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.

V. ERSPAMER and M. B. NOBILI

Istituto di Farmacologia, Università di Parma (Italy), April 28, 1961.

[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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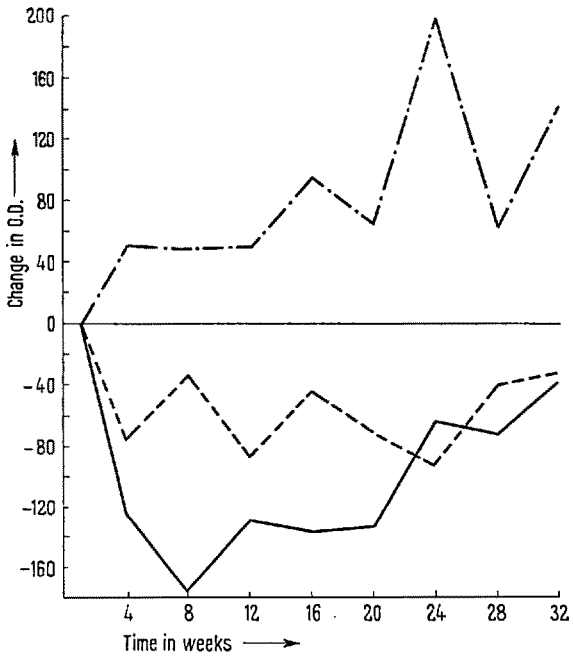

Fig. 1. Phenylene diamine oxidase: changes in rat serum during dietary treatment with DMAAB (——), DMAAB + CuAc (----). Compared with basic maize diet (-----). (Average of 5 rats.)

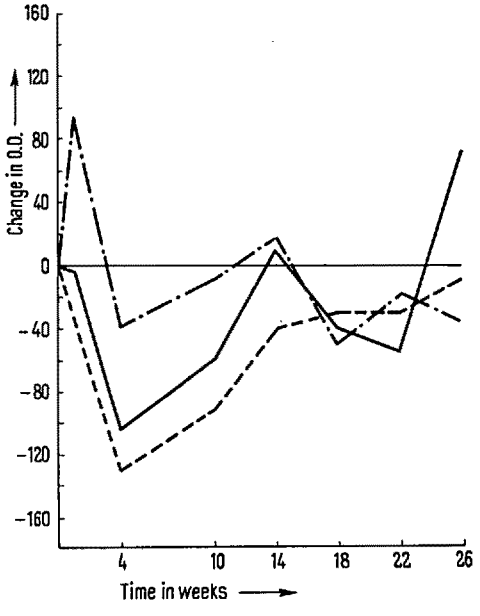

Fig. 2. Phenylene diamine oxidase: changes in rat serum during dietary treatment with DMAAB (——), N-methyl AAB (----), AAB (-----). (Average of 5 rats.)

0.04% in the food to groups of 5 rats along with the DMAAB. Blood assays were carried out before commencing the dye feeding and after 1 and 4 weeks. The oxidase values were significantly lower after one week and considerably decreased after four weeks to as great an extent as was produced by giving the carcinogenic dye only. Thus the cancer-inhibiting action of these dye metabolites does not appear to be associated with systems connected with copper oxidase.

Short term tests. Metabolic products of azo dyes. The following metabolites of DMAAB *p*-aminophenol, *p*-phenylene diamine, N-methylaminoazobenzene, and *p*-aminoazobenzene were investigated for their effects using

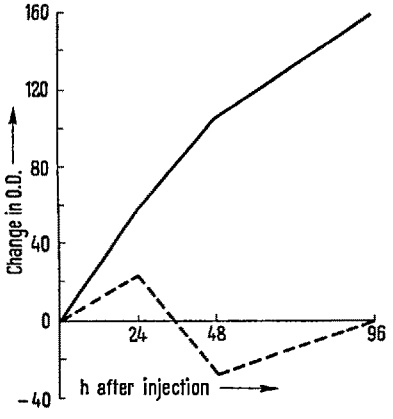

Fig. 3. Effect of single arachis oil injection on rat serum phenylene diamine oxidase 0.6 ml/100 g body weight (——). 0.3 ml/100 g (----). (Average of 2 rats.)

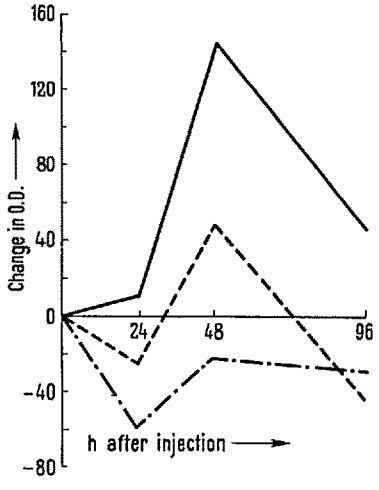

Fig. 4. Effect of sing'e injection of N-methyl AAB (-----) and AAB (----) compared with DMAAB. In arachis oil 0.3 ml/100 g rat body weight. (Average of 2 rats.)

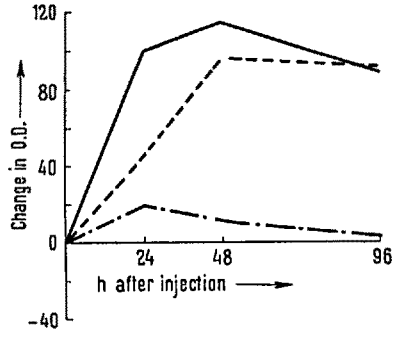

Fig. 5. Effect of single injections of *p*-amino phenol (——). *p*-phenylene diamine (----). In arachis oil 0.3 ml/100 g rat body weight. Aqueous *p*-amino benzoic acid (-----). (Average of 2 rats.)

the injection technique with the slight modification that only 0.3 mg/100 g rat of arachis oil was used as vehicle. It has been noted previously that the injection of this quantity of arachis oil did not produce as great an increase in the serum oxidase values of otherwise untreated animals, as does the amount (0.6 ml/100 g rat) injected by NEISH[1] so that the main effect of the chemical under test could be more accurately assessed (Figure 3). The effect of *p*-aminobenzoic acid was also tested but was given in aqueous solution.

The amounts of the various compounds injection per 100 g rat were: *p*-aminophenol 3.6 mg, *p*-phenylene diamine 4.0 mg, *p*-aminobenzoic acid 1.5 mg, DMAAB (for comparison) 7.8 mg. The results of typical tests are given in the Figures 4 and 5.

It will be noted that N-methylaminoazobenzene caused less depression than DMAAB while *p*-aminoazobenzene induced a temporary depression followed by a marked rise with a subsequent fall to the original value at 96 h. On the whole, *p*-amino phenol and *p*-phenylene diamine caused increased values during the 96 h observation period, and *p*-aminobenzoic acid caused slightly raised values[6].

Résumé. Dans le sérum sanguin, on a constaté une diminution de l'activité de la *p*-phénylènediamine-oxydase pendant l'administration *per os* de diméthylaminoazobenzène. En ajoutant de l'acétate de cuivre dans l'alimentation, l'éffet sur le sérum sanguin est moindre. Le monométhylaminoazobenzène, qui est aussi cancérigène sur le foie, a produit une oxydase réduite, mais la aminoazobenzène non cancérigène n'a provoqué qu'une diminution très peu sensible.

Chez les rats nourris avec de l'acide *p*-aminobenzoïque ou *p*-toluidine additionné d'azoïque, le sérum sanguin a continué d'offrir un pourcentage moindre d'oxydase. Les métabolites des azoïques, *p*-aminophénol et *p*-phénylènediamine, provoquent une augmentation de l'oxydase après injection intra-péritonéale dans l'huile d'arachide.

D. L. WOODHOUSE

Cancer Research Laboratories, Department of Pathology, Medical School, Birmingham (England), December 12, 1960.

[6] These investigations were carried out with the financial support of the Birmingham Branch of the British Empire Cancer Campaign.

Vincanorin und Vincarein, zwei neue Alkaloide aus *Vinca minor* L.

In *Vinca minor* wurden bisher 7 Alkaloide festgestellt[1–9]. Wir haben aus der ganzen Pflanze (Stengel, Blätter und Wurzeln) mit dem von SCHEINDLIN und RUBIN[4] beschriebenen Verfahren zwei neue Alkaloide isoliert. Bei der Chromatographie an Aluminiumoxyd wurde eine mit Äther eluierbare Fraktion erhalten, die beim Einengen ein kristallisiertes Gemisch von Alkaloiden (Fraktion A, 0,015% bezogen auf getrocknete Droge) neben amorpher Mutterlauge (Fraktion B, 0,01%) lieferte. Die Fraktion A zeigte im Papierchromatogramm neben einem Flecken, der Vincamin und Isovincamin entsprach, einen solchen mit kleinerem Rf-Wert (0,37 im System Isobutylalkohol/0,2 *M* KH_2PO_4). Zur präparativen Isolierung wurde die Fraktion A mit Hilfe der Gegenstromverteilung im System Benzol/*n*-Butanol/85% Ameisensäure/McIlvains Puffer pH = 3,5 (Verhältnis 25:5:1:31) zerlegt, wobei das bisher unbekannte Alkaloid *Vincarein* in Form einheitlicher Kristalle (1,5% der Fraktion A) gefasst wurde.

Die Gegenstromverteilung der Fraktion B im System Benzol/0,1 *M* Zitronensäure lieferte neben amorphen Fraktionen ein weiteres neues Alkaloid in kristalliner Form, das wir *Vincanorin* nennen. Das neue Alkaloid unterscheidet sich vom isomeren Vincamin ausser durch den Schmelzpunkt auch im Papierchromatogramm (im selben System, das für die Gegenstromverteilung der Fraktion A verwendet wurde).

In der Tabelle sind die Eigenschaften der beiden Alkaloide zusammengestellt.

Summary. The isolation of two new alkaloids from *Vinca minor* L. (Apocynaceae) is described. Vincarein, $C_{21}H_{24}N_2O_4$, and Vincanorin, $C_{19}H_{22}N_2O$, both probably belong to the group of indol alkaloids.

J. MOKRÝ, I. KOMPIŠ, O. BAUEROVÁ, J. TOMKO und Š. BAUER

Abteilung für Chemie der Naturstoffe des Chemischen Institutes der Slowakischen Akademie der Wissenschaften, Bratislava (Tschechoslowakei), 13. März 1961.

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	Smp.	$[\alpha]_D^{20°}$	UV[a] λ_{max} logε	λ_{inflex} logε	λ_{mix} logε	IR[b] in Nujol	Summenformel	Analysen C ber.	C gef.	H ber.	H gef.	N ber.	N gef.	Salze
Vincarein[c]	205–206°	−28,2±2°	*227*	*286*	*250*	5,71 μ	$C_{21}H_{24}N_2O_4$	68,46	68,70	6,57	6,60	7,60	7,89	Methojodid
	aus Aceton	c = 1,48	4,35	3,66	3,41	5,83 μ								Smp. 221–222°
		$CHCl_3$	*274*											
			3,76											
Vincanorin	201–202,5°	0±2°	*227*		*256*	5,86[d]	$C_{19}H_{22}N_2O$	77,52	77,65	7,53	7,70	9,52	9,51	Perchlorat
	aus Methanol	c = 1,02	4,49		3,41									Smp. 243–245°
		$CHCl_3$	*287*		*292*									
			3,89		3,85									
			294											
			3,87											

[a] Vincarein in Methanol, Vincanorin in Äthanol. [b] C=O-Streckschwingungsgebiet. [c] Mit konz. H_2SO_4 wird schwach gelborange, mit Vanillin in konz. H_2SO_4 gelbe, mit $K_2Cr_2O_7$ in konz. H_2SO_4 rotbraune und mit NH_4VO_3 in konz. H_2SO_4 grünbraune Färbung erhalten; das UV-Spektrum ist typisch für ein Indolderivat. [d] Sechsgliedriges, eventuell offenes Keton, ausserdem deutet eine Bande bei 14,5 μ auf ein 1,2-disubstituiertes Benzolderivat.