

No appreciable reaction took place when NaF, in place of KF, was heated with 1:2:4-chlorodinitrobenzene employing the conditions given above.

Physiological examination

(1) A batch of 23 rabbits, rats, guinea-pigs and mice were exposed to a Ct* of 5000 mg. min./m.³ in a 1.87m.³ static chamber. The material was sprayed in ether (25 ml.). During exposure the animals suffered from irritant action, but recovered after being removed from the chamber. At the end of a week there were no deaths. The batch was then re-exposed to the same Ct. The irritation seemed more intense and all four guinea-pigs died in 48 hr. The post-mortem examination showed changes typical of those caused by a lung irritant.

(2) Subcutaneous injection into mice, using propylene glycol as solvent: 100 mg./kg. killed 1/6.

(3) *Vesicancy on human skin.* Benzene solutions of 1:2:4-fluorodinitrobenzene (F) were dropped on to the right arms of two subjects, and similar solutions of 1:2:4-chlorodinitrobenzene (Cl) were dropped on to the left arm. There was free evaporation.

5 mg. drops of 1% solution (i.e. 50 µg.) had no effect within 4 days. 5 mg. drops of 10% solutions (i.e. 500 µg.) produced considerable irritation as follows:

* Ct is the dose expressed as concentration mg./m.³ × time of exposure (min.).

Subject 1. *F patch.* Smarting and swelling in 7 hr., blister without surrounding erythema in 36 hr. No change when blister was pricked on 3rd day. Scar almost healed by 9th day. *Cl patch.* During the first 9 days there was no more than a red weal. By the 12th day there was irritation and swelling which subsided on the 15th day.

Subject 2. *F patch.* Immediate smarting and swelling which persisted for 1 day, and subsided the next day without a blister. After 3 days the skin broke down with formation of pus and scabs. After 8 days only a scar remained. *Cl patch.* Slight redness during first 8 days. Swelling on 9th day and weal rash of vesicles on 10th day. The spots oozed freely on the 14th day. Recovery on 20th day.

SUMMARY

1. A convenient method of preparing 1:2:4-fluorodinitrobenzene is described.

2. The compound is relatively non-toxic in comparison with the highly toxic fluoroacetates. It produces, however, a slight vesication on human skin.

Our thanks are due to the Director General of Scientific Research (Defence) for permission to publish the above and to Mr K. J. Carpenter who carried out the physiological examination of the action of the compound on animals.

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* Information available on application to Ministry of Supply, London.

The Utilization of Nicotinamide Derivatives and Related Compounds by Mammals, Insects and Bacteria

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This investigation was prompted by the desire to find an antipellagra drug of the efficiency of nicotinamide, but of a greater lasting effect similar to that of nicotindimethylamide (nikethamide) (Ellinger & Coulson, 1944). For this purpose a number of nicotinamide derivatives in which one or both hydrogen atoms of the amide were replaced by alkyl, cyclo-

hexyl, or aryl groups were tested for their ability to form nicotinamide and to exhibit antipellagra activity. This aim was not reached, but the investigation gave results which are of interest, since they give some information about the relation of chemical constitution to pharmacological action and about the ability of widely different species to transform

nicotinamide derivatives and related compounds into nicotinamide or nicotinic acid which are essential to all of them.

Anti black-tongue activity of a large number of such compounds has been studied by Woolley, Strong, Madden & Elvehjem (1938); SubbaRow, Dann & Meilman (1938); Strong, Madden & Elvehjem (1938); SubbaRow & Dann (1938); Waisman, Mickleson, McKibbin & Elvehjem (1940); Smith, Margolis & Margolis (1940) and Teply, Krehl & Elvehjem (1945). These authors found the following to be active: nicotinic acid, nicotinamide, nicotinmethylnamide, nicotindiethylamide, nicotinamide glucosidoiodide, nicotinuric acid and to a lesser degree β -picoline, whilst trigonelline, nicotinamide methochloride, pyridine, pyridine-3-sulphonic acid, 6-methylnicotinic acid, 3-acetylpyridine, 3-aminopyridine, quinolinic acid, picolinic acid, isonicotinic acid, hexahydronicotinic acid, nicotinonitrile, pyrazine mono- and 2:3-dicarboxylic acids, and thiazole-5-carboxylic acid were found to be inactive in canine black-tongue. Nicotinuric acid was found inactive by Dann & Handler (1941) and nicotinamide methochloride active by Najjar, Hammond, English, Wooden & Deal (1944).

Antipellagra activity has been mainly studied by Spies and his co-workers (Spies, Bean & Stone, 1938; Spies, Grant & Huff, 1938; Vilter & Spies, 1939; Vilter, Bean & Spies, 1938; Bills, McDonald & Spies, 1939), and by Smith *et al.* (1940). The results are less definite than those obtained for black tongue activity. It is certain, however, that nicotinic acid, nicotinamide, nicotinethylamide and -diethylamide, ethyl nicotinate, quinolinic acid, and pyrazine mono- and 2:3-dicarboxylic acids are active against pellagra. α -Picoline, trigonelline, and 3-aminopyridine are inactive, whilst β -picoline, 2:6-dimethylpyridine-3:5-dicarboxylic acid, and pyridine-3:5-dicarboxylic acid are of doubtful activity.

Nicotinamide methochloride has been found to be formed in the mammalian body after administration of nicotinic acid, nicotinamide or nicotindiethylamide (Najjar & Holt, 1941; Ellinger & Coulson, 1944). The former authors found no increased nicotinamide methochloride elimination after administration of trigonelline, 2:6-dimethylpyridine-3:5-dicarboxylic acid, quinolinic acid, pyridine-3:5-dicarboxylic acid and pyrazine mono- and 2:3-dicarboxylic acids.

Very little is known of the pharmacological and toxicological effects of nicotinamide and related compounds in mammals. The lethal doses of nicotinic acid, nicotinamide, nicotindiethylamide, pyridine, pyridine methochloride, trigonelline, nicotinamide methochloride, and nicotindiethylamide methochloride have been studied for the rat, mouse, and guinea-pig by Chen, Rose & Robbins (1938), Unna (1939) and Brazda & Coulson (1946). Whilst Ackermann (1913) failed to observe any toxic effect of nicotinic acid 'in medium-sized dogs after ingestion of 9 g. during 5 days', Chen *et al.* (1938) report lesions of the intestinal mucosa and central nervous system in dogs after large doses of nicotinic acid.

The need of insects for nicotinic acid seems to be general. This has been demonstrated, e.g. for *Droso-*

phila (Tatum, 1939, 1941), mosquitoes (SubbaRow & Trager, 1940), *Galleria* (Rubinshtein & Shekun, 1941), *Tribolium* and many other beetles and moths (Fraenkel & Blewett (1943*b*, 1946*a, b*), Blewett & Fraenkel, 1944). The effects of nicotinamide and other derivatives of nicotinic acid on insects have, however, never been investigated.

The utilization of a number of compounds related to nicotinic acid by various bacteria (*Staphylococcus aureus*, *Proteus vulgaris*, dysentery bacilli, *Lactobacillus arabinosus*) has been studied by Landy (1938), Fildes (1938), Lwoff (1938), Lwoff & Querido (1939), Dorfman, Koser, Reames, Swingle & Saunders (1939), Pelczar & Porter (1940), Elvehjem & Teply (1943) and v. Euler, Högberg, Karrer, Salomon & Ruckstuhl (1944). Of the compounds tested, nicotinic acid, nicotinamide, methyl, ethyl, propyl and butyl nicotinate, and nicotinuric acid could be utilized by all bacteria examined, nicotinethylamide and -diethylamide and quinolinic acid were reported to be utilized by *Proteus* and dysentery bacilli. β -Picoline, nicotinonitrile, pyridine-3-sulphonic acid only by *Proteus*, tetrahydro-, and hexahydronicotinic acid (nipecotic acid) by *Staphylococcus aureus* and *Proteus*, and picolinic acid and isonicotinic acid by none of the bacteria. Hexahydronicotinic acid had only very little activity in promoting growth of *Lactobacillus arabinosus* (Teply *et al.* 1945). Najjar, Scott & Holt (1943) considered nicotinamide methochloride a powerful growth promoter for *Escherichia coli* and *Haemophilus influenzae*.

In the present investigation a number of new and known nicotinamide derivatives have been examined for their general pharmacological action and toxicity on mice or rats. Their ability to form nicotinamide and nicotinamide methochloride and the speed of elimination was also investigated. Attempts were also made to study the mechanism of the breakdown of the substances in the body. The utilization of nicotinamide derivatives by insects was tested by their effects upon growth and development of larvae of *Tribolium confusum*, and utilization by bacteria by effects on growth and acid formation of *Lactobacillus arabinosus*, *Proteus* and dysentery strains. The formation of nicotinamide methochloride from nicotinamide by *Tenebrio* larvae and by some *Proteus* strains has been investigated.

METHODS

The drugs used in the experiment are shown in Table 1. The purity of hitherto unknown substances made by Bayer was checked by analysis. Hydrochlorides were dissolved in water, bases in equivalent amounts of dilute HCl and acids in equivalent amounts of dilute NaOH. Nikethamide was obtained as 25% aqueous solution.

Experiments on mammals. General pharmacological action and toxicity were observed in mice, hooded rats (Lister stock), and albino rats (own stock of P.E.). The drugs were administered in 5-20% solution by intraperitoneal injections to mice of 20-30 g. and rats of 150-300 g. To find the LD₅₀ the region was first explored with doses differing from each other by 50% and, when a suitable

order of dose had been established, groups of 4 mice or rats (2, if material was limited) were given doses differing by 10% from each other; at the same time the general pharmacological effect of the various drugs was studied.

To investigate the increased urinary elimination of nicotinamide methochloride after administration of the various drugs, rats of 200–300 g. were kept in metabolism cages, 24 hr. urine samples were collected and their nicotinamide methochloride content estimated by the method of Coulson, Ellinger & Holden (1944). During this period the rats were on the following diet: 10 g. of a mixture composed of 4.0 g. of a grain mixture, 3.5 g. of extracted liver (British Drug Houses), 1.0 g. of wheat germ, 0.3 g. of dried yeast, 0.5 g. of McCollum's salt mixture, no. 185 (McCollum, Simmonds & Pitz, 1917), 0.6 g. of maize oil and 0.1 g. of cod-liver oil, 13 g. of bread and 1.5 g. of dried milk (Glaxo) in 30 ml. of water. The content of nicotinamide in the diet, estimated chemically (Ellinger & Abdel Kader, 1947), was 1.0 mg. and of nicotinic acid 0.8 mg. A number of 24 hr. urine samples (3–5) were examined for nicotinamide methochloride (N.M.), then nicotinamide (20 mg.) was injected intraperitoneally and the response to this dose examined by determinations continued until the predosing level was regained. From the extra output (extra N.M.) in mg. the response x , was calculated by the following formula:

$$x = \frac{\text{mg. extra N.M.}}{\text{mg. } D \text{ ingested}} \times 100 \times \frac{\text{mol. wt. } D}{\text{mol. wt. N.M.}},$$

where D is the compound ingested. This gives the percentage of the nicotinamide dose which appears in its methylated form in the urine. After an interval of 7 days one of the other drugs was injected in equimolar dose to the rats calibrated with nicotinamide and the response was calculated in a similar manner. All drugs examined were tested in duplicate at least.

In order to gain further information on the transformation of the substituted nicotinamide compounds into nicotinamide methochloride, the following experiments were carried out similarly to those described by Ellinger (1946). Rats were stunned and bled, the various organs were removed, pieces were cut off and weighed in wet state, then cut into slices of 0.2–0.4 mm. thickness. These were suspended in 10 ml. of modified Ringer solution (Krebs & Henseleit, 1932), aerated with an O_2 - CO_2 mixture, and 10 μ g./ml. of the various substituted nicotinamide derivatives were added. The tubes contained either liver alone, or slices of one of the following organs alone or with liver; kidney, brain, spleen, skeletal muscle, heart muscle, pancreas or intestinal mucous membrane. The mixture was agitated at 37° for 2–3 hr. After cooling to room temperature, the supernatant liquid was separated from the tissue, protein was removed with trichloroacetic acid and by heating to 70–80° followed by centrifuging. Nicotinamide methochloride was estimated in the supernatant liquid by the method of Coulson *et al.* (1944).

Experiments on insects. The experiments were carried out on larvae of *Tribolium confusum* by the method of Fraenkel & Blewett (1943a, b). The nicotinic acid-free diet consisted of 50 g. of acid-extracted casein (Glaxo), 50 g. of anhydrous glucose, 5 g. of the water-insoluble fraction of yeast (exhaustively extracted with hot water), 1 g. of cholesterol, 2 g. of McCollum's salt mixture no. 185 (McCollum *et al.* 1917) and 10 ml. of water. The following vitamins of the B complex were added (μ g./g. dry diet): aneurin, 25; ribo-

flavine, 25; pyridoxin, 12.5; pantothenic acid, 25; choline chloride, 250; inositol, 250. All tests were performed at 25° and 70% relative humidity. The compounds to be tested were added to the diet in various quantities and their effect compared with that of nicotinic acid and nicotinamide. The growth of the larvae, the number of pupae, and the average time of pupation formed the basis for a quantitative comparison.

To study the question whether nicotinamide methochloride is formed by insects, 60 g. of larvae of *Tenebrio* grown in white flour (75% extraction) with the addition of 5% yeast were killed, frozen, ground with sand, dried to about 20 g. weight, extracted with 0.1N-HCl, and filtered. The filtrate (150 ml.) was dialyzed three times for 2 days against 200 ml. of distilled water each time. The dialysates were combined, reduced to 50 ml. by evaporation *in vacuo* and then examined for nicotinamide methochloride by the method of Coulson *et al.* (1944). 'F₂' was tested for by reactions with 12N-KOH, K₃Fe(CN)₆ and alkaline acetone (Najjar *et al.* 1943).

Experiments on bacteria. The various drugs were tested on the following bacteria which require nicotinic acid or nicotinamide for growth: *Lactobacillus arabinosus*, obtained from Dr T. F. Macrae; *Proteus vulgaris* 401, 3156, 5887, from the National Collection of Type Cultures; *Proteus morgani*, isolated from rat faeces; *Proteus X 13 syriac*, from Dr A. Lwoff, Paris; *Shigella sonnei* 2941; Flexner 4832; and Shiga 4837, all from the National Collection of Type Cultures. In addition, the effect of nicotinamide methochloride on growth and acid formation of *Esch. coli* was studied in an ammonium-glucose medium on five strains isolated from human and rat faeces. The medium used for *Lactobacillus arabinosus* was that described by Barton-Wright (1944); for the other bacteria a medium of the following composition was used; K₂HPO₄, 2.25 g.; NH₄Cl, 0.25 g.; (NH₄)₂SO₄, 0.25 g.; FeSO₄·(NH₄)₂SO₄·6H₂O, 0.01 g.; MgSO₄·7H₂O, 0.01 g.; the mixture was made up with distilled water to 250 ml. The pH was adjusted to 7.6 with N-NaOH. The solution was boiled and filtered while hot through a no. 50 filter. The volume was brought to 400 ml. after cooling and the solution was then autoclaved; 0.25 ml. of sterile 0.5M-glucose solution was added to 8 ml. of the salt solution. After adding the sterile test solutions of the nicotinamide derivatives the volume was made up to 10 ml. with sterile water. Two drops of a thin emulsion of the inoculum were added to the medium. After 2–3 days of incubation at 37°, growth was measured by visual estimation of the turbidity and by determination of the acid produced using 0.1N-NaOH and bromothymol blue as internal indicator. In each experiment blanks were done without inoculum and without the addition of nicotinamide compounds, and the values of the latter were deducted from the experimental values. Tests with nicotinic acid and nicotinamide at different concentrations were carried out as standards for comparison. The compounds to be tested were used in at least two concentrations. In each case the efficiency of the tested compound in forming acid was compared with that of nicotinic acid.

To examine for possible formation of nicotinamide methochloride from nicotinamide or nicotinic acid, *Proteus vulgaris* 3156 was incubated for 48 hr. both aerobically and anaerobically in a casein hydrolysate medium (Barton-Wright, 1944) containing 1 μ g./ml. nicotinamide or nicotinic acid. Samples were autoclaved and examined for

nicotinamide methochloride. The remainder was centrifuged, and the supernatant liquid also examined for nicotinamide methochloride by the method of Coulson *et al.* (1944). Controls were done by incubating the medium alone and by estimating the nicotinamide methochloride content immediately after inoculation without incubation.

RESULTS

Pharmacological action. The pharmacological effect of all the compounds tested was qualitatively almost identical, viz. an action on the central nervous system, excitation with convulsions, narcosis, paralysis of the respiratory and the heat

regulation centres. In some cases, nos. 12-16, the body temperature of the animals was little above that of their surroundings and changed with it, if the poisoning lasted for more than 24 hr.; none of these animals recovered. With some of the compounds (1, 4, 6, 7, 18) the exciting action predominated; mice developed a 'morphia tail' and jumped like kangaroos; both mice and rats were very restless, convulsions developed and frequently the animals died as a result of one of these. With most of the other compounds the narcotic action was predominant, the animals fell asleep, respiration deteriorated and after a few convulsions death super-

Table 1. *Toxicity and utilization of nicotinic acid, nicotinamide and related compounds in mice and rats*

(The values given in the column for nicotinamide methochloride response are averages of four experiments; the limiting values are given in brackets.)

No.	Name of compound	Toxicity (LD50)				Nicotinamide methochloride response* (% of theoretical)	Period of elimination (hr.)
		(a) Mouse		(b) Rat			
		(g./kg.)	(mmol./kg.)	(g./kg.)	(mmol./kg.)		
1	Nicotinic acid	4.5	36.55	3.75	30.90	11.8 (7.9-13.3)	48
4	Nicotinamide	1.75	14.32	1.50	12.28	22.7 (18.2-27.8)	48
5	Nicotinethylamide†	0.65	4.34	0.52	3.47	17.2 (14.3-19.1)	48
6	Nicotindiethylamide	0.42	2.36	0.45	2.55	16.8 (14.7-18.2)	72
7	Nicotinallylamide	0.55	3.40	0.45	2.78	15.7 (13.6-16.9)	48
8	Nicotincyclohexylamide	0.35	1.71	0.45	1.99	0	—
9	Nicotin-(2'-methylcyclohexyl)-amide	0.175	0.80	0.20	0.91	0	—
10	Nicotin-(3'-methylcyclohexyl)-amide	0.12	0.51	0.13	0.60	0	—
11	Nicotin-(4'-methylcyclohexyl)-amide	0.18	0.83	0.20	0.91	0	—
12	Nicotinphenylamide	0.375	1.90	0.40	2.02	6.5 (4.7-8.3)	72
13	Nicotindiphenylamide	0.07	0.26	0.05	0.18	0	—
14	Nicotin-(4'-methoxyphenyl)-amide	0.35	1.53	0.40	1.76	6.2 (3.8-7.6)	72
15	Nicotinbenzylamide	0.08	0.38	0.07	0.34	5.8 (4.2-6.9)	72
16	Nicotindibenzylamide	0.035	0.12	0.035	0.12	0	—
17	Trigonelline hydrochloride	4.80	27.68	5.20	30.00	0	—
18	Nicotinamide methochloride	0.48	2.78	0.95	5.50	62.7 (56.2-68.3)	24
19	Nicotinonitrile	0.15	1.44	0.20	1.92	0	—
23	Quinolinic acid	0.45	2.69	0.47	2.81	5.2 (3.7-5.9)	48
24	2-Carboxypyridine-3-carboxylic acid cyclohexylamide	0.35	1.41	0.35	1.41	0	—
29	β-Picoline	0.45	4.85	0.48	5.16	41.3 (35.7-53.4)	48-72

* See p. 561.


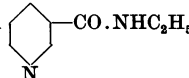
† The prefix "nicotin-" is used in this paper for the radical . Nicotinethylamide, therefore, stands for the compound .

Table 2. Utilization of nicotinic acid, nicotinamide and related compounds by *Tribolium*

No.	Name of compound	Amount of drug in diet ($\mu\text{g./g.}$)	Surviving larvae		No. of pupae	Average time of pupation (days)
			No.	Size (mm.)		
1	Nicotinic acid	2	—	—	14	44.6
		4	—	—	16	37.4
		8	—	—	20	37.6
		16	—	—	20	35.8
		32	—	—	18	35.4
2	Methyl nicotinate		Same activity as nicotinic acid			
3	Ethyl nicotinate	8	—	—	19	45.0
		16	—	—	18	36.1
		32	—	—	20	35.8
		64	—	—	18	35.6
4	Nicotinamide	2	20	3-5	0	0
		4	—	—	18	41.1
		8	—	—	17	37.0
		16	—	—	19	35.4
		32	—	—	19	36.1
5	Nicotinethylamide	100	19	3-5	0	0
		300	—	—	20	49.8
		900	—	—	15	35.8
6	Nicotindiethylamide	5	5	2-3	0	0
		25	12	2-3	0	0
		125	16	2-3	0	0
		625	—	—	19	40.4
7	Nicotinallylamide	20	17	4-5	0	0
		40	—	—	20	39.5
		80	—	—	17	34.3
8	Nicotincyclohexylamide	1000	—	—	Few	>100
9	Nicotin-(2'-methylcyclohexyl)-amide	1000	—	—	Few	>100
11	Nicotin-(4'-methylcyclohexyl)-amide	1000	—	—	Few	>100
12	Nicotinphenylamide	100	—	—	18	35.5
		300	—	—	19	37.3
		900	—	—	18	38.0
14	Nicotin-(4'-methoxyphenyl)-amide	20	—	—	17	37.1
		40	—	—	19	36.1
		80	—	—	13	35.3
15	Nicotinbenzylamide	100	19	3-4	0	0
		1000	—	—	17	36.7
18	Nicotinamide methochloride	100	No growth		0	0
		500	No growth		0	0
		2500	—	—	Few	>100
19	Nicotinonitrile	100	—	—	Few	>100
		1000	—	—	Few	>100
23	Quinolinic acid	20	No growth		0	0
		100	No growth		0	0
		500	20	4	0	0
		2500	—	—	17	39.0
29	β -Picoline	250	17	4	0	0
		2500	—	—	16	60
		5000	—	—	19	49
		10000	—	—	15	44
		20000	—	—	18	40

vened from paralysis of the respiratory centre. Some of the compounds (nos. 1, 7 and 13) produced a peripheral vasodilatation, another (no. 29) a distinct hypersensitivity to touch of tail and legs. Generally, death occurred within 2 hr. of the injection of the compound. If this critical period was overcome there was generally complete recovery. In a few cases, however, an apparent recovery was followed after 24–48 hr. by collapse and death from respiratory paralysis. In nearly all the compounds, the LD₅₀ was very well defined and frequently one quarter or one half of the lethal dose had no recognizable effect on the animals.

Nicotinamide methochloride formation by animal tissues in vitro. The experiments carried out with tissue slices to identify the organ responsible for the breakdown of the drug to nicotinamide or nicotinic acid gave negative results. Nicotinamide methochloride was produced only, when either nicotinamide was shaken with liver slices, or nicotinic acid with liver and kidney, or liver and brain slices.

The utilization of various pyridine derivatives by insects

The following compounds produced neither growth of larvae nor pupation in doses up to 1 mg./g. dried food: nicotin-(3'-methylcyclohexyl)-amide, nicotindiphenylamide, nicotindibenzylamide, trigonelline hydrochloride (prepared according to Hantzsch, 1886), 6-hydroxynicotinic acid (Light and Co.), picolinic acid (Roche), pyridine-4-carboxylic acid (Light and Co.), 2-carboxypyridine-3-carboxylic acid cyclohexylamide (Bayer), pyridine-3:4-dicarboxylic acid (Light and Co.), pyridine-2:5-dicarboxylic acid (Roche), pyridine, α -picoline (B.D.H.) and γ -picoline (Light and Co.).

There was no growth at all when the larvae were fed the basal diet. Usually the minimum concentration of nicotinic acid in the diet required for optimal growth was 8 μ g./g., but on one occasion optimal growth was obtained with 4 μ g./g. In all experiments the efficiency of methyl nicotinate equalled that of nicotinic acid, whilst the ethyl ester had about one quarter and nicotinamide about one half of the activity of nicotinic acid.

Nicotinamide methochloride content of Tenebrio larvae. An extract obtained by the addition of dilute HCl (450 ml.) to *Tenebrio* larvae (60 g.) contained 7.4 μ g. nicotinamide methochloride/60 g. wet or 20 g. dry larvae. The alkaline isobutanol solutions of the Coulson, Ellinger & Holden test gave a greenish fluorescence with 12N-NaOH which faded on standing at room temperature, turning yellow. With dilute ferricyanide a deep violet fluorescence developed and the addition of alkali and acetone produced a yellow colour with green fluorescence. This proved the identity of the fluorescent substance with 'F₂'.

The utilization of various pyridine derivatives by bacteria

Compounds no. (7) nicotinylamide (Bayer), (8) nicotincyclohexylamide (Bayer), (9) nicotin-(2'-methylcyclohexyl)-amide (Bayer), (10) nicotin-(3'-methylcyclohexyl)-amide (Bayer), (11) nicotin-(4'-methylcyclohexyl)-amide (Bayer), (12) nicotinphenylamide (Bayer), (13) nicotindiphenylamide (Bayer), (14) nicotin-(4'-methoxyphenyl)-amide (Bayer), (15) nicotinbenzylamide (Bayer), (16) nicotindibenzylamide (Bayer), (17) trigonelline hydrochloride (Hantzsch, 1886), (18) nicotinamide methochloride (Karrer, Schwarzenbach, Benz & Solmsen, 1936) (Roche), (19) nicotinonitrile (Dr J. Walker, National Institute for Medical Research) and (24) 2-carboxypyridine-3-carboxylic acid cyclohexylamide (Bayer) were tested. They produced no growth, but a small amount of acid, generally less than 1/100th of that produced with nicotinic acid by *Lactobacillus arabinosus* and neither growth occurred nor acid was produced by any of the other bacteria tested when the various drugs were applied in doses up to 0.03 or 0.05 μ g./ml.

The effects of nicotinic acid and nicotinamide on the growth of *Proteus vulgaris* 5887, *Shigella dysenteriae* (Shiga) 4837 and Flexner 4832 were so small that tests of the related compounds on these bacteria were abandoned. No increased growth or acid formation resulted from addition of nicotinamide methochloride to cultures of *Esch. coli* in doses of 0.0001–10 μ g./ml., but a distinct inhibition was observed which gradually increased with rising concentration.

The amounts of nicotinamide methochloride formed when *Proteus vulgaris* 3156 was incubated with nicotinamide or nicotinic acid in Barton-Wright medium for 48 hr. are shown in Table 4. No nicotinamide methochloride was found in culture-free medium after 48 hr. incubation or in the unincubated inoculum. Thus no nicotinamide methochloride was released from intact cells into the medium. More nicotinamide methochloride was formed from nicotinamide than from nicotinic acid and an increase in concentration of the substrate from 0.1 μ g./ml. to 1.0 μ g./ml. did not significantly increase the formation of nicotinamide methochloride. Nicotinamide methochloride formation from nicotinic acid and nicotinamide was greater under anaerobic than under aerobic conditions. The tests of the fluorescent alkaline isobutanol solutions with 12N-NaOH, ferricyanide and alkaline acetone indicated the presence of 'F₂'.

DISCUSSION

Toxicity. The general pharmacological effects on mice and rats, namely excitement and narcosis of the central nervous system and particularly of the

Table 3. Utilization of nicotinic acid, nicotinamide and related compounds by various bacteria

No.	Name of compound	Drug content of medium ($\mu\text{g./ml.}$)	Growth (arbitrary units)	Acid formed (ml. 0.1 N-NaOH)	Efficiency compared with that of nicotinic acid	Drug content of medium ($\mu\text{g./ml.}$)	Growth (arbitrary units)	Acid formed (ml. 0.1 N-NaOH)	Efficiency compared with that of nicotinic acid
<i>Lactobacillus arabinosus</i>									
1	Nicotinic acid	0.005	+	0.67	1	0.0025	+	0.16	1
		0.01	++	1.37		0.005	++	0.21	
		0.02	+++	2.69		0.01	++	0.26	
		0.04	5+	4.46		0.02	+++	0.31	
		0.08	6+	4.91					
		0.12	8+	5.99					
<i>Proteus vulgaris</i> 401									
4	Nicotinamide	0.005	+	0.70	1	0.0025	+	0.15	1
		0.01	++	1.37		0.005	++	0.19	
		0.02	+++	2.73		0.01	++	0.25	
		0.04	5+	3.86		0.02	+++	0.31	
		0.08	6+	4.37		0.86			
		0.12	8+	5.48		0.89			
				0.92					
5	Nicotin-ethylamide	0.08	0	0.045	0.01	0.02	\pm	0.07	0.2
		0.12	0	0.065		0.03	+	0.09	
		0.20	0	0.155					
6	Nicotin-diethylamide	0.08	0	0.045	<0.01	0.02	0	0	0
		0.12	0	0.045		0.03	0	0	
		0.20	0	0.090					
23	Quinolinic acid	0.08	\pm	0.14	c. 0.012	0.02	+	0.06	0.2
		0.12	\pm	0.19		0.03	+	0.07	
		0.20	+	0.33					
29	β -Picoline	0.08	\pm	0.03	c. 0.008	0.02	+	0.06	0.2
		0.12	\pm	0.11		0.03	+	0.07	
		0.20	+	0.24					
<i>Proteus X</i> 13									
1	Nicotinic acid	0.0025	+++	0.18	1	0.0025	+	0.11	1
		0.005	++++	0.31		0.005	++	0.45	
		0.01	6+	0.41		0.01	+++	0.80	
		0.02	8+	0.52		0.02	++++	1.79	
						0.04	6+	2.12	
						0.06	8+	2.24	
4	Nicotinamide	0.0025	++	0.11	0.79	0.0025	+	0.15	1
		0.005	+++	0.23		0.005	++	0.38	
		0.01	6+	0.39		0.01	+++	0.79	
		0.02	8+	0.45		0.02	++++	1.75	
						0.04	6+	2.23	
						0.06	8+	2.26	
5	Nicotin-ethylamide	0.05	\pm	0.05	0.02	0.05	0	0	0
		0.1	+	0.09					
6	Nicotin-diethylamide	0.05	\pm	0.05	0.02	0.05	0	0	0
		0.1	\pm	0					
23	Quinolinic acid	0.02	+	0.17	0.12	0.02	+	0.40	0.15-0.25
		0.03	+	0.21		0.03	+	0.41	
29	β -Picoline	0.02	+	0.15	0.1	0.02	+	0.37	0.12-0.16
		0.03	+	0.20		0.03	+	0.40	
<i>Proteus morgani</i>									

Table 4. Nicotinamide methochloride formation from nicotinamide and nicotinic acid by *Proteus vulgaris* 3156 after aerobic and anaerobic incubation at 37° for 48 hr.

Substrate	Concentration of substrate ($\mu\text{g./ml.}$)	Nicotinamide methochloride formed ($\mu\text{g./10 ml.}$) on			
		(a) Aerobic incubation		(b) Anaerobic incubation	
		In medium	In organisms	In medium	In organisms
Nicotinamide	0.1	0	1.71	0	2.18
	1.0	0	1.71	0	2.12
Nicotinic acid	0.1	0	0.62	0	0.82
	1.0	0	0.75	0	1.02

respiratory centre, seem to be quite separate from the metabolic action of nicotinamide. They occur immediately after the injection and disappear after a few minutes, when sublethal doses are given. It is almost certain that in consequence of this action on the central nervous system, no single dose can be administered which can seriously diminish the methyl donors of the body. The toxicity observed in these experiments is similar to that reported by Chen *et al.* (1938), Unna (1939) and Brazda & Coulson (1946) for nicotinic acid, nicotinamide, nikethamide, and trigonelline; but there is a discrepancy as to the relative toxicity of nicotinamide and its methochloride. Brazda & Coulson found the latter to be less toxic than nicotinamide (by 33%), while our results indicate that nicotinamide methochloride is twice as toxic as nicotinamide itself; this finding is not in accordance with the generally accepted idea that this methylation is a detoxication (Perlzweig & Huff, 1945). The toxicity of each of the compounds examined is almost identical for mice and rats with the exception of nicotinamide methochloride which is about twice as toxic for the mouse as for the rat. The toxicity of the various compounds examined rises continuously from the fairly hydrotropic nicotinic acid, trigonelline, nicotinamide and nicotinamide methochloride by more than 100 times to the lipotropic nicotin-diphenyl- and -dibenzyl-amides. Since the main point of attack is presumably on the lipids of the central nervous system, the increased toxicity of the phenyl and benzyl compounds might be related to the partition coefficients between aqueous and lipid phases. This factor is likely to favour concentration of the more toxic compounds in lipids.

Conversion to nicotinic acid in mammals. The ability to increase the formation and elimination of nicotinamide methochloride is based on the intermediary formation of nicotinamide. Antiblack-tongue and antipellagra activities are certainly a property of nicotinamide. Therefore one may attribute antiblack-tongue and antipellagra activities to those compounds which give rise to an intermediary formation of nicotinamide. There is general agreement among the various investigators mentioned in the introduction, that nicotinamide as well as nicotinic acid, its salts and esters and nicotinic acid possess antiblack-tongue and antipellagra activities. Our experiments have shown a nicotinamide methochloride response to all of these substances which were examined. If the activities against black-tongue and against pellagra respectively are identical, then the results with regard to quinolinic acid and nicotinamide methochloride are difficult to explain. Woolley *et al.* (1938) did not find any antiblack-tongue activity with quinolinic acid which seemed to have a marked antipellagra effect in the experiments of Vilter & Spies (1939). Our experiments are in agreement with

the findings of the latter workers. Nicotinamide methochloride was found to be inactive against canine black-tongue by Woolley *et al.* (1938), and later by Teply *et al.* (1945), but was claimed to be active by Najjar *et al.* (1944). The experiments of Elvehjem and co-workers are, however, not fully conclusive because the compound was given orally and with this route of administration only a small proportion is absorbed from the intestine (Ellinger & Coulson, 1944; Ellinger, 1947). Najjar's work needs confirmation and our results are not able to decide the matter in question. β -Picoline has antiblack-tongue activity, but to a much lower degree than the other compounds examined and found active by Woolley *et al.* (1938). In our experiments the response to β -picoline was greater than to all other compounds tested except nicotinamide methochloride. It is difficult to explain this fact in the present state of our knowledge of the mechanism of the intermediary process. Unfortunately the method used by Perlzweig, Bernheim & Bernheim (1943) and Ellinger (1946) to elucidate the mechanism of methylation of nicotinamide and of the formation of nicotinamide from nicotinic acid yielded no results. We do not know definitely, whether the substituted nicotinamide derivatives are broken down to nicotinic acid or nicotinamide. However, the quantitative nicotinamide methochloride response to the various compounds yields some information. The response by the rat to extradietary doses is 22.7% to nicotinamide and 11.8% to nicotinic acid, similar to the values found in man by Ellinger & Coulson (1944). A breakdown to nicotinic acid and intermediary formation of nicotinamide and nicotinamide methochloride would be limited by the nicotinic acid response to values below 11.8%; the alkyl derivatives, mono- and di-ethyl- and monoallylamides show, however, responses of 17.2, 16.8 and 15.7% respectively, which are intermediate between those to nicotinamide and nicotinic acid. For these three compounds dealkylation to the amide is most probable; that such a reaction can occur follows from the observations of Elson, Goulden & Warren (1946) who found that dimethylaniline is partly eliminated as *p*-aminophenylsulphonic acid by the rat.

The monoaryl derivatives of nicotinamide (nos. 12, 14 and 15) too produced an increase of nicotinamide methochloride elimination, the response being about 6%. This low value might be due to the fact that these monoaryl derivatives break down to nicotinic acid and not to nicotinamide. The diaryl derivatives (nos. 13 and 16) and the cyclohexyl derivatives (nos. 8, 9, 10, 11 and 24) and nicotinonitrile (no. 19) do not appear in the urine as nicotinamide methochloride, and it is concluded that their breakdown does not lead to either nicotinamide or nicotinic acid. Quinolinic acid is decarboxylated in the 2-position and about 5% of it appears in the

urine as nicotinamide methochloride, a response similar to that of the monoaryl derivatives. From trigonelline no nicotinamide methochloride is formed. On the average 62.7% of the administered nicotinamide methochloride is eliminated in the urine; similar values were observed in man after parenteral ingestion (Ellinger & Coulson, 1944; Perlzweig & Huff, 1945). The remainder is probably eliminated with the bile and destroyed by the intestinal flora (Ellinger, 1947). There remains only β -picoline (no. 29) which appears in the urine as nicotinamide methochloride to the extent of 35.7–53.4%, a response much higher than with nicotinamide. This, together with its low antiblack-tongue and anti-pellagra activity, can only be understood, if it is assumed that its metabolic transformation into nicotinamide methochloride occurs in such a manner that nicotinamide is not involved as an intermediate; it may be suggested that methylation in the 1-position precedes oxidation of the 3-methyl group.

Conversion to nicotinic acid by insects. While in the rat, besides nicotinic acid and nicotinamide, the alkyl and monoaryl derivatives were comparatively well utilized, *Tribolium* was found to be more exacting. Nicotinic acid, its esters, and nicotinamide were well utilized, but most of the other compounds tested had little activity. The only exceptions were nicotinylamide (no. 7), nicotin-(4'-methoxyphenyl)-amide (no. 14) and nicotinphenylamide (no. 12), whose activity was about 10–20% of that of nicotinic acid. A definite, but much lower activity was shown by nicotinmono- and -di-ethylamides (nos. 5 and 6), nicotinbenzylamide (no. 15), quinolinic acid (no. 23) and β -picoline (no. 29). This activity might be due to a slight contamination of about 2% with nicotinamide or nicotinic acid from which all the compounds tested except β -picoline and quinolinic acid are made. This is unlikely since most of these substances showed no activity in bacteria. It appears therefore, that all these substances are utilized by *Tribolium*, if only to a small extent.

Utilization of the various pyridine derivatives by bacteria. In most of the bacteria examined nicotinamide and nicotinic acid were utilized almost equally well except for *Proteus* X 13 which utilized the former by about 20% less well than the latter. All the other compounds were utilized, if at all, to a much lower degree. *Lactobacillus arabinosus* utilized only nicotinic acid and its amide, in agreement with the results of Elvehjem & Teply (1943), quinolinic acid, β -picoline and nicotinonitrile. The small effect of other derivatives might be explained by contamination with one of the two active compounds. In *Proteus vulgaris* 401 and 3156, nicotinethylamide (no. 5) quinolinic acid (no. 23) and β -picoline (no. 29) showed an activity of about 20% of that of nicotinic acid. In *Proteus* X 13 these compounds

and nicotindiethylamide (no. 6) showed an even smaller activity which, in the case of nicotinmono- and -di-ethylamides, can be attributed to a slight contamination with nicotinamide or nicotinic acid. The slight effect of these compounds is in general agreement with the findings of Lwoff & Querido (1939). The positive results reported by the French workers for nicotinonitrile may be due to impurities. *Shigella sonnei* utilized, of all compounds tested, only nicotinic acid and its amide and, to a much smaller extent, quinolinic acid and β -picoline. This confirms the findings by Dorfman *et al.* (1939) also with regard to nicotinonitrile. The positive effect found by these workers for nicotindiethylamide can be attributed to contamination with nicotinic acid or amide. The findings of Najjar *et al.* (1943) that nicotinamide methochloride promotes the growth of *Esch. coli* could not be confirmed. Najjar's results may also have been caused by heavy contamination with nicotinamide or nicotinic acid. The antiblack-tongue effect of nicotinamide methochloride (Najjar *et al.* 1944) can be explained in a similar manner. Nicotinic acid and nicotinamide promote growth with *Esch. coli* (Ellinger & Abdel Kader, 1947).

General conclusion. Comparing the utilization by rat, insect and bacterium of the different compounds tested one realizes that different mechanisms play a role in the transformation of the various compounds into either nicotinamide or nicotinic acid. The rat is able to convert most of the compounds, all alkyl and monoaryl derivatives of nicotinamide and quinolinic acid and β -picoline, into either nicotinamide or nicotinic acid. The mechanisms involved consist in either dealkylation, deamination, decarboxylation or oxidation. The two latter processes seem to occur in insects and in those bacteria tested, whilst dealkylation and deamination are much more restricted in insects and almost entirely absent in bacteria.

SUMMARY

1. The general pharmacological action and toxicity of a number of substituted nicotinamide derivatives and of a few other related compounds have been examined in the mouse and rat. Their conversion of these compounds into nicotinamide methochloride by the rat has also been tested. Relations between physico-chemical properties and toxicity and between chemical constitution and metabolic transformation into nicotinamide methochloride have been discussed.

2. This utilization by the rat has been compared with that by insects and bacteria. It was found that all those compounds which could be utilized by bacteria were also utilized by the higher species, similarly all the substances which were utilized by insects were utilized by rats, but not vice versa.

3. In addition to nicotinamide and nicotinic acid, rats utilized all alkyl and monoaryl nicotinamides, quinolinic acid and β -picoline, but not dialkyl or cyclohexyl nicotinamides or nicotinonitrile. Insects utilized nicotinamide and nicotinic acid and their esters, and less well the mono- and di-alkyl and monoaryl nicotinamides, and still less quinolinic acid and β -picoline. Of the bacteria examined, all utilized nicotinic acid and nicotinamide, quinolinic acid and β -picoline, but only *Proteus vulgaris* and *Proteus X13* utilized the monoethylamide; the latter also utilized the diethylamide and *Lactobacillus arabinosus* also nicotinonitrile.

4. Nicotinamide methochloride was found to be present in *Tenebrio* larvae and was formed by *Proteus vulgaris* from nicotinic acid and nicotinamide.

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