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The Fate of Nicotinamide Methochloride and the Effect of Liver Poisons on its Elimination Rate in the Rat*

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(*Received* 3 *December* 1946)

The object of this work was to investigate two obscure aspects of the metabolism of nicotinamide, namely the fate of nicotinamide methochloride in the body and the effect of liver poisons on its formation and elimination. An important factor determining the rate of methylation of nicotinamide is probably the methylating efficiency of the liver. There are indications that in certain liver diseases in humans the urinary elimination of nicotinamide methochloride is affected (Najjar, Hall & Deal, 1945; Ellinger, 1945). The effect of carbon tetrachloride poisoning on the elimination of ingested nicotinamide methochloride by the rat has been studied by Perlzweig, Huff & Rosen (1946), who found elimination to be increased during the first days of poisoning. Ellinger & Coulson (1944) have shown that the recovery of nicotinamide methochloride from human urine is about 15% after oral and about 60% after parenteral administration; the remainder is unaccounted for. These findings have been confirmed by Perlzweig & Huff (1945), who postulated partial destruction in the liver (Perlzweig, Huff & Rosen, 1946), while Najjar & Deal (1946) suggested that hydrolysis of nicotinamide methochloride took place in the liver to yield methyl groups and nicotinamide.

To investigate these factors, the following experiments were carried out. The possible destruction of nicotinamide methochloride in the liver was studied (*a*) with liver slices and (*b*) with perfused surviving livers; (*c*) faeces and (*d*) bile were tested for nicotinamide methochloride; (*e*) the effect of faecal bacteria on the compound was examined and (*f*) the effect of liver poisons on the elimination of nicotinamide methochloride and protein nitrogen was investigated.

* Some of the results presented in this paper were communicated to the Biochemical Society on 4 May and 6 December 1946 (*Biochem. J.* **40**, xxxi and **41** (in the press)).

METHODS

(*a*) *Liver slices.* Albino rats of 250–400 g. of both sexes from our own stock, kept since weaning on a mixed diet and with a known daily nicotinamide methochloride output, were stunned and killed by bleeding. The liver was removed immediately after death and kept in a covered glass bowl. Pièces of 300–500 mg. wet weight were cut off, weighed, sliced into slices of 0·2–0·4 mm. thickness and suspended in 10 ml. of modified Ringer solution (Krebs & Henseleit, 1932) in sufficient weighing bottles to form a duplicate series; the solutions contained in addition 0, 1, 3, or 10 μg. of nicotinamide methochloride per ml. Suspensions were aerated with a mixture of 5% CO_2 and 95% O_2 before stoppering and controls prepared of each concentration without the addition of liver. All bottles were agitated at 37°. Never more than 30 min. elapsed between the death of the animal and commencement of shaking. Pairs of bottles containing liver were opened after 2, 30, 60, 120 or 240 min. respectively; after 4 hr. the blanks were opened. The supernatant liquids were decanted into graduated centrifuge tubes, the residues washed twice with 0·5 ml. water which was added to the centrifuge tubes. 1·0 ml. of 20% (w/v) trichloroacetic acid was added. After heating to 75° for 30 min. and adjusting the volume to 12 ml., the precipitate was centrifuged off and nicotinamide methochloride in the liquid determined by the method of Coulson, Ellinger & Holden (1944). On two occasions liver slices were agitated in Krebs-Ringer solution at 37° for 2 hr. before 3 μg./ml. nicotinamide methochloride were added. Determinations of this compound were made 2, 30, 60, and 120 min. subsequently.

(*b*) *Liver perfusion.* Rats of the same type were killed as in (*a*); the peritoneal cavity was opened, a cannula inserted

into either the hepatic artery or portal vein and the liver perfused with Krebs-Ringer solution at 37° after the hepatic vein had been severed. The liver was then removed, placed on a funnel and perfused first with Krebs-Ringer solution alone, and 20 min. later with the same solution containing 1, 3 or 10 μg./ml. of nicotinamide methochloride. The same 200 ml. of perfusion liquid were used during the whole experiment. The solution was examined at intervals for its nicotinamide methochloride content. The whole procedure was carried out at 37°. The perfusion pressure was 90 cm. water when the liver was perfused through the hepatic artery and 40 cm. when the perfusion was done through the portal vein.

(*c*) *Urinary and faecal elimination.* Rats of both sexes were kept in metabolism cages for about 2 months. Urine was collected daily and its nicotinamide methochloride content was measured. In addition, 24 hr. specimens of faeces were collected every other morning and dried *in vacuo* over $CaCl_2$ to constant weight. 3 g. or less of the dried faeces were suspended in 50 ml. 0·02% (w/v) HCl and kept in a boiling water bath for 2 hr. After cooling to about 40°, 10 ml. of a freshly made 5% (w/v) taka-diastase emulsion in sodium acetate-acetic acid buffer (0·1 M) of pH 4·8 were added and the mixture kept at 45° for 12 hr. After cooling to room temperature, the supernatant liquid was siphoned off, and nicotinamide methochloride estimated in measured portions. After a preliminary observation period, the rats were given either 10 mg. of nicotinamide or 10 mg. nicotinamide methochloride orally or intraperitoneally. In order to ascertain whether the bluish fluorescence of the *iso*butanol solution of the tests on faeces and bile was due to its content of the nicotinamide methochloride degradation product F_2 (Ellinger & Coulson, 1943; Coulson & Ellinger, 1943), its reaction towards strong alkali, potassium ferricyanide and alkaline acetone (Najjar, Scott & Holt, 1943) was examined.

(*d*) *Biliary excretion.* Male rats (150–450 g.) from various sources were anaesthetized by intraperitoneal administration of 0·07 ml. of nembutal solution (1 grain/ml.)/100 g. body weight. In some cases the dose had to be increased and small amounts had to be given about every hour to keep the animals fully unconscious. The peritoneal cavity was opened by a cut about 1 cm. long in the lower hepatic region and the duodenum carefully taken out on a gauze moistened with warm saline. The entrance of the common bile duct which is generally found near the distal end of the duodenum was then located. Ligatures were placed a few mm. on each side of the entrance and a cannula was fixed so that its tip almost touched the entrance of the bile duct. The intestines were then returned as completely as possible into the peritoneal cavity and the animal covered with gauze moistened with warm saline and heated by a lamp. The bile, free from blood, was collected in a test tube and another small test tube was fixed with plaster over the penis to collect urine. After some time, 10 mg. of either nicotinamide or of nicotinamide methochloride were given intraperitoneally.

(*e*) *Effect of faecal bacteria.* An emulsion was made from freshly passed rat faeces, corresponding to about 25 mg. dry weight, in 5 ml. peptone water, and filtered through a sterile paper filter. The filtrate was diluted 100 times with peptone water and 2 drops of this dilution were inoculated into tubes containing a synthetic ammonium lactate medium (Fildes, 1938), or a casein hydrolysate medium (Barton-Wright, 1944), both containing 5 μg./ml. nicotinamide methochloride. Controls were made without nicotinamide methochloride and without bacteria. The tubes were incubated aerobically for 4 days at 37°, the solid was then removed by centrifugation and nicotinamide methochloride was measured in the supernatant fluid. A König (1904) reaction using metol as aromatic amine (Bandier & Hald, 1939) was carried out before and after incubation, and before and after hydrolysis with strong alkali (12 N-KOH), in the presence of ammonia (Perlzweig, Levy & Sarett, 1940).

(*f*) *Effect of hepatic poisons.* Eight rats of both sexes and weighing between 250 and 400 g. were given intraperitoneally 0·2 ml. of a mixture of equal parts of chloroform and carbon tetrachloride twice at 11-day intervals. Four other rats received subcutaneously a dose of 2 ml. of a 0·25% (w/v) solution of yellow phosphorus in peanut oil which was repeated 11 days later. The basal urinary nicotinamide methochloride elimination, the response of these rats to 20 mg. of extra nicotinamide and, in some instances, the urinary total and protein-N output had been measured before the beginning of the poisoning; N estimations were continued during the experiment. The separation of protein and non-protein-N was done by phosphotungstic acid precipitation; N was estimated by a semi-micro-Kjeldahl method.

RESULTS

Most of the results are presented in Tables 1–6 in which all values for nicotinamide methochloride, N and König reactions are averages of two or three separate determinations. There was in both types of experiments reported in Tables 1 and 2 an initial loss, the rate of which is inversely proportional to the concentration. In the liver slice experiments, at any one concentration of added nicotinamide methochloride the loss was greater, the more liver tissue was used. All the loss occurred in the first 2–15 min., no significant further loss being observed during the next 4 hr. This absence of further change after the initial loss cannot be due to an inactivation of the liver tissue by agitation in Krebs-Ringer at 37°, since a corresponding initial loss is seen in exps. 7 and 8, when nicotinamide methochloride was added to the liver-Krebs-Ringer system after a preliminary shaking at 37° for 2 hr.; moreover liver tissue treated in the same manner retains its ability to methylate nicotinamide (Perlzweig, Bernheim & Bernheim, 1943; Ellinger, 1946). The only possible explanation of these results is that an initial adsorption of some nicotinamide methochloride on the liver tissue occurs.

The results on the elimination of nicotinamide methochloride in the faeces (Table 3) are confusing. The daily faecal output varied widely in the same rat, kept under constant dietary and environmental conditions, and the mean figures vary from rat to rat. Considerable doses of either nicotinamide or nicotinamide methochloride, which cause a large rise in the urinary output, did not generally increase the

Table 1. *Effect of sliced liver on nicotinamide methochloride (NM)*

Exp. no.	Wet wt. of liver used (mg.)	Concentration of NM before shaking (μg./ml.)	NM concentration (μg./ml.) after min. shaking 2	30	60	120	240	Average loss (%)		NM concentration (μg./ml.) of controls after 240 min. Without NM	Without liver
1	267 (253–278)*	1	0·89	0·90	0·91	0·89	0·90	10·8	12·3	0·02	0·99
2	383 (370–397)	1	0·86	0·85	0·88	0·83	0·87	13·8		0·01	1·02
3	282 (269–289)	3	2·82	2·86	2·81	2·86	2·84	5·4	7·0	0·3	3·03
4	392 (378–403)	3	2·74	2·72	2·78	2·71	2·76	8·6		0·0	2·96
5	258 (247–266)	10	9·72	9·67	9·77	9·64	9·73	2·9	3·25	0·02	10·03
6	314 (299–326)	10	9·65	9·70	9·60	9·58	9·67	3·6		0·01	10·05
7	255 (243–262)	3 NM added after 2 hr. shaking	2·87	2·83	2·84	2·90	—	5·1	7·0	0·03	3·05
8	382 (371–395)	3 NM added after 2 hr. shaking	2·72	2·76	2·69	2·74	—	8·9		0·04	3·02

* Values in parentheses are the limiting values.

Table 2. *Nicotinamide methochloride (NM) content of perfusion liquid after passing through a surviving liver preparation*

Exp. no.	Perfusion from	NM content after 20 min. perfusion without NM (μg./ml.)	NM content of perfusion liquid (μg./ml.)	NM content (μg./ml.) of perfusate during min. perfusion 0–10	10–30	30–60	60–90	Average loss (%)	
9	Hepatic artery	0·03	1	0·88	0·90	0·88	0·87	11·8	10·1
10		0·02	1	0·92	0·89	0·93	0·92	8·5	
11	Portal vein	0·00	1	0·93	0·90	0·91	0·90	9·0	
12		0·02	1	0·87	0·89	0·90	0·89	11·2	
13	Hepatic artery	0·05	3	2·78	2·80	2·82	2·78	6·8	7·0
14		0·02	3	2·83	2·76	2·79	2·80	6·8	
15	Portal vein	0·00	3	2·77	2·81	2·77	2·79	7·2	
16		0·01	3	2·80	2·76	2·78	2·81	7·1	
17	Hepatic artery	0·00	10	9·61	9·58	9·63	9·57	4·0	4·1
18		0·03	10	9·57	9·59	9·55	9·56	4·3	
19	Portal vein	0·02	10	9·63	9·65	9·60	9·59	3·8	
20		0·04	10	9·58	9·55	9·62	9·53	4·3	

faecal excretion significantly. The mode of administration of the drugs, oral or intraperitoneal, made no difference to the faecal output, while the urinary elimination was about 50 % higher after intraperitoneal than after oral administration in the case of nicotinamide, and 200–300 % higher in the case of nicotinamide methochloride.

The fluorescent substance developed from the KCl eluate by alkali and *iso*butanol when faeces extracts or bile were tested for nicotinamide methochloride (Tables 3 and 4) gave a greenish fluorescence with strong alkali which faded on standing at room temperature to form a yellowish colour. With dilute potassium ferricyanide the fluorescence became deep violet. Addition of alkali and acetone produced a yellow colour with green fluorescence, thus proving that the fluorescence was due to the presence of 'F_2'.

The examination of bile for nicotinamide methochloride (Table 4) showed clearly that some of this compound formed in the liver was eliminated in the bile, the amount varying between 12·8 and 56 % of that in the urine collected simultaneously. After administration of nicotinamide or nicotinamide methochloride, the output in the bile rose considerably, relatively more in the former than in the latter case, biliary elimination being 92 and 37 %, and 21·7 and 9·3 % of the urinary output, respectively.

Table 3. *Daily faecal elimination of nicotinamide methochloride (NM) before and after oral (O.) or intraperitoneal (Ip.) dosage of 10 mg. nicotinamide (NA) or NM*

Exp. no.	Weight (g.)	Observation period	Limits of daily NM output during pre-dosing period: Urinary (μg.)	Limits of daily NM output during pre-dosing period: Faecal (μg.)	Average NM output on 3 predosing days: Urinary (μg.)	Average NM output on 3 predosing days: Faecal (μg.)	Output 24 hr. after dosing 10 mg. NA: Urinary (mg.)	Output 24 hr. after dosing 10 mg. NA: Faecal (μg.)	Output 24 hr. after dosing 10 mg. NM: Urinary (mg.)	Output 24 hr. after dosing 10 mg. NM: Faecal (μg.)	Route
21	304	19. x.–	78·0–	303·0–	287·2	576·3	1·632	683·0	—	—	O.
		22. xii. 44	366·8	1390·0	313·5	721·4	—	—	1·970	744·0	
					268·3	471·7	2·347	617·2	—	—	Ip.
					303·2	517·3	—	—	5·753	640·2	
22	329	19. x.–	137·3–	278·9–	186·4	346·8	1·287	337·2	—	—	O.
		22. xi. 44	288·7	1017·2	198·6	327·2	—	—	1·246	321·2	
					217·6	412·8	1·964	398·3	—	—	Ip.
					149·6	362·5	—	—	5·483	393·1	
23	362	22. i.–	578·8–	397·2–	672·1	447·6	1·874	430·7	—	—	O.
		14. iii. 45	862·2	768·5	691·7	483·6	—	—	2·168	477·6	
					712·8	507·4	2·583	471·9	—	—	Ip.
					728·6	433·7	—	—	6·821	482·0	
24	369	2. i.–	782·3–	313·6–	848·3	393·6	2·178	425·7	—	—	O.
		27. iii. 45	1104·5	584·7	1009·7	496·2	—	—	2·470	528·3	
					1089·6	557·6	3·176	798·3	—	—	Ip.
					1066·4	568·2	—	—	7·284	673·3	

Experiments nos. 25–28: 4 additional rats treated in exactly the same manner yielded results which did not differ significantly from those of experiments nos. 21–24.

Table 4. *Biliary elimination of nicotinamide methochloride (NM) before and after intraperitoneal dosage with nicotinamide (NA) or NM*

Exp. no.	Weight (g.)	Rate of bile flow during observation period (ml./hr.)	Duration of observation period: Before dosing (min.)	Duration of observation period: After dosing (min.)	Drug dosed	NM output in bile: Before dosing (μg./hr.)	NM output in bile: After dosing (μg./hr.)	Simultaneous urinary output of NM: Before dosing (μg./hr.)	Simultaneous urinary output of NM: After dosing (μg./hr.)
29	257	0·86	30	210	NA	11·3	183·7	32·3	198·3
30	313	0·61	30	180	10 mg.	3·8	86·2	29·7	232·0
31	287	0·83	30	240		6·7	98·0	22·4	450·7
32	186	0·69	30	180	NM	18·0	35·1	14·3	378·6
33	153	0·71	30	150	10 mg.	8·9	83·2	—	—
34	211	0·77	15	90		7·2	77·3	—	—

Table 5. *Effect of incubation with faecal bacteria on nicotinamide methochloride (NM) solutions*

Exp. no.	Dry wt. of faeces (mg.)	NM content of ammonium lactate medium: Before incubation: C.E.H.* (μg./ml.)	NM content of ammonium lactate medium: Before incubation: König† (μg./ml.)	NM content of ammonium lactate medium: After incubation: C.E.H.* (μg./ml.)	NM content of ammonium lactate medium: After incubation: König† (μg./ml.)	Medium alone after incubation (μg./ml.)	NM content in Barton-Wright medium: Before incubation: C.E.H.* (μg./ml.)	NM content in Barton-Wright medium: Before incubation: König† (μg./ml.)	NM content in Barton-Wright medium: After incubation: C.E.H.* (μg./ml.)	NM content in Barton-Wright medium: After incubation: König† (μg./ml.)	Medium alone after incubation (μg./ml.)
35	24·7	5·13	5·32	1·09	1·23	4·89	—	—	—	—	—
36	27·8	5·22	5·27	0·39	0·45	5·11	—	—	—	—	—
37	29·6	5·13	5·44	0·53	0·55	5·21	—	—	—	—	—
38	24·1	—	—	—	—	—	5·18	5·52	0·84	0·90	5·32
39	30·6	—	—	—	—	—	4·98	5·63	0·53	0·58	4·97
40	28·7	—	—	—	—	—	4·86	5·49	0·29	0·35	4·91

All König (1904) reactions before hydrolysis gave no measurable colour.

* Determined by the method of Coulson, Ellinger & Holden (1944).

† Determined by the König method after hydrolysis with strong alkali in presence of ammonia.

Table 6*a*. *Effect of liver poisons on the basal urinary elimination of nicotinamide methochloride (NM) and on the response to ingested nicotinamide (NA)*

Exp. no.	Weight (g.)	Poison used	Urinary pre-poisoning NM output: mean of 3 days (μg./day)	Response before poisoning to 20 mg. NA (mg./day)	First poisoning period Urinary NM output (μg./day) after poisoning on day 1	2	3	4	5	6*	7	8–10 aver.
41	287	$CHCl_3$ (0·1 ml.) + CCl_4 (0·1 ml.)	168·7	5·037	517·0	179·0	134·3	121·2	138·7	2264·0	490·7	155·6
42	318		328·6	4·807	867·0	432·0	310·1	151·7	183·9	1976·2	702·0	322·1
43	396		276·0	4·708	1068·0	632·1	260·3	210·7	201·6	1876·3	658·3	316·0
44	274		874·3	5·237	1924·0	1160·1	840·1	621·5	578·2	2174·0	917·3	790·6
45	343	P (2 ml. of 0·25 % soln.)	763·5	5·198	1632·0	1072·3	694·3	584·2	579·0	1870·0	937·4	780·7
46	317		212·8	4·317	1216·2	485·7	208·3	154·7	137·8	1674·2	516·2	198·6

Exp. no.	Weight (g.)	Poison used	Second poisoning period Urinary NM output (μg./day) after poisoning on day 1	2	3	4	5	6*	7
41	287	$CHCl_3$ (0·1 ml.) + CCl_4 (0·1 ml.)	552·0	216·0	140·7	110·3	121·0	2603·0	461·2
42	318		759·0	388·6	217·1	209·3	224·3	2016·0	754·6
43	396		923·3	472·4	240·3	198·7	196·8	1948·0	834·0
44	274		1763·0	1064·0	742·0	533·1	542·8	1843·2	767·0
45	343	P (2 ml. of 0·25 % soln.)	1974·0	Dead	—	—	—	—	—
46	317		1374·0	873·2	Dead	—	—	—	—

Nos. 47–52: 4 additional rats were poisoned with the chloroform-carbon tetrachloride mixture and 2 with yellow phosphorus in exactly the same way. N elimination was not measured. The NM elimination and the response to NA was not significantly different from those reported above.

* After intraperitoneal injection of 20 mg. NA.

Table 6*b*. *Effect of liver poisons on the urinary elimination of total and protein nitrogen (TN and PN)*

Exp. no.	Poison used	Urinary N before poisoning mean of 3 days TN (mg./day)	PN (mg./day)	First poisoning period Urinary N output (mg./day) after poisoning on day 1 TN	PN	2 TN	PN	3 TN	PN	4 TN	PN	5 TN	PN	6 TN	PN	7 TN	PN	8–10 aver. TN	PN
41	$CHCl_3$ (0·1 ml.) + CCl_4 (0·1 ml.)	81·2	4·9	92·3	14·8	87·2	6·8	82·0	5·0	—	—	—	—	—	—	82·1	4·7	83·2	4·8
42		90·5	5·8	107·2	17·2	95·6	10·4	91·6	6·1	—	—	—	—	—	—	91·0	6·1	90·7	6·3
43		132·2	7·3	148·7	22·6	136·3	10·9	133·1	9·0	—	—	—	—	—	—	133·7	7·5	131·5	7·2
44		76·3	4·4	89·3	12·4	81·2	6·2	77·0	4·1	—	—	—	—	—	—	75·8	4·2	74·0	4·3
45	P (2 ml. of 0·25 % soln.)	117·8	6·5	129·8	17·9	123·2	11·5	118·0	6·8	—	—	—	—	—	—	118·3	6·9	121·2	7·2
46		92·5	6·3	107·3	18·4	96·8	10·3	91·9	6·5	—	—	—	—	—	—	92·3	6·5	93·1	6·3

Exp. no.	Poison used	Second poisoning period Urinary N output (mg./day) after poisoning on day 1 TN	PN	2 TN	PN	3 TN	PN	4 TN	PN	5 TN	PN	6 TN	PN	7 TN	PN
41	$CHCl_3$ (0·1 ml.) + CCl_4 (0·1 ml.)	94·7	17·3	89·3	8·3	81·4	5·2	—	—	—	—	—	—	82·0	4·8
42		104·3	14·8	96·8	8·7	91·4	5·8	—	—	—	—	—	—	91·8	6·1
43		146·3	18·7	135·2	9·7	133·6	7·2	—	—	—	—	—	—	134·2	7·5
44		186·2	10·8	79·6	7·2	77·0	4·3	—	—	—	—	—	—	75·8	4·4
45	P (2 ml. of 0·25 % soln.)	131·3	19·4	Dead	—	—	—	—	—	—	—	—	—	—	—
46		109·4	19·2	99·3	14·6	Dead	—	—	—	—	—	—	—	—	—

To obtain the six results quoted in Table 4, 15 animals had to be used. In some cases extra nembutal had to be given to keep the animal quiet and this sometimes caused death from respiratory paralysis. Moreover, in some animals it was almost impossible to find the common bile duct which was hidden in the mesenteric fat. An uninterrupted flow of bile could only be obtained if the intestines were handled very gently. Urine was passed spontaneously only by one animal; in three more it was possible to obtain urine by pressure on the bladder. It is noteworthy that the relative biliary output is much higher when nicotinamide methochloride is formed in the liver from nicotinamide than when the methylated compound is ingested.

Table 5 shows that an emulsion of intestinal bacteria growing aerobically in various media destroyed between 80 and 94% of added nicotinamide methochloride within 4 days.

In exps. 41–44 (Tables 6*a*, 6*b*) rats were killed on the 8th day after the last dose of chloroform-carbon tetrachloride mixture. No pathological lesions could be observed in the livers. In exps. 45 and 46, the livers were examined immediately after death and found on macroscopical examination to be diffusely yellow and swollen; microscopical examination (magnification 16 ×) indicated that the acini were indistinct.

The experiments on the effect of liver poisons (Table 6*a*, 6*b*) showed, instead of the expected diminished output after poisoning, a transitory rise in the urinary nicotinamide methochloride elimination and a simultaneous rise in that of protein-N. The former then fell below the predosing level, the latter to the predosing level; 8–10 days after the poisoning, both were back to normal. The same occurred when the rats were poisoned for a second time with chloroform-carbon tetrachloride 11 days after the first dose, the animals recovering. But a second poisoning with phosphorus caused death with severe hepatic degeneration. If on the 5th day after poisoning, 20 mg. of nicotinamide were injected, the response in all cases was less than half of that observed before poisoning, indicating a severe damage to the methylating mechanism.

DISCUSSION

The fate of nicotinamide methochloride in the mammalian body is still obscure. There is no direct evidence for the statement by Perlzweig, Huff & Rosen (1946) that nicotinamide methochloride is destroyed in the liver. In man, parenterally administered nicotinamide methochloride is eliminated in the urine to about 60% and the remainder cannot be traced; in the rat, the urinary elimination of orally ingested nicotinamide methochloride is temporarily increased by about 50% after poisoning of the liver with carbon tetrachloride. These findings led Perlzweig, Huff & Rosen (1946) to conclude that poisoning of the liver diminished a normal hepatic destruction of nicotinamide methochloride. Najjar & Deal's (1946) suggestion of a demethylation of nicotinamide methochloride in the liver is based on even more indirect evidence. From Tables 1 and 2 it is clear that surviving liver tissue or perfused surviving liver do not destroy significant amounts of nicotinamide methochloride. Destruction of nicotinamide by liver *in vivo* would thus appear improbable.

It is difficult to relate the results reported in Table 4 of liver and kidney function in the rat on a time basis. The rat has no gall bladder and no bile is stored; some urine, however, may be stored for many hours in the bladder and a complete collection is difficult. It is, therefore, impossible to obtain over a short period a complete balance sheet of the relative elimination of nicotinamide methochloride in bile and urine; collection of bile over a 24 hr. period seems hardly possible in the rat.

The experiment reported in Table 5 on bacterial destruction of nicotinamide methochloride *in vitro* suggests that a similar destruction may occur in the intestinal tract during life. The great difference in urinary elimination of nicotinamide methochloride when the drug is given by mouth as compared with the values obtained on injection, which is shown here for the rat (Table 3) and has been demonstrated for man in earlier papers (Ellinger & Coulson, 1944; Perlzweig & Huff, 1945), is probably due to this destruction in the intestines. The negative König reaction in the medium before hydrolysis shows that even the pyridine ring is disrupted by the bacteria. There is in addition some formation of nicotinamide methochloride from nicotinamide by *Proteus vulgaris* (Ellinger & Abdel Kader, 1947). This extensive destruction and small scale formation of nicotinamide methochloride by the intestinal bacteria explain the results of the examination of the faecal output. Whether such destruction accounts for the balance of nicotinamide methochloride, eliminated neither in urine nor in faeces, cannot be decided from these experiments, but it is probable that most or all of the nicotinamide methochloride not accounted for by the urinary output is eliminated in the bile and then for the most part destroyed by the bacteria of the lower intestines; some may be reabsorbed and the remainder eliminated in the faeces. A complete balance of the nicotinamide methochloride metabolism requires information about the factors which determine the relative elimination in bile and urine and as to whether this distribution between bile and urine is constant or varies between different individuals and changes in the same animal under various conditions. Such knowledge cannot be gained from the experiments reported;

this could only be done if arrangements can be made to collect bile and urine simultaneously over a long period. The increased spontaneous nicotinamide methochloride output reported in Table 6*a* during the first day following administration of poison is probably due to an increase of available nicotinamide caused by tissue destruction and the liberation of coenzymes. Such tissue disintegration is proved by the increased output of protein nitrogen. No indication of a decreased destruction of nicotinamide methochloride can be found and the temporary increase in urinary elimination of ingested nicotinamide methochloride after carbon tetrachloride poisoning found by Perlzweig, Huff & Rosen (1946), can be fully explained by the increase of the circulating nicotinamide from tissue breakdown and the diminished elimination of the methylated compound in the bile from the damaged liver.

SUMMARY

1. Experiments carried out *in vitro* produced no evidence that destruction of nicotinamide methochloride occurred in the liver.

2. It could be shown that a considerable portion of nicotinamide methochloride formed in the liver or administered to a rat, was eliminated with the bile into the intestinal tract. Whether this accounts for the total amount, not eliminated in the urine, could not be decided.

3. An emulsion of intestinal bacteria destroyed a large part of the nicotinamide methochloride present in the medium.

4. The faecal elimination of nicotinamide methochloride depends on the elimination in the bile, its destruction and synthesis by intestinal bacteria and perhaps on reabsorption from the intestines. Faecal output affords no conclusive evidence regarding the nicotinamide state of the body.

5. Liver poisons such as chloroform, carbon tetrachloride or yellow phosphorus cause a temporary rise in nicotinamide methochloride output, probably due to tissue destruction, but they also reduce temporarily the efficiency of the methylating mechanism of the liver.

I wish to acknowledge my thanks to Miss J. Vaughan Morgan and Mr W. Wolff for technical assistance, to Roche Products Ltd. for the generous supply of nicotinamide, and the Ella Sachs Plotz Foundation, Boston, Mass., for a grant for technical assistance.

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The Cystine and Methionine Contents of Liver Protein in Acute Hepatic Necrosis

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(*Received* 18 *November* 1946)

Acute necrosis of the liver has been produced in rats by many workers using diets deficient in the sulphur-containing amino-acids. This work has recently been reviewed by Glynn, Himsworth & Neuberger (1945). All have found that there is a delay of 20–100 days, during which the animal appears normal, before the sudden onset of the disease. The condition has been attributed to a simple deficiency of cystine, although methionine can replace cystine in preventing the disease. It has been suggested that during the latent period the animal is living on endogenous stores of cystine and methionine and that the onset of disease follows their final depletion. It is possible that an unknown