Metabolism of Bladder Carcinogens

THE METABOLIC PATH OF 2-[8-¹⁴C]NAPHTHYLAMINE IN THE RAT

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2-Naphthylamine is one of the few compounds which are known to lead to cancer in man (Goldblatt, 1947, 1949). It has also been shown to be carcinogenic in dogs (Hueper, Wiley & Wolfe, 1938; Bonser, 1943). In both man and dog the tumours to which it (or its metabolites) gives rise are almost entirely confined to the bladder. The metabolic path of the material is not well known. Various metabolites have been identified in the urine of test animals (Wiley, 1938; Dobriner, Hofmann & Rhoads, 1941; Manson & Young, 1950), but the pure compounds isolated have accounted for only a few per cent of the administered dose. Bonser, Clayton & Jull (1951), using a colorimetric method for the estimation of 2-amino-1-naphthol conjugates, have detected considerably larger fractions of the dose, finding between 30 and 70% in this form in the urine of dogs, and less in other animals. They have demonstrated an apparent correlation between the proportions of the dose excreted in the urine as 2-amino-1-naphthol derivatives in various animal species, and the biological response of the species to treatment with the amine, and therefore strongly suggest that 2-amino-1-naphthol is the true carcinogen.

Before attempting to identify and measure all the metabolites it appeared to be important to determine quantitatively the distribution of the material in various animals, in order to know where to look for the metabolites, and also in the hope of obtaining some information on the varying activity of the carcinogen in different species. This paper presents the results obtained with rats, and a similar study on rabbits is in progress.

MATERIALS AND METHODS

Throughout this paper the terms 'activity' and 'specific activity' refer to radioactivity. M.p.'s are uncorrected.

Albino rats were used from our colony bred and maintained in the Industrial Hygiene Research Laboratories for years in excellent health, fertility and uniformity. All injections were intraperitoneal in animals of 200 g. or a little more in weight. The 2-naphthylamine was dissolved in 0.05 ml. ethanol and 0.15 ml. propylene glycol. After

injection the syringe was rinsed out with a further 0.2 ml. propylene glycol which was injected; the residual radioactivity in the syringe was determined and allowed for in calculating the injected dose.

Purification of 2-[8-¹⁴C]naphthylamine. The synthesis of 2-naphthylamine labelled with ¹⁴C in the 8-position has already been described (Catch, Huggill & Somerville, 1953). Impurities so far detected in commercial β -naphthylamine include α -naphthylamine, pyrene, 3:4:5:6-dibenzcarbazole, 2:2'-dinaphthylamine and 1:2:5:6-dibenzphenazine (Case & Pearson, 1952), but material prepared by our method may reasonably be expected to be free from the first three of these. Dinaphthylamine is produced during the conversion of 2-naphthol into 2-naphthylamine by the Bucherer reaction, and dibenzphenazine by air oxidation of the naphthylamine so that both of these may be present.

The material received from the Radiochemical Centre, Amersham, was mixed with 10% by weight of silica gel (100-200 mesh) and sublimed *in vacuo* at 80-95°/10⁻⁶ mm. This procedure was repeated and the naphthylamine finally sublimed alone to ensure the complete removal of silica. The material thus obtained was pure white, m.p. 108.4-109.9°; it was stored *in vacuo* and protected from light. Some material has now been kept in this way for 18 months without change, but the exposure to air necessitated by the removal of samples has caused the material used for experiments to discolour slightly after about 6 months. This is probably due to traces of dibenzphenazine and it is not regarded as important in the present investigation, as dibenzphenazine is inevitably formed when the naphthylamine is dissolved and injected into the animal.

Measurement of ¹⁴C. Samples were oxidized to CO₂ which was then counted in CO₂-CS₂-filled Geiger counters which can be used up to a true rate of 15 000 counts/min. When the activity was expected to be greater than this the CO₂ was diluted with inactive gas and a portion taken. The technique has been fully described by Henson (1953).

Nineteen counters of the standard design have been made and intercalibrated. 'Memory' effects are found to be very slight, but to minimize the risk of contamination several counters are kept exclusively for very low activity work.

The efficiency of these counters for ¹⁴C should be 100% within the sensitive volume, which is about 75% of the total volume. A comparison with the Radiochemical Centre standard for ¹⁴C was made by oxidizing five samples of labelled polymethylmethacrylate estimated by the Radiochemical Centre to have an activity of 10.37 μ C/g. $\pm$ 3% (new standard, September, 1952). The oxidation was carried out by dry combustion in a standard microanalytical

combustion tube and a portion of the CO_2 taken for counting. The mean value was $1.659 \pm 0.012 \times 10^4$ counts/min./mg. which corresponds to a specific activity of $7.473 \pm 0.054 \mu\text{Ci/g}$. This represents an efficiency of 72.1% for the counters.

Sensitivity of detection. The minimum detectable activity can be defined as that which differs from zero by a statistically significant amount. Assuming a background rate of about 30 counts/min., and counting both background and sample for 40 min., it can be calculated that there is a 95% probability that a single reading of 2 counts/min. differs significantly from zero. This is therefore a borderline case. For a sample activity of 3 counts/min. the probability of a single reading differing from zero is about 99%, and this is therefore taken as the minimum detectable activity under these conditions. Since a standard counter can contain up to 1 m-mole CO_2 , the minimum detectable specific activity is 3 counts/min./m-mole.

Oxidation techniques. For the oxidation of animal tissues to CO_2 , the method of Van Slyke was adopted (Van Slyke & Folch, 1940; Van Slyke, Steele & Plazin, 1951). In its original form this involves heating the sample with a mixture of chromic, phosphoric and fuming sulphuric acids and potassium iodate to 170–180°, collecting the CO_2 in alkali and liberating it with acid. For our purpose it was considered sufficient to transfer the CO_2 to a Geiger counter without measurement, and some modifications were made to simplify the procedure and at the same time ensure that no gaseous by-products were carried over with the CO_2 . The method adopted was to heat the sample for 10 min. at 150–160° with the oxidizing acid, omitting the potassium iodate. The CO_2 was collected by freezing in a trap (any oxygen formed by decomposition of the acid was pumped away), and the product then fractionated by surrounding the trap with a dry-ice-acetone bath at –70 to –80° which allowed the CO_2 to evaporate while retaining any moisture, acid vapour or other impurities which are solid at this temperature. The CO_2 was then condensed into a counter or a storage vessel.

The efficiency of the method was tested by oxidizing samples of inactive naphthylamine and analytically pure fluorene, and measuring the CO_2 formed. About 95% of the theoretical was obtained which could be raised 2–3% by the addition of potassium iodate during the oxidation, but this causes considerable frothing when used with animal tissue, and also gives rise to some iodine. Although the iodine remains behind after evaporation of the CO_2 , its accumulation in the trap over a series of oxidations could cause trouble. It was therefore decided not to use iodate in the oxidizing mixture.

A further estimate of the efficiency of the method was obtained by oxidizing samples of standard labelled polymethylmethacrylate and counting the activity of the CO_2 formed. The mean of three determinations was $1.547 \pm 0.013 \times 10^4$ counts/min./mg. which is 93.2% of the figure obtained above by dry combustion. Similar measurements using labelled naphthylamine (described below) gave a value of 92.2%.

Model experiments were carried out on various tissue samples and figures for their carbon content obtained. In most cases 25 mg. samples give 10–15 cm. pressure of CO_2 in the counter, which is a convenient amount.

Assay of 2-[8- ^{14}C]naphthylamine. Samples of about 1 mg. were used for assay. As previously described (Catch *et al.* 1953), dry combustion of the naphthylamine gave a mean value of $2.984 \pm 0.027 \times 10^7$ counts/min./mg. or, assuming

72% efficiency for the counters, an activity of 2.67 mCi/m-mole. As the tissue samples were to be assayed by the wet oxidation method, however, it was thought preferable to use this method for the assay of the naphthylamine. Four samples were oxidized giving a mean value of $2.751 \pm 0.014 \times 10^7$ counts/min./mg., which is the value that has been used for all the animal experiments.

Preparation of animal tissues and excreta. Urine and faeces were collected separately by a method due to Dr J. C. Gage, to be described elsewhere, which ensured minimal intercontamination. The cage floor, collection funnel, etc. were washed out and the washings added to the urine, which was made up to a standard volume. At the end of the experiment the animal was anaesthetized and as much blood as possible withdrawn from the jugular vein or the heart. The various organs required were then removed and the animal was skinned. The small organs and bone samples were disintegrated by grinding under liquid nitrogen in a steel mortar, while the liver, intestines and faeces were mixed with measured amounts of water and homogenized in a small top-drive macerator (MSE Nello 10). The carcass was treated similarly in an Ato-Mix 100 blender, and the skin can also be prepared in this way, though not so efficiently since the hair is not disintegrated.

RESULTS

Activity of expired CO_2 after intraperitoneal injection of 2-[8- ^{14}C]naphthylamine. Although the metabolic formation of CO_2 by the oxidation of the C-8 of naphthylamine is not very probable, it was, nevertheless, necessary to make sure that this does not occur. A rat of 200 g. was kept in a desiccator and was supplied with CO_2 -free air, the expired air being passed through traps cooled in liquid O_2 to freeze out the CO_2 , which could then be dried and counted. A control experiment was first carried out in which non-radioactive naphthylamine was injected and the expired CO_2 collected for 80 min. Samples showed no significant increase in the counting rate above background. Active material (0.7 mg.) was injected into the same animal and CO_2 collected continuously for 2 hr., and then for short periods after 3, 20, 26, 44 and 188 hr. from the time of injection. No significant increase in count rate was observed in any sample. Thus, five determinations on the first 2 hr. excretion gave a mean count rate of 1.19 counts/min./m-mole CO_2 . Therefore, assuming an excretion of 10 mg. CO_2 /min. the amount of activity in the CO_2 expired in 1 hr. is less than 0.0002% of the injected dose. (See assay of 2-[8- ^{14}C]naphthylamine.)

It appears, therefore, that no volatile product containing C-8 is detached from the molecule in the metabolism of 2-naphthylamine by the albino rat.

Activity in the blood after intraperitoneal injection of approximately 1 mg. 2-[8- ^{14}C]naphthylamine. Measurements were made to determine as far as possible the changes in blood activity as the amine enters and leaves the blood. Eight experiments of this kind were carried out and the data are given in

Table 1. *Percentage of the dose present in the blood at times up to 3 hr. after intraperitoneal injection of approx. 1 mg. 2-[8-¹⁴C]naphthylamine*

It is assumed that the total blood is 5% of the body wt. and that it contains 80% of water.

Time after injection (min.)	Rat (no.)							
	1	5	6	17	18	8*	20†	21†
5	2.5	1.4	1.7	0.1	2.6	—	1.5	0.2
15	—	2.6	2.9	0.9	3.1	—	1.7	0.7
20	6.3	—	—	—	—	3.1	—	—
25	—	4.0	3.1	1.8	3.65	—	2.0	1.6
30	—	—	3.2	1.8	2.8	—	2.8	1.55
35	21.9	4.5	3.3	1.7	3.1	—	2.8	1.7
40	—	—	3.0	1.7	2.8	—	—	—
45	—	3.7	2.9	—	—	3.15	2.6	2.6
50	6.5	—	2.8	1.85	2.7	—	—	—
55	—	3.8	—	—	—	—	—	—
60	—	—	3.1	1.5	2.7	—	2.7	—
65	2.5	—	—	—	—	—	—	—
75	—	—	2.9	1.2	2.4	3.4	—	—
80	3.6	—	—	—	—	—	—	—
90	—	—	1.9	0.9	2.2	—	3.1	2.0
95	—	—	—	—	—	3.2	—	—
100	3.1	—	—	—	—	—	—	—
105	—	—	2.2	0.8	2.4	—	—	—
110	2.8	2.4	—	—	—	3.1	—	—
120	1.6	—	—	0.6	1.8	—	—	2.1
180	—	—	—	0.6	1.5	—	3.1	2.0

* Bile duct ligated 24 hr. before injection.

† Bile duct ligated 5 days before injection.

Tables 1 and 2. Samples of blood varying in volume from 20 to 80 μ l. were withdrawn from the tail vein; these were dried and weighed and the total activity present in the animal's blood estimated on the assumption that whole blood contains 80% water and that the total weight of blood in the rat is 5% of the body weight.

Partition of activity between blood plasma and blood cells. Two groups of four rats and three rats respectively were used and each rat was injected intraperitoneally with 0.25 mg. 2-[8-¹⁴C]naphthylamine. The first group was killed after 10 days and the second after 15 days. Heart blood was withdrawn, pooled and oxalated in each case. Determinations were made on whole blood, plasma and cells and the results are shown in Table 3.

Excretion of radioactivity after intraperitoneal injection of approximately 1 mg. 2-[8-¹⁴C]naphthylamine. The daily excretion in the urine and faeces of the above two groups of animals was measured for periods up to 15 days after injection: the results are shown in Fig. 1. 90% of the dose is excreted in 3 days, 60–65% in the urine and 25–30% in the faeces. After 10–14 days the excretion is less than 0.1%/day.

In order to throw some light on the metabolic path of the substance, nine experiments were carried out, six on normal rats and three on rats whose common bile duct had been ligated. The

Table 2. *Percentage of the dose present in the blood at times up to 63 days after intraperitoneal injection of approx. 1 mg. 2-[8-¹⁴C]naphthylamine*

It is assumed that the total blood is 5% of the body wt. and that it contains 80% of water.

Time after injection (days)	Rat (no.)	
	17	18
1	0.3	0.3
9	0.2	0.2
16	0.1	0.2
23	0.1	0.1
30	0.05	0.1
37	0.03	0.05
42	0.01	0.03
49	0.003	0.01
63	0	0.003

Table 3. *Partition of radioactivity between blood plasma and blood cells after intraperitoneal injection of 0.25 mg. 2-[8-¹⁴C]naphthylamine*

	Activity in counts/min./mg. wet wt.	
	Three rats (pooled), 15 days after injection	Four rats (pooled), 10 days after injection
Whole blood	2.05	1.71
Plasma	0.045	0.028
Cells	4.12	2.87

animals were killed 2, 16 and 26 hr. after injection and the radioactivity of the alimentary tract, liver, blood, carcass and excreta determined. The results are shown in Table 4 together with the total percentage of the dose accounted for which was over

Fig. 1. Activity in urine (U) and faeces (F) after intra-peritoneal injection of 0.25 mg. 2-[8-¹⁴C]naphthylamine/200 g. rat; ●—●, group of three rats; ○—○, group of four rats.

90% in each case. The low values obtained for the alimentary tract and the high urinary excretion in the case of the bile duct ligated animals clearly shows that the material is normally conveyed to the gut in the bile. The total amount present in faeces and alimentary tract in normal animals appears to decrease with time, which would indicate a re-absorption from the intestine. Some further experiments were made to measure this effect more accurately. Twelve female rats, all of approximately 200 g. weight, were each injected with 0.05 mg. of the labelled naphthylamine after being separated into three groups of four. These groups were killed at intervals of 2 hr., 7 hr. and 26 hr. after the injections, the urine and faeces having been collected in these periods. The radioactivity in the urine, faeces and gastro-intestinal tract was measured for each group.

Three such experiments were carried out: Table 5 sets out the data obtained on the thirty-six animals, and it is clear that considerable re-absorption from the gut does take place.

The possibility that the size of the dose would affect the distribution between gut and urine was investigated. Groups of four rats were given doses varying from 0.005 to 2.0 mg./animal and killed after 26 hr. The results are shown in Table 6. No significant variation in distribution with dose was observed.

Table 4. Percentage of the dose present in tissues and excreta after injection of approx. 1 mg. 2-[8-¹⁴C]naphthylamine

Animal no.	...	...	...	Time after injection								
				2 hr.			16 hr.			26 hr.		
				6	15	8*	4	10	9†	12	16	20‡
Urine				21.1	8.7	28.5	66.4	50.9	86.2	75.0	77.3	85.4
Collected faeces				None available			8.7	29.3	1.6	7.4	10.6	3.1
Gastro-intestinal tract and contents				43.9	38.3	7.9	17.8	15.7	1.1	11.4	7.6	0.9
Liver				3.5	6.2	7.9	0.9	1.5	1.1	0.7	0.7	1.0
Carcass (total remainder excluding skin and fur)				29.6	38.5	49.8	1.3	1.7	4.4	3.0	1.7	1.6
Estimated % of dose in total blood				1.3	1.3	3.1	0.3	0.3	(2-3)	0.3	0.3	—
% of dose accounted for				99.4	93.0	97.2	95.4	99.4	96+	97.8	98.2	92.0
% of dose in faeces and alimentary tract				41.1 (av.)			35.8 (av.)			18.5 (av.)		

* Bile duct ligated about 20 hr. before injection.

† Bile duct ligated about 9 days before injection.

‡ Bile duct ligated about 5 days before injection.

Table 5. Percentage of dose present in urine and gastro-intestinal tract and faeces at successive times after intraperitoneal injection of 0.05 mg. 2-[8-¹⁴C]naphthylamine/rat

Expt. no.	No. of animals	Urine			Gastro-intestinal tract and faeces		
		2 hr.	7 hr.	26 hr.	2 hr.	7 hr.	26 hr.
1	12	19.5	48.9	60.2	61.6	45.4	38.5
2	12	20.8	43.4	58.8	56.8	45.8	37.7
3	12	26.9	50.1	69.3	58.6	40.1	22.0
Means		22.4	47.5	62.8	59.0	43.8	32.7
Standard errors		±2.3	±2.1	±3.3	±1.4	±1.8	±5.4

Long-term retention of activity after intraperitoneal injection of 2-[8-¹⁴C]naphthylamine. Four rats (nos. 14, 17, 18, 0, Table 7) were injected intraperitoneally with about 1 mg. of the radioactive naphthylamine and killed 3, 9, 10, 29 weeks thereafter. Since at the longer periods the observed activity in the samples of organs used (varying weights) approached the limiting significant count, we have given the actual observed activity of each

sample as well as the specific activity. (Specific activity = percentage of dose present in the whole organ divided by the weight of the organ.)

DISCUSSION

The principal biological interest in naphthylamine is the carcinogenic action on the bladder epithelium after its absorption over long periods by man or dog. The selective action on the bladder has led to two views of the mechanism involved: one that the amine is or gives rise to a carcinogen in the body, which is carried to the bladder by the renal route and exerts its action on the epithelium from the inside of the organ; the other that the amine is or gives rise to a carcinogen in the body which exerts its action on the bladder epithelium through the blood supply to the bladder wall. The greater number of investigators of this problem tend to the urinary carriage of the carcinogen, and the recent work of Bonser *et al.* (1951) gives strong support to this view.

The only metabolites of 2-naphthylamine hitherto detected in a variety of animals are derivatives of

Table 6. *Effect of varying dose on distribution of radioactivity between gut and urine after 26 hr.*

Four rats were used in each experiment and the excreta pooled.

Dose to each rat (mg.)	% of dose	
	Urine	Intestines and faeces
0.005	70.8	30.0
0.005	64.5	30.4
0.005	64.2	33.9
0.5	69.6	25.6
2.0	70.3	23.9
2.0	60.6	32.9

Table 7. *Retention of activity after varying periods following the intraperitoneal injection of 2-[8-¹⁴C]naphthylamine (specific activity = % of dose present in the whole organ divided by the wt. of the organ)*

Four rats, nos. 14, 17, 18 and 0, were injected with 1 mg. of radioactive naphthylamine and killed after 3, 9, 10 and 29 weeks, respectively.

Organ	Rat no. 14		Rat no. 17		Rat no. 18		Rat no. 0
	Observed activity counts/min.	Mean specific activity	Observed activity counts/min.	Mean specific activity	Observed activity counts/min.	Mean specific activity	Observed activity counts/min.
Liver	90	0.005	5	0.0003	3	0.0002	3
	85		0		3		1
			10				
Kidneys	71	0.004	10	0.0005	9	0.0006	2
	78		9		20		1
			8		8.5		0
Bladder	5	0.0006	7.5	0.0007	13	0.0006	2
	6		6		2		7
	(13)*		(11)*		(15)*		
Brain	10	0.0006	0	0.001	2	—	0
	10		16		0		0
			6				
Scapula	11.5	0.0006	—	—	—	—	9
	6						7
							5
Femur	11	0.0005	3	0.0001	19	0.0004	0
	13		5		5		3
					1		
Carcass	162	0.007	—	—	—	—	—
	43						
	73						
Blood	43	—	0	0	13	0.0004	—
	—						

* The figures in parentheses give counts for two samples when combined.

naphthylamine, and no evidence of opening of the condensed ring system in animals has been described. To the extent that we find no indication of activity in the carbon dioxide expired by rats injected with the labelled compound, we may affirm that disruption at the 8-9 bond has not taken place. If the naphthalene ring has not been broken then the radioactivity measured in all our experiments is that of the original naphthylamine or such derivatives of it as are usual in the metabolic transformations of aromatic compounds, e.g. hydroxylation, acetylation, sulphation and glycuronide formation.

Table 1 shows that there is little variation in the rate at which maximum blood concentration is reached after intraperitoneal injection or in the value of the maximum concentration (except in the case of rat no. 1, to which we attach little significance as this high maximum has not been observed again). In normal animals the concentration falls off markedly in 1-2 hr. after reaching the maximum, whereas the animals with ligated bile ducts maintain the maximum concentration during this period. This is to be expected since about 60% of the material normally enters the gut with the bile in the first 2 hr. (Table 5), and if this route is blocked elimination from the blood must be slower.

From the data given in Tables 4 and 5 it is possible to visualize something of the dynamics of disposal of the activity of the injected 2-[8- 14 C]-naphthylamine. The compound is first absorbed into the blood stream and is carried to the kidneys, liver and other organs and tissues. In the first 2 hr. there is a considerable excretion into the urine and, with intact common bile duct, a large excretion into the gut, a large deposition in the body tissues, and a relatively small retention in the liver. The pattern of this distribution is dynamic, so that after 16 hr. it has become entirely different. In this period the

Fig. 2. Activity in urine (U) and in gastro-intestinal tract with contents (G.I.) after intraperitoneal injection of 0.05 mg. 2-[8- 14 C]naphthylamine/rat. Each point represents pooled result from twelve rats.

carcass activity has been mobilized almost completely and the liver activity has been greatly diminished: the amount of activity now accountable for in the faeces and intestines has dropped considerably and the urinary excretion has enormously increased. The distribution continues to change in the same sense in the succeeding 10 hr. (26 hr. period). The carcass activity continues to fall as does the liver activity, and by this time the activity in the faeces and intestine has dropped to some 50% of that detectable in normal animals after 16 hr. and the urinary excretion has continued to rise. This changing pattern is graphically illustrated in Fig. 2. When excretion into the gut by the bile duct is impossible, the urinary excretion rises more rapidly. The interpretation we place upon the progressive fall in the activity in the gut is that there must be re-absorption of the compound carrying the activity. Since the ultimate excretion in the faeces is only 25-30%, it is clear that at least 30-35% of the material must be re-absorbed and eventually excreted in the urine.

The long retention of the amine in the body is of considerable significance in the general problem of the induction of tumours by 2-naphthylamine and hence it was of particular importance to obtain information, if possible, on the location of the long-retained material.

Measurable radioactivity was found in the blood in two cases (nos. 17 and 18) 49 and 68 days after injection of a single dose of 1 mg. and in both there was still 0.1% of the dose in the blood after 23 days. In the two groups examined 10 and 15 days after injection, the overwhelmingly greater part of the activity was present in the blood cells. If this applies also to the blood cells in the bone marrow, then the long period of activity in the blood may be due to the replenishment of the circulating blood with cells 'labelled' in the marrow.

Three weeks after the injection there was still a total of well over 1% of the dose in the whole animal with manifestly rich residues in the kidneys, liver and carcass, whereas bladder, brain, scapula and femur were relatively and absolutely very much poorer. At 9 weeks the activities in the samples taken were well above the limit of detectability by our method except in the case of the femur. At 10 weeks significant radioactivity was detectable in the kidneys, bladder and in the femur, but at 29 weeks the observed activity was too near background to permit any conclusions as to specific activity.

The demonstration that detectable radioactivity is present in the blood, kidneys and bladder of animals injected with a single dose, even after 9 weeks, is significant as probably indicating the difficulty of ridding the body completely of traces of foreign aromatic amines or derivatives of them. This fact may have some bearing on the clinical

problem of bladder cancer in the organic dye industry, since it would indicate that a workman exposed to carcinogenic aromatic amines may in the course of his working life never be free of them even after considerable periods away from exposure. The largest dose used in the above reported experiments was 5 mg./kg. (approximately), which is probably higher than a workman would be likely to inhale in moderately satisfactory conditions of work, but is certainly not higher than would be inhaled in notably bad conditions of work.

Our findings strongly suggest that cumulation of 2-naphthylamine or derivatives of it in the body must have occurred in the conditions of work formerly associated with the development of carcinoma or papilloma of the bladder. The following cycle is suggested as the mode of excretion of injected naphthylamine:

Fig. 3. Cycle of 2-naphthylamine (radioactivity) after intraperitoneal injection.

Presumably, having entered the upper part of the small intestine, the active compound is partly carried with the bile pigments along the gut and is partly detached from them and re-absorbed in the small intestine. Of that portion which is re-absorbed, most must be disposed of in the urine and a smaller proportion is returned to the gut via the liver.

Having accounted for well over 90 % of the injected material even at doses of 0.05 mg., it will be necessary to discover what proportions of this amount are attributable to the unchanged amine,

known metabolites (some of which we have synthesized) and unknown metabolites.

SUMMARY

1. Rats injected intraperitoneally with 2-[8-¹⁴C]-naphthylamine eliminate no radioactivity in the expired CO₂.

2. Rats injected intraperitoneally with 2-[8-¹⁴C]-naphthylamine (approximately 1 mg.) retain radioactivity in the blood for periods up to about 9 or 10 weeks. The activity in the blood after 10–15 days is attached almost entirely to the blood cells.

3. Excretion of the radioactive compound or its metabolites is readily demonstrated into the urine and through the bile into the gut.

4. Re-absorption of the amine or its metabolites from the gut after intraperitoneal injection has been demonstrated.

5. Excretion of 90–95 % of the radioactivity of a single dose in the urine and faeces occurs in about 3 days after intraperitoneal injection, but thereafter excretion is very slow so that activity is clearly demonstrable in various organs up till at least 10 weeks after injection.

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