60. THE URINARY EXCRETION OF MANDELIC ACID ADMINISTERED INTRAVENOUSLY TO CATS

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MANDELIC acid, when administered to animals, may be recovered almost quantitatively from the urine. Moreover, when mandelic acid appears in the body as the result of metabolic processes, it is supposed to be excreted without further change [Knoop, 1905; Dakin, 1922; Quick, 1928].

Neubauer [1909], Neubauer & Fischer [1910], Neubauer & Gross [1910] and Neubauer & Warburg [1910] claimed that after giving *r*-phenylaminoacetic acid to dogs by mouth and perfusing dog livers, the following substances could be recovered from the urine and perfusate:

When, however, $(-)$ -phenylaminoacetic acid was given to dogs in similar fashion it appeared in the urine largely unchanged, while $(+)$ -phenylaminoacetic acid was metabolized with relative ease. Both benzoylformic acid and $(-)$ -mandelic acid were recovered from the urine in the latter case. When benzoylformic acid was administered to dogs, $(-)$ -mandelic acid appeared in the urine. *r*-Mandelic acid, when given by mouth, was practically all recovered in the urine as such, showing, if anything, a slight dextrorotation, there being a mere trace of benzoylformic acid.

In man the results were the same as in the dog, but in rabbits administration of *r*-phenylaminoacetic acid by mouth gave rise to urinary excretion of optically pure $(-)$ -phenylaminoacetic acid, benzoylformic acid, hippuric acid, but no mandelic acid.

Neubauer therefore argued that mandelic acid could not be an intermediary between phenylaminoacetic acid and benzoylformic acid. He also believed that $(+)$ -phenylaminoacetic acid, the form attacked in the body, could not yield $(-)$ -mandelic acid directly because of stereochemical dissimilarity. This latter conclusion is, however, untenable, since Clough [1918] showed that $(+)$ -phenylaminoacetic acid has the same stereochemical configuration as $(-)$ -mandelic acid.

Neubauer put forward the following scheme for the metabolism of phenylaminoacetic acid:

For many years Neubauer's hypothesis has been accepted without question. Wrede [1938], Scholz [1938] and Montenbruck [1938], however, claim that mandelic acid does undergo appreciable metabolic change both in man and in dogs. They gave *r*-mandelic acid as the ammonium salt by mouth, collected

the urine for 72 hr. and recovered an appreciable amount of (–)mandelic acid and of benzoylformic acid. (–)Mandelic acid was also recovered when the ammonium salt of benzoylformic acid was given in similar fashion. Two further experiments were carried out on man. In one case (–)mandelic acid, in the other (+)mandelic acid was given. In neither case was the optically pure acid used. When the laevo-acid was given, 73 % was recovered as such, and 7 % as benzoylformic acid, while, with the dextro-acid, only 65 % was recovered and 14 % appeared as benzoylformic acid. These workers therefore suggest that the dextro-form of mandelic acid is more readily metabolized in the body than the laevo-form.

When we started the present investigation early in 1938 we were unaware of the results of these German workers. We accepted the evidence of the previous literature that mandelic acid is resistant to attack in the body [Garry & Smith, 1938].

With the exception of the perfusion experiments on isolated organs, all the above results were obtained by oral administration of the substances under examination. McKenzie [1902] showed that when optically inactive potassium and sodium β -hydroxybutyrates respectively were injected subcutaneously into dogs a small proportion of the β -butyrates was recovered in the urine, and this product was laevorotatory. This partial resolution cannot be ascribed to the action of bacteria and does not therefore fall under the head of any of Pasteur's methods for resolving externally compensated compounds. We are indebted to Prof. McKenzie for the suggestion that it might be of interest to examine the behaviour of mandelic acid when given parenterally. Would it be possible for the living organism, under these conditions, to resolve *r*-mandelic acid into its antimerides?

METHODS

Administration. In order to introduce as few variables as possible we gave the mandelic acid intravenously. Decerebrate cats were used to avoid interference from anaesthesia. The urine was collected through a silver cannula tied into the urinary bladder.

To promote an ample flow of urine throughout the experiment, warmed Ringer's fluid, or a 1 % solution of NaCl, was slowly run into the external jugular vein. Mandelic acid, as a solution either of the free acid or of the sodium salt, was delivered from a burette into the main stream of fluid entering the jugular vein. The total volume of fluid given varied between 1000 and 1500 ml.; the volume of urine recovered was usually less by several 100 ml.

The urine was collected in small measured amounts, varying from 25 to 100 ml. A few drops of toluene were added to each collecting flask to prevent bacterial action. The pH of each sample was determined colorimetrically.

The sodium salt of mandelic acid could be given rapidly in large quantity, for example 10 g. as a 10 % solution in 15 min., without distress to the cat. When the free acid was given the administration had to be slow and the solution dilute.

Chemical extraction. The individual samples of urine were evaporated on a water bath to smaller bulk in the presence of $(\text{NH}_4)_2\text{SO}_4$ and filtered. Thereafter, the filtrate was acidified with H_2SO_4 saturated with $(\text{NH}_4)_2\text{SO}_4$ and extracted 6 times with ether. Continuous extraction was not used since a large number of individual samples had to be dealt with. The ethereal extracts were dried over anhydrous Na_2SO_4 ; the bulk of the ether was removed by distillation and the remainder allowed to evaporate at room temperature.

The residues were dried in a vacuum desiccator until constant in weight and then, without further purification, examined polarimetrically at room temperature in acetone using the mercury green line.

RESULTS

Control experiments

Pure optically active (–)mandelic acid has $[\alpha]_{5461}^{20} - 189.9^\circ$ for $c = 5.046$ in acetone solution [Roger, 1932].

No lowering of specific rotation was caused when pure (–)mandelic acid was dissolved in water, treated with $(\text{NH}_4)_2\text{SO}_4$ and extracted in the manner described above. The dextro-acid was similarly unaffected.

When laevo- or dextro-mandelic acid was added to cats' urine in amounts comparable with those recovered during an experiment the rotation of the recovered material was slightly less than the value for the pure forms of the active acid.

Collection of urine from cats during intravenous injection of Ringer's fluid alone showed that the early samples of urine had a small amount of ether-soluble material; this material was optically inactive. Later samples of urine had a negligible amount of extract.

Sodium r-mandelate

When the sodium salt of the racemic acid was given there was initially preferential excretion of the laevo-form, the maximum activity being observed in the early samples. Thereafter the material from subsequent samples showed a decreasing laevorotation, usually passing through optical inactivity to dextro-rotation (Table 1).

Table 1

4.9 g. *r*-mandelic acid in 1% solution as the sodium salt given intravenously in 554 min.

Time min.	Vol. of urine ml.	Wt. material extracted g.	$[\alpha]_{5461}$	($l=2$)
118	50	0.32	-31.1°	($c=1.3035$)
155	50	0.18	-12.9°	($c=0.777$)
194	100	0.26	-5.2°	($c=1.145$)
229	100	0.23	-2.5°	($c=1.015$)
263	100	0.22	$+1.6^\circ$	($c=0.9315$)
299	100	0.23	$+3.2^\circ$	($c=0.938$)
336	100	0.19	$+6.5^\circ$	($c=0.8425$)
371	100	0.18	$+7.4^\circ$	($c=0.678$)
396	100	0.16	$+6.6^\circ$	($c=0.687$)
423	100	0.16	$+6.2^\circ$	($c=0.643$)
449	100	0.15	$+5.3^\circ$	($c=0.656$)
476	100	0.17	$+4.9^\circ$	($c=0.71$)
501	100	0.21	$+4.2^\circ$	($c=0.9445$)
543	100	0.24	$+2.8^\circ$	($c=1.0545$)
577	100	0.24	$+4.2^\circ$	($c=1.0765$)
608	100	0.19	$+8.6^\circ$	($c=0.756$)

The smaller the amount of salt given in unit time, the more obvious the preferential excretion of the laevo-form of the acid in the early samples of urine (Table 2).

When using the sodium salt of the racemic acid we never obtained a rotation greater than $[\alpha]_{5461} - 65.0^\circ$ ($c=0.676$) in acetone, and that was in 0.1 g. of recovered material.

In one case, where 6 g. of *r*-mandelic acid were given in 150 ml. water as the sodium salt, 83 % was recovered in 436 min. In another case 92 % was recovered in 592 min. when 7.5 g. (+)mandelic acid were given.

Table 2

Rapid administration

6 g. *r*-mandelic acid in 4 % solution as the sodium salt given intravenously in 24 min.

Time min.	Vol. of urine ml.	Wt. material extracted g.	$[\alpha]_{5461}$	(<i>l</i> = 2)
15	25	0.23	- 18.8°	(<i>c</i> = 0.876)
26	25	0.36	- 17.4°	(<i>c</i> = 1.462)
40	25	0.35	- 17.2°	(<i>c</i> = 1.545)
52	25	0.29	- 15.8°	(<i>c</i> = 1.107)
79	100	0.59	- 13.0°	(<i>c</i> = 2.3455)

Slow administration

2 g. *r*-mandelic acid in 1 % solution as the sodium salt given intravenously in 277 min.

Time min.	Vol. of urine ml.	Wt. material extracted g.	$[\alpha]_{5461}$	(<i>l</i> = 2)
104	100	0.21	- 55.0°	(<i>c</i> = 0.717)
163	100	0.20	- 34.5°	(<i>c</i> = 0.884)
209	100	0.17	- 21.8°	(<i>c</i> = 0.6645)
253	100	0.16	- 13.7°	(<i>c</i> = 0.6215)
325	125	0.22	- 12.3°	(<i>c</i> = 0.811)

Table 3

2 g. *r*-mandelic acid in 1 % solution as the sodium salt given intravenously in two separate lots of 1 g. each.

Time min.	Vol. of urine ml.	Wt. material extracted g.	$[\alpha]_{5461}$	(<i>l</i> = 2)
95	50	0.06	- 57.0°	(<i>c</i> = 0.395)
152	50	0.15	- 42.9°	(<i>c</i> = 0.6405)
Administration of acid stopped				
214	100	0.15	- 14.7°	(<i>c</i> = 0.6115)
256	100	0.07	+ 6.5°	(<i>c</i> = 0.382)
Administration of acid resumed				
294	100	0.07	+ 10.8°	(<i>c</i> = 0.277)
333	100	0.13	- 2.6°	(<i>c</i> = 0.576)
372	100	0.16	- 3.5°	(<i>c</i> = 0.71)
Administration of acid finally stopped				
416	100	0.19	Inactive	(<i>c</i> = 0.7785)
455	100	0.11	+ 12.1°	(<i>c</i> = 0.4135)

In several cats administration of the racemic acid was delayed until active diuresis was in progress; the initial preferential excretion of the laevo-form was unaffected. Administration of the acid in two separate quantities, with an interval of 2 hr. between, showed that the optical activity of the excreted material from the first injection followed the usual course. On resumption of the injection of the acid the preferential excretion of the laevo-form became once more apparent (Table 3).

Free r-mandelic acid

Administration of free *r*-mandelic acid gave very similar results (Table 4).

Table 4

3.2 g. free *r*-mandelic acid in 1% solution given in 292 min.

Time min.	Vol. of urine ml.	Wt. material extracted g.	$[\alpha]_{5461}$	($l=2$)
76	50	0.07	-82.2°	($c=0.523$)
103	50	0.06	-58.1°	($c=0.516$)
134	100	0.14	-34.2°	($c=0.584$)
165	100	0.14	-26.3°	($c=0.665$)
196	100	0.16	-17.4°	($c=0.631$)
222	100	0.17	-14.7°	($c=0.7485$)
250	100	0.17	- 8.8°	($c=0.7425$)
294	200	0.34	- 6.5°	($c=0.824$)
335	200	0.24	Inactive	($c=0.591$)
382	200	0.16	+ 9.4°	($c=1.067$)
434	200	0.12	+16.9°	($c=0.8$)
503	200	0.15	+19.0°	($c=0.948$)
581	200	0.11	+23.6°	($c=0.954$)

Optically active mandelic acids

We obtained these acids in optically pure form by resolution of the racemic acid with (-)ephedrine [Roger, 1935].

Typical results with the sodium salts of the active acids are shown in Tables 5 and 6, and with the free acids in Tables 7 and 8.

Table 5

7.5 g. (+)mandelic acid in 4% solution as the sodium salt given intravenously in 120 min.

Time min.	Vol. of urine ml.	Wt. material extracted g.	$[\alpha]_{5461}$	($l=2$)
75	100	0.72	+183.9°	($c=2.624$)
95	100	0.71	+186.6°	($c=2.7085$)
116	100	0.83	+185.3°	($c=3.0525$)
176	200	1.51	+184.4°	($c=2.5325$)
248	200	1.10	+181.6°	($c=2.745$)
323	200	0.84	+181.3°	($c=3.086$)
470	300	0.86	+181.6°	($c=3.216$)
587	300	0.34	+170.5°	($c=1.4955$)

Table 6

5 g. (-)mandelic acid in 4% solution as the sodium salt given intravenously in 125 min.

Time min.	Vol. of urine ml.	Wt. material extracted g.	$[\alpha]_{5461}$	($l=2$)
70	100	0.60	-180.7°	($c=2.628$)
98	100	0.56	-183.0°	($c=2.47$)
145	200	0.90	-179.7°	($c=2.5345$)
215	300	0.87	-180.9°	($c=2.501$)
296	300	0.60	-176.7°	($c=2.575$)
420	400	0.54	-172.5°	($c=2.5415$)

Table 7

3.7 g. free (+)mandelic acid in 1% solution given in 246 min.

Time min.	Vol. of urine ml.	Wt. material extracted g.	$[\alpha]_{5461}$	($l=2$)
38	50	0.09	+ 169.4°	($c=0.121$)
57	50	0.10	+ 180.8°	($c=0.224$)
72	50	0.08	+ 185.3°	($c=0.17$)
86	50	0.10	+ 182.4°	($c=0.2385$)
107	100	0.16	+ 185.1°	($c=0.543$)
129	100	0.16	+ 183.6°	($c=0.55$)
171	200	0.36	+ 186.6°	($c=0.614$)
210	200	0.39	+ 187.8°	($c=0.724$)
247	120	0.29	+ 185.3°	($c=1.152$)

Table 8

4.0 g. free (-)mandelic acid in 1% solution given in 295 min.

Time min.	Vol. of urine ml.	Wt. material extracted g.	$[\alpha]_{5461}$	($l=2$)
38	50	0.08	- 157.7°	($c=0.333$)
62	50	0.13	- 165.7°	($c=0.67$)
93	100	0.25	- 178.8°	($c=0.285$)
148	200	0.45	- 180.0°	($c=0.715$)
293	400	0.89	- 181.9°	($c=1.2815$)
360	200	0.32	- 178.7°	($c=1.1925$)

The rotation of the extracted material was usually slightly lower than the full rotation of the injected acid.

Material recovered in the ethereal extract

Droller [1938] found that taking by mouth 4.5 g. "Mandecal", containing roughly 3 g. mandelic acid as calcium mandelate, lowered the pH of his urine to 5, and he states that free mandelic acid was present. In our cats, however, when the sodium salt was used the pH of the urine ranged from 6.7 to 7.4, and with the free acid the pH of the urine never fell below 6.2. It would appear that the cat is able to excrete most of the mandelic acid as a salt. This was borne out by extraction of the urine with ether before and after acidification (Table 9).

Table 9

Administration of the sodium salt of <i>r</i> -mandelic acid			
Material extracted before acidification		Material extracted after acidification	
Wt. (g.)	$[\alpha]_{5461}$	Wt. (g.)	$[\alpha]_{5461}$
0.02	—	1.51	- 17.1°
0.01	—	0.86	- 4.8°
0.00	—	0.74	- 1.2°
Administration of free <i>r</i> -mandelic acid			
0.05	- 40.8°	0.34	- 56.1°
0.07	- 19.4°	0.24	- 17.7°
0.07	- 6.3°	0.18	- 5.4°
0.08	+ 6.0°	0.12	+ 6.7°

The material recovered by routine extraction was a dry powder with a slight yellowish colour which was removed completely by boiling the solution of the

acid with charcoal (Norite). Such treatment gave the following results in two cases:

Before decolorization	After decolorization
$[\alpha]_{5461} - 58^\circ$ ($c=0.548$)	$[\alpha]_{5461} - 61^\circ$ ($c=0.638$)
$[\alpha]_{5461} - 180^\circ$ ($c=2.169$)	$[\alpha]_{5461} - 184^\circ$ ($c=2.281$)

Contamination with benzoylformic acid was looked for by means of the colour reaction with benzene (containing thiophene) and conc. H_2SO_4 ; there was no sign of the characteristic violet-red colour. Moreover, the extracted material gave no hydrazone when treated with phenylhydrazine in acid solution. Further, when material with a rotation of -184° was dissolved in ether and treated with a saturated solution of sodium bisulphite to trap benzoylformic acid, the rotation was unchanged.

This same sample showed a melting point of $129\text{--}132^\circ$, with softening at 124° . (–)Mandelic acid melts at 133° , *r*-mandelic acid at 118° and benzoylformic acid at $65\text{--}66^\circ$.

Three separate samples, A, B and C, of material extracted in routine fashion were submitted to analysis without further purification. A was obtained from an early sample of urine when *r*-mandelic acid was given, B when pure dextro-acid was given and C when pure laevo-acid was given.

	% of		
	C	H	N
Found A	63.2	5.1	Nil
Found B	63.3	5.7	Nil
Found C	62.3	5.5	0.9
Calculated for pure mandelic acid	63.2	5.3	Nil

Equivalent determinations with baryta, using phenolphthalein as indicator, were carried out on three samples of recovered material. One sample from an early stage in an experiment where the racemic acid was given showed marked laevorotation, the second sample was optically inactive, while the third was from a late stage in an experiment and showed dextrorotation. In all three cases the titration values gave equivalents essentially in agreement with the theoretical value for mandelic acid.

DISCUSSION

There is little doubt that the material extracted from the urine by our routine method was in all cases practically entirely mandelic acid. In the experiments of which the results are given in Tables 5–8, the percentages of active mandelic acid in the entire recovered material were, using optical activity as the criterion, 96, 94, 97 and 94 respectively. The assumption that the impurities were optically inactive is supported by the fact that the degree of purity was very similar with both laevo- and dextro-forms.

To obtain such results if the contaminant had been optically active, it must have been of opposite sign in the two contrasting experiments. Thus, the impurity, if derived from mandelic acid, would have to be generated without destruction of the asymmetry of the mandelic acid molecule. On the other hand, if mandelic acid were oxidized partially to benzoylformic acid, and if the latter were to undergo metabolic change back to mandelic acid, the hypothesis of Neubauer would lead us to expect preferential formation of the laevo-form independently of the nature of the mandelic acid originally administered.

The improbability of benzoylformic acid playing any marked part in our experiments extending over a relatively short time is also supported by failure to detect it in the ethereal extract.

There is no support in our results for the hypothesis of Montenbruck [1938] that the dextro-form of mandelic acid is more rapidly metabolized than the laevo-form. We were, however, probably not concerned with metabolic derivatives of mandelic acid to the same extent as other workers, who gave the acid by mouth and collected the urine for several days thereafter. The time allowed for metabolic change and for the excretion of metabolic products was, in our experiments, relatively short.

The use of intravenous administration in our technique eliminated the possibility of bacterial action in the gut and avoided passage through the intestinal mucous membrane. McKenzie & Harden [1903] showed that the laevo-form of mandelic acid is preferentially attacked by micro-organisms.

Actually, within the duration of our experiments there was little evidence for ultimate differential behaviour of the body towards either of the active forms of mandelic acid. Without fractional collection of the urine and optical examination of the excreted fractions, the initial preferential excretion of the laevo-form would have been missed. When the experiment lasted a reasonable length of time the early preferential excretion of the laevo-form was nearly compensated by an excretion of the dextro-form (Fig. 1). In both cases the curves show that

Fig. 1. The curves are drawn from the data recorded in Tables 1 and 4. Each point represents the rotation of the total amount of material excreted up to that time.

the rotation is approaching optical inactivity. Here again, in the cat at least, there is no good evidence to support Montenbruck's hypothesis that the dextro-form is preferentially metabolized in the body when the acid is given intravenously.

There must, however, be an early temporary differential reaction of the cat's body to the two forms of mandelic acid. The preferential excretion of the laevo-form at the outset must leave a preponderance of the dextro-form in the body which finds its expression in the subsequent excess of the dextro-form in the urine. One could postulate preferential metabolic attack on the dextro-form at the outset, but then one would need to invoke the conception of subsequent regeneration of that form to explain the preponderance of this isomer in the later samples of urine. The results from administration of active acids do not support this view.

Henderson & Rule [1939] have shown that percolation of a solution of racemic *p*-phenylenebisiminocamphor through a column of lactose leads to partial resolution due to the dextro-isomeride being more strongly adsorbed on the lactose. Westenbrink [1936] and Westenbrink & Gratama [1937] found that the intestines of pigeons and of frogs absorb the naturally occurring (+)xylose more rapidly than the (-) form. It is conceivable that such phenomena may occur with respect to mandelic acid in the kidney or in some other site in the body.

Still another possible explanation depends on the well-known principle of Marckwald & McKenzie [1899]. These workers showed that when mandelic acid is esterified with (-)menthol, the (-)menthyl (+)mandelate is more rapidly formed than the (-)menthyl (-)mandelate. Moreover, the rate of hydrolysis of (-)menthyl (+)mandelate is greater than that of (-)menthyl (-)mandelate. That such phenomena can occur in the body is strongly suggested by the observations of Dakin [1903, 1, 2; 1905]. He found that, when esters of *r*-mandelic acid are hydrolysed by liver lipase, the rate of hydrolysis of the ester of (+)mandelic acid is greater than the rate of hydrolysis of the ester of (-)mandelic acid. The inference was that the ester of the dextro-form was attacked more rapidly than the ester of the laevo-form. However, when this hypothesis was tested at a later date, Willstätter *et al.* [1928], by taking the dextro- and laevo-esters separately, found that the enzyme attacked the ester of the laevo-form of mandelic acid more rapidly. To explain this apparent contradiction they postulated that there are two processes with distinct reaction rates, first the formation of the ester-ferment compounds and secondly the actual hydrolysis of the esters themselves.

However this may be, there are probably in the body optically active substances capable of forming compounds with mandelic acid without destruction of the stereochemical identity of the acid. If the formation and subsequent destruction of such compounds have different reaction rates according to the nature of the mandelic acid concerned, then in some such way may be explained the rapid initial preferential excretion of the laevo-form of mandelic acid.

SUMMARY

1. *r*-Mandelic acid was given intravenously to decerebrate cats, either as the sodium salt or as the free acid.
2. The urine was collected fractionally and the mandelic acid recovered with little contamination by extraction of the urine with ether.
3. The early samples of urine had a marked excess of the laevo-form of the acid. In subsequent samples of urine the preponderance of the laevo-form of the acid became progressively less. In still later samples the recovered material was optically inactive, and ultimately, in experiments lasting a sufficient length of time, the samples of excreted material showed a steadily increasing dextro-rotation. A partial resolution was thus effected without the agency of bacteria.
4. The total excreted material in long experiments showed a rotation only slightly to the laevo-side.
5. The total amount of recovered acid in long experiments was between 80 and 90% of the acid administered.
6. When pure active dextro- or laevo-acid was administered, the rotation of the recovered material was slightly below the theoretical value.
7. Possible interpretations of these results are discussed.

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