BIOCHEMICAL STUDIES ON SHOCK

IV. THE OXYGEN CONSUMPTION OF LIVER AND KIDNEY TISSUE FROM RATS IN HEMORRHAGIC SHOCK *

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In the preceding papers of this series (1–3) it has been indicated that one of the consequences of hemorrhagic shock in the rat is a failure of hepatic function due to diminished blood flow and oxygen supply to the liver. The object of the experiments reported in this paper was to examine the effects of hemorrhage on the liver and kidney more directly by comparing the rates of respiration of liver and kidney slices from normal rats and from rats in progressively severe states of shock. In addition, experiments were carried out to test how far any tissue changes taking place after hemorrhage might be accounted for simply by anoxia.

Methods and Materials

Male albino rats of the Sprague-Dawley strain, weighing 250 to 300 gm., were used throughout these experiments. The animals were fed a diet of purina dog chow and in all instances were fasted for 24 hours before being studied. All experiments were performed under light anesthesia with sodium pentobarbital (nembutal) administered intraperitoneally, in a dose of 4 mg. per 100 gm.

Shock was induced by bleeding from the cut tail. An amount of blood equivalent to 3 per cent of the body weight was removed, usually over a period of 1 hour. The animal was kept warm under a lamp, and, at the end of an hour after bleeding (or earlier, if the animal appeared to be near collapse) the liver and in some experiments the kidneys were excised, washed free of blood, blotted upon a hardened filter paper, and placed in a covered dish on ice. In some experiments, samples of blood were taken at the onset of bleeding and again just before the animal was sacrificed, in order to determine the blood amino nitrogen. Normal control animals were kept under anesthesia for periods comparable to those endured by the bled rats.

The respiration of thin slices of the liver and kidney tissue was studied in the Warburg apparatus. The duration of these experiments was 2 hours, the temperature was 37.5°C., and the vessels were filled with oxygen. The main chamber of each vessel contained 2.5 ml. of the "physiological salt solution" of Krebs (4), buffered with phosphate, pH 7.4. The side bulbs contained 0.5 ml. of the buffer solution, either plain, or containing suitable amounts of glucose, sodium succinate (neutral), or

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liver *Kochsaft*, as the design of the experiment required. These additions were tipped in at the end of a 15 minute equilibration period, so that the operating volume for the experiments was 3.0 ml. Carbon dioxide was absorbed in 0.1 ml. of 30 per cent potassium hydroxide placed in the central well of each vessel.

All of the tissue required for a single experiment (usually enough to serve 12 vessels and to provide parallel samples for dry weight determinations) were quickly sliced by hand, by the method of Deutsch (5), and collected in a Petri dish with a slotted top, lined with moisted filter paper, and cooled with ice. This cold moist chamber keeps the tissue in good condition and prevents any marked changes in the original water content of the tissues. From this pool of slices samples of about 200 mg. weight were measured on a torsion balance and placed in the Warburg vessels. Five smaller samples of 50 to 100 mg. were weighed and placed in tared vials to be dried in the oven at 110°C. for the determination of initial dry weight. After the tissues were distributed, the vessels were attached to their manometers, filled with oxygen, mounted in the bath, and shaken for a 15 minute equilibration period at 100 to 110 double excursions per minute.

In the experiments in which the effects of anoxia *in vitro* were studied, liver or kidney slices from a normal fasted rat were weighed and distributed in 12 vessels as described above. Four vessels were filled with oxygen, the remaining 8 with nitrogen and all were placed in the bath and shaken for 15 minutes. Four of the nitrogen-filled vessels were then removed, refilled with oxygen, and replaced in the bath. At the end of another 15 minutes, the taps of the 8 vessels now filled with oxygen were closed and the measurement of the respiration in these vessels was begun. After 1 hour, the 4 remaining nitrogen-filled vessels were removed from the bath, refilled with oxygen, and replaced. At 1 hour, 15 minutes, the taps of these vessels were closed and the respiration was measured for the ensuing hour. At this time, additions were made from the side bulbs of all the vessels, and the effects of the additions on the oxygen uptake were measured for another hour. The duration of these experiments was 3 hours, 15 minutes, and the tissues were equally disposed (*a*) in oxygen continuously, (*b*) in nitrogen for 15 minutes, then in oxygen, or (*c*) in nitrogen for 60 minutes, then in oxygen.

In some experiments the final dry weight of the tissues was determined. The slices of 5 vessels were removed, washed briefly in distilled water, blotted on a No. 1 Whatman filter paper, placed in tared vials, and dried in the oven at 110°C.

Liver *Kochsaft* was prepared by mincing several livers of fed normal rats, suspending the mince in 5 times its weight of distilled water, and heating the mixture to 80 to 90°C. for 10 minutes. The cooked material was chilled and thoroughly centrifuged, and the supernatant solution was frozen and dried in the lyophil apparatus. A fluffy, soluble, yellow powder was obtained which could be conveniently weighed out and dissolved in the buffer solution used in these experiments. In each instance an amount of *Kochsaft* was taken which was equivalent to the weight of liver tissue slices which it was intended to reinforce. This was calculated from the yield of dry material extracted from the livers. In these experiments it amounted to about 10 mg. of the powder for each 200 mg. sample of liver slices.

Blood amino nitrogen was determined by the method of Frame, Russell, and Wilhelmi (6) on 0.2 ml. samples of whole blood. Oxygen uptakes are expressed as "Q_{O_2}", cubic millimeters of oxygen per milligram *initial* dry weight of tissue per hour.

The initial dry weights were calculated from the weights of the samples and the per cent dry weight of the parallel tissue samples taken at the beginning of the experiment.

RESULTS

Although the standard bleeding procedure did not produce a uniform degree of shock in the experimental animals, every bled rat exhibited some signs of shock in the period after bleeding. By observations of pallor, cyanosis, depth and rate of respiration, depth of anesthesia, and ease of bleeding, the animals could be classed as being in good, fair, or poor condition at the time the tissues

TABLE I

Oxygen Utilization of Liver Slices from Bled Rats

Condition	Normal rat controls		Bled rats					
	—		I (good)		II (fair)		III (poor)	
Increase in blood amino N, *mg. per cent*	—		+1.7		+3.0		+7.2	
	No.	Q_{O_2}	No.	Q_{O_2}	No.	Q_{O_2}	No.	Q_{O_2}
No substrate								
1st hr.	25	5.60 ±0.10	16	5.00 ±0.16	14	3.63 ±0.17	12	1.82 ±0.08
2nd hr.	25	4.69 ±0.10	16	4.05 ±0.13	14	2.83 ±0.14	12	1.30 ±0.09
Glucose, 0.1 per cent								
1st hr.	28	5.39 ±0.13	19	5.00 ±0.14	23	3.60 ±0.12	24	1.92 ±0.05
2nd hr.	28	4.51 ±0.12	19	4.10 ±0.13	23	2.90 ±0.10	12	1.23 ±0.04
Glucose, 0.1 per cent, *Kochsaft*								
1st hr.	8	5.4 ±0.2	2	6.0 ±0.2	4	3.6 ±0.1	6	2.3 ±0.3
2nd hr.	8	4.3 ±0.2	2	4.7 ±0.2	4	2.8 ±0.1	6	1.5 ±0.2
Succinate, 0.01 M								
1st hr.	19	10.05 ±0.18	19	9.58 ±0.14	11	7.93 ±0.22	8	7.28 ±0.14
2nd hr.	19	5.46 ±0.16	19	4.60 ±0.13	11	2.93 ±0.16	8	1.54 ±0.13
Succinate, 0.04 M								
1st hr.	6	17.6 ±0.1			6	18.8 ±0.2	3	20.8 ±0.4
2nd hr.	6	9.3 ±0.2			6	6.3 ±0.2	3	3.3 ±0.1
Succinate, 0.04 M, *Kochsaft*								
1st hr.	6	18.4 ±0.5			6	19.3 ±0.3	3	21.5 ±0.4
2nd hr.	6	10.2 ±0.7			6	8.0 ±0.2	3	5.1 ±0.2

were taken. These appraisals were supported by the determinations of the rise in blood amino nitrogen, the extent of which is proportional to the severity of shock (1).

The oxygen uptake of the liver tissue of bled rats exhibited a mild (but significant), moderate, or profound depression, in accordance with the estimates of the rat's condition and the increases in the blood amino nitrogen. The presence or absence of glucose as substrate had no influence on the oxygen uptake of liver slices either from normal rats or from rats in states of shock. The data are presented in Table I. The inverse relationship between the Q_{O_2} of the liver tissue and the increase in the blood amino nitrogen is shown in Fig. 1.

The depressed respiration of the liver slices after hemorrhage was not a con-

sequence of prolonged subnormal body temperature, since the rectal temperature of most of the animals was maintained throughout the period of observation. The depression in oxygen uptake of the liver tissue was correlated only with the severity of shock, and not necessarily with the speed with which the animals were bled or with the rapidity with which the symptoms developed after bleeding.

Since one of the important factors in maintaining normal tissue respiration is the concentration of coenzyme substances, a series of experiments was carried out to test whether the low rate of respiration of liver tissue after hemorrhage might not be due to a deficiency of these agents. The effects on the liver slices of the addition of liver *Kochsaft* in amounts equivalent to the size of the

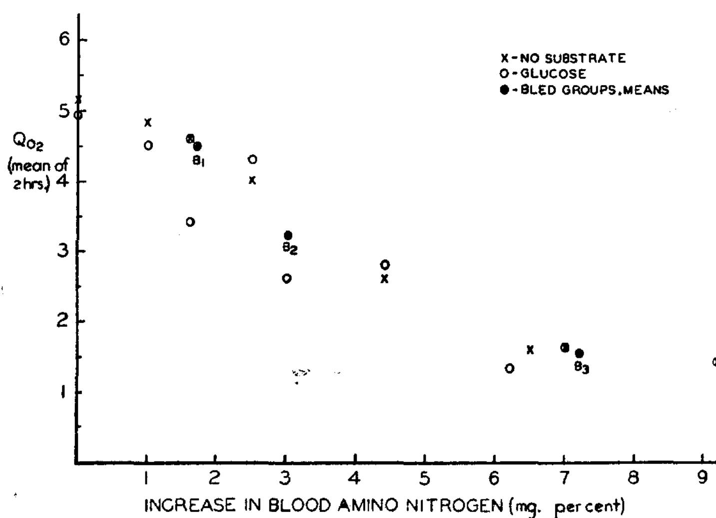

FIG. 1

tissue sample taken were studied in a group of normal and bled rats. The data (Table I) indicate that the supplement of soluble coenzyme factors had little or no effect on the level of the oxygen uptake of liver slices from normal rats or from animals in moderate or severe states of shock, but that there was a strong suggestion of improvement in the performance of the liver tissue from bled rats in "good" condition. Loss or destruction of coenzyme factors after hemorrhage might therefore contribute in part to the depression of liver respiration, but it is evidently not the sole cause of the depression.

Succinate was selected as a test substrate in another series of experiments because it is readily oxidized by normal tissues, and because the complex succinoxidase system may play an important part in the normal respiration of the tissues. The oxygen uptakes of liver slices from a number of normal and bled rats, in the presence of 0.01 M and of 0.04 M succinate, are presented in Table I.

With 0.01 M succinate, the levels of oxygen uptake of the different classes of tissue differ in the same order as they do in the absence of substrate; the increment in oxygen uptake over the no-substrate level during the first hour is in every instance nearly the same (normal, + 4.4; class I, +4.6; class II, +4.3; class III, + 5.4). In the 2nd hour the respiration of all classes has returned to the no-substrate level. The increased respiration with succinate has therefore not resulted in improvement or repair of the basal respiration of the liver tissue from the bled rats.

The responses of the tissues to 0.04 M succinate are somewhat different. In the 1st hour, there is a greater oxygen uptake by the liver slices from the bled rats, and if the increments over the no-substrate levels are taken, the increases in oxygen uptake are significantly greater in the liver tissue from the bled rats

TABLE II

Percentage Dry Weight of Liver and Kidney Slices from Normal and Bled Rats

	Dry weight			
	No.	Initial	No.	Final
Liver		*per cent*		*per cent*
Control	24	27.7 ±0.2	3	17.0 ±0.8
Bled (I)	5	27.7 ±0.5	—	
(II)	7	26.7 ±0.4	2	16.6
(III)	6	25.7 ±0.4	1	13.6
Kidney				
Control	4	23.9 ±0.3		
Bled (I)	1	25.0		
(II)	2	23.3		
(III)	1	23.1		

(normal, +12.8; class II, +15.7; class III, +20.7). This may be an expression of a tendency towards disorganization of the liver tissue from bled rats. It is well known that the rate of oxidation of succinate is greatly increased by mincing or homogenizing the tissue. An effect of moderate or profound shock upon the integrity of the liver is indicated by the data on the initial and final dry weights of the tissues (Table II). The original water content of the liver increases steadily with increasing severity of shock, and the final dry weights suggest that the liver slices from the bled rats lose more substance during the experiments than liver slices from normal rats. It is difficult to decide whether this is a consequence of, or a factor contributing to, the low rates of respiration of liver tissue from bled animals. The data do suggest that the initial dry weight is a somewhat safer measure of the comparative activities of different tissue samples than the dry weight based upon the amount of tissue recovered from the vessels at the end of the experiments. The effects of liver *Kochsaft* on

the oxygen uptake of the various classes of liver tissue in the presence of 0.04 M succinate are not significant in the 1st hour. In the 2nd hour, however, the oxygen uptake of the liver slices from the bled rats was better maintained in the presence of *Kochsaft.*

The experiments with succinate indicate that there is little or no impairment of the succinoxidase system in the liver tissue of rats in hemorrhagic shock. With both concentrations of succinate the course of the respiration was nearly the same in all classes of liver tissue, and the same maximum rates were attained in the first few minutes after the addition of the substrate. The low rates of respiration of rat liver tissue after hemorrhage cannot be ascribed to the development of defects either in the cytochrome system or in the succinic dehydrogenase.

TABLE III

Oxygen Utilization of Kidney Slices from Bled Rats

	Normal rat controls		Bled rats					
Condition			I (good)		II (fair)		III (poor)	
	No.	Q_{O_2}	No.	Q_{O_2}	No.	Q_{O_2}	No.	Q_{O_2}
No substrate								
1st hr.	4	16.1	2	15.4	4	16.5	2	14.3
2nd hr.	4	13.9	2	13.1	4	14.0	2	13.0
Glucose, 0.1 per cent								
1st hr.	5	15.9	2	15.3	6	17.7	2	14.9
2nd hr.	5	15.0	2	14.4	6	16.2	2	14.1

The effects of hemorrhage upon the oxygen uptake of rat kidney tissue are relatively small. In Table III, it will be seen that kidney slices from rats even in profound shock exhibit only a slight depression in rate of oxygen consumption. With glucose the initial rate of respiration is better maintained in all classes of kidney tissue.

In hemorrhage shock in the rat there is a sharp reduction in the portal blood flow and oxygen supply (3). In these circumstances the liver, which receives a large part of its oxygen supply by the portal vein, may be subjected to severe anoxia. It therefore seemed of interest to compare the effects of lack of oxygen *in vitro* on the respiration of normal rat liver slices with the effects of hemorrhage. In Table IV are presented the results of experiments in which normal rat liver slices were exposed to nitrogen at 37.5°C. for 15 minutes and 60 minutes. The effects of this treatment on the oxygen uptake are quantitatively and qualitatively similar to the effects of moderate and severe shock after hemorrhage. The succinoxidase system appears to be relatively unaffected in these experiments, and there is a slight improvement in the respiration of the anoxic

TABLE IV

The Effect of Anoxia in Vitro on the Oxygen Uptake of Liver Slices of Fasted Rats (Glucose, 0.2 Per Cent, Present in All Vessels)

1st hr.	Oxygen throughout		Nitrogen 15 min., then oxygen		Nitrogen 60 min., then oxygen	
	No.	Q_{O_2}	No.	Q_{O_2}	No.	Q_{O_2}
2nd hr.	14	4.69 +0.13	12	3.05 +0.11	11	1.45 +0.06
3rd hr.						
No addition	3	4.7	3	2.7	3	0.9
Kochsaft added	5	4.8	4	3.4	4	1.4
Succinate, 0.02 M	4	12.4	3	12.4	3	11.9

TABLE V

The Effect of Kochsaft and Anoxia on the Oxygen Uptake of Rat Liver Slices (in 0.2 Per Cent Glucose)

	Oxygen throughout	Nitrogen 15 min., then oxygen	Nitrogen 60 min., then oxygen
	Q_{O_2}	Q_{O_2}	Q_{O_2}
No addition			
1st hr.	5.4	3.2	—
2nd hr.	5.3	2.9	1.5
3rd hr.	4.9	2.6	0.9
Kochsaft added			
1st hr.	5.2	4.9	—
2nd hr.	5.0	3.9	2.3
3rd hr.	4.6	3.5	1.4

These figures were obtained simultaneously on slices from the liver of one fasted rat. Each figure is the mean of closely agreeing duplicate determinations.

TABLE VI

The Effect of Anoxia in Vitro on the Oxygen Uptake of Rat Kidney Slices

	Oxygen throughout	Nitrogen 15 min., then oxygen	Nitrogen 60 min., then oxygen
	Q_{O_2}	Q_{O_2}	Q_{O_2}
No substrate			
1st hr.	17.1	14.3	—
2nd hr.	14.5	13.5	5.6
3rd hr.	12.3	11.5	4.2
Glucose, 0.1 per cent			
1st hr.	17.7	14.8	—
2nd hr.	16.3	15.5	10.0
3rd hr.	15.3	14.7	10.4

Each figure is the mean of 4 determinations which agreed closely.

tissue in the presence of *Kochsaft*. The data of a single experiment in Table V illustrate more clearly the course of the respiration and the effects of *Kochsaft*.

The data of a similar series of experiments with rat kidney slices are presented in Table VI. The effects of the shorter period of anoxia are much less marked in kidney tissue than in liver tissue, but after 60 minutes in nitrogen, the respiration of both tissues is depressed in about the same proportion. Glucose, which is without effect on the oxygen uptake of liver slices, supports nearly complete recovery of the oxygen uptake of kidney slices after 15 minutes in nitrogen, and nearly doubles the rate of respiration of kidney tissue after 60 minutes in nitrogen. The more moderate effects of hemorrhage upon the rate of oxygen consumption of rat kidney tissue may therefore be due to the fact that the blood flow and oxygen supply to this organ are not so severely diminished as compared to the liver, and to the fact that, by using glucose more readily, the kidney is better able to withstand and overcome the effects of anoxia.

DISCUSSION

The data presented here indicate that the respiration of liver tissue is severely depressed in shock following hemorrhage in the rat, and that this change may be largely a consequence of lack of oxygen. The blood chemical changes in the bled rat described in earlier papers of this series (1–3) also indicate that an important metabolic factor in hemorrhagic shock in the rat is a decrease in hepatic function due to diminished blood flow and oxygen supply to the liver. The behavior of the isolated tissue provides a direct confirmation of the other evidence showing that the liver is seriously affected in hemorrhagic shock. The most consistent sign of hepatic failure is the rise in blood amino nitrogen, which can now be correlated with blood pressure (1), venous oxygen tension (3), and rate of oxygen uptake of the liver tissue *in vitro*.

The nature and sequence of the effects of hemorrhage and anoxia upon the respiration of liver tissue remain to be explained. From the experiments with *Kochsaft* it is suggested that one of the first effects is the loss or partial destruction of one or more coenzyme factors necessary for normal tissue function. The failure of the supplement of *Kochsaft* to increase the rate of respiration of liver tissue from moderately or profoundly shocked rats indicates that enzyme systems as well as coenzymes may become disorganized as shock progresses, but the primary loss or breakdown of coenzymes essential to the production and transfer of energy within the cell may influence critically the rate of decline of other tissue functions.

The experiments with succinate indicate that an increased rate of oxygen uptake in the tissue following the addition of a readily oxidizable substrate does not of itself lead to an improvement in the basal rate of respiration. In every instance, the terminal rate of respiration of the tissue to which succinate had

been added was identical with the rates of oxygen uptake of the slices respiring without added substrate. Thus, although the oxidation of succinate has been linked with phosphorylation of glucose (7) and may be regarded as an energy-producing reaction, there is in these experiments no evidence that the energy has been used to restore the conditions for normal basal respiration. It may be significant that the combination of succinate and *Kochsaft* did result in better maintenance of the respiration of liver tissue from shocked animals in the period following the complete oxidation of succinate to fumarate. Both the extra supplement of coenzyme factors and the increased rate of energy production may be required to initiate the restoration of normal tissue respiration.

The apparent integrity of the succinoxidase system suggests that the ability to take up oxygen and to carry out the terminal steps of hydrogen and electron transport is not a limiting factor in the respiration of liver tissue from rats in states of shock. In drawing this inference from the behavior of isolated tissues a note of caution is required. The oxidation of succinate proceeds vigorously in minced and even in homogenized liver, so that rapid removal of succinate is no guarantee of the integrity of the tissue. While it is interesting to find that the elements of the succinoxidase system are intact, even in severe shock, there is no assurance in this evidence that the enzyme complex is still in proper relation to the other enzyme systems of the liver. It is evidently in this relationship, as well as in the functional integrity of other liver enzymes, that an explanation of the depressed oxygen uptake of liver tissue from bled rats is to be sought.

The excellent correlation of the rise in blood amino nitrogen with the rate of oxygen uptake of the liver indicates that one consequence of shock is a failure of the liver to deal adequately with amino acids. At the moment there is no evidence to indicate whether this involves a failure of the liver to deaminate amino acids or whether the assimilation of amino acids may not also be affected. An accelerated protein breakdown in the peripheral tissues, in consequence of shock, has been described in a preceding paper (2). It is probable that the proteins of the liver are similarly affected by diminished blood flow and oxygen supply. In these circumstances the liver deaminases, if still intact, may be saturated with substrate of hepatic origin, and the normal assimilatory mechanisms, involving continued protein synthesis and breakdown, may fail for lack of the oxidative energy required to maintain these processes in proper equilibrium.

The different effects of glucose on the respiration of liver and of kidney, in shock and in anoxia, lend support to the interesting point that the order of resistance to damage of the tissues in these circumstances—liver<kidney<muscles—is the same as that of their ability to utilize glucose, both aerobically and anaerobically. The respiratory quotient of normal liver tissue is about 0.5; it does not use glucose readily as a substrate. This suggests that the low

rate of oxygen uptake of the liver in shock may be due to damage to enzyme systems responsible for the utilization of some primary substrate other than glucose.

Profound effects of hemorrhagic shock on the metabolism of the liver may not be found in every circumstance or in every species. Beecher and Craig (8) have carried out experiments similar to those reported here, using cats fasted for 24 hours and inducing shock by hemorrhage. They did not observe any effects of their treatment on the oxygen uptake of liver or kidney slices from cats in which the blood pressure had been held below 70 mm. of Hg for 1.3 to 3.0 hours. Our own experiments on cats are as yet incomplete, but it appears that while the liver respiration is not seriously affected in hemorrhagic shock in the cat fasted for 24 hours, it is depressed to a marked degree if the cat is fasted for 48 hours before the experiment. The cat, a large carnivore, and the rat, a small omnivore on a high carbohydrate diet, cannot be expected to be in similar nutritive states after a 24 hour fast. Fasting may certainly affect the resistance of liver tissue to anoxia. Beecher and Craig (9) have found that liver tissue from fed rats maintains a nearly normal rate of oxygen uptake after exposure to nitrogen *in vitro* for even 60 minutes, and we have been able to confirm this in a preliminary experiment.

SUMMARY

1. With increasing severity of shock following hemorrhage in fasted rats there is an increasing depression in the rate of oxygen uptake, in oxygen, of liver slices from the bled animals. The respiration of kidney tissue is only slightly depressed even in severe states of shock.

2. The rates of oxygen uptake of liver tissue from bled rats are nicely correlated with the increases in blood amino nitrogen that follow severe hemorrhage.

3. A supplement of coenzyme factors, in the form of a hot water extract of normal rat liver, increases the oxygen uptake of liver tissue from rats in mild shock, but is without effect on the respiration of liver slices from rats in moderate or severe shock.

4. The ability of rat liver to oxidize succinate is not impaired even in severe shock, but the extra oxygen uptake does not improve the basal rate of respiration of the tissue.

5. Effects on the rate of oxygen uptake of normal rat liver slices comparable to those seen after hemorrhage could be produced by exposing the tissue to an atmosphere of nitrogen for periods of 15 and 60 minutes. This treatment had more marked effects on the respiration of kidney slices than are found after hemorrhage, but the kidney, unlike the liver, exhibited a marked degree of recovery in the presence of glucose.

6. The significance of these findings is briefly discussed.

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