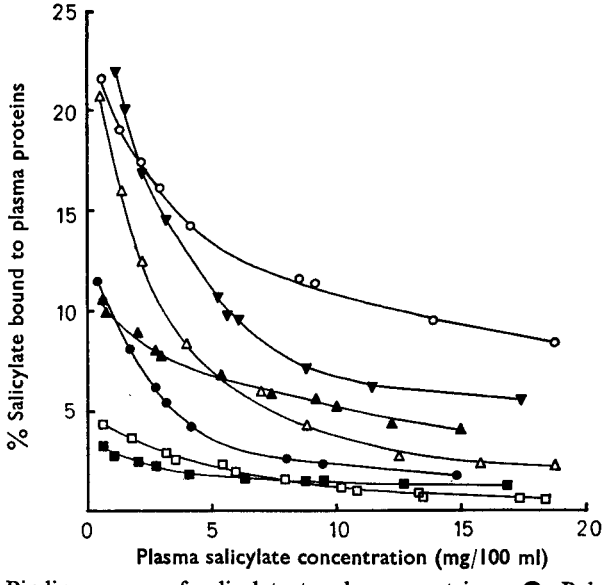

FIG. 1. Binding curves of salicylate to plasma proteins. ●, Baboon; ■, rat; ▲, mouse; ▼, horse; ○, dog; □, toad; △, turkey.

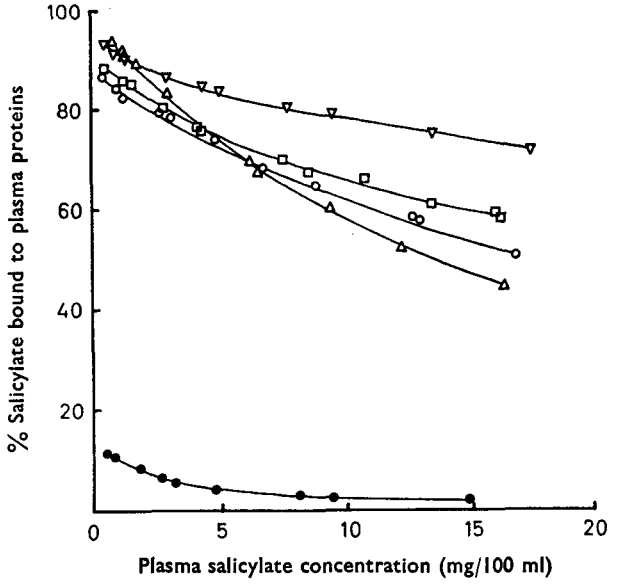

FIG. 2. Binding curves of salicylate to plasma proteins. ●, Baboon; ○, monkey; □, man; △, guinea-pig; ▽, rabbit.

possessing a much greater affinity for binding salicylate to the plasma protein (Fig. 2). The binding curve for the baboon, one of the first group, has been included in Fig. 2 to illustrate this point. No species giving intermediate values for protein binding of salicylate was found in the present work and there was an apparently clear differentiation between the two groups.

In human plasma, salicylate principally binds to the albumin fraction (Reynolds & Cluff, 1960) and there is some evidence that this involves the ε-amino- and possibly the guanidino-group of the protein (Davison & Smith, 1961). The present results show that the plasma proteins of some species are less capable of binding salicylate and this may be due to a relative deficiency of ε-amino- and guanidino-groups in the molecules of their albumin fractions.

The present work also suggests that greater proportions of free salicylate would occur in the circulation of the low-binding species after the administration of the drug. In these species there would be an increased entry of free salicylate into the cells from the blood and an increased rate of elimination of the drug from the circulation. Thus the species which show a low capacity to bind salicylate to their plasma proteins may be at higher risk, i.e. the LD50 values for salicylate would be lower, and may show lower values for the circulating half-life of salicylate than the species which possess a much greater affinity for binding salicylate to the plasma proteins.

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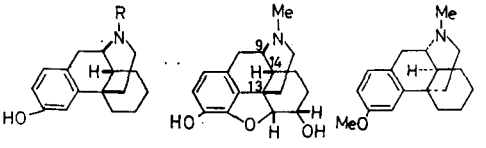
Steric correlation between (−)-3-hydroxy-*N*-methylmorphinan and (−)-morphine and related compounds

SIR,—The recently published investigation (Casy & Hassan, 1967) of the optical rotatory dispersion characteristics of (−)-3-hydroxy-*N*-methylmorphinan (levorphanol, I; R = Me) and (−)-morphine (II) gave strong evidence that the configuration of the C-9, 13 and 14 asymmetric centres of I are the same as those of the corresponding centres of II. These results are in agreement with previous conclusions (Beckett & Anderson, 1960) based on work involving stereoselective adsorbents.



I II III

May we draw your attention to the fact that the stereochemical problem discussed above has already been unambiguously solved by chemical degradation studies (Corrodi, Hellerbach & others, 1959). These findings proved further that the morphine antagonist (−)-3-hydroxy-*N*-allylmorphinan (levallorphan, I; R = $-CH_2-CH=CH_2$) has the same configuration as levorphanol (I; R = Me), while the cough-relieving compound (+)-3-methoxy-*N*-methylmorphinan (dextromethorphan) corresponds to the enantiomeric structure III.

In addition, the degradation experiments showed that the structural formulae I, II, III represent the absolute configurations, thus providing a more fundamental basis for understanding of the biological actions of these substances and the responses of their biological receptors.

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