through the hydrolysis of an hydroxyindanyl sulphuric acid intermediate. *trans*- 1-Hydroxyindanyl-2-sulphuric acid was identified as a metabolite of *cis*- and *trans*- indane-1,2-diol. The mechanism for the chemical hydrolysis of *trans*- 1-hydroxyindan-2-yl sulphuric acid appears to differ from the mechanism for the enzymic hydrolysis. Boiling aqueous solvents hydrolyses the individual hydroxyindanyl sulphates, to a mixture of the *cis*- and *trans*- dihydrodiols,[4] but the enzymic hydrolysis of *trans*-1-hydroxyindan-2-yl sulphuric acid produced only *trans*- indane-1,2-diol. It is possible that the chemical hydrolysis occurred through an epoxide intermediate since some 1,2-dihydroxy groups in sugars are converted to epoxides through treatment with aqueous reagents.[11] Indene epoxide is hydrolysed *in vivo* and *in vitro* to a mixture of *cis*- and *trans*- diols.[4] The enzymic hydrolysis of *trans*-1-hydroxyindan-2-yl sulphate was carried out over 12 hr at 37° and yielded only *trans*- indane-1,2-diol. As this reaction was at physiological temperature it is unlikely that *trans*- 1-hydroxyindan-2-yl sulphate was the intermediate in the conversion of *trans*- indane-1,2-diol to *cis*- indane-1,2-diol. The direct chemical interconversion of one distereoisomer to the other is unlikely since the chemical interconversion of the dihydrodiol forms requires elevated temperature and low pH.[1] The most likely route for the interconversion of *cis*- and *trans*- indane-1,2-diol is through 2-hydroxyindan-1-one as previously suggested.[4]

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Inhibition of rat liver homogenate arginase activity *in vitro* by the hepatotoxic amino acid indospicine

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IT HAS previously been reported that acute administration of the hepatotoxic amino acid indospicine (L-2-amino-6-amidino hexanoic acid) to rats depressed the incorporation of ^{14}C-leucine into liver and serum protein *in vivo*.[1] Subsequently it was shown, using cell-free reaction systems, that a competitive antagonism existed between indospicine and arginine such as to limit the incorporation of the latter into protein.[2] Further, because esterification of arginine to transfer RNA (*t*RNA) was inhibited in

the presence of indospicine, it was suggested that indospicine might be competing with arginine for the binding site on arginyl-*t*RNA synthetase.

These considerations indicated that the effect of indospicine on the activities of enzymes other than the synthetase, having arginine as a substrate, should be investigated. Arginase (L-arginine ureohydrolase; EC 3. 5. 3. 1) activity was chosen for study because of the importance of this enzyme in protein catabolism. Because the chemical synthesis of DL-indospicine was recently achieved,[3] it was also of interest to compare the effects of the synthetic and natural products in a biological test system.

Liver samples were obtained from female Sprague–Dawley rats (220–250 g) which were fed a complete pellet diet *ad lib.* till killing. The samples were homogenized in 9 vol. of ice-cold deionized water. After brief centrifugation to remove debris, the homogenates were diluted 25 times with deionized water. The standard assay medium contained in a final volume of 1·0 ml: L-arginine, brought to pH 9·5 with 1N NaOH, 6·25 mM; $MnCl_2$, 0·25 mM; glycine, pH 9·5, 25 mM; L- or DL-indospicine pH 9·5, where indicated; and diluted homogenate (equivalent to 0·5 mg wet liver) with which the reaction was initiated. Incubation was carried out at 38° for 10 min. The reaction was stopped by the addition of 3 ml of 0·5 M $HClO_4$. After centrifugation, the urea content of the sample supernatants was determined automatically (Technicon Auto-Analyzer) using the method described by the manufacturers of the instrument for blood urea nitrogen. Preliminary experiments showed that the urea content of zero-time samples was negligible. Also, the presence of indospicine was found not to influence the reaction of urea with the colour-forming reagents.

The effects of natural L-indospicine or synthetic DL-indospicine on liver homogenate arginase activity are shown in Table 1. It was found that activity was inhibited by indospicine from either

TABLE 1. EFFECT OF NATURAL L-INDOSPICINE OR SYNTHETIC DL-INDOSPICINE ON LIVER HOMOGENATE ARGINASE ACTIVITY*

Indospicine present (mM)	Arginase activity†	% of control value
none	2·60	
(L-indospicine)		
3·125	1·36	52
6·25	1·00	38
15·625	0·56	22
31·25	0·34	13
(DL-indospicine)		
6·25	1·22	47
12·5	0·88	34

*The standard assay procedure was used as described in the text, with indospicine added where shown.

†Enzyme activity is expressed as m-moles urea produced per g wet liver per 10 min at 38°.

source, and that the degree of inhibition was dependent upon the concentration of indospicine present. It is noteworthy that twice the concentration of the DL-form of the toxin was required to produce the same order of inhibition as the L-form. Since a similar finding occurred with regard to the inhibition by indospicine *in vitro* of ^{14}C-arginine incorporation into protein in cell-free systems,[4] it may be concluded that only the L-form of indospicine is active as an inhibitor.

Further experiments were carried out to elucidate the mode of action of L-indospicine on arginase activity. Urea formation was determined, using 0·625–5·0 mM arginine as substrate, in the absence and presence of 1·25 mM indospicine. A Lineweaver–Burk plot of a typical experiment is shown in Fig. 1. The point of intersection of the lines shows that indospicine is a competitive inhibitor of arginase. K_m and K_i values were calculated from the plot and the equations described in Dixon and Webb,[5] and were found to be 1·14 mM and 1·49 mM respectively. In a separate experiment plotted by the method of Dixon,[5] it was confirmed that the inhibition was competitive with a K_i value of 1·88 mM. Thus it is concluded that indospicine competes with arginine for the active centre of arginase and that

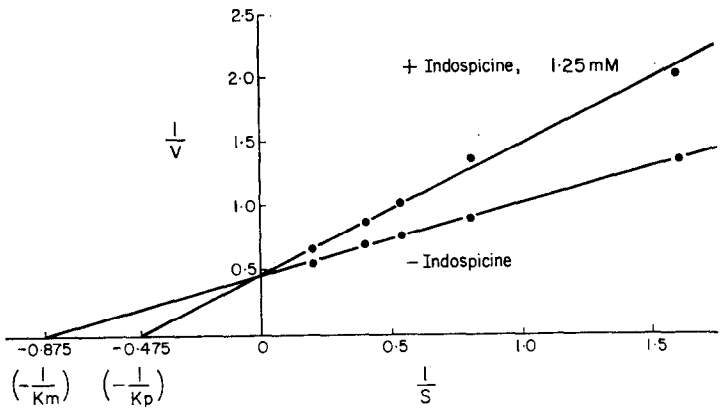

FIG. 1. Lineweaver–Burk plot of the inhibition of arginase activity by L-indospicine. *s* refers to the mM concentration of the substrate L-arginine. *v* is expressed as m-moles urea produced per g wet liver per 10 min at 38°. The standard assay procedure described in the text was used with concentration variations where shown.

the affinity of arginase for indospicine is of an order approaching that of the enzyme for arginine.

The competitive inhibition of arginase by indospicine suggests that other enzymes involved in arginine metabolism may be similarly affected by the toxin. The finding of the inhibitory effect of indospicine on arginyl-*t*RNA formation[2] would be compatible with such an action. The recent report of a procedure for the purification of rat liver arginyl-*t*RNA synthetase[6] will enable this postulate to be fully tested. Another implication is that, if arginase activity is also inhibited by indospicine *in vivo*, there may be a disturbance in ammonia metabolism in animals treated with the toxin. Studies are in progress to determine whether such an effect occurs. The fact that, with respect to arginase, the K_i value for indospicine is of the same order as the K_m value for arginine may at least partially explain why a relatively large dose of indospicine is required to induce toxic changes *in vivo*, as discussed in the preliminary paper.[1]

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