Metabolism of Nicotinic Acid and Related Compounds in Man and Rat

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The earliest attempts to study the fate of ingested nicotinic acid were confined to the isolation of various metabolites from urines (Ackerman, 1912; Komori & Sendju, 1926). It is now accepted that N^1-methylnicotinamide (Huff & Perlzweig, 1943; Najjar, White & McNair Scott, 1944) and N^1-methyl-2-pyridone-5-carbonamide (Knox & Grossman, 1946, 1947; Holman & de Lange, 1949) are important excretory products of nicotinic acid metabolism. The excretion of tertiary nicotinyl compounds, which give a colour by König's reaction (1904), has also been repeatedly reported, but the data were obtained by techniques based on complicated differentiation procedures (Melnick, Robinson & Field, 1940; Perlzweig, Levy & Sarett, 1940; Huff & Perlzweig, 1941; Sarett, Huff & Perlzweig, 1942; Johnson, Hamilton & Mitchell, 1945; Ellinger & Kader, 1949). Recently, more refined and accurate techniques were used by Johnson & Lin (1951) and by Leifer, Lloyd, Hogness & Corson (1951) who gave labelled nicotinic acid and nicotinamide to rats and partitioned the metabolites in the urines by paper chromatography. This procedure, however, is difficult to apply to man.

The present communication deals with the differentiation and estimation of various metabolites of nicotinic acid and related compounds in the urines of man and the rat, by employing chromatographic techniques recently developed in this laboratory for both tertiary and quaternary nicotinyl compounds (Kodicek & Reddi, 1951). No attempt has been made to determine the N^1-methyl-2-pyridone-5-carbonamide, which the above mentioned procedure cannot detect.

MATERIALS AND METHODS

Human subjects

Four healthy male subjects on a normal diet were selected for these studies.

Collection of urines. The subjects were given various nicotinyl compounds (Table 1) in turn at weekly intervals, together with 400 ml. of water, and their urines were collected for 3 hr. The dose was administered after breakfast, except in the case of the second dose of nicotinic acid which was given before breakfast to induce maximum flushing of the skin.

The control urines from the undosed subjects were collected in the same way after giving 400 ml. of water. The urines were stored at 4° under toluene.

Rats

Sixteen male piebald rats, weighing 55–60 g., were divided into two groups and housed in individual cages. One group received the low-protein maize diet of Harris & Kodicek (1950), which is deficient in nicotinic acid, and the other group received an adequate synthetic diet containing 18% casein.

Low-protein maize diet (Harris & Kodicek, 1950). Casein (Genatosan, vitamin-free) 3·5, sucrose 51·4, maize meal 40; salt mixture 3 (Kodicek & Carpenter, 1950), cotton-seed oil 2, cystine 0·1.

Synthetic casein diet. Casein (Genatosan, vitamin-free) 18, sucrose 73·85, salt mixture 5, cotton-seed oil 3, cystine 0·15.

Each kg. of diet also contained: choline chloride 2 g., thiamine 3 mg., riboflavin 3 mg., pyridoxin 3 mg., calcium D-pantothenate 20 mg., biotin 0·1 mg.

In addition, each rat received *per os*, once a week, 0·5 mg. tocopherol acetate, 0·1 mg. 2-methyl-1:4-naphthaquinone and 0·1 g. Radiostoleum (British Drug Houses Ltd.) containing 1500 i.u. vitamin A and 300 i.u. vitamin D.

The rats were maintained on these diets for 19 days during which time the deficient group on the low-protein maize diet gradually decreased in weight. At the end of this period they were transferred to metabolism cages. Two rats were placed in each cage and their urines were collected for 48 hr. while they were maintained on the diets. The animals were then given various nicotinyl compounds, listed in Table 2, on palettes for 2 days and the urines were again collected.

Estimation of nicotinyl compounds

Total alkali-hydrolysable tertiary nicotinic acid derivatives, which include nicotinic acid, nicotinamide and nicotinuric acid (nicotinylglycine), were estimated chemically according to the method of Wang & Kodicek (1943). The estimation of N^1-methylnicotinamide was carried out by the method of Carpenter & Kodicek (1950).

In view of certain differences between the chemical and chromatographic data, human urines (see Table 1) were also studied by using the microbiological procedure of Clegg, Kodicek & Mistry (1952). Chromatograms of the urine from an undosed subject (no. 2) were also tested microbiologically by using a technique similar to that described by Leifer, Langham, Nyc & Mitchell (1950). These estimations were kindly carried out by Miss K. M. Clegg.

Separation of nicotinyl compounds in urines by paper chromatography and their estimation

The chromatographic procedures for the separation of tertiary and quaternary nicotinyl compounds (Kodicek & Reddi, 1951) were applied to urines, and the concentrations of the tertiary nicotinyl substances were evaluated by visual

comparison of the coloured spots by a procedure similar to that described by Berry, Sutton, Cain & Berry (1951).

Procedure. 10 ml. of urine were evaporated to dryness under reduced pressure at 55–60°. The residue was extracted with 1 ml. of distilled water and centrifuged. The clear supernatant was used for chromatographic studies, in which 10 μl. were placed on Whatman filter paper no. 1 and descending chromatograms were run overnight. In some cases, where the quantity of substance to be detected was very low, four spots of 10 μl. each were placed one over the other after drying each spot thoroughly. Pure nicotinyl compounds, in amounts ranging from 0·1 to 2·0 μg., were run on the same paper along with the urines.

Tertiary nicotinyl compounds. For these compounds *n*-butanol saturated with water was found to be the best solvent system. After development, the papers were dried at room temperature. The dry papers were placed in a closed glass tank for 1 hr. at room temperature over CNBr, prepared according to the method of Wang & Kodicek (1943). They were then sprayed with 2% *p*-aminobenzoic acid (2 g. of *p*-aminobenzoic acid dissolved in 75 ml. 0·75 N-HCl and made up to 100 ml. with 96% ethanol). The different compounds could be identified by their colours and R_F values (Kodicek & Reddi, 1951). All the spots fluoresced bright yellow under ultraviolet light, and quantities as low as 0·1 μg. could be detected under these conditions. Since within a range of 0·1–2·0 μg. the area of the spots remained the same, the gradation of the colour intensity, especially when viewed in ultraviolet light, allowed a fairly accurate comparison with the standards. The most suitable concentration for evaluation was within the range of 0·25–0·5 μg., and by averaging the estimates of two independent observers a difference of 0·1 μg. could be detected. Duplicate chromatograms sprayed only with the *p*-aminobenzoic acid reagent, without exposing them to CNBr, served as controls to eliminate non-specific colour spots which were found to be present both in dosed and undosed urines. In the present studies it was not found necessary to adjust the pH of the urinary concentrate to neutrality, since the R_F values of the metabolites corresponded well with those reported earlier on pure substances (Kodicek & Reddi, 1951). However, it may be advisable in general to control the pH of the urinary concentrates to eliminate the possibility of shifts of R_F values.

The results obtained by the above procedure are given in the last three columns of Tables 1 and 2.

Quaternary nicotinyl compounds. The solvent system *n*-propanol-water (60:40) was found to be the best for developing chromatograms of quaternary nicotinyl compounds. The dry papers after development were placed over a mixture of ethyl methyl ketone and NH_3 (1:1) for 1 hr. in a closed glass tank at room temperature. The relative positions of various compounds could be detected by their bluish white fluorescence, and quantities as low as 0·05 μg. (nicotinic acid equivalents) could be detected.

Detection of tryptophan metabolites in urines of humans dosed with L-*tryptophan*

Urinary concentrate (10 μl.) was placed on Whatman filter paper no. 1 and developed in *n*-butanol-acetic acid-water (50:10:40) overnight and dried at room temperature. The chromatograms of urines from both dosed and undosed subjects were viewed under ultraviolet light and the R_F values of the fluorescent metabolites determined (Fig. 1). 3-Hydroxyanthranilic acid and kynurenic acid were identified by running pure samples on the same papers. Tryptophan and kynurenine were identified by spraying with the *p*-dimethylaminobenzaldehyde reagent (0·5 g. *p*-dimethylaminobenzaldehyde, 1 ml. conc. HCl and 100 ml. absolute ethanol), and by their behaviour on exposure to vapours of conc. HCl (Tabone, Robert, Thomassay & Mamounas, 1950). The R_F values of other metabolites were compared with those reported by Dalgliesh, Knox & Neuberger (1951).

RESULTS

Man

The urines from undosed subjects, collected for a period of 3 hr., contained 0·5–0·6 mg. of N^1-methylnicotinamide, with an average of 0·53 mg. (Table 1). The total content of tertiary alkali-hydrolysable derivatives of nicotinic acid, as determined chemically, ranged from 0·2 to 0·3 mg. within the 3 hr. period. However, no tertiary nicotinyl derivatives could be detected on chromatograms. In view of this discrepancy, microbiological estimations were carried out on urinary concentrates hydrolysed with N-sodium hydroxide. The microbiological values (Table 1) were much lower than those determined chemically, ranging from 0·04 to 0·12 mg. of nicotinic acid/3 hr. Similar results were obtained when the urines were tested without a prior alkaline hydrolysis.

When urine from undosed subjects, concentrated 10 times, was chromatographed and the strips were analysed microbiologically for nicotinic acid activity, according to the procedure of Leifer *et al.* (1950), the R_F region corresponding to nicotinamide showed some activity. The concentration of nicotinamide amounted to 40% of the total nicotinic acid derivatives as determined microbiologically. The rest of the activity remained at the place of application of the spots (40 μl.) and spread to a distance of 3 cm. from the origin.

Dosing with nicotinic acid. After doses of 100 mg. nicotinic acid the excretion of alkali-hydrolysable nicotinic acid derivatives and of N^1-methylnicotinamide increased to 6·0–14·6 mg. and 2·8–5·7 mg./3 hr., respectively (Table 1). The chromatographic results showed that the major metabolite in the urines was nicotinuric acid, forming 92–99% of the alkali-hydrolysable derivatives; the rest could be accounted for by a small amount (1–4%) of nicotinamide.

There was no free nicotinic acid in the urines except in one subject (no. 3) who had intense flushing of the skin soon after the ingestion of nicotinic acid. This individual had, incidentally, the highest excretion of tertiary nicotinyl compounds and the lowest of N^1-methylnicotinamide. The vasodilatory effect of nicotinic acid, especially when taken ante-prandially, is well known. It was of interest to verify whether the appearance of free

Table 1. *Excretion of nicotinyl compounds in human urines and their distribution*

Subject	Age (years)	Dose	Excretion (mg./3 hr.): N^1-Methylnicotinamide determined chemically	Total alkali-hydrolysable nicotinic acid derivatives determined: Chemically	Total alkali-hydrolysable nicotinic acid derivatives determined: Microbiologically	Approximate relative distribution of tertiary nicotinyl compounds (%)*: Nicotinic acid	Nicotinamide	Nicotinuric acid†
1	29	None	0·5	0·3	0·12	Nil	Nil	Nil
2	43	None	0·5	0·2	0·04	Nil	Nil	Nil
3	32	None	0·5	0·2	0·04	Nil	Nil	Nil
4	20	None	0·6	0·2	0·06	Nil	Nil	Nil
1	29	100 mg. nicotinic acid (after breakfast)	4·5	7·3	—	Nil	4	96
2	43	100 mg. nicotinic acid (after breakfast)	3·7	6·0	—	Nil	2	98
3	32	100 mg. nicotinic acid (after breakfast)	2·8	14·6	—	7	1	92
4	20	100 mg. nicotinic acid (after breakfast)	5·7	8·6	—	Nil	1	99
1	29	100 mg. nicotinic acid (before breakfast)	5·4	40·3	—	10	0·5	89·5
2	43	100 mg. nicotinic acid (before breakfast)	2·7	17·3	—	5	0·5	94·5
1	29	100 mg. nicotinamide	16·2	1·5	—	Nil	100	Nil
2	43	100 mg. nicotinamide	16·6	1·8	—	Nil	100	Nil
3	32	100 mg. nicotinamide	6·9	1·8	—	Nil	100	Nil
4	20	100 mg. nicotinamide	15·6	0·9	—	Nil	100	Nil
2	43	3 g. L-tryptophan	1·0	—	0·09	Nil	Nil	Nil
3	32	3 g. L-tryptophan	0·7	—	0·04	Nil	Nil	Nil
4	20	3 g. L-tryptophan	1·0	—	0·05	Nil	Nil	Nil

* Determined by paper chromatography (Kodicek & Reddi, 1951).
† The values for nicotinuric acid were obtained by difference.

nicotinic acid in the urine after a dose of nicotinic acid was associated with flushing and the other vasodilatory symptoms. Therefore, two individuals (nos. 1 and 2), who normally did not experience any flushing, were given before breakfast 100 mg. nicotinic acid and the urines, collected for 3 hr., were analysed in the usual way. Both subjects experienced intense flushing which was more pronounced in subject no. 1. As will be seen from Table 1, the urinary excretion of N^1-methylnicotinamide did not show any appreciable change, but that of the tertiary nicotinyl compounds increased considerably, to 40·3 and 17·3 mg. respectively. In addition to the usual appearance of nicotinuric acid (89·5–94 %) and of nicotinamide (0·26–0·5 %), free nicotinic acid also appeared in the urines in amounts of 5–10 % of the total tertiary nicotinyl compounds excreted.

Dosing with nicotinamide. After the ingestion of 100 mg. nicotinamide, there was a large increase in the excretion of N^1-methylnicotinamide, which varied from 6·9 to 16·6 mg./3 hr. The small rise in the tertiary nicotinyl derivatives (0·9–1·8 mg.) was solely due to nicotinamide, since no other nicotinyl compound could be detected on the paper chromatograms.

Dosing with tryptophan. After the ingestion of 3 g. L-tryptophan, there was an increase in the excretion of N^1-methylnicotinamide. The tertiary nicotinyl compounds, tested microbiologically, did not increase. On chromatograms tested by chemical means no nicotinyl compounds except N^1-methylnicotinamide were observed.

Tryptophan metabolites. When 10 times concentrated urines, obtained after dosing with tryptophan, were chromatographed using *n*-butanol-acetic acid-water (40:10:50), a number of tryptophan metabolites could be identified by their fluorescence and also by spraying with the *p*-dimethylaminobenzaldehyde reagent (Fig. 1). Spots 4 and 6 were identified as kynurenic acid and 3-hydroxyanthranilic acid, respectively, by running the pure substances side by side on the same paper. Spot 3 was identified as kynurenine. It corresponded to the spot *F* (kynurenine) of Dalgliesh *et al.* (1951). Spraying with *p*-dimethylaminobenzaldehyde produced an orange spot (S_2) which faded on exposure to fumes of concentrated hydrochloric acid. This behaviour was in keeping with the observations of Tabone *et al.* (1950). It was placed immediately above tryptophan (spot S_3) whose yellow colour turned violet on exposure to concentrated hydrochloric acid. The R_F values of spots S_2 and S_3 in *n*-butanol-water were 0·26 and 0·28, respectively, which agreed well with values reported by Tabone *et al.* (1950) for kynurenine and tryptophan. Spots 1, 2 and 5 had R_F values similar to those of spots *C*, *E* and *I* of Dalgliesh *et al.* (1951).

Spot S_1 turned bright yellow on spraying, and there was no change in colour on exposure to con-

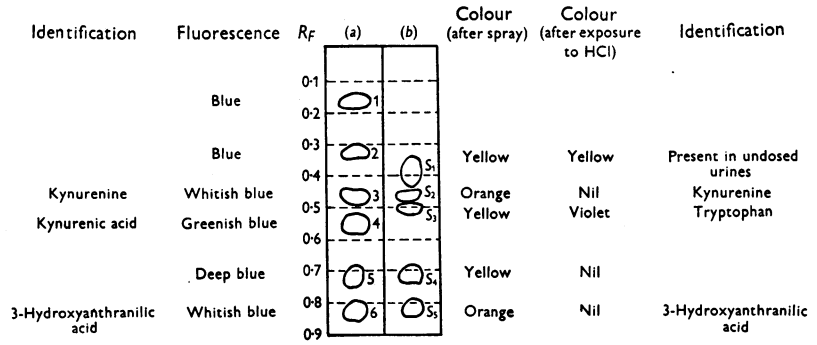

Fig. 1. Diagram showing the approximate R_F values of tryptophan metabolites in human urines after ingestion of L-tryptophan. (Solvent system: *n*-butanol-acetic acid-water, 40:10:50.) (*a*) Fluorescent spots obtained by chromatographing 10 times-concentrated urines; (*b*) colour spots of the same after spraying with *p*-dimethylaminobenzaldehyde.

centrated hydrochloric acid. However, a similar spot was observed in the urines from undosed subjects. It cannot therefore be considered a metabolite derived from tryptophan.

Rats

It will be seen from Table 2 that rats given a maize diet deficient in nicotinic acid excreted a very small amount of N^1-methylnicotinamide, nicotinic acid and nicotinamide, while those given an adequate casein diet excreted daily 0·24 mg. N^1-methylnicotinamide and 0·04 mg. total tertiary nicotinyl compounds per rat. Both deficient and normal rats excreted nicotinic acid and nicotinamide in equal proportions.

Dosing with nicotinic acid. After dosing with 2·5 mg. nicotinic acid, the deficient rats excreted 37 % and the normal animals 90 % of the dose in the form of N^1-methylnicotinamide, nicotinuric acid, nicotinic acid and nicotinamide. In the deficient animals, the major excretion products, N^1-methylnicotinamide and nicotinuric acid, were in equal proportions, each being about 16 % of the dose. There was a definite rise in the excretion of nicotinic acid, but only a small rise in nicotinamide, about 3·5 and 0·3 % of the dose respectively. In the normal animals, the main excretion products in descending order were N^1-methylnicotinamide 41 %, nicotinuric acid 23 %, nicotinic acid 12·2 %, and nicotinamide 2·5 % of the dose (Fig. 2). As will be seen from Table 2, nicotinuric acid formed 81 and 63 % of the total tertiary nicotinyl compounds found in the urines of deficient and normal animals respectively.

Dosing with nicotinamide. After dosing with 2·5 mg. nicotinamide, the deficient and normal rats excreted 52 and 95 % of the dose respectively. N^1-Methylnicotinamide, nicotinamide and nicotinic acid, but no nicotinuric acid, could be found on the chromatograms. The relative distribution of the tertiary nicotinyl compounds, namely nicotinamide and nicotinic acid, was in both groups in a ratio of 3 : 1

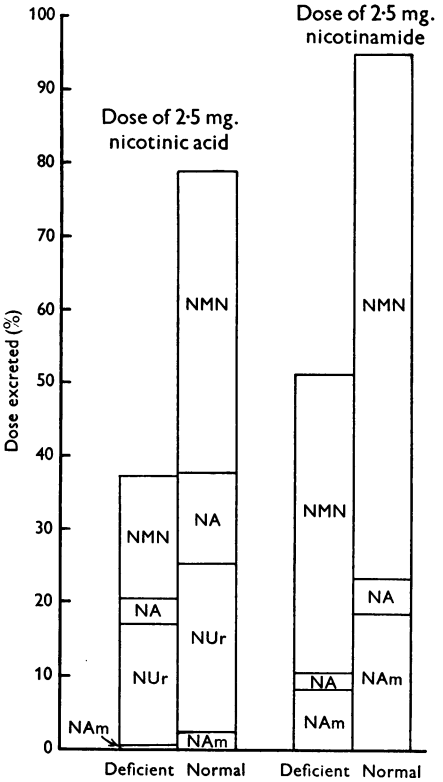

Fig. 2. The percentage of ingested nicotinic acid and amide excreted by nicotinic acid-deficient and normal rats. NMN, N^1-methylnicotinamide; NA, nicotinic acid; NUr, nicotinuric acid; NAm, nicotinamide.

(Table 2). The major excretion product was N^1-methylnicotinamide, forming in the deficient group 40 % and in the normal group 70 % of the dose.

Table 2. *Excretion of nicotinyl compounds in urines of normal and deficient rats before and after dosing with nicotinic acid and related substances*

Group	Diet	No. of rats	Average wt. (g.)	Dose	Average daily excretion per rat (mg.)		Approximate relative distribution of tertiary nicotinyl compounds (%)*		
					N^1-Methyl nicotinamide	Total alkali-hydrolysable nicotinic acid derivatives	Nicotinic acid	Nicotin-amide	Nicotinuric acid
1 (deficient)	Low-protein maize (Harris & Kodicek, 1950)	8	78	None	0·01	0·01	43	57	Nil
		2	78	2·5 mg. nicotinic acid	0·42	0·53	17	2	81
		2	87	2·5 mg. nicotinamide	1·03	0·27	26	74	Nil
		2	74	100 mg. quinolinic acid	0·01	0·01	46	54	Nil
		2	88	100 mg. L-tryptophan	0·10	0·03	71	29	Nil
2 (normal)	Synthetic casein diet	8	139	None	0·24	0·04	49	51	Nil
		2	147	2·5 mg. nicotinic acid	1·27	0·98	31	6	63
		2	210	2·5 mg. nicotinamide	2·03	0·62	25	75	Nil
		2	132	100 mg. quinolinic acid	0·57	0·14	86	14	Nil
		2	130	100 mg. L-tryptophan	1·74	0·20	90	10	Nil

* Determined by paper chromatography (Kodicek & Reddi, 1951).

Nicotinamide was excreted in significant amounts, namely 8 % of the dose in the deficient animals and 18·6 % in the normal rats. The excretion of nicotinic acid was only about 2·5 and 4·6 % of the dose respectively (Fig. 2).

Dosing with quinolinic acid. After dosing with 100 mg. quinolinic acid the deficient animals showed no rise in the excretion of N^1-methylnicotinamide, and an insignificant increase in the tertiary nicotinyl compounds. The distribution of nicotinic acid and amide was the same as in urine obtained before dosing, namely 46 and 56 %. In the normal group the excretion of N^1-methylnicotinamide increased from 0·24 to 0·57 mg. and that of the tertiary nicotinyl compounds rose from 0·04 to 0·14 mg./day/rat. This rise was mainly due to an increased excretion of nicotinic acid, which formed 86 % of the total tertiary nicotinyl compounds (Table 2).

Dosing with L-*tryptophan.* After dosing with 100 mg. L-tryptophan, there was, in the deficient rats, a slight increase in the excretion of N^1-methylnicotinamide and of tertiary nicotinyl compounds. A similar but more pronounced rise of these substances was found in the normal group. The increase in the tertiary nicotinyl compounds, both in the deficient and normal rats, was mainly due to a rise in nicotinic acid, which formed in the deficient group 71 % and in the normal rats 90 % of these derivatives (Table 2).

DISCUSSION

The paper-chromatographic technique used for partitioning nicotinic acid derivatives, besides being simple and easily performed, has many advantages over the chemical methods employed by earlier workers (Melnick *et al.* 1940; Perlzweig *et al.* 1940; Huff & Perlzweig, 1941). Especially in the case of the tertiary nicotinyl compounds, the estimations based on König's colour reaction may be interfered with by unspecific colour formation in the urines which has to be corrected by the use of proper blanks. In the chromatographic procedure, one compares the colour of pure compounds separated on the chromatograms and thus eliminates errors due to interference by other substances. It was found advantageous to spray a duplicate chromatogram with the *p*-aminobenzoic acid reagent alone, without the cyanogen bromide treatment, and to distinguish in this way several coloured non-specific spots which usually appear on the chromatograms. One other advantage of the procedure is the possibility of employing it in elucidating the fate of ingested nicotinyl compounds in man, where one could not easily use radioactive substances employed successfully in the rat (Johnson & Lin, 1951; Leifer *et al.* 1951). The method is sufficiently sensi-

tive to detect amounts of importance, although in certain cases one has to take recourse to the more sensitive microbiological technique (Leifer *et al.* 1950), as will be discussed below. One drawback of the method is that it could not be used for the detection of N^1-methyl-2-pyridone-5-carbonamide which is of particular importance in human urines. Furthermore, the method does not detect quinolinic acid.

The fluorescent procedure for detecting quaternary nicotinyl compounds is highly sensitive and would allow the differentiation of a number of compounds besides N^1-methylnicotinamide, should they appear in the urines. It is interesting that in no case could any other quaternary nicotinyl derivative, such as the pyridine nucleotide coenzymes or N^1-methylnicotinuric acid betaine, be found.

Metabolites in normal urine

In urine from undosed human subjects, no nicotinyl compound other than N^1-methylnicotinamide could be detected on the chromatograms. The high values of the total alkali-hydrolysable nicotinyl derivatives obtained by chemical methods may have been due to interfering substances which the blank did not correct. This is supported by the relatively low values found microbiologically. No such interference in the chemical method was found in the urines after dosing because of the small amount of urine used for assay. The analysis of chromatograms by the microbiological procedure showed the presence of a small amount of nicotinamide, of the order of 0·075 μg./ml. of urine. This concentration was below that detectable by the spraying procedure. The rest of the microbiological activity remained at the origin of the chromatograms and we were unable to identify these unknown active substances. Since large amounts of urine concentrate had to be applied to the paper to obtain a satisfactory microbiological response, and since the original spots spread to the region where normally nicotinuric acid appears, we could not eliminate the possibility of an occurrence of small amounts of nicotinuric acid; however, no nicotinic acid seemed to be present. Perlzweig *et al.* (1940) and Sarrett *et al.* (1942) reported the presence of small amounts of nicotinuric acid, while Melnick *et al.* (1940) could not find any in normal urine. Ellinger & Kader (1949) reported the presence of both nicotinic acid and nicotinamide. These discrepancies may be due to defects inherent in chemical methods. In contrast to the human urines, the urines both of the nicotinic acid-deficient and normal rats, which were not given any doses, contained detectable amounts of nicotinic acid and nicotinamide, in addition to small amounts of N^1-methylnicotinamide. These metabolites were present in a significantly higher concentration in the normal group.

Metabolites of nicotinic acid

The ingestion of nicotinic acid by man is followed within the first 3 hr. by the excretion of a glycine conjugate, nicotinuric acid, and of the N^1-methylated nicotinyl derivatives, of which N^1-methylnicotinamide could be detected in these studies. It is interesting to find a small rise in the excretion of nicotinamide, indicating not only that amidation has taken place, but also that a portion of this metabolite is being lost to the organism. It appears from our results that free nicotinic acid is not a normal constituent in the human urine after dosing, but is found only under certain conditions, associated with the known pharmacological effects of this compound. It seems that it is due to a sudden occurrence in the organism of a high concentration of nicotinic acid which cannot be dealt with satisfactorily by the normal metabolic mechanisms. The large excretion of nicotinic acid was particularly pronounced when the dose was taken on an empty stomach. It seems probable that the liver is mainly concerned with the formation of the glycine conjugate, and if it cannot perform this function free nicotinic acid circulating in the blood causes the vasodilatory symptoms and is ultimately excreted in the urine. The appearance of free nicotinic acid in the urine may possibly be a test for the efficiency of liver function, although the effect on the vessels of the kidney may be a complicating factor. A distribution of metabolites similar to that in man was found in the rat, but in this case nicotinic acid appeared consistently in appreciable amounts in the urines. This may have been due to the relatively large dose administered to the animals. The deficient animals excreted a smaller amount of nicotinyl metabolites than the normal group, but the excretion pattern did not change. It would be of interest to see if similar results were obtained in human deficiency.

Our results on human urines are in keeping with those of Holman & de Lange (1950*a*) who found that ingestion of nicotinic acid resulted in a greater excretion of non-methylated derivatives of nicotinic acid than after ingestion of nicotinamide. In either instance 76–80 % of the dose could be accounted for, when the excretion of N^1-methyl-2-pyridone-5-carbonamide was included. The presence of free nicotinic acid in the urines of individuals dosed with nicotinic acid, was reported by Ellinger & Kader (1949) who, on the other hand, could not find in human and rat urines any nicotinuric acid which in our studies formed the main excretory product. Our results on rat urines are in good agreement with those of Johnson & Lin (1951) and Leifer *et al.* (1951) who fed radioactive nicotinic acid to rats.

Metabolites of nicotinamide

The spectrum of metabolites appearing in human urines after dosing with nicotinamide was different from that found after ingestion of nicotinic acid. N^1-Methylnicotinamide appeared to be the major excretion product after dosing with nicotinamide, but we have no data for N^1-methyl-2-pyridone-5-carbonamide which is known to be an important metabolite (Knox & Grossman, 1946, 1947; Holman & de Lange, 1949). Of the tertiary nicotinyl compounds only nicotinamide was found in the urine. It thus seems that no appreciable deamidation occurred, sufficient to cause an excretion of free nicotinic acid or its glycine conjugate. Our results differ from those of Johnson *et al.* (1945) who found, by microbiological methods, free nicotinic acid in the urines. Ellinger & Kader (1949) could detect chemically only an increase in N^1-methylnicotinamide. Our results are based on direct evidence from chromatograms and are therefore more specific than those obtained by indirect methods.

In contrast to man, both deficient and normal rats excreted nicotinamide and a relatively smaller amount of nicotinic acid as well as N^1-methylnicotinamide. No nicotinuric acid was found, suggesting that also in the rat conjugation with glycine does not play any role in the disposal of nicotinamide. Our findings are in good agreement with data obtained from the isotope experiments of Leifer *et al.* (1951). On the other hand, Johnson & Lin (1951) by tracer techniques, and Perlzweig, Rosen & Pearson (1950), reported the presence of nicotinuric acid in rat urines after dosing with nicotinamide.

While only 50% of the dose of nicotinamide appeared in urines of deficient rats, almost the entire dose was recovered in the normal animals, the major part as N^1-methylnicotinamide. Similarly, a higher proportion of the dose of nicotinic acid was excreted by the normal than the deficient group. It would appear that in the presence of sufficient dietary tryptophan the rat either has preference for the tryptophan-nicotinic acid conversion or the ingestion of nicotinyl compounds may influence favourably this conversion of tryptophan. This may be the reason why in normal rats such a high recovery of the dose of nicotinic acid or amide was observed. This is in agreement with the findings of Spector (1948).

Metabolites of L-*tryptophan*

In man, after ingestion of tryptophan, an increased excretion of N^1-methylnicotinamide was found, but not of other nicotinyl derivatives. Similar observations were earlier reported by Sarrett & Goldsmith (1947, 1949, 1950) and by Sarrett (1950*a*). Perlzweig, Rosen, Levitas & Robinson (1947) observed by microbiological methods a small increase in the excretion of nicotinyl compounds. The excretion of N^1-methyl-2-pyridone-5-carbonamide, which rises significantly after administration of tryptophan (Holman & de Lange, 1950*b*), and of quinolinic acid, which forms a major excretion product in rats (Henderson, 1949*a*), and in man (Sarrett, 1950*a*), was not determined since these substances could not be identified on paper chromatograms with the present procedures.

In the rat, possibly due to its more efficient conversion of tryptophan to nicotinic acid, there was a small but definite increase in the excretion of nicotinic acid, while that of nicotinamide did not rise. This was the case both in the deficient and normal rats, but the latter excreted more of the nicotinyl metabolites than the deficient ones. The finding of nicotinic acid rather than the amide could be taken as circumstantial evidence that tryptophan is converted primarily into nicotinic acid. This is in keeping with the conclusions of Heidelberger, Abraham & Lepkovsky (1948, 1949) who showed the conversion by rats of labelled tryptophan into N^1-methylnicotinamide.

Sarrett (1950*a*, *b*) found an increase in the excretion of xanthurenic acid (4:8-dihydroxyquinoline carboxylic acid) in man after administration of tryptophan and of an ether-soluble tryptophan-like metabolite which seemed to be derived from D-tryptophan (Sarrett & Goldsmith, 1950). So far as we are aware, no other metabolites have been reported in the urine of humans dosed with tryptophan. In the present study, 3-hydroxyanthranilic acid, kynurenic acid and kynurenine have been found in the human urines. It seems, therefore, that the conversion of tryptophan in man follows the same pathway as was found for the rat (Heidelberger *et al.* 1949). An appearance of tryptophan in the urines was not unexpected, but there were several other tryptophan metabolites which could not be definitely identified. It was only possible to compare them with published paper-chromatographic data recently reported by Dalgliesh *et al.* (1951) and there is not sufficient evidence for a definite identification.

Metabolites of quinolinic acid

Dosing with large amounts of quinolinic acid in normal rats resulted in an increased excretion of N^1-methylnicotinamide and of nicotinic acid. This is in keeping with the findings of Henderson (1949*a*, *b*) and would indicate that the rat can convert quinolinic acid into nicotinic acid. However, no such effect was observed in deficient rats, but the declining growth of the rats was reversed and they increased in weight by 17 g. within 13 days after a single dose of 100 mg. quinolinic acid. It is possible

that the conversion into nicotinic acid was of such low order that all the vitamin was retained by the deficient animal and none was available for excretion. We would agree with Krehl, Bonner & Yanofsky (1950) that the metabolism of quinolinic acid is of the order of a side reaction rather than forming a keystone in the nicotinic acid turnover.

SUMMARY

1. The excretion of metabolites by man and the rat after administration of nicotinic acid, nicotinamide and tryptophan has been studied by paper-chromatographic techniques. The procedure did not allow for the detection of N^1-methyl-2-pyridone-5-carbonamide.

2. After a dose of 100 mg. nicotinic acid to man, the main nicotinyl metabolites in the urine were, in decreasing order, nicotinuric acid, N^1-methylnicotinamide and nicotinamide. Nicotinic acid appeared in the urine only when flushing of the skin and other vasodilatory symptoms occurred. In the rat, nicotinic acid appeared consistently in the urines, but otherwise the pattern of excretion remained the same as in man.

3. The administration of 100 mg. nicotinamide to human subjects caused only an increased excretion of N^1-methylnicotinamide and of nicotinamide. Rat urine contained, in addition, an increased amount of nicotinic acid. Nicotinuric acid was not excreted by either of the species.

4. After ingestion of 3 g. L-tryptophan, human urines showed an increase in the excretion of N^1-methylnicotinamide, while rat urines had in addition an increased output of nicotinic acid. In human urines, a number of tryptophan metabolites, namely kynurenic acid, 3-hydroxyanthranilic acid, kynurenine and tryptophan, were identified.

5. Administration of 100 mg. quinolinic acid to normal rats resulted in an increased excretion of N^1-methylnicotinamide and nicotinic acid, while rats deficient in nicotinic acid showed no such rise.

6. The differences in the excretion patterns between nicotinic acid-deficient and normal rats after dosing with the various substances have been discussed.

7. In urines from undosed human subjects, microbiological techniques instead of the chemical procedure had to be used to detect on chromatograms the tertiary nicotinyl compounds because of the small amounts of nicotinyl derivatives present. The urines contained a small amount of nicotinamide and of N^1-methylnicotinamide. Urines from undosed rats, both deficient and normal, contained N^1-methylnicotinamide, nicotinamide and nicotinic acid.

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The Sugar Content of Nectars

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Observations of the selection by honeybees of solutions of sugars which occur in nectar showed that sucrose, glucose and fructose were not equally attractive to bees, which appeared to prefer a mixture of the three sugars (Wykes, 1952*a*). Few determinations of individual sugars present in different nectars have been carried out, and relatively large volumes were necessary for the analytical methods used by Beutler (1930), Vansell (1944) and others. However, as the flowers of most plants secrete nectar sparingly, such methods cannot be applied to investigations of nectar available from a wide range of honey-producing species. By using the technique of paper partition chromatography it has been possible to carry out qualitative determinations of sugars present in nectar from many species (Wykes, 1952*b*). These analyses confirmed the earlier work of Beutler (1930), showing that sucrose, glucose and fructose, sugars readily accepted by honeybees (von Frisch, 1934), are normal constituents of nectar; they also showed that other sugars may occur, which is in agreement with the work of Täufel & Reiss (1952). During the summer of 1951 this technique was applied to quantitative investigations of nectar sugars secreted by flowers of some important British honey-producing species and others which appeared from the earlier qualitative work to secrete nectar of unusual sugar composition.

METHODS

At intervals during the flowering period of species studied, samples of nectar were gathered at random from plants in the field. Shortly after collection determinations of the sugar content of each nectar sample were carried out as follows:

(*a*) *Glucose and fructose.* Individual sugars were separated on paper chromatograms according to the general method of Partridge (1948), using modified methods adopted by Jermyn & Isherwood (1949) for quantitative analyses.

Six spots of every nectar sample, each 2·4 μl., were introduced with a micropipette (Hawthorne, 1947) 5·5 cm. apart on a sheet of Whatman no. 1 filter paper. The solvent was the *n*-butanol-acetic acid-water mixture described by Partridge (1948). After 72–96 hr. the chromatograms were removed from the tank and air-dried. Two vertical strips, each containing the separated component sugars of the two outside spots, were cut from the sides of the papers and sprayed with either a benzidine-trichloroacetic acid reagent (Bacon & Edelman, 1951), or a *p*-anisidine hydrochloride reagent (Hough, Jones & Wadman, 1950) with a few crystals of $SnCl_2$ added. By reference to the two guide strips, the four areas of paper each containing one of the separated sugars (glucose and fructose) were cut from the chromatogram. The quadruplicate papers for each sugar and a corresponding paper blank were refluxed (Flood, Hirst & Jones, 1947) and the reagents of Somogyi (1945) and Nelson (1944) were used for quantitative determinations of glucose and fructose. Because of practical limitations of time and equipment, no sucrose estimations were carried out.

(*b*) *Total sugars.* Measurements of total sugars present in each sample were made with a refractometer giving sugar concentration, expressed as percentage sucrose, on a direct-reading scale. As the work of Schneller (1926), Zerban & Martin (1944) and others suggested that a close agreement exists between the refractive indices of the main constituent sugars of nectar, no corrections were applied to the readings.

RESULTS

It will be seen from Table 1 that the proportions of the constituent sugars varied markedly in nectar secreted by different species. For any one species, however, the figures for the ratio of fructose/glucose and of (total sugars)/(fructose + glucose) were reasonably constant. The greatest variation in the fructose/glucose ratio was shown in nectar from mountain mint (*Pycnanthemum pilosum* Nutt.), but for this species glucose was present in amounts which were too small to be estimated reliably by the methods used.