an den Pyridinkern (IV). Das äusserst kurzlebige[5], spirocyclische BENDER-Zwischenprodukt (V) stabilisiert sich jetzt unter Rückbildung des Pyridin-Systems zu den Endprodukten, Benzoesäure und Hydroxymethyl-N-methylpyridiniumchlorid (VI).

Präparative Durchführung der alkalischen Hydrolyse in $H_2{}^{18}O$-haltigem Wasser ergab die vom skizzierten Mechanismus geforderte, normale Isotopenverteilung; in der isolierten Benzoesäure findet man durch Decarboxylierung des Silbersalzes[2] und massenspektrometrische Analyse des CO_2 die Hälfte des im $H_2{}^{18}O$ enthaltenen schweren Sauerstoffs (vergleiche Tabelle II). Die Ähnlichkeit dieser Esterspaltung mit der kürzlich entdeckten oxydativ-bromierenden Hydrolyse von Tyrosin- und Tryptophan-Peptiden[6,7], unter Beteiligung des desaromatisierten Ringsystems, ist augenfällig.

Tab. II. 2-Benzoyloxymethyl-N-methyl-pyridiniumchlorid (I), alkalische Hydrolyse in $H_2{}^{18}O$ bei Zimmertemperatur, 22 und 70 h. Ansatz: 1 mMol Ester + 10 ml 0,1-*n* NaOH–O^{18} (1,09% ^{18}O); CO_2 aus Bombe: 0,207% ^{18}O.

Versuch Nr.	1	2	3	4
h	22	22	70	70
Silbersalz, mg	138	137	142	112
% ^{18}O	0,51	0,50	0,54	0,53

Substrate: 1. Benzoylcholin-chlorid, bezogen von Hoffmann-La Roche. 2. Pyridyl-2-carbinylbenzoat-methochlorid (I). Aus Pyridyl-2-carbinylbenzoat-hydrochlorid (Ia; F. 134–136°, unkorr.) erhielt man das Methojodid (Ib; F. 185–186°, unkorr.), und durch Ionen-Austausch schliesslich das Chlorid I (F. unscharf, Zers. bei ca. 180°); für Ia und I liegen gut stimmende CH, N- und Halogen-Analysen vor.

Summary. A structural analogue of benzoylcholine is prepared in which the trimethylamino group is replaced by a pyridine ring. Rate measurements are presented to demonstrate that the pyridinium ester I undergoes alkaline hydrolysis faster than the choline ester II. It is suggested that the abnormal rate is due to an unusual type of neighbouring group effect involving anchimeric participation of the pyridine ring.

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Observations on the Fate of Oral 4-Hydroxytryptophan in Man[1]

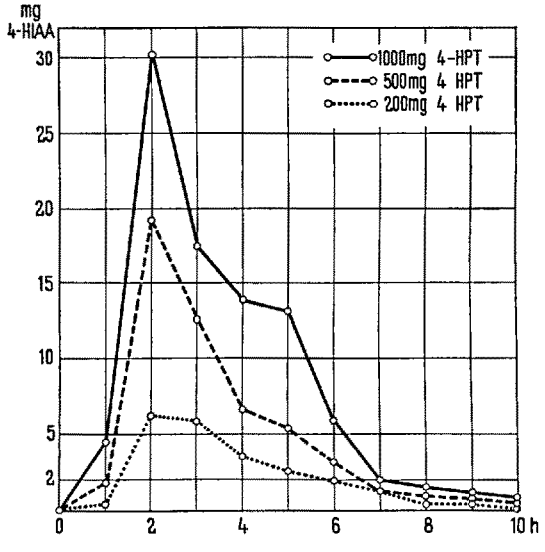
It is known that 4-hydroxytryptophan (4-HTP) is an excellent substrate for mammalian dopadecarboxylase[2]. The urine of rats given 4-HTP, by oral or subcutaneous route, contains a number of 4-HTP metabolites, among which are 4-hydroxytryptamine (4-HT), 4-hydroxyindoleacetic acid (4-HIAA), and their O-glucuronides[3].

This communication is intended to give a concise account of the fate of 4-HTP given by mouth in man. Three doses of 200, 500, and 1000 mg of *DL*-4-HTP, dissolved in a little water, were ingested by one of us (E.) at intervals of 7 days. Urine was collected and measured every hour for 12 h, and then in the following 12 h period.

Chromatograms of suitably concentrated or diluted urine were run, using prevalently the *n*-butanol : acetic acid : water mixture (4 : 1 : 5) and 4-hydroxyindole spots were developed with the NNCD reagent (2-chloro-4-nitro-1-diazobenzene-α-naphtalene sulphuric acid) or with diazotized *p*-nitroaniline. Semi-quantitative estimation of 4-HTP and 4-HIAA was carried out on paper chromatograms by visual comparison of the spots given by different amounts of urine with the spots given by known amounts of pure synthetic 4-HTP or 4-HIAA. 4-HT was estimated, both by paper chromatography and by bioassay.

In order better to separate and individualize the 4-HTP metabolites, pooled urine was passed through an alumina column and eluted with descending concentrations of ethanol.

The main results may be summarized as follows: (a) oral *DL*-4-HTP, in doses up to 1 g, had no obvious pharmacological effects in man. (b) 4-HIAA was by far the most important metabolite of 4-HTP found in urine after the oral administration of the amino acid. The total amount of free 4-HIAA was 95–100 mg after 1000 mg



Urinary excretion of 4-HIAA after oral administration, at 0 time, of 200, 500, and 1000 mg of *DL*-5-HTP.

[1] Supported by a grant from the Rockefeller Foundation, New York.
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DL-4-HTP, 53–55 mg after 500 mg *DL*-4-HTP, and 22–24 mg after 200 mg *DL*-4-HTP. The excretion curve of 4-HIAA in urine is shown in the accompanying Figure. (c) Only a very small aliquot (1.3–2.2%) of the administered amino acid was recovered unchanged in the urine, and less than 1% as 4-HT. This is in contrast with the relatively high amounts of 4-HT found in rat urine under similar experimental conditions. The presence of *O*-glucuronides of 4-HTP, 4-HT, and 4-HIAA, as well as of the *O*-sulphate of 4-HT, is questionable. (d) In addition to the above metabolites, human urine contained large amounts of another important 4-HTP metabolite, possibly an acid conjugate, the identification of which is in progress.

The above results suggest that oral 4-HTP and the 4-HT originating from it are already metabolized to a great extent in the intestinal wall and in the liver. Very little 4-HTP seems to be available for the renal decarboxylase, as shown by the small amount of 4-HT found in urine[4].

Riassunto. È stato studiato nell'uomo il destino del 4-idrossitriptofano (4-HTP) somministrato per bocca. Fra i metaboliti urinari dell'aminoacido quello di gran lunga più importante è risultato l'acido 4-idrossiindolacetico (10–12% riferito al *DL*-4-HTP). Scarsa è stata l'eliminazione urinaria di 4-HTP inalterato (1,3–2,2%) e ancora più scarsa quella della 4-idrossitriptamina (meno dell'1%). Un ulteriore importante metabolita del 4-HTP, presumibilmente un coniugato acido, è in corso di identificazione.

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[4] The 4-hydroxyindoles used in the present investigation were synthetized at the Farmitalia S.p.A. Research Laboratories, Milan, by Dr. C. PASINI and Dr. V. COLÒ.

Rat Serum *para*-Phenylene Diamine Oxidase Reaction During Azo Dye Carcinogenesis

NEISH[1] reported that the *para*-phenylene diamine oxidase (copper oxidase) activity of rat serum, as evaluated by the method of RAVIN[2], was greatly diminished for a period of four days after injections of the strong hepatocarcinogens 3′-methyl and 4′-ethyl-dimethylaminoazobenzene (3′-MeDMAAB and 4′-et-DMAAB). The feeble carcinogen 4′-MeDMAAB did not have this effect and injection of 16 mg/100 g rat in 0.6 ml arachis oil caused an increase over a 2–4 day period. He also showed that the injection of powerful carcinogenic azo dyes greatly reduced the copper levels in the rat serum after 72 h. From a further study NEISH[3] concluded that any compound which is found to suppress the copper oxidase under the experimental conditions described may be suspect as a potential liver carcinogen.

In our laboratory HOWELL[4] showed that feeding copper acetate to rats (6 mg/day in the cancer-inducing diet containing DMAAB) greatly inhibited the production of liver tumours. Because of the relevance of this finding to the work of NEISH we have conducted experiments on the effects of long-term feeding of the dye and copper acetate, separately and in combination, on the serum copper oxidase, and also on the effects of administering the primary metabolic products, viz; monomethylaminazobenzene and aminoazobenzene, and of the further metabolites, *p*-aminophenol and *p*-phenylene diamine.

Preliminary tests confirmed NEISH's observations on the effects of azo dye injection in arachis oil.

During the course of these investigations it was observed that the enzyme reaction was very sensitive to the concentration of acetate in the buffer. Thus, at constant pH 5.5, a change in the concentration of the sodium acetate from 4.15% to 0.95% resulted in the decrease of the optical density from 0.43 to 0.21 at 530 mμ, although an alteration in the pH with this buffer system from pH 6.1 to 5.1 made practically no difference to the enzyme activity. It was important, therefore, in the long term experiments to check the properties of new buffer solutions against the existing stock.

Long term azo dye feeding tests. In one experiment 3 groups of 5 rats were employed. One group was fed a basic maize diet similar to that utilized by HOWELL[4], the second group was fed DMAAB, 6 mg/day in this diet, and the third group was given the same amount of dye with the addition of 6 mg copper acetate/day. A careful check on the daily consumption of the food, and periodic weighing of the animals, which were 120–160 g weight initially, demonstrated that the chemicals were satisfactorily consumed and that there was no undue inhibition of the growth of the animals, all of which survived more than 20 weeks.

It was found that after one week, and thereafter over the whole period, the serum oxidase values were consistently lower in the dye-fed group than in the control maize-fed animals and that the copper acetate opposed the effect of the carcinogenic dye on the oxidase so that the enzyme values were near normal. The results are given in Figure 1, where the levels of the oxidase above or below the values prior to the dye feeding are expressed in arbitrary units derived from the optical density of the coloured solutions estimated at 530 mμ as described by NEISH. These values are the averages for 5 animals in each group at intervals of 4 weeks but the effects were regularly found in every individual animal of the experimental groups.

In a second experiment three groups of 5 rats were fed NN-dimethylaminoazobenzene (DMAAB), the N-methyl derivative and *p*-aminoazobenzene, respectively. The results are shown in Figure 2 from which it is evident that the monomethyl compound, which is regarded as similar in carcinogenicity to the dimethyl compound, also produced lower values in the serum oxidase reaction during the whole period, while the effect of the non-methylated, non-carcinogenic compound was much less marked.

From experiments in which DMAAB was fed to rats along with a second azo dye, which inhibited the cancer producing effect of the butter yellow, CRABTREE[5] concluded that this was attributable to the metabolic production of *p*-aminoazobenzoic acid in the liver by breakdown of the second dye. He pointed out that *p*-aminobenzoic acid itself did not inhibit azo dye carcinogenesis but that azo dyes capable of forming *p*-toluidine or *p*-aminobenzoic acid were inhibitory.

It was thus of interest to see whether these two compounds would inhibit the serum oxidase. They were given

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