Br. J. Pharmac. Chemother. (1968), 32, 483–492.

EFFECT OF MERCURY DERIVATIVES, IMPLANTED INTO THE HYPOTHALAMUS, ON THE WATER INTAKE OF ALBINO RATS

BY

F. BERGMANN, M. CHAIMOVITZ, Y. GUTMAN AND A. ZERACHIA

From the Department of Pharmacology, Hebrew University—Hadassah Medical School, Jerusalem, Israel

(*Received September* 22, 1967)

When drugs affecting active sodium transport are deposited bilaterally into the hypothalamus, they have a pronounced influence on water consumption. Thus chlorothiazides suppress both the spontaneous drinking of rats and their response to hypertonic saline (Gutman & Chaimovitz, 1965). Cardiac glycosides are even more effective, for only microgram quantities are needed to produce complete adipsia (Bergmann, Chaimovitz, Costin, Gutman & Ginath, 1967) while with ethacrynic acid slightly higher doses are necessary (Bergmann, Zerachia, Chaimovitz & Gutman, 1968).

Both cardiac glycosides and ethacrynic acid are typical inhibitors of sodium and potassium-dependent transport ATPases (Skou, 1965 ; Duggan & Noll, 1965). The antidipsic effect of these drugs was therefore tentatively ascribed to inhibition of the enzyme and thus of the active sodium transport in osmoreceptors and other cells in the hypothalamus concerned with the regulation of water intake. Similarly, the diuretic effect of organic mercurials has been related to inhibition of renal transport ATPase (Jones, Lockett & Landon, 1965), because *in vitro* this enzyme is strongly inhibited by derivatives of mercury (Landon & Norris, 1963 ; Taylor, 1963). Furthermore, it has been shown by Gussin & Cafruny (1965) that ethacrynic acid competes with chlormerodrin for uptake by renal cortical slices. It was thus of interest to determine whether mercurials, implanted into the hypothalamus, would influence drinking.

Clarkson, Rothstein & Sutherland (1965) showed that the diuretic action of organic mercurials was caused by inorganic mercuric ions liberated in both renal and hepatic tissue by splitting of the mercury-carbon bond. Indeed, in proper experimental conditions, inorganic mercury salts are more potent diuretics than organic mercurials (Levy, Weiner & Mudge, 1958). A similar splitting reaction may take place in nerve cells. In the present study therefore we have compared the action of an inorganic and an organic mercury derivative on spontaneous and stimulated water consumption.

METHODS

Male albino rats of the Hebrew University strain, weighing 200–300 g, were kept in groups of four per cage. The rats lived on a standard diet of Amrod pellets (Ambar Inc., Emek Hefer, Israel). Daily food and water consumption was measured both before and after implantation into the hypothalamus.

Surgical procedures

Food and water were withheld 3 hr before surgery. Implantation was performed under ether anaesthesia, at the following co-ordinates: hypothalamus—1.5 mm from the midline, 2.5 mm caudal to the centre of the coronary suture and 8.5 mm below the surface of the skull; thalamus—same co-ordinates, but at a depth of 6 mm.

Talc powder was impregnated with a methanolic solution of mercuric acetate or chlormerodrin and the solvent evaporated at 30° C with occasional stirring.

Chlormerodrin was a gift of Zori Pharmaceuticals Inc., Tel Aviv.

A pellet of 100 μg was ejected through a 21 gauge cannula by pushing down a No. 27 gauge needle which was sealed at its end, and whose length was adjusted to fit exactly the muzzle of the outer 21 gauge tube. After completion of the experiment the animals were killed with ether and the brain fixed in formalin. The sites of implantation were identified in serial sections of 80 μ thickness. In Fig. 1, the pellets can easily be recognized because charcoal was added to the talc. The sites of the deposits were plotted on charts according to the atlas of Massopust (1961) (see Fig. 2).

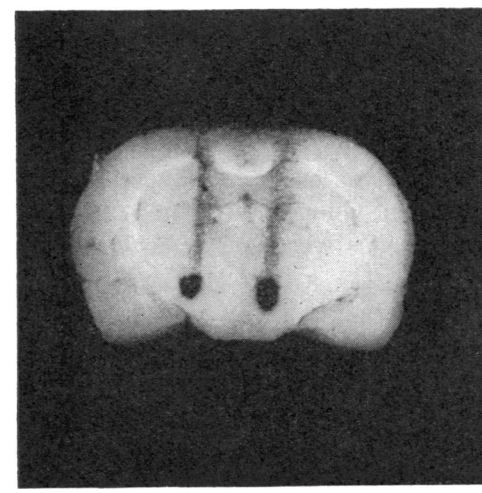

Fig. 1. Transverse cut through rat brain showing (as a black stripe) the trajectory of the needle used for implantation and the two hypothalamic deposits of talc, in a mixture with charcoal. Note that the pellets differ in size because they were not implanted in exactly parallel sites, so that the cut is placed nearer to the pole of the left pellet.

Bilateral nephrectomy was performed under ether anaesthesia through a dorsal midline cut. The animals had free access to water and food during the 24 hr following the operation. They were then used to measure the effect of hypertonic saline on drinking.

For acute stimulation of drinking, the rats were given subcutaneous injections of 3% sodium chloride 50 ml./kg. Thereafter, water intake was measured for 3 hr at hourly intervals.

RESULTS

Effects of chlormerodrin on water intake

When the drug was deposited bilaterally into the hypothalamus, the minimal effective amount was 20 μg. This dose reduced spontaneous drinking markedly for 3–4 days, the adipsia waning gradually (Table 1). After larger doses of the mercurial, complete adipsia (and aphagia) ensued and body weight declined steadily.

A remarkable feature was the delay in onset of action. This was especially conspicuous with small doses (20–40 μg), where the full pharmacological effect sometimes became

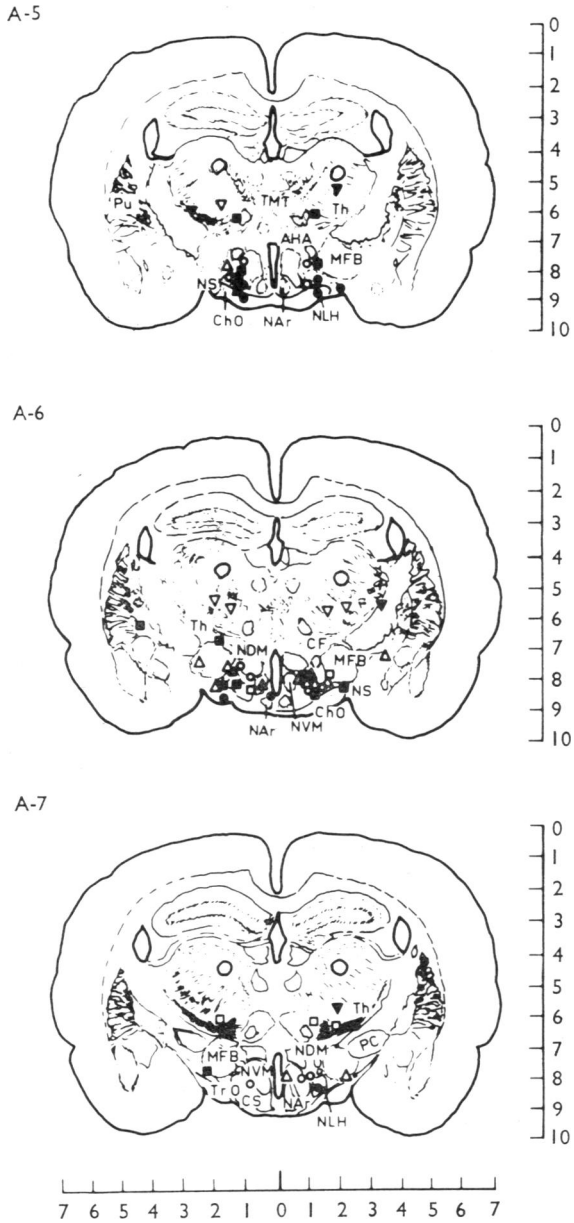

Fig. 2. Transverse sections of rat brain showing the localization of mercurials implanted bilaterally into the hypothalamus or thalamus. Planes A-5 to A-7 according to the atlas of Massopust (1961). Symbols: A, for hypothalamic implantation: ●, RHg+ 15 μg; ▲, RHg+ 30 μg; ■, RHg+ 50 μg; ○, Hg++ 5 μg; △, Hg++ 10 μg; □, Hg++ 20 μg; B, for deposition in the thalamus: ⊡, RHg+ 40 μg; ▽, Hg++ 20 μg; ▼, Hg++ 40 μg. (RHg+ is chlormerodrin; Hg++ is mercuric acetate.) Abbreviations: AHA, area hypothalamica anterior; CF, columna fornicis; ChO, chiasma opticum; CS, commissura supraoptica; MFB, medial forebrain bundle; NAr, nucleus arcuatus; NDM, nucleus dorsomedialis hypothalami; NLH, nucleus lateralis hypothalami; NS, nucleus supraopticus; NVM, nucleus ventromedialis hypothalami; PC, pedunculus cerebri; Pu, putamen; Th, thalamus; TMT, tractus mammillo-thalamicus; TrO, tractus opticus.

TABLE 1

WATER AND FOOD INTAKE AFTER BILATERAL IMPLANTATION OF CHLORMERODRIN INTO THE HYPOTHALAMUS

Groups of four rats of about 1 kg body weight were used for each dose. Water (W) (ml./24 hr) and food (F) (g/24 hr) consumption were measured every 24 hr. (The figures in the table express the actual water or food consumption of the surviving animals.) Figures in parentheses indicate number of animals found dead. Dose indicates the amount of drug deposited on each side of the hypothalamus. * Measurements on day before implantation. † This group received implants of talc 100 μg. ‡ Note that the only survivor has returned to normal consumption after about 8 days.

Dose (μg)	—†		20		40		60	
Days after implantation	W	F	W	F	W	F	W	F
Control*	115	80	140	82	145	75	140	80
1	21	19	25	24	40	10	3	0
2	82	71	8	6	0	0	8	3
3	77	56	12	0	6	2	0	2
4	81	58	28	25	19	6	4	0
5	100	60	30	17	10	0	6	0
6	80	62	32	25 (1)	20	0	4	0
7	112	95	25	8 (2)	0	0 (1)	6	0
8	114	90	25	20‡	12	0	5	0 (2)
9	100	91	28	21	8	0 (1)	8	0
10	98	74	30	27	0	0 (1)	4	0 (1)
11	106	78	30	24	4	0	0	0
12	116	96	30	26	0	0	0	0 (1)
13	130	98	35	28	2	0 (1)		

manifest only 2–3 days after implantation of chlormerodrin. With higher doses, adipsia was usually apparent on the first day after application of the drug. In parallel with the effect on spontaneous drinking, the mercurial also prevented the response to hypertonic saline (Table 2).

TABLE 2

DRINKING RESPONSE TO HYPERTONIC SALINE AFTER HYPOTHALAMIC IMPLANTATION OF CHLORMERODRIN

Groups of four rats of about 1 kg body weight were used for each dose. Dosage indicates the amount deposited on each side of the hypothalamus. Chlormerodrin was implanted bilaterally into the hypothalamus. After the periods indicated, the rats received subcutaneous injections of 3% sodium chloride 50 ml./kg. Water consumption was measured every hour for 3 hr and was expressed as ml./kg body weight. After a dose of chlormerodrin 60 μg, no rat survived for the test on the thirteenth day.

Drug implanted	None	Talc				Chlormerodrin (40 μg)				Chlormerodrin (60 μg)		
Days after implantation	—	2	5	8	13	2	5	8	13	2	5	8
Water consumed during												
1 hr	52	25	35	36	42	0	5	4	25	0	0	0
2 hr	6	7	9	16	15	0	0	0	10	0	10	12
3 hr	2	6	2	2	0	2·5	1	0	25	0	4	0
Total	60	38	46	54	57	2·5	6	4	60	0	14	12

The rats succumbed to continuous lack of water intake after 7–10 days (Table 3). With 150 μg the survival period was somewhat shortened and after 200 μg it was reduced to half. Thus large doses exerted acute toxic effects.

While rats receiving chlormerodrin 20–80 μg behaved normally when they awakened from ether narcosis, some of the rats subjected to doses of 100–200 μg showed unrest, motor excitation and in rare cases epileptic fits, about 60 min after hypothalamic implan-

TABLE 3

TOXICITY OF CHLORMERODRIN IMPLANTED BILATERALLY INTO THE HYPOTHALAMUS

Numerator, number of rats which died; denominator, day of death after implantation. All animals which survived more than 13 days were considered to have recovered from drug action because they consumed food and water in more or less normal quantities.

Dosage (μg)	10	15	20	30	40	50	60	100	150	200
Number of rats	4	4	8	16	16	8	8	8	8	8
Died			2/5	1/3	2/5	1/6	1/1	1/1	2/1	1/1
			1/6	3/6	1/7	4/7	1/5	1/6	1/4	3/1
			1/8	1/7	3/8	1/8	1/6	2/7	2/7	3/7
			1/10	2/8	3/9	1/11	1/8	1/8	1/8	1/10
			1/12	3/9	2/10	1/14	3/11	3/10	1/10	
					3/11				1/13	
					2/13					
Overall mortality	0/4	0/4	6/8	10/16	16/16	8/8	7/8	8/8	8/8	8/8
Average survival time (days) of fatal cases			7·7	7·1	9·2	8·4	7·6	7·4	6·4	4·3

tation. Those rats which survived the application of high doses for more than 1 day, thereafter exhibited quiet behaviour.

Effect of mercuric acetate on water consumption

The mercuric salt was more effective than chlormerodrin. An amount of 5 μg, deposited on each side of the hypothalamus, markedly reduced water and food intake, but about half the rats receiving this dose recovered. With 10 μg or more, however, the effect was practically irreversible, and the animals succumbed to dehydration and inanition (Table 4). It is noteworthy that in all experiments with mercuric acetate the adipsic

TABLE 4

WATER AND FOOD INTAKE AFTER BILATERAL DEPOSITION OF MERCURIC ACETATE INTO THE HYPOTHALAMUS

The figures in the table express actual water and food consumption of the surviving rats. Conditions as in Table 1.

Dose (μg)	5		10		15	
Days after implantation	W	F	W	F	W	F
Control	135	75	110	65	130	45
1	0	5	7	0	3	0
2	4	2	5	1	2	0
3	5	1	0	3 (1)	4	3 (1)
4	4	1 (1)	0	0	4	2
5	7	0	0	0	0	0
6	23	11	4	0	8	0 (2)
7	31	17	3	0 (2)	0	0
8	30	29	3	0	8	8
9	30	30	2	0	0	0
10	34	30 (1)	0	0 (1)	0	0 (1)
11	32	31				
12	40	31				

effect appeared without delay. Suppression of spontaneous drinking was again accompanied by a lack of response to hypertonic saline (Table 5).

With a dose of up to 30 μg, the average length of the survival period was approximately 7 days. With doses of the mercuric salt above 30 μg, early death appeared with increasing frequency and the survival period was markedly shortened (Table 6).

TABLE 5

RESPONSE TO HYPERTONIC SALINE AFTER IMPLANTATION OF MERCURIC ACETATE INTO THE HYPOTHALAMUS OF RATS

For controls, see Table 2. All figures represent water intake/kg body weight. In the group receiving 20 μg of the mercuric salt, no rat survived for the test on the fourteenth day.

Dose	10 μg			20 μg	
Days after implantation	2	8	14	2	5
Water consumed (ml.) during					
1 h	10	26	75	5	0
2 h	0	3	4	0	0
3 h	0	0	0	0	0
Total	10	29	79	5	0

TABLE 6

TOXICITY OF MERCURIC ACETATE, AFTER IMPLANTATION INTO THE HYPOTHALAMUS

For explanation, see Table 3.

Dose (μg)	5	10	15	20	30	40	50	80	100
Number of rats	8	15	8	8	8	8	4	4	4
Died	1/4	1/2	1/1	1/6	2/1	3/1	3/1	3/1	2/1
	1/5	1/3	1/3	3/7	1/6	1/2	1/14	1/8	1/4
	1/10	1/6	2/6	2/8	2/8	1/3			1/5
	1/14	2/7	3/10	1/11	2/10	3/6			
		2/8			1/11				
		1/9							
		1/11							
Overall mortality	4/8	9/15	7/8	7/8	8/8	8/8	4/4	4/4	4/4
Average survival time (days) of fatal cases	8·3	6·8	6·6	7·7	7	3·2	4·2	3·8	3·8

Implantation of doses above 30 μg frequently produced convulsions within 1 hr. Those animals which survived the high doses for more than 1 day, subsequently showed normal behaviour until their death.

Histological examination of the implants showed that they were distributed between 1 and 3 mm from the midline (Fig. 2).

Unilateral implantation of mercurials into the hypothalamus

Bergmann *et al.* (1967) observed that unilateral implantation of ouabain produced adipsia only after doses of 10–20 μg—which are about ten times larger than those required in bilateral application—and that even then the effect was transient. On the other hand, unilateral deposition of ouabain 10 μg into the hypothalamus of the rat was followed by strong convulsions and later by a characteristic disturbance of body equilibrium. The rats kept their heads bent to the contralateral side and therefore had difficulties in licking water. Most of the animals that did not succumb to the initial convulsions, however, also recovered from the adipsia (see Table 7).

We have therefore studied the effect of unilaterally applied mercurials. Here again, the marked tendency to side-positioning of the head interfered with drinking. Nevertheless, seven out of eight rats implanted with chlormerodrin 100 μg recovered after 3–4 days and subsequently consumed normal amounts of water and food (Table 7). Simi-

TABLE 7

UNILATERAL IMPLANTATION OF MERCURIALS OR OUABAIN INTO THE HYPOTHALAMUS OF RATS

The figures express water (ml.) or food (g) consumption/kg body weight.

Substance	Chlormerodrin				Mercuric acetate						Ouabain	
Dose (μg)	100		40		60		80		100		10	
Number of rats	8		4		4		4		4		8	
Days after implantation	W	F	W	F	W	F	W	F	W	F	W	F
1	154	97	30	11	23	24	49	42	6	0	4	2 (2)
2	35	58	46	37	20	6	46	30	14	0	10	6 (1)
3	72	39	54	27	19	14	63	37	30	3	94	11
4	180	77	50	32	38	23	55	44	22	0	120	40
5	154	117	76	50	27	19	67	51	4	0	144	61
6	180	126	90	70	52	34 (2)	80	59	40	12 (1)	119	100
7	195	135	123	86 (1)	66	56	101	68	56	20	141	109 (1)
8	194	140 (1)	109	68	96	75 (1)	78	46	49	26	80	88
9	243	159	128	93	110	82	104	77	52	28	149	120
Mortality	1/8		1/4		3/4		0/4		2/4		5/8	
Survival time (days) of fatal cases	1/8		1/7		2/6 1/8				1/6 1/10		2/1 1/2 1/7 1/13	
Average survival period (days)	8		7		6·7				8		4·8	

larly, after unilateral implantation of mercuric acetate, marked effects on water and food intake were observed only with doses close to 100 μg, which also caused side-positioning of the head; 50% of the animals recovered within 7 days although initially some of them had convulsions.

Thus it seems that high doses of mercurials applied unilaterally do not have a lasting adipsic effect.

Deposition of mercurials into the thalamus

In order to test the specificity of the effect in the hypothalamus, the mercurials were also implanted bilaterally into the thalamus. Here, chlormerodrin in doses of 40–60 μg, which are highly effective in the hypothalamus, had practically no effect on water or food consumption. Mercuric acetate was inert up to doses of 30 μg; but food and water intake were markedly reduced by bilateral deposition of 40 μg and were completely suppressed by 60 μg of the compound. These quantities are six to eight times higher than those necessary to produce adipsia by hypothalamic application. The sites of thalamic application are shown in Fig. 2.

Intravenous application of mercurials to nephrectomized rats

Because the above experiments demonstrated the antidipsic effect of mercurials deposited directly into the hypothalamus, it became of interest to see whether a similar effect results when these drugs are present in the circulation.

In order to test the effect of systemically applied mercurials on water consumption, it is necessary to use nephrectomized rats so as to avoid any possible diuretic action of large doses with the concomitant increase in water intake, and likewise to prevent too rapid excretion of the drugs. When doses about 1,000 times higher than those effective in the hypothalamus were administered intravenously (chlormerodrin 80 mg/kg and mercuric acetate 10 mg/kg), no clear-cut adipsic effect could be established. Larger amounts could not be tested because of acute toxic effects.

DISCUSSION

The data presented demonstrate the strong inhibitory effect of mercurials applied to the hypothalamus, both on spontaneous drinking and on the response to a hypertonic stimulus. Evidently the rats do not react to an increase in plasma sodium concentration. The data, however, do not show whether this refractoriness is absolute or relative—that is, whether the animals might respond if the plasma concentration were raised sufficiently by the use of higher salt concentrations for stimulation.

While large doses of mercurials, deposited in the hypothalamus, produce toxic signs (excitation, convulsions, early death), small amounts do not evoke any overt behavioural effect. It thus seems that the action of low doses of mercurials is specific, preferentially affecting food and water consumption.

Certain differences are apparent between the organic mercurial and the mercuric-salt used. Chlormerodrin in low doses exhibits a delayed onset of action. This suggests that chlormerodrin is transformed slowly and progressively into an " active " form, presumably an inorganic mercury derivative. In addition, larger quantities of the organic mercurial are required to produce adipsia than are needed with the inorganic salt, and the toxicity of the organic compound is much lower. Apparently only part of the latter becomes pharmacologically active.

Figure 2 demonstrates that the location of the pellet, containing the mercurial, in the hypothalamus is not critical. This indicates that the active substance spreads from the deposit to reach the sensitive receptors wherever they may be situated. At present it is not clear whether only a single type of cell connected with the drinking process is affected. In the hypothalamus, both osmoreceptors and a lateral " drinking centre " have been defined (Montemurro & Stevenson, 1957). The drinking centre receives stimuli from a variety of sensory receptors in the mouth, the digestive tract and other organs, and presumably also from the hypothalamic osmoreceptors. It can be assumed that all thirst stimuli, whether emanating from peripheral or central receptors, are ultimately propagated along the same pathway, because the act of drinking is the common response. This pathway runs through the hypothalamus and it is here that drugs inhibiting active sodium transport affect the thirst response. Different thirst stimuli may therefore be blocked by drugs in the hypothalamus. Experiments corroborating this assumption will be reported elsewhere.

For a positive drinking response, it is sufficient that one side of the hypothalamus remains intact and capable of transmitting the thirst stimulus. This is shown by the ineffectiveness of unilateral application of the mercurials or of ouabain (Table 7). The regulation of thirst thus falls in line with many other autonomous functions, which can be maintained by one side of the brain.

In contrast to the results with hypothalamic deposition, systemic application of mercurials exerts only a slight effect on water consumption. It is known that injected mercurials disappear rapidly from the plasma and become bound to the tissue. In the plasma itself they are only partially free, for they react rapidly with sulphydryl groups—for example, those of mercaptalbumin (Hughes, 1947). In the kidney cortex, the mercurials are concentrated several hundred-fold over the plasma concentration (Landon & Norris, 1963) and therefore exert their principal action in this tissue. If the hypothalamus lacks a similar concentration mechanism, circulating mercurials may not reach an effective level in this organ. The failure to suppress thirst may explain why systemically applied mercurials do not show a constant effect on the polyuria of diabetes insipidus in man (Heinemann & Becker, 1958 ; Havard & Wood, 1961) and in experimental animals (Miller & Riggs, 1961), although earlier investigators have claimed that such an action is present (Bauer & Aschner, 1924).

SUMMARY

1. Mercurials were implanted bilaterally into the hypothalamus or the thalamus of rats, and the effect on water and food consumption was measured.

2. Chlormerodrin, in quantities of 40 μg or more, completely suppressed water and food intake, after a delay of 1–2 days. Concomitantly with the abolition of spontaneous drinking, the response to hypertonic saline also failed.

3. Mercuric acetate was more effective, the minimal dose for complete adipsia being 10 μg. The action became manifest on the first day. Larger amounts of the salt often caused excitation and convulsions.

4. Unilateral implantation of large doses of the mercurials into the hypothalamus did not produce a lasting effect on water or food consumption.

5. In bilateral thalamic applications, much larger doses of mercurials were required for suppression of thirst, than were effective in the hypothalamus.

6. Intravenous injection of chlormerodrin 80 mg/kg or mercuric acetate 10 mg/kg into nephrectomized rats did not produce a constant antidipsic effect.

7. It is suggested that chlormerodrin is converted in the hypothalamus into an inorganic mercury derivative, which represents the active form, this process resembling the breakdown of the organic mercurial in the kidney.

This work forms part of a Ph.D. thesis, submitted by A. Zerachia to the Faculty of Medicine, the Hebrew University, Jerusalem, Israel.

The authors thank Mr. R. Knafo for preparation of the figures.

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