SEPARATION OF HORMONAL AND EXOGENOUS IODINE IN SERUM BY MEANS OF A CATION EXCHANGE RESIN

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SUMMARY

The influence of iodine-containing compounds on the determination of iodoamino acids (IAA) and hormonal iodine (HI) in serum with a cation exchange resin has been studied. The IAA values were elevated by most of the compounds, though to a lesser degree than the protein-bound iodine. Of 15 chemically different iodinated radiopaques, only three caused false elevation of the HI, for periods varying from 2 to 14 days after the administration of the drug. Of a number of other iodine-containing compounds, only iodochlorhydroxyquin appeared to give variable results during its administration, all others being eliminated from the HI.

The false elevation of protein-bound iodine (PBI) levels, produced by iodinated radiopaques, forms the principal deficiency of this determination (review see [1]). The duration of this effect varies from a few days (*e.g.*, after diatrizoate) to tens of years (after iophenoxic acid), being of the order of months in most cases[1,2]. Methods using butanol extraction[3] (butanol-extractable iodine, BEI) or Dowex-1 chromatography[4] are not influenced by some of these drugs, but are ineffective in the presence of several others[4–7]. As most of these compounds are organic acids and do not contain free amino groups, they were thought probably not to interfere with the *cation* exchange technique, described in a previous communication[8].

The present paper deals with the influence of these and other exogenous iodine compounds on the determination of iodoamino acids (IAA) and, especially, hormonal iodine (HI) in serum with this method. Most exogenous iodine compounds which spuriously elevate PBI values will be shown to be eliminated in the determination of HI.

EXPERIMENTAL

IAA and HI were determined as reported previously[8]. Briefly, 0.5 ml serum is allowed to run through a small column filled with a cation exchange resin (Dowex

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50W × 2). Selective elution, followed by chloric acid digestion and colorimetric assay, permits the quantitation of either all four of the iodoamino acids together—the iodotyrosines MIT and DIT and the hormones T_3 and T_4—or the hormonal iodine only. Normal values are 3.6–7.2 and 3.0–6.0 μg per 100 ml for IAA and HI, respectively.

In some HI determinations, a small salt residue appeared after chloric acid digestion of the eluate. If necessary, isolation of the HI was modified as follows in these cases: after washing with 8 ml borate buffer pH 8.5, the column was washed with 1 ml water, 4 ml 1 *N* HCl, and 4 ml water. The HI was then eluted with 2 ml 5 *N* ammonia as usual.

Washing with more water proved to be effective in only a minority of highly contaminated samples, and has, therefore, not been tried routinely in the greater part of this study.

Washing with more borate buffer was generally found necessary in the presence of high levels of exogenous iodine. A total of 15 ml buffer was sufficient in most cases; extremely high levels required up to 20 ml.

Very large amounts of inorganic iodide (over 1000 μg per 100 ml) were not always adequately removed from the resin with water or borate buffer (though better with buffer than with water). Washing with 1 *N* HCl or 1 *N* $HClO_4$ proved equally inadequate in these cases. Pretreatment with Amberlite IRA 400 anion exchange resin is recommended for these samples. The following procedure has been found to remove 98–99% of as much as 4000 μg iodine per 100 ml. Two ml serum, 2.0 ml water and about 1 g dry Amberlite are placed in a test tube and shaken for at least 20 sec on a mechanical test tube mixer. The remaining iodide does not interfere with the subsequent HI determination.

The determination of total iodine in diluted serum (1:10 or more) was carried out by placing 0.5 ml samples of the diluted serum in colorimeter tubes, adding carborundum and 1.0 ml chloric acid, and carrying out digestion and colorimetry as usual.

RESULTS AND DISCUSSION

Influence of iodinated radiopaques on IAA and HI

In some patients the effect of an iodinated radiopaque could be studied by comparing values for IAA and HI before and after the administration (Table I).

In most patients only contaminated sera could be obtained. In this group, the radiopaque was considered not to interfere with the determination when (a) the same HI values were obtained after washing the column with 8, 10 and 12 ml borate buffer, and/or (b) IAA or HI correlated with the clinical status of the patient. The results are shown in Table II. Samples with more than one contaminant and cases in which the contaminants could not by identified have been omitted.

As appears from Tables I and II, most of the contrast media produce elevated IAA values. Washing with more than the usual 4 to 6 ml water was effective in a few cases, but has not been tried routinely. Apparently, non-specific adsorption of organic compounds lacking basic groupings is not uncommon. This kind of adsorption, however, appeared to be undone by washing with borate buffer: the HI was unaffected by most of the radiopaques investigated. Only iocetamic acid, iopanoic acid and

TABLE I

IODOAMINO ACIDS (IAA) AND HORMONAL IODINE (HI) PRIOR TO AND AFTER ADMINISTRATION OF IODINATED RADIOPAQUES *in vivo*

All values are in μg I per 100 ml serum. Values separated by a semicolon (;) refer to two samples obtained from the same patient with an interval of 2–11 days. Trade names are given in Table IV. When not stated otherwise, patients were euthyroid.

Radiopaque	*Total iodine*	*IAA*		*HI*	
	after	*before*	*after*	*before*	*after*
Diatrizoate	43000	—	—	6.2	6.8
	3000	4.9	9.8	4.3	4.3
	3000	3.7	10.1	3.2	3.7
	>100	5.0	7.2	4.5	4.5
	70	4.9	5.2	4.4	4.6
	12.8*	—	6.2	4.2	4.2
	11.8*	5.0	—	3.9	4.2
Iocetamic acid	1650; 93	—	—	4.2	>20; 4.9
	1530; 65	—	—	2.7	>20; 2.9
	1150	—	—	3.8	>20
	1010; 41	—	—	6.8	9.4; 6.2
	990; 26	—	—	2.6	8.2; 2.8
	960	—	—	2.9	6.9
	730	—	—	4.4	7.6
	730; 37	—	—	6.0	11.2; 7.1
	660; 113	—	—	3.8	7.8; 5.4
	510; 29	—	—	6.6	9.0; 6.0
	400; 19	—	—	3.2	7.4; 2.0
	390; 55	—	—	4.0	3.8; 2.8
	300	—	—	4.3	5.2
Iodipamide	30000	—	—	3.8	4.7
	2300	—	—	4.3	4.7
Iodopyracet	77; 42	7.1	—	—	4.0; 4.1
	15**;26**	8.3**	12.6**	—	9.0**; 7.9**
Iopanoic acid	500	—	—	4.7	>20
	110	8.1	15	—	9.0
Ipodate	6800; 860	6.8	>20; >20	—	>20; 11.0
Methiodal	50	7.2	7.3	6.3	6.1
Propyliodone	13.4*	3.4	5.2	—	3.9

* PBI rather than total iodine was determined in these cases.
** Clinically hyperthyroid patient.

ipodate were not effectively removed from the column when present in high concentrations (Fig. 1). In practice, this was found to mean that the HI is invalidated by the administration of iopanoic acid and ipodate for about 1–2 weeks. Values obtained 2, 4 or 7 days after iocetamic acid (Fig. 2) did not surpass pretreatment values by more than 1.4 μg/100 ml, the average difference (12 cases) being 0.2 μg/100 ml. Preliminary results obtained with a new cholecystographic drug, AG 54-463 (Guerbet, Paris) suggest that it does not invalidate the HI, at any rate at the 100 μg/100 ml level (N. J. Poulie, pers. comm.).

In considering the HI data, it should be kept in mind that several of the patients were gravely ill, and may, therefore, have had lower HI values than other euthyroid subjects as a result of lower binding capacity of thyroxine-binding prealbumin[9]. For example, in a patient with metastatic carcinoma of the prostate, total iodine was 1000 μg/100 ml shortly after diatrizoate, and HI 2.2 μg/100 ml; in another patient, who had obstructive jaundice, the HI was 2.7 and 2.9 μg/100 ml prior to and 7 days after iocetamic acid, respectively.

TABLE II

IAA AND HI AFTER ADMINISTRATION OF RADIOPAQUES *in vivo*

In these cases, no serum was obtained prior to the administration of the iodinated radiopaque. With few exceptions, only one sample was obtained from each patient. All values are in μg I per 100 ml. Trade names are given in Table IV. Unless stated otherwise, patients were either euthyroid or their clinical thyroid status could not be ascertained.

Radiopaque	*Number of samples*	*Total iodine*	*IAA*		*HI*	
			range	*mean*	*range*	*mean*
Bunamiodyl	8	12.5*–1000	5.0–40	15	3.6–7.0	5.3
Diatrizoate	7	8*–1000	3.7–15.2	6.6	2.2–5.7	3.7
Iodinated oil	3	25*,**–36	7.5–18.5**	—	5.0; 11.0**; 16.4**	—
Iodipamide	7	16.5*–750	4.7–>15	—	4.2–7.8	5.5
Iodopyracet	17	8.0–950	4.7–>20	—	2.8–7.5	4.7
Ioglycamate	1	17*	5.8	—	4.9	—
Iopanoic acid	21	16–6300	10.1–>20	>20	3.0–>20	—
Iophenoxic acid	2	230; 1100	>20; >20	>20	4.4; 4.6	4.5
Iopydol+iopydone	2	600; 600	>20; >20	>20	5.2; 6.8	6.0
Iothalamate	1	54000**	>20**	—	9.6**	—
Ipodate	3	100–11500	13.8–>20	—	4.3–>20	—
Propyliodone	3	10.6*–780	7.5–>20	—	4.9–5.5	5.3

* PBI rather than total iodine was determined in these cases.
** Clinically hyperthyroid patient.

Effect of iodinated medications on IAA and HI

Only contaminated sera were obtained from a group of patients who had received iodinated medications. The effect on PBI, IAA and HI is shown in Table III, with the exception of serial determinations during and after Lugol's solution (see below).

As has been found for the radiopaques, iodinated medicaments falsely elevate the IAA, though to a lesser extent than the PBI. Inorganic iodine does so only when present in large doses such as are used in cough mixtures: hundreds of milligrams if not grams per day. On the other hand, the contaminants proved to be effectively eliminated from the HI. The HI values after inorganic iodide, shown in Table III, were obtained without pretreatment with Amberlite (*cf.* EXPERIMENTAL).

After iodochlorhydroxyquin the success of the method appeared to be variable. This compound has been found to be metabolized in the body[10,11], but its fate may well differ from patient to patient[12]. In the patient with the highest total iodine value, the last sample was taken 20 months after cessation of the drug and 14 months after the first sample; the high total iodine concentration, even in the last sample (630 μg/100 ml), makes additional contamination with iophenoxic acid probable, but this could not be ascertained. In another patient (not included in Table III because of prior administration of radiopaques), elevated HI values were found during a course of iodochlorhydroxyquin although total iodine values did not surpass 55 μg/100 ml. Within six days after cessation of the drug, the HI returned to the pretreatment level (3.4 μg/100 ml) in this patient.

During preparation for thyroidectomy with Lugol's solution (elemental iodine dissolved in potassium iodide solution), hyperthyroid patients rapidly become euthyroid in most cases. Due to the exogenous iodine, however, the PBI remains elevated; on the other hand, IAA and HI, like BEI[13], closely reflect the hormonal iodine con-

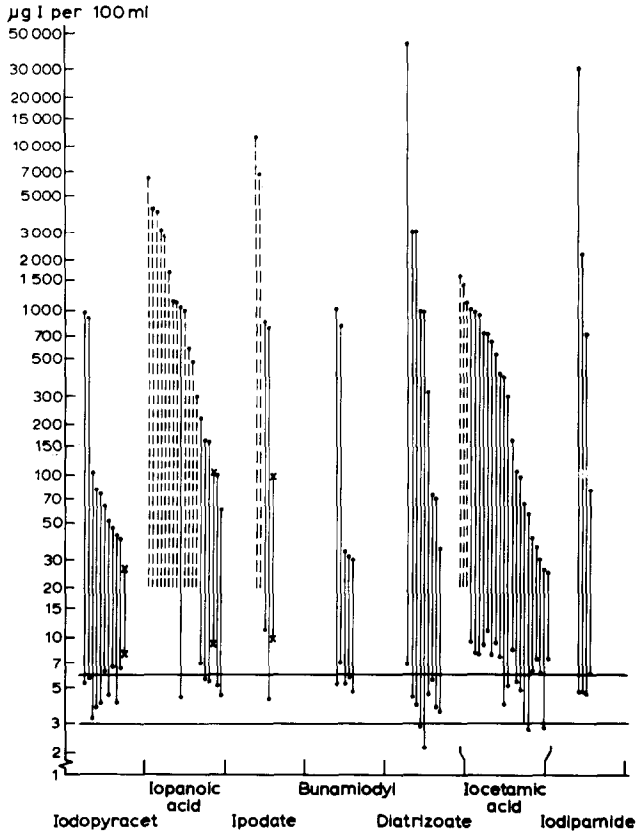

Fig. 1. Total iodine and hormonal iodine (HI) in serum samples obtained after the administration of some iodinated radiopaques. Samples with total iodine values under 25 µg/100 ml have been omitted. Broken lines: HI values over 20 µg/100 ml. Dots: patients clinically euthyroid or thyroid status unknown. Crosses: hyperthyroid patients. Horizontal lines: normal limits for HI.

centration (Fig. 3). In one of the patients (A.B.-B.), the hyperthyroidism was not sufficiently controlled with Lugol's solution to warrant operation; therefore, carbimazole was given and Lugol's solution gradually stopped; carbimazole treatment was interrupted for a short time to permit the performance of a tracer study and the administration of a therapeutic dose of radioiodine.

Effect of iodinated compounds added in vitro

Radioiodinated human serum albumin and [^{131}I]hippurate were added to serum in trace amounts. In each case, over 90% of the radioactivity was removed by two 2-ml water washings, and over 99.0% by washing with 3×2 ml borate buffer followed by 2 ml water.

HI after unidentified iodinated compounds

In about 30% of all samples sent for routine IAA determination and found to be contaminated, the source of the contamination was not detected. In some of these cases, no detailed information could be obtained, whereas in others scrutiny of the

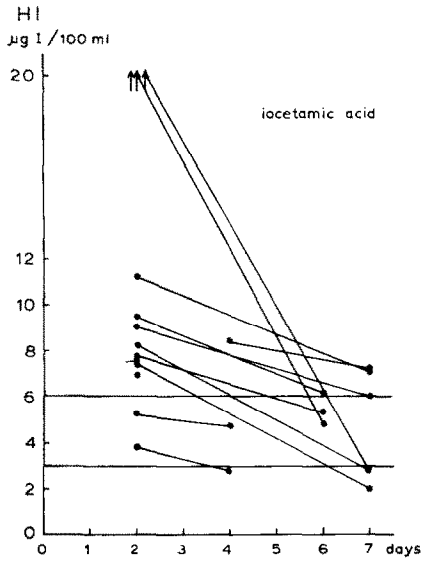

Fig. 2. Dependence of HI on time after the administration of iocetamic acid. Values connected by a line pertain to the same patient. Arrows: HI over 20 µg/100 ml. All patients were euthyroid. Horizontal lines: normal limits.

TABLE III

IAA AND HI AFTER ADMINISTRATION OF IODINATED MEDICATIONS *in vivo*

All values are in µg I per 100 ml serum. Values separated by a semicolon (;) or a hyphen (–) refer to two or more samples obtained from the same patient with an interval of 6 days to 14 months. Unless stated otherwise, patients were euthyroid. Samples from hyperthyroid patients, obtained during preparation for thyroidectomy with Lugol's solution (Fig. 3) are not included.

Iodinated medication	*Total iodine*	*PBI*	*IAA*	*HI*
KI (cough mixtures)	4800	—	>20	11.3+
	3300–1800	—	>20	9.0–7.8
	755	—	>20	5.0
	—	—	13.5	6.2
	—	—	13.1	4.2
	—	18.1	6.1	3.8
	—	14	8.4	4.3
	—	8.2	3.1	—
KI + I_2 (Lugol's soln.)	—	16.5	7.9	5.5
Iodochlorhydroxyquin tablets	810–630*	—	>20	3.2–5.1
	210; 43	—	>20; —	>20; 5.3
ointment	—	19.1; —	—; 11.2	3.1; 3.6**
Chiniofon tablets	—	54	8	6.8

+ Possibly hyperthyroid.
* Additional contamination probable though not proved (see text).
** Probably hypothyroid.

patient's record was unyielding. In most of the latter cases—nine samples, with total iodine and HI values of 190 to 800 and 3.8 to 5.3 µg/100 ml, respectively—ingestion of iophenoxic acid for cholecystography years before was considered the most likely cause.

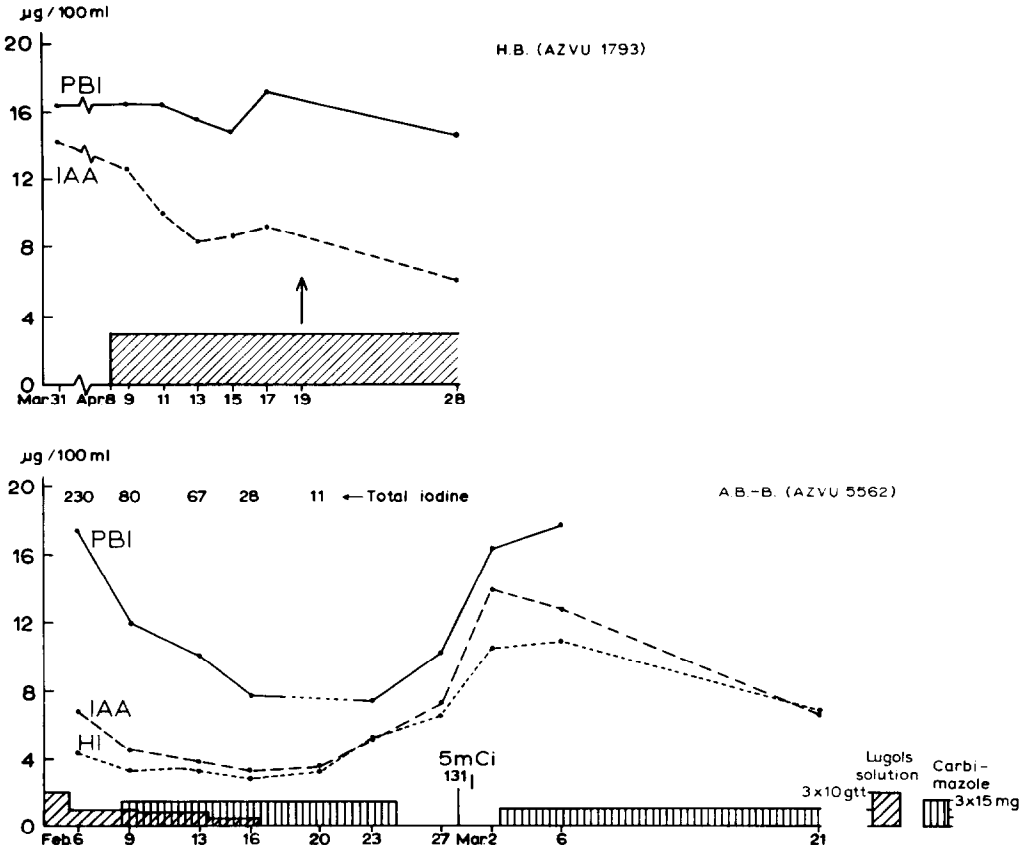

Fig. 3. Influence of treatment with Lugol's solution on PBI, IAA and HI in two hyperthyroid patients. Arrow indicates subtotal thyroidectomy. A.B.-B. received Lugol's solution from Jan. 13, but was finally not operated upon (see text).

Comparison with other methods

The influence of various iodine compounds on the PBI, the BEI, the Dowex-1 technique and the present method has been summarized from literature and present data in Table IV. Where results of experiments *in vivo* and *in vitro* were available, only the former have been included. As for the Dowex-1 technique, the original ashing procedure[4,6] has been referred to if successful, and the non-incineration method (ref. 20, also including personal communication from V. J. Pileggi) in other cases. It will be seen that PBI, BEI, Dowex-1 and present methods are increasingly selective in the order named. Furthermore, the present method is as simple and reproducible as any PBI method, and simpler than BEI and Dowex-1 techniques.

Combination with countercurrent distribution[6] appears to make the BEI technique more specific (though probably no more specific than the present method) but also more laborious.

Methods using thin-layer chromatography (ref. 21 and Th. J. Postmes, pers. comm.) possibly have about the same selectivity but are certainly more complicated

TABLE IV

SEPARATION OF NON-HORMONAL FROM HORMONAL IODINE IN SERUM WITH VARIOUS METHODS

Trade names in the second column are those stated in one or more of the papers from which the data have been compiled; [p] denotes present paper. In the third and following columns, f = failure (level not always specified), s = success at any level, fs = failure or success depending on level, s or fs = no data available on level of 1000 μg I per 100 ml. Symbol in brackets denotes results with chemically closely related compound when no data are available on compound itself, *e.g.*, (s) with acetrizoate refers to success with diatrizoate. Whenever possible, results after administration *in vivo* are given.

Compound		*PBI*	*BEI*	*Dowex-1*	*Dowex-50 W*
generic name	*trade name*				*(HI)*
Acetrizoate	Salpix[4,6,7], Urokon[4,6]	f	fs	s	(s)
Bunamiodyl	Orabilex[6,7], Orabilix[p]	f	f	f	s
Chiniofon[p]		f	—	—	s or fs
Diatrizoate	Gastrografin[6,p], Hypaque[6,7], Urografin[14,p]	fs	s	fs	s
Di-iodotyrosine[3,4,8,15]		f or fs+	s*	s*	s*
Di-iodohydroxyquin	Floraquin[5,20]	f	f	f*	(fs)
Diprotrizoate	Miokon[4,7,20]	f	s*	s*	(s)
Ethiodized oil	Ethiodol[4,6]	f	s or fs	s or fs	—
Ethyliodophenyl undecylate	Pantopaque[6]	f	f	f	—
Iocetamic acid	Cholebrine[p]	f	—	—	fs
Iodide[3,4,p]		fs	s	s	s**
Iodinated oil	Lipiodol[6,p], Viscidol[20]	f	s or fs	fs*	s or fs
Iodinated serum albumin[16,17,p]		f	s or fs	s or fs	s*
Iodine (elemental)″[4,13,p]		f	s	s	s
Iodipamide	Biligrafin[14,p], Cholografin[6,7]	f	fs	f	s
Iodoalphionic acid	Priodax[20]	f	fs*	f*	—
Iodobrassid	Lipoidine[20]	f	—	s*	—
Iodochlorhydroxyquin	Entero-Vioform[12,p], Vioform[p]	f	fs‴	***	fs‴
Iodohippurate	Hippuran[20,p]	f*	f*	s*	s*
Iodomethamate	Neo-Iopax[4]	f*	s*	s*	—
Iodopropylidene glycerol	Organidin[4]	fs*	f*	s*	—
Iodopyracet	Diodrast[5,6], Pyelombrine[p]	f	f	s*	s
Iodothiouracil	Itrumil[4,5]	f	f	fs	—
Ioglycamate	Bilivistane[p]	f	(fs)	(f)	s or fs
Iopanoic acid	Bilijodon-Na[p], Felombrine[p], Telepaque[6,7,12,18,p]	f	fs	f	fs
Iophenoxic acid	Teridax[4,18], Trilombrine[p]	f	f	f	s
Iopydol + iopydone	Hytrast[p]	(f)	—	—	s
Iothalamate	Conray[p]	(f)	(s)	(fs)	s
Ipodate	Biloptine[p], Oragrafin[20]	f	—	s*	fs
Methenamine tetraiodine	Mirion[11]	f	s	—	—
Methiodal	Leo U[p], Skiodan[4]	f*	s*	s*	s or fs
Monoiodotyrosine[4,8,15]		f or fs+	s or fs*	s*	s*
Propyliodone	Dionosil aquosum[20,p]	f*	f*	fs*	s

* Only data of addition *in vitro* available.
\+ Depending on technique[19].
** Extremely high levels: see EXPERIMENTAL.
″ Administration to patients or addition *in vitro* results in formation of iodinated proteins.
*** Unsuccessful in our hands in two cases where Dowex-50 W failed.
‴ Success or failure probably varying from patient to patient (see text).

than the present method. Modifications[21,22] resulted in methods which were simpler (though still more laborious than ours) but somewhat less specific.

Very recently, a method using Sephadex filtration has been published[23]. Iopanoic acid, among other compounds, was found not to interfere up to at least 1600

μg/100 ml. This technique, therefore, may be somewhat more specific than ours; it appears to have a comparable precision but is less simple.

The determination of thyroxine which makes use of the property of protein binding[24,25] has a better selectivity than the present technique. This method, however, is more laborious and less precise than ours, and requires the use of radioactive thyroxine.

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