faeces and intestinal contents of rats of series 2 consisted mostly of the corresponding aglycones, but small amounts (< 8%) of the glycosides were also detected.
These results indicate that a significant level of reabsorption of [^{14}C] labelled hydroxyethylrutosides occurs after biliary excretion. It is not possible, on the evidence obtained, to state whether metabolic hydrolysis of the hydroxyethulrutoside glucuronides by the intestinal microflora precedes reabsorption although this appears probable. Evidence that hydroxyethylrutoside glucuronides are susceptible to bacterial glucuronidase has been presented earlier[3,8] and the ^{14}C activity of rat faeces following HR administration has previously been shown to be mainly attributable to unabsorbed hydroxyethylquercetins[3,4].

The demonstration that free and conjugated hydroxyethylrutosides can be recovered from the bile and urine of each second animal following reabsorption of the biliary metabolites indicates that a significant level of enterohepatic cycling is operating in animals receiving hydroxyethylrutosides. These findings may be of significance in relation to the maintenance of therapeutic levels of the drugs in man but the possibility of species differences should not be excluded.

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Affinity chromatography of monoamine oxidase

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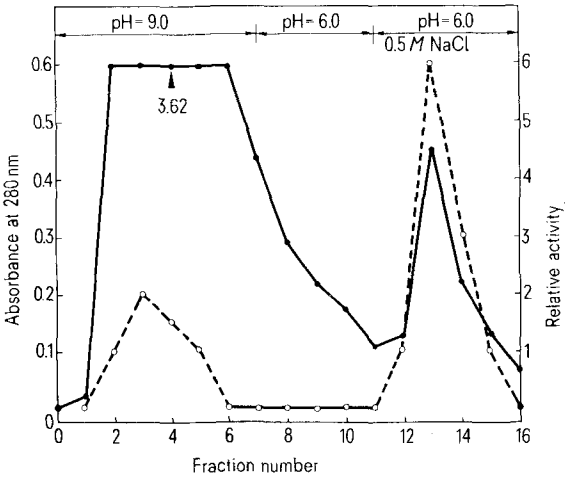
Summary. A method is described for the purification of pig liver monoamine oxidase by affinity chromatography, using a column with covalently bound pargyline.

Liver mitochondrial monoamine oxidase (MAO) has been isolated from a variety of mammalian sources including the rat[2–5], human[6], beef[7–9] and pig[10–13]. This high molecular weight protein catalyzes the oxidative deamination of primary, secondary, and tertiary amines with a methylene group adjacent to the nitrogen as follows:

$$RCH_2NHR' + O_2 + H_2O \rightarrow RCHO + R'NH_2 + H_2O_2.$$

We recently reported the successful substitution of various divalent metals for copper(II) in pig liver MAO[14]. The preparation of such pseudo-MAOs requires essentially pure enzyme, which prompted the development of the affinity chromatography system described herein.
Monoamine oxidase is inhibited by a variety of basic substances, many of which were included in a recent structure-activity study[15]. Unfortunately, the mode of action of the inhibitors are not completely understood, even with regard to reversibility. Pargyline (N-methyl-N-propargylbenzylamine) is known to react stoichiometrically and irreversibly with bovine kidney monoamine oxidase[16] and the structures of photoaddition products of pargyline and flavoquinone model compounds have been determined[17]. Nevertheless, the inhibition of pig liver monoamine oxidase by pargyline appears to act initially in a reversible manner[18]. Therefore, we attempted to use the pargyline inhibitor for purification by



Elution of MAO from pargyline affinity column in the dark at 4 °C. Absorbance at 280 nm (●) and relative activity (○) using benzylamine as substrate at 30 °C.

1 Acknowledgment. The authors wish to thank the University Research Council for financial support.

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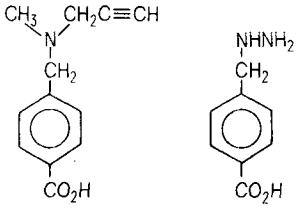
affinity chromatography in the dark and without prolonged contact with the column. For comparison, a benzylhydrazine inhibitor was also studied.

The required molecules for this work were the p-carboxy derivatives (**I** and **II**) of pargyline and of benzylhydrazine, which were prepared by nucleophilic displacement of bromine from p-carboxybenzyl bromide[19]. Heating excess N-methyl-N-propargylamine or hydrazine with p-carboxylbenzyl bromide gave, after concentration, extraction with benzene (**I** is soluble, while **II** is not), and conversion to the hydrochlorides with 3N HCl, 70% **I** · HCl, m.p. 209–210 °C (decomp.), or 70% **II** · HCl, m.p. 240 °C (decomp.).



(I) (II)

The inhibitors were coupled to AH-Sepharose 4B (Pharmacia, Inc.) using N-ethyl-N'-(3-dimethylaminopropyl)-carbodiimide hydrochloride and pH 8 Tris and pH 3 acetate buffers. A large excess of **II** was employed in the coupling step, as it undergoes self-condensation under the reaction conditions.

Pig liver MAO was prepared as described earlier[12] up to and including treatment with Triton X-100. The specific activity as defined by Tabor et al.[20] was seen to be 300. At this point, 2 ml of enzyme solution was added to the 1 × 5 cm affinity column containing pargyline, previously equilibrated with 50 mM phosphate buffer, pH 9.0. In view of the photochemical properties of the MAO inhibitor, all operations were performed in the dark at 4 °C. MAO adhered to the column which was further washed with 50 ml of 50 mM pH 9.0 phosphate buffer. The enzyme was then eluted from the column with either a salt gradient (0–1.0 M in NaCl) in pH 6.0 50 mM phosphate buffer or by the direct application of a 50 mM phosphate buffer (pH 6.0) 0.50 M in NaCl (figure). As is shown therein, the majority of the activity was eluted from the column as a single protein peak. The specific activity of this latter MAO peak was 3000 using 50 mM glycine buffer, pH 9.0, and benzylamine as the substrate[12] at 30 °C. This specific activity of the MAO obtained by a single affinity column treatment was thus identical to that of MAO obtained by the previously described method[13] involving two recrystallizations and 2 column treatments. Acrylamide gel electrophoresis (4%) showed a single dense band at pH 9.0. The affinity column is reusable if kept in a cool, dark environment and the enzyme purification process has been repeated 6 times with the same column.

All attempts to elute pig liver monoamine oxidase from an affinity column containing benzylhydrazine were without success. The enzyme readily attached itself to the column and resisted elution at high and low pH as well as with varying buffer and salt gradients.

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The role of sialic acid in determining the survival of circulating interferon

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Summary. Rabbit interferon has been extensively desialylated and its metabolic behaviour has been evaluated in the rabbit. The half-life of asialointerferon is significantly shorter than the native interferon and its urinary excretion becomes negligible. Moreover the rapid uptake of asialointerferon by the isolated and perfused rabbit liver, suggests a hitherto unsuspected catabolic pathway for this glycoprotein.

A striking feature of interferon (IF), either actively induced or passively administered, is its rapid disappearance from plasma[1,2]. While therapeutically this may appear somewhat disadvantageous[3], it reveals a deeper significance if it is considered that one of the functions of IF is to unleash the synthesis of the antiviral protein[4], and that a long-lasting plateau of IF in the body fluids could exert a depression of the immune response[5] or other deleterious effects[6]. It would then appear that catabolic or excretory systems have developed in order to remove rapidly the circulating IF as soon as this has been able, in some degree, to derepress the synthesis of the antiviral protein by interacting with the cell receptor[7,8].

In the past we and others have evaluated the possible catabolic role of the kidney[9], of the intestinal tract[9], of liver[10] and of body fluids[11], but nonetheless the main catabolic pathway has remained elusive.

The concept developed by Ashwell and Morell[12] that the terminal NAN has a role in regulating the survival time of glycoproteins in the circulation, and the fact that IF is a glycoprotein containing sialic acid (NAN)[13–17], may contribute a new approach to the study of the catabolism of this protein. Infact once NAN is cleaved by neuraminidase, galactose becomes exposed as the terminal sugar residue and acts as a specific determinant for recognition of the asialoglycoprotein by the hepatic receptor[18,19]. Thus, if desialylated IF is recognized and bound to the liver, its plasma disappearance should be considerably faster than that of native IF.

The problem has been approached by studying the behaviour in vivo and in vitro of native and desialylated rabbit IF and the results indicate that desialylation may represent an important first step in the catabolism of IF. Rabbit urinary and serum IF are obtained as previously described[9], but urinary IF has now been purified by