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THE DYNAMICS OF DISTRIBUTION AND EXCRETION OF BUTYL-(2,3-^{14}C)-ACRYLATE IN MALE WISTAR ALBINO RATS

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Abstract. The disposition of butyl-(2,3-^{14}C)-acrylate has been studied following intraperitoneal and oral administration to rats. Most of the administrated acrylate underwent rapid metabolism and excretion with expired air (more than 70% of the dose) and urine (15 – 22%). Most of ^{14}C found in tissues was associated with the liver and kidneys. The level of ^{14}C associated with most of the examined tissues remained unchanged, at least, for the first 8–10 hours, followed by its fairly rapid loss. The only exception was erythrocytes, fat and the sciatic nerve. Significant differences in the rate of ^{14}C loss from tissues were found in relation to the route of its administration.

INTRODUCTION

Esters of acrylic acid are widely used in the production of polymers and synthetic resins. Butyl acrylate in a mixture with other acrylates is also used in the textile and leather industries, as well as in the production of paints and lacquers (5, 7, 10). The respiratory tract and skin (2) are the main routes of exposure to butyl acrylate under industrial (occupational) conditions. An animal study performed on several species has proved that in both acute and chronic exposures, the compound in question exerts irritant effects on the skin and mucuous membranes (8, 11). Experiments with guinea pigs have also demonstrated the allergic potentials of butyl acrylate (8, 19). No carcinogenic effect of this compound in the area of its administration has been reported (4).

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The metabolism of butyl acrylate in rats has not been fully clarified. As is known from earlier studies, this compound, added to the homogenates of rat liver, kidneys and lungs, undergoes hydrolysis to acrylic acid, due to the participation of nonspecific carboxylesterases (13, 14). There are no literature data concerning the distribution of this compound and the routes of its excretion. The mechanism of its *in vivo* metabolism also remains unknown.

The purpose of the present study was to examine the distribution of ^{14}C in the routes of its elimination after the administration of a single dose of butyl-(2,3-^{14}C)-acrylate in rats.

MATERIALS AND METHODS

Butyl-(2,3-^{14})-acrylate (specific activity 48 mBq/mmol) was purchased from the Institute of Nuclear Research, (Dept of Reactors and Isotope Production) Swierk, Poland. Analytical grade butyl acrylate (BA) was supplied by BDH, England. All the other reagents were obtained from POCh, Gliwice, Poland.

Preparation of samples and measurements of ^{14}C activity.

Blood cells and 20% tissue homogenates were digested according to Mahin and Lofberg (12). Urine samples, diluted with water to the volume of 100 ml, and plasma samples were directly measured. Determination of ^{14}C, permanently bound in tissues was performed according to Peter and Bolt (15).

The activity of samples, placed in glass scintillation vials with 15 ml of the scintillator (Tritosol, Fricke) (6), was measured with the use of the LKB Wallac – 81000 scintillation counter. The results were automatically corrected by the channel ratio method.

Description of experiments

The experiments were performed on 72 male Wistar rats (approximate body weight 250 g ± 10%). The rats were divided into 2 groups. The animals were put individually in glass metabolism cages (Simax, Czechoslovakia) and

TABLE 1. The excretion of ^{14}C after a single administration of butyl-[2,3^{-14}C]-acrylate in rats[1].

Route of administration	expired air (% of a dose)		urine (% of a dose)		faeces (% of a dose)	Total excretion (% of a dose)
	Time intervals (in hours)					
	0–24	24–48	0–24	24–48	0–24	
intraperitoneal	73.8 ± 4.6	0	13.7 ± 1.2	7.9 ± 0.6	1.6 ± 0.5	97.00
intragastric	83.7 ± 10.2	0	14.6 ± 1.4	2.2 ± 0.3	0.7 ± 0.2	101.20

[1] values represent mean for 6 rats ± SD

TABLE 2. Distribution of ^{14}C in rat tissues after a single i.p. administration of butyl-[2,3-^{14}C]-acrylate[1].

Tissue	Time after administration (in hours)											
	0.5		4		8		12		24		48	
	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue
liver	1.51	3.91	1.28	2.88	1.50	3.07	0.96	2.06	0.59	1.14	0.28	0.56
kidneys	1.95	0.89	2.39	1.55	2.70	1.34	0.76	2.08	0.55	0.25	0.23	0.10
lungs	0.50	0.17	0.51	0.13	0.76	0.21	0.49	0.15	0.33	0.08	0.17	0.05
brain	0.39	0.15	0.39	0.15	0.35	0.13	0.25	0.10	0.21	0.08	0.11	0.05
spleen	0.53	0.06	0.46	0.06	0.58	0.06	0.59	0.07	0.30	0.02	0.20	0.01
heart	0.46	0.09	0.35	0.08	0.37	0.06	0.28	0.06	0.18	0.05	0.10	0.05
stomach	0.59	0.20	0.63	0.24	0.77	0.28	0.56	0.20	0.40	0.11	0.21	0.07
epididymal fat	0.31	–	0.41	–	0.45	–	0.47	–	0.46	–	0.36	–
sciatic nerve	0.33	–	0.31	–	0.34	–	0.27	–	0.33	–	0.27	–
erythrocytes[2]	0.38	0.83	0.42	0.92	0.40	0.87	0.46	1.00	0.44	0.95	0.40	0.87
plasma[2]	0.88	2.55	0.34	0.90	0.37	0.98	0.32	0.85	0.27	0.72	0.19	0.05
Total		8.85		6.91		7.00		6.57		3.40		1.08

[1] all results are presented as a mean for 6 rats; SD $\leqslant \pm 10\%$
[2] the measurements of ^{14}C activity are presented in kBq/g/ml

TABLE 3. The content of ^{14}C permanently bound in rat tissues after a single intraperitoneal administration of butyl-[2,3-^{14}C]-acrylate[1].

Tissue	Time after administration (in hours)											
	0.5		4		8		12		24		48	
	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue
liver	0.34	0.88	0.55	1.20	0.71	1.45	0.58	1.24	0.51	0.99	0.29	0.58
kidneys	0.10	0.05	0.28	0.14	0.47	0.23	0.40	0.19	0.37	0.17	0.21	0.09
lungs	0.05	0.02	0.17	0.04	0.32	0.09	0.20	0.06	0.21	0.05	0.15	0.04
brain	0.02	0.01	0.08	0.03	0.13	0.05	0.11	0.05	0.12	0.04	0.09	0.04

[1] all results are presented as a mean for 6 rats; SD $\leqslant \pm 10\%$

acclimatised for 48 hours. Subsequently, both groups of animals were administered (100 mg/kg of BA-^{14}C (about 435 kBq/rat) intraperitoneally (group I) and intragastrically (group II). The BA-^{14}C solution, with a specific activity of 870 kBq/ml was prepared directly before administration by adding an appropriate amount of the medium (BA without an isotope marker) and dissolving it in 0.1% Tween 80. Immediately after the administration, the rats were placed in individual metabolism cages which enabled collection of separate samples of urine and faeces. The expired air was pulled out of cages with the use of a water pump through 3 washers, fixed one after another and containing 25 ml of isopropanol (I) and 25 ml of ethanolamine (II and III). The washers were exchanged every hour during the first 10 hours of the experiment and then every 12 hours.

Blood samples were collected from the tail veins by means of calibrated, heparinized capillaries: 0.5, 1, 1.5, 2, 2.5, 3, 4, 8, 12, 24 and 48 hours following the administration of the compounds; 30 μl of blood was collected each time. After centrifugation (haematocrite centrifuge, Unipan) the amount of haematocrite was checked; the capillary was cut at the division line. Plasma and blood cells were put separately into scintillation tubes. In order to determine ^{14}C content in the whole blood, about 7 ml of blood/100 g body weight were used (1).

Rats were killed at time intervals given in Tables 2 and 3, by means of decapitation, under light-ether narcosis. The liver, kidneys, lungs, brain, spleen, heart, stomach, a fragment of sciatic nerve and a section of fat from the abdomen were examined.

RESULTS

The excretion of ^{14}C from the rats' organisms in their expired air and urine during the first 48 hours are presented in Table 1. The expired air proved to be the main route of excretion of ^{14}C. More than 70–80% of the given dose of ^{14}C was eliminated in this way during 24 hours following the administration of butyl acrylate, irrespective of the route of administration. The maximum excretion took place in the 2nd hour following the intoxication in the case of intraperitoneal – and in the 3rd hour – after intragastric administration (Fig. 1). The excretion of ^{14}C in the expired air was monophasic, and the half-lives, estimated with the use of graphic technique ($T\frac{1}{2}$) for both routes of administration, were approximately 1.5 hours. Lack of ^{14}C activity in the washers containing isopropanol excluded the presence of unchanged BA. Apart from the expired air, ^{14}C was also excreted in considerable amount (17–21%) in urine, mainly during the first 24 hours, and partly during the following day and night. After 48 hours (i.p. and p.o. administration) the total amount of the excreted ^{14}C reached almost 100%. In the case of intraperitoneal administration, the loss of ^{14}C in the plasma was biphasic. Half-lives of ^{14}C, counted by means of graphic technique, for the fast and slow phase ($T\frac{1}{2}$), were: 1.7 and 44 hours, respectively. The fast phase of ^{14}C loss clearly correlated with ^{14}C

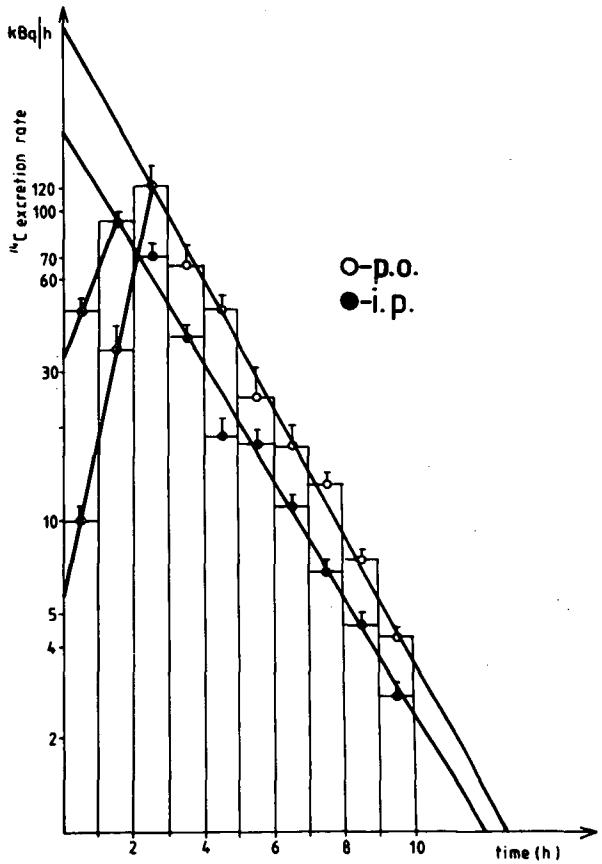

Fig. 1. The rate of ^{14}C excretion in the expired air after a single administration of butyl[2,3-^{14}C]-acrylate in rats (each point represents the mean for 6 rats ± SD).

excretion with the expired air (Fig. 1). In the case of intragastric administration, however, the loss of ^{14}C from the plasma was monophasic ($T\frac{1}{2}$ = 24 hours). No loss of ^{14}C from erythrocytes was reported (Tables 2 and 4). The distribution of ^{14}C in tissues, following intraperitoneal administration of BA-^{14}C was demonstrated in Table 2.

The highest ^{14}C concentration, half an hour after its administration, was detected in the liver and kidneys. In all the examined tissues it remained at the level of initial concentrations during the first 8 hours. In the kidneys and lungs, however, a significant increase of ^{14}C content was observed. During the next few hours following the intoxication, a significant decrease of BA-^{14}C activity was reported in all tissues, except erythrocytes, fat and the sciatic nerve, where the concentration of ^{14}C remained unchanged.

The lowest concentration of ^{14}C was detected in the brain. The determination of ^{14}C permanently bound in the liver, kidneys, lungs and brain, showed

TABLE 4. Distribution of ^{14}C in rat tissues of rats after a single intragastric administration of butyl-[2,3^{-14}C]-acrylate[1].

Tissue	Time after administration (in hours)											
	0.5		4		8		12		24		48	
	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue
liver	2.16	5.87	1.54	3.41	1.55	3.55	1.33	3.16	0.96	1.97	0.38	0.88
kidneys	1.10	0.51	1.06	0.51	1.07	0.46	0.94	0.46	0.74	0.30	0.32	0.20
lungs	0.57	0.15	0.61	0.19	0.70	0.20	0.63	0.20	0.51	0.13	0.32	0.10
brain	0.34	0.11	0.36	0.13	0.30	0.11	0.20	0.08	0.15	0.05	0.11	0.04
spleen	0.50	0.05	0.46	0.05	0.58	0.08	0.72	0.08	0.51	0.05	0.27	0.03
heart	0.43	0.08	0.40	0.08	0.37	0.07	0.28	0.06	0.25	0.04	0.14	0.03
stomach	0.82	0.71	1.54	0.49	1.38	0.67	0.92	0.36	1.10	0.36	0.25	0.06
epididymal fat	0.30	–	0.35	–	0.42	–	0.46	–	0.45	–	0.61	–
sciatic nerve	0.44	–	0.25	–	0.54	–	0.44	–	0.33	–	0.82	–
erythrocytes[2]	0.23	0.59	0.28	0.61	0.22	0.48	0.25	0.54	0.23	0.50	0.25	0.54
plasma[2]	0.90	2.38	0.82	2.17	0.77	2.04	0.69	1.83	0.47	1.25	0.27	0.72
Total		11.36		7.64		7.66		6.77		4.65		2.60

[1] all results are presented as a mean for 6 rats; SD $\leqslant \pm 10\%$
[2] measurements of ^{14}C activity are presented in kBq/g/ml

TABLE 5. Concentration of ^{14}C permanently bound in rat tissues after a single intragastric administration of butyl-[2,3-^{14}C]-acrylate[1].

Tissue	Time after administration (in hours)											
	0.5		4		8		12		24		48	
	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue	kBq/g	% of a dose in tissue
liver	0.82	2.23	0.91	2.01	0.95	2.17	0.73	1.73	0.57	1.17	0.38	1.11
kidneys	0.42	0.20	0.53	0.26	0.56	0.24	0.45	0.22	0.40	0.16	0.32	0.15
lungs	0.25	0.07	0.42	0.13	0.36	0.11	0.21	0.07	0.21	0.05	0.27	0.09
brain	0.14	0.05	0.19	0.07	0.15	0.06	0.12	0.05	0.07	0.02	0.10	0.05

[1] all results are presented as a mean for 6 rats; SD $\leqslant \pm 10\%$

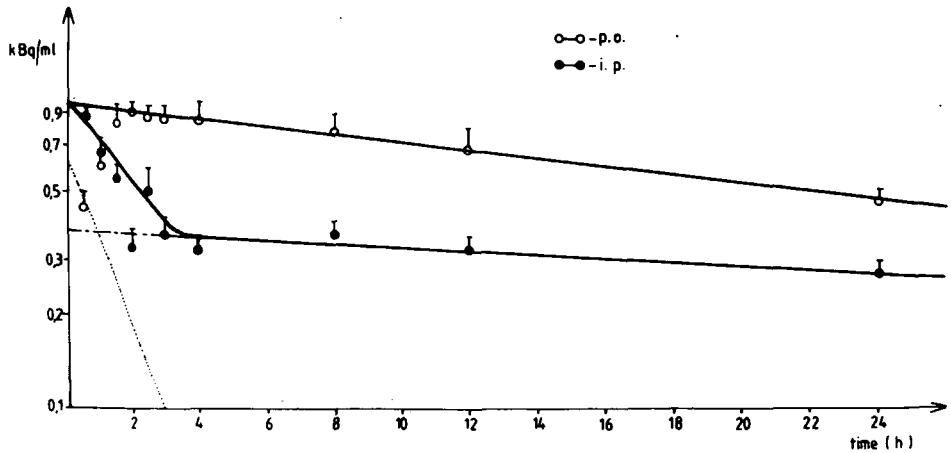

Fig. 2. Loss of ^{14}C carbon from the plasma after a single administration of butyl[2,3-1^{14}C]acrylate to rats (each point represents the mean for 6 rats ± SD).

that between 0–0.5 hour after the administration, its participation was minute in comparison with the whole amount of ^{14}C in these tissues (Tables 2 and 3). It was not until between 4 and 8 hours after the administration that we observed an increase, followed by a slow decrease of ^{14}C content. After 48 hours the whole of the examined ^{14}C was permanently bound in tissues.

Table 4 presents the distribution of ^{14}C in the tissues following p.o. administration of BA-^{14}C. As in the case of i.p. administration, the highest ^{14}C concentration, between 0–2 hours after the intoxication, was detected in the liver and kidneys as well as in the stomach, as a result of the route of administration. The loss of ^{14}C from the examined tissues 12h after administration was insignificant, whereas between 24–48 hours, a significant decrease of ^{14}C content was noticed. Again, the erythrocytes fat and sciatic nerve were the exception.

Table 5 shows the concentrations of ^{14}C permanently bound after p.o. administration of BA-^{14}C. In all the tested tissues, we observed high initial concentrations of permanently bound ^{14}C, a slight increase in the next time intervals, and the subsequent slow decrease, as in the case of i.p. administration. Fourty eight hours after p.o. administration, the whole ^{14}C determined in the examined tissues was permanently bound.

DISCUSSION

As the results in the presented data show, the expired air proves to be the main route of ^{14}C excretion after a single dose of BA-^{14}C, irrespective of the route of administration. The absorbing solution used in the washers indicates the route of excretion of $^{14}CO_2$ (Table 1). Most of the expired CO_2 was

excreted during the first 8 hours: despite an hour difference in reaching the peak excretion on the route of administration, the half-life ($T\frac{1}{2}$) was the same in both cases (Fig. 1).

Approximately 1/5 of a dose of BA-^{14}C was excreted in the urine, mainly during the first 24 hours following administration. The above-quoted data basically do not differ from the data concerning other acrylates (methyl acrylate, 2-ethylhexyl acrylate) given to rats (16). The expired air and urine were the main routes of ^{14}C excretion after ^{14}C-methyl acrylate administration in guinea pigs (17). In the case of all the three quoted acrylates we could observe a correlation between the increase of ^{14}C excretion in the expired air and a decrease in the ^{14}C excretion in urine as a result of the increasing molecular weight of the acrylates (16).

The experiments on the distribution of ^{14}C in tissues and blood have shown significant differences in the rate of ^{14}C loss, depending on the route of administration of BA-^{14}C (Fig. 2, Tables 2 and 4) which might concern the first, initial phase and the first 8 hours of ^{14}C loss from the plasma (Fig. 2). However, despite the evident correlation between the rate of ^{14}C loss in the plasma and its excretion in the expired air after i.p. administration of BA-^{14}C, it is difficult to explain the lack of a similar correlation (this is to be expected because of a similarly proceeding excretion with the expired air) after p.o. administration (Figs 1 and 2). Thus, the only plausible explanation might be the prolonged absorption of BA-^{14}C after intragastric administration, and simultaneous increase of radioactivity in the blood, originating both from $^{14}CO_2$ as well as from the given compound.

The high concentration of ^{14}C in the liver and kidneys, with considerable participation of ^{14}C permanently bound, confirms the metabolic processes taking place in these tissues. Evident differences in the rate of ^{14}C loss in the majority of the examined tissues, depending on the route of administration, most probably result (as in the case of blood) from the difference in the rate of absorption of butyl acrylate (Tables 2 and 4). The initially high level of ^{14}C in the stomach, significantly higher after p.o. administration, is also worth noticing. Similar results were observed by Kutzman et al. (9) after inhalatory or intragastric administration of ^{11}C acrylic acid. The later data suggest that most of BA-^{14}C administrated intragastrically undergoes hydrolysis in the presence of muriatic acid in the stomach, and then is absorbed in the blood as acrylic acid.

High radioactivity in the stomach walls following p.o. administration of the examined compound can be explained by the process of its absorption, whereas high radioactivity after i.p. administration suggests selective accumulation of ^{14}C in peritoneal walls. High retention of ^{14}C in erythrocytes, fat and sciatic nerve (Tables 2 and 4) constitutes an important problem. Repeated exposures can lead to the accumulation of this compound. It is worth emphasizing that the discussion on levels of ^{14}C determined in tissues concerns only a small part of the administered ^{14}C. Most of the radioactivity is excreted in the expired air during the first 8 hours, and in urine during the first 24 hours

following the administration (Table 1, Fig. 1). The balance of excretion for both the routes of administration approximated 100%.

The high ratio between permanently bound ^{14}C and total ^{14}C content should also be emphasized. Permanent binding of ^{14}C in tissues and erythrocytes can be explained by the binding of some acrylates to low molecular weight –SH groups, as it was demonstrated by Miller et al. (14) in *in vitro* experiments, and by Silver and Murphy (18). The experiments quoted suggest the existence of two metabolic pathways of acrylates in the organism. The first consists in the conjugation with glutatione, and the excretion of the resulting compounds in urine. The second is the process of hydrolysis of the acrylates in the liver, lungs, kidneys and plasma with the participation of non-specific carboxylesterases, to form acrylic acid and the appropriate alcohol. Carbon dioxide (CO_2), expired from the lungs would probably be a further product of the acrylic acid metabolism.

The existence of the two metabolic pathways for acrylates was confirmed by De Bethizy et al. (3). They examined *in vivo* the metabolism of acrylic acid and ethyl acrylate marked with ^{14}C. Apart from $^{14}CO_2$ present in the expired air (about 68% of the dose), they proved the presence of 3 metabolites in the urine of rats: 3-hydroxypropionic acid, N-acetylo-S(carboxyethylo)cysteine and N-acetylo-S(carboxyethylo)-cysteine ester. The experiments performed by these authors on the metabolism of acrylic acid and ethyl acrylate suggest the possibility of a similar metabolic pathway for butyl acrylate. We hope that the experiments being conducted presently on the metabolism of butyl acrylate in rats will confirm this hypothesis.

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