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Effects of dietary nitrogen level on electrolyte and water metabolism in sheep, (part of a coordinated programme on tracer techniques in studies on the use of non-protein nitrogen in ruminants)

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Effect of Dietary Nitrogen Level on Electrolyte and Water Metabolism in Sheep

K^+ Metabolism II.

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Potassium has obtained an increasing importance in animal feeding, detailed investigations into its metabolism are of immediate interest. The widespread use of potassium containing fertilizers has caused a considerable rise of potassium levels in fodders. At the same time the dietary protein and NPN supplies are being increased continuously in ruminant feeds, for reasons of improving performance. The absulute abundance of K in the diets brings about a relative Na-deficiency, and a shift of the alkali /Na^+/K^+/ ratio in the organism of the ruminant. Abundant N-supply, on the other hand, enhances the formation of ammonia in the rumen; this imposes a continuous strain on the liver, and occasional rises above the hepatic ammonia threshold result in temporary or permanent rise of the blood ammonia level. Thus excess dietary N supply is in itself disadvantageusly affecting metabolism, and along with the similar influence of excess dietary K, the concomitant

metabolic disorder is often accounting for a considerable depression of performance.

In earlier experiments /Juhász et al. 1974. and 1975./ the distribution of ^{42}K between whole blood, plasma and erythrocytes was studied after intraruminal administration of urea, at rising blood levels of ammonia. In sheep given urea, the ^{42}K activity of the plasma decreased more markedly than in the controls and there was a simultaneous reduction in the ^{42}K activity of whole blood. Unlike controls, the erythrocytic ^{42}K activity also fell. It appears that the rise of the blood ammonia level either inhibits the transport of K^+ from the extracellular space into the cells, or promotes the release of K^+ from the cells by diffusion.

In the folloing experiments we had examined in vitro the precise effect of ammonia on the cell membrane and other side in vivo we would like to see further precise date on K^+ metabolism in sheep after i.v. infusion of ammonium-acetate.

Materials and methods

In vitro experiments

For the measuring of the ingression of ^{42}K into the erythrocytes, the erythrocytes from freshly withdrawn sheep blood were washed in Ringer-Locke solution and were resuspended in the same medium so as to correspond with the original haematocrit reading. To this suspension was added the ^{42}K isotope /1 mCi/ml ^{42}KCl inj. steril, isotonic solution; Institute for Radioisotopes, Hungarian Academy of Sciences, Budapest/ and after its distribution the suspension was divided into two equal parts. One part was adjusted to the required high ammonia level. The suspensions were incubated at 38° C. The ammonia concentration, haematocrit value and ^{42}K activity of the suspension and after centrifugation the activity of Ringer-Locke solution were determined at predetermined time intervals.

In the case of measuring of the egression of ^{42}K from the erythrocytes the ^{42}K was administered intravenously to the experimental animals /donor sheep/ 14-16 hours before the in vitro experiments, in order to allow the physiological distribution of the radioisotope to take place between erythrocytes and plasma. The experiment was then conducted

on the same schedule as above, except that no ^{42}K was added to the Ringer-Locke suspension, it being already present in the erythrocytes.

In both case the dose of ^{42}K was so established that the samples taken at the begining of the experiment had an activity of about 1 /uCi/100 ml.

In vivo experiments

Twelwe sheep provided with ruminal fistula, urinary catheter and unilateral parotid gland fistula were given intravenously ^{42}K-labeled KCl solution /27 /uCi/kg bodyweight/, 16-20 hours before the experiment, which lasted 8-10 hours. During the experiment blood and rumen fluid samples were taken every hour, and discharged saliva and urine were secured continuously. A 0.95 mM/ml ammoniumacetate solution of pH 7.20 was used for slow intravenous infusion over 4 hours, at constant rate of 0.79 ml/min; the total amount of the solution was 190 ml per animal.

The potassium content of the diet fed to the experimental animals provided for a normal K^+- supply. Part of the animals were not given drinking water for 16 hours, part were given about half a litre before the experiment.

In the in vitro and in vivo experiments the ammonia contents of samples were determined by the microdiffusion method /Juhász and Szegedi, 1958/. The activity of the samples was determined using a hollow scintillation detector and

a NK 108 counter /GAMMA, Budapest/.

Results

In vitro experiments

The precise nature of ammonia effect on cell membrane function was examined in vitro, because in such systems the concentration changes, viz. activity changes of radiopotassium can be followod up until physiological equalization. Since this equalization is an exponential process taking place in 14-16 hours, the active influx of ^{42}K into erythrocytes, viz. the inhibitory effect of ammonia on this process, can be read only during the first 5-6 hours. These conditions were borne in mind at planning the experiments /Juhász et al. 1974. and 1975/.

The high initial ammonia level /914±210 /ug/100 ml/ tended to rise further in the in vitro systems. This phenomenon was consistently observed in all experimental series, also occurred in the control lot, from 95±20 /ug/100 ml to 200±16 /ug/100 ml. This phenomenon did not interfere with the accuracy of the reading, because the degree of concentration increase remained well below the toxic level /Table I./.

The experiments having been conducted in a closed system, the ^{42}K activity of the erythrocyte suspension did not change, apart from minor differences presumably due to technical error. Accordingly, the ^{42}K activities of control and ammonia-treated samples did not differ from one another in these series. The activity of the Ringer-Locke solutions separated from the suspension by centrifugation decreased gradually in both control and ammonia-treated series. The two series showing activity change do not notably differ from one another if the standard deviations are also taken into consideration. It follows that the calculated ^{42}K activities of the erythrocytes do not differ between the control and ammonia-treated systems. The distribution of ^{42}K between erythrocytes and Ringer-Locke solution also did not differ among in vitro systems treated and not treated with ammonia. /Table I./.

It is concluded from the above findings that elevated concentrations of ammonia do not inhibit those erythrocyte membrane processes which promote the intracellular ingression of ^{42}K. It follows that the change of ^{42}K distribution with the rise of the ammonia concentration cannot be explained by inhibition of membrane function. The alteration of K-distribution is in all probability due to an enhanced rediffusion of K, this being the sole explanation for the decrease of erythrocytic ^{42}K-activity.

We speculated that a check-up of this process might be possible with the above method, if the erythrocytes were saturated *in vivo* with ^{42}K prior to the *in vitro* experiment. Thus it was possible to assure the exit of ^{42}K from erythrocytes by determination of activities in the full suspension and in the Ringer-Locke solution separated from it. In the system used, the activity of the erythrocytes tended to decrease, that of the Ringer-Locke solution to increase, and the degree of activity changes corresponded with the rate of the rediffusion of K^+. Fig. 1. shows the percentual decrease of erythrocyte activity, as related to initial activity. The slopes of the two curves permit the conclusion that in those samples in which the ammonia concentration was elevated, the release of ^{42}K increased over the controls. The results of the *in vitro* experiments indicate that in media containing approx. 600-900 /ug/100 ml ammonia, the NH_4-ion enhancing the passive diffusion of K^+ from the erythrocytes, but it does not interfere with the active influx of K^+ into the cells.

In vivo experiments

In vivo studies have also been carried out to obtain more information about K-metabolism, namely about the factor responsible for the fall of blood or plasma potassium level, while the blood ammonia concentration rises. These experiments were planned on the basis of earlier investigations into the water metabolism, and saliva and urine production of sheep fed different doses of urea. /Juhász and Szegedi 1968, 1969; Szegedi and Juhász 1971/. The rise of the blood ammonia level was found to be accompanied by decrease in the rate of saliva secretion, and a gradual increase of diuresis. The newest investigations have been centered on the influence of the above change on K-metabolism and on K-elimination with saliva and urine.

The changes of blood ammonia level under the influence of the 4-hour infusion can be seen from Fig. 2. The average concentration was 600 /ug/100 ml after one hour, and 800 /ug/100 ml after two hours, but did not rise further during the rest of the infusion period and thereafter it fell abruptly.

Fig. 3. shows that the infusion caused an approx. 15- 20 % decrease in the plasma potassium activity, related to preinfusion activity as 100 %.

As can be seen from Fig. 4.saliva secretion decreased by about 80 % during the infusion. The secretion normalized abruptly during the first hour after infusion.
Changes in diuresis are shown in Fig. 5. It increased markedly by approx. 80 % on the average during infusion, on which followed an abrupt decrease.
The concentration of ^{42}K activity /Fig. 6./ did not change in the saliva during the first hour of infusion, trereafter it rose abruptly, by 60 % on the average, but began to decrease immediately after the infusion had been finished.
It is remarkable /Fig. 7./ that the concentration of urinary ^{42}K activity did not become altered during infusion, but it decreased abruptly by about 40 % after it.
The amount of ^{42}K eliminated with the saliva is shown in Fig. 8. The shape of the curve bears a close resemblance to that of the curve for saliva secretion. The elimination of ^{42}K decreased by approx. 80 % already during the first hour, but subsequently it rose so that the average measure of decrease was by about 50 %. The amount of salivary K^+ secration normalized soon after the infusion.
Fig. 9. shows that the urinary elimination of ^{42}K rose by 50-60 % under the influence of the infusion.

Discussion

Investigation of Funder and Wieth /1974/ show that sodium accumulated in red cells during alkalosis. Red cell potassium increased when the plasma concentration of potassium was normal but decreased when the alkalosis was complicated by low extracellular potassium.
Beal et al. /1974/ found that the salivary flow rate was depressed during the infusion of potassium chloride into both intact sheep and adrenalectomized sheep. As the salivary flow was unchanged by sodium chloride infusion it was concluded that the potassium ion was responsible for the decrease in flow and that this effect was not mediated through any of the adrenal hormones. The highly significant negative correlation between plasma potassium concentration and salivary flow throughout all potassium infusions indicated that the extent to which the salivary flow was depressed varied with the degree of hyperkaleamia.

Slow intravenous infusion of a dilute ammonium-acetate solution for 4 hours caused rise of the blood ammonia level on the average to 800 µg/100 ml, while the blood level of ^{42}K decreased significantly, as assessed from activity measurements in blood plasma. Rise of the blood ammonia level was followed by decrease of saliva secretion and

marked increase of diuresis. The concentration of ^{42}K activity rose in the saliva, but it did not notably change in the urine. The amount of ^{42}K secreted in the saliva in unit time decreased considerably, while that excreted with the urine increased.

Sheep normally provided with drinking water showed a salivary K^+ secretion of approx. 130 mg/hour before infusion, and an average decrease to 90 mg/hour during it. The urinary elimination of K^+ was on average 180 mg/hour before infusion, followed by a rise to 320 mg/hour during it. Further calculations based on the experimental data have disclosed information about the extent of reduction of K^+ in blood, viz. plasma during the abrupt rise of the ammonia level.

The above in vitro and in vivo ~~and in vivo~~ experiments support the conclusion that the presence of approx. 800 µg/100 ml ammonia in the blood does not interfere with the active ingression of K^+ into the cells, but it results in a notable increase of K^+- elimination with the urine, while the amount of K^+ discharged into the rumen with the saliva is decreasing. Accordingly, the decrease in the K^+-content of the erythrocytes can be regarded as a passive process, brought about by equalization of the plasma and cellular concentration gradients.

The i.v. infusion of ammonium-acetate caused no alteration in the ammonia concentrations of saliva and ruminal fluid, but it caused a marked rise in urinary NH_3 excretion, which still persisted at a high level after infusion. The pH of the ruminal fluid was slightly acid /6.5/, while the pH-s of saliva and urine were alkaline during the experiment. The urine pH rose slightly during infusion. The experimental results show that in sheep, deprivation of water for 10-16 hours depress not only diuresis, but also the secretion of saliva in the intervals between feeding.

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Table I.

The changes of blood ammonia concentration and influx of ^{42}K into the erythrocytes

		Time 0	0,5	1,0	1,5	2,5	3,5	4,5	6,5 hours
Blood ammonia /ug/100 ml	Control	95±22	75±15	77±9	107±47	120±17	142±49	156±61	200±16
	Ammonia treated	914±210	898±177	904±192	926±228	909±204	956±234	978±199	998±209
Distribution of ^{42}K %	Control	0	1,2±0,5	3,9±1,2	7,2±3,4	15,6±3,6	22,2±8,5	28,5±11,2	33,4±4,4
	Ammonia treated	0	3,4±2,0	6,7±2,8	16,7±6,7	15,2±9,4	29,7±14,5	28,6±16,3	39,0±6,0

Figures.

/ [redacted] /

Fig. 1. Percentual decrease of erythrocyte ^{42}K activity

Fig. 2. The changes of blood ammonia level under the i.v. infusion of ammonium-acetate

Fig. 3. Changes in the plasma ^{42}K activity

Fig. 4. Salivary secretion rate

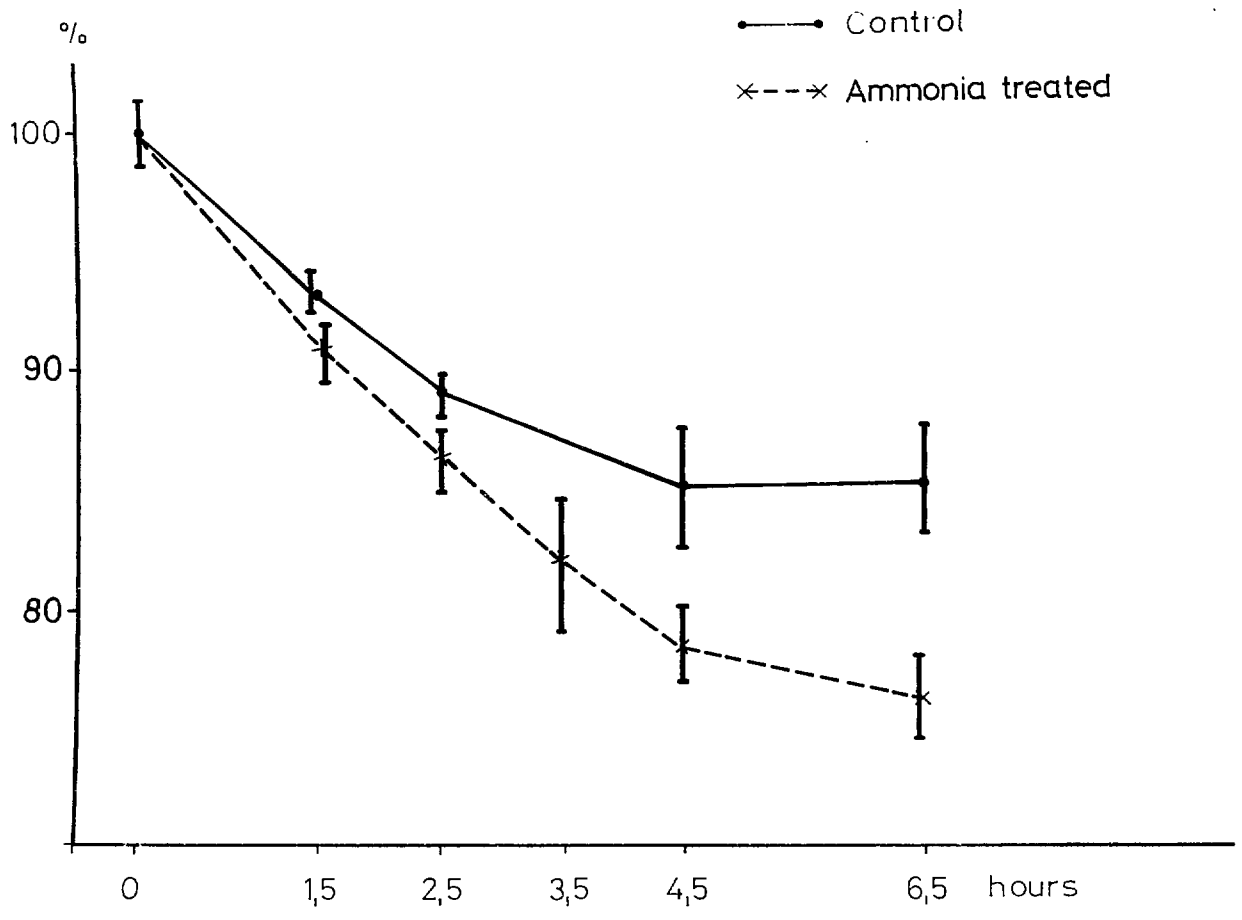
Fig. 5. Changes in diuresis

Fig. 6. Changes of ^{42}K activity in the saliva

Fig. 7. The concentration of urinary ^{42}K activity

Fig. 8. The amount of ^{42}K eliminated with the saliva

Fig. 9. The urinary excretion of ^{42}K


%
Control
Ammonia treated
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90
80
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1,5
2,5
3,5
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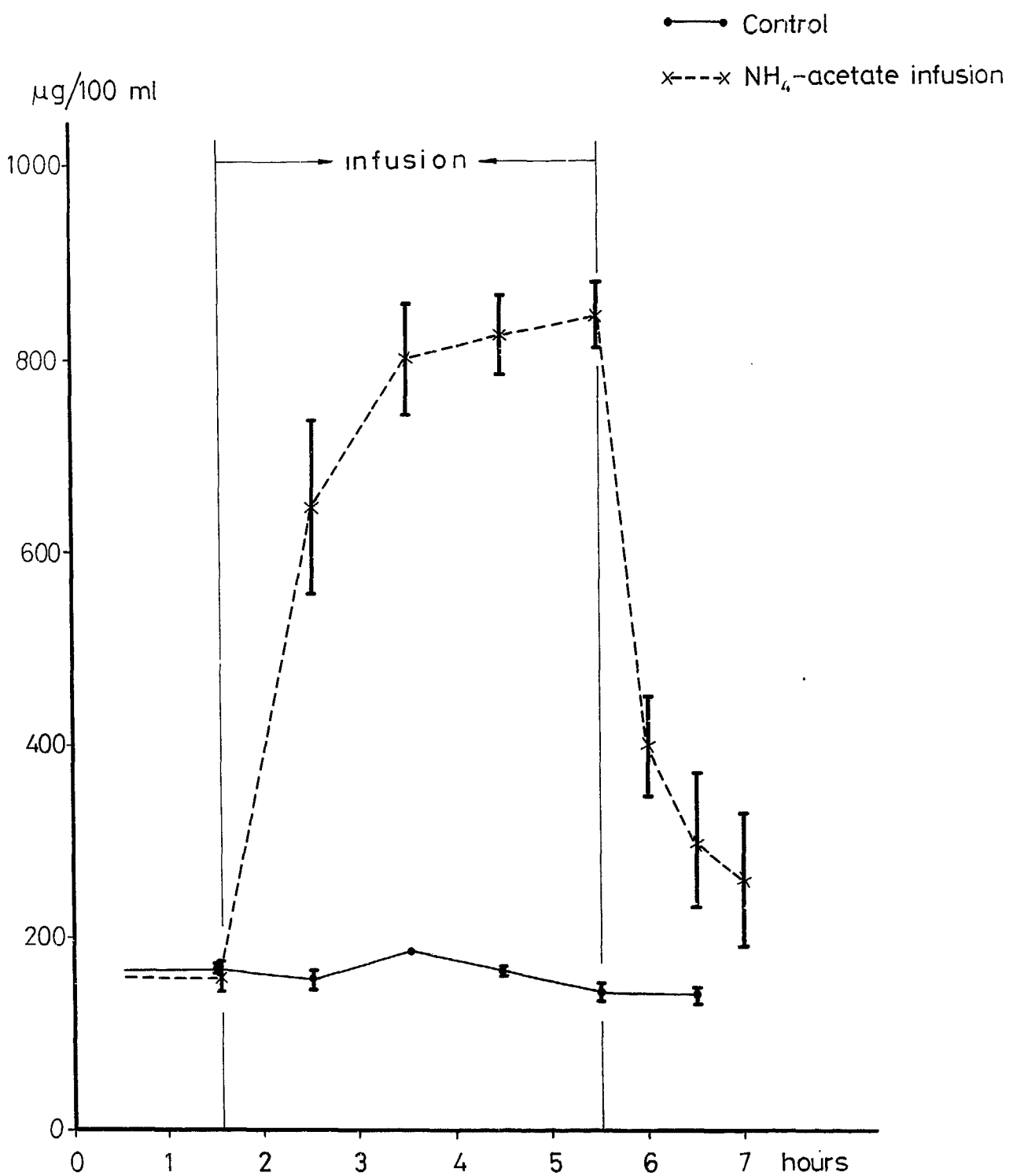
hours


Control
NH4-acetate infusion
µg/100 ml
infusion
1000
800
600
400
200
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1
2
3
4
5
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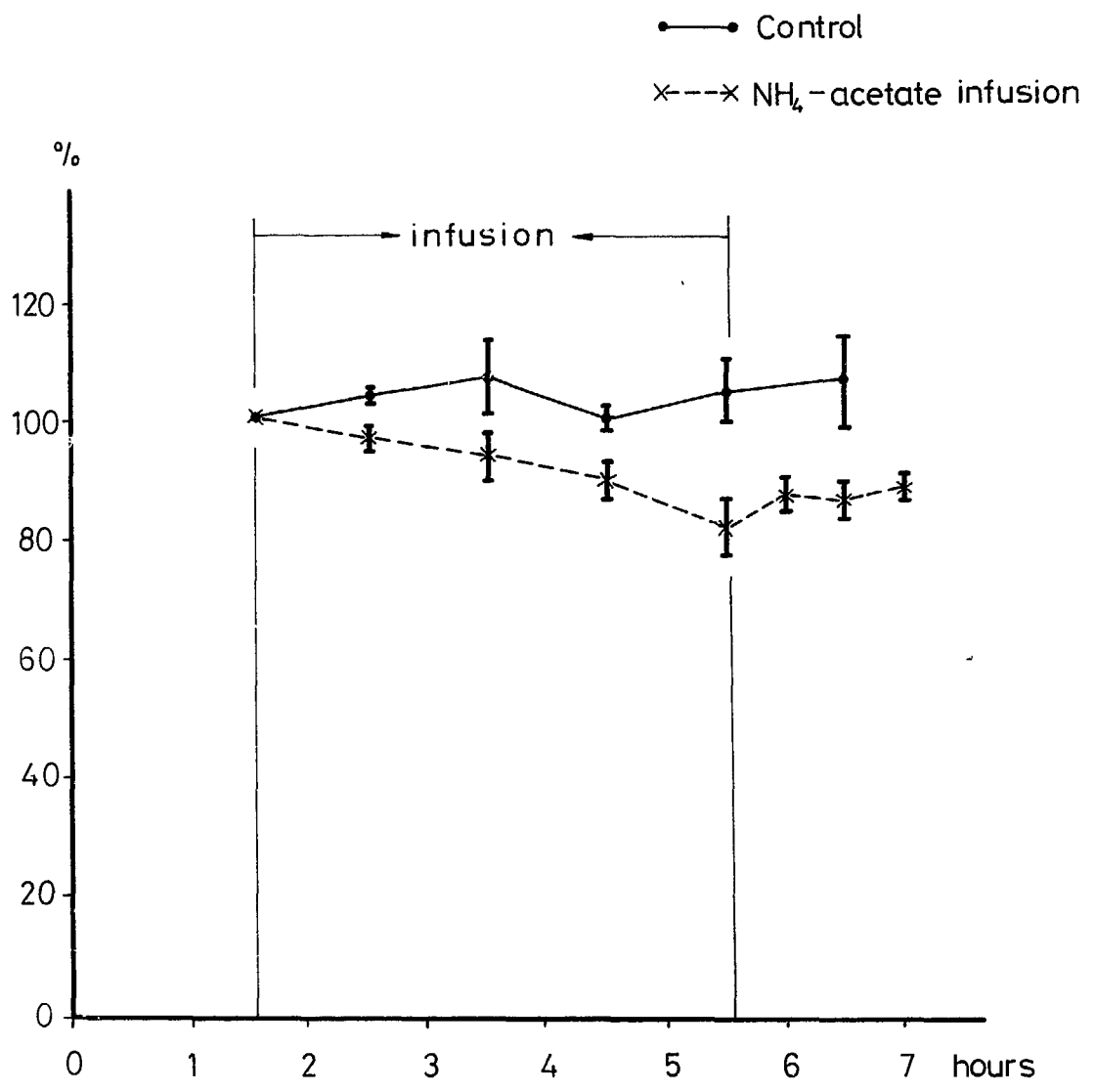
7
hours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NH4-acetate infusion
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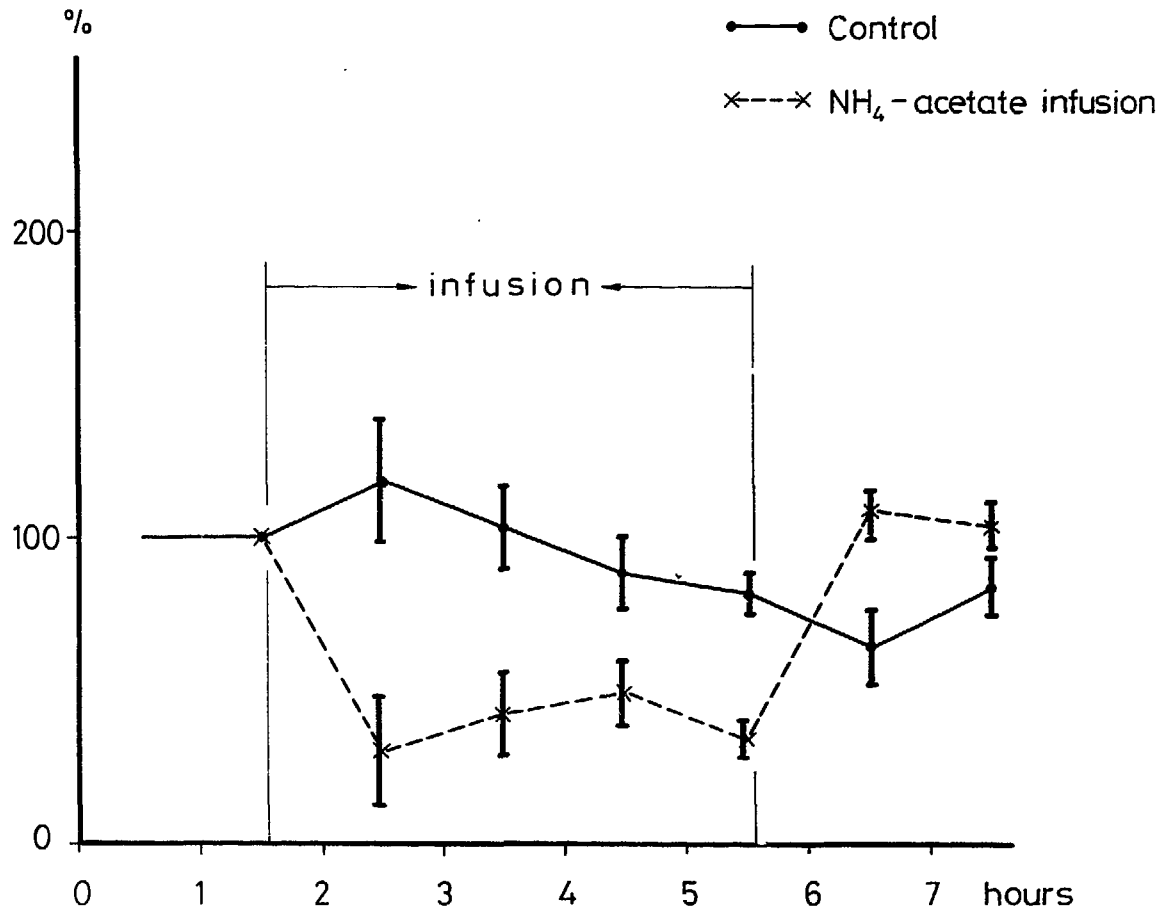
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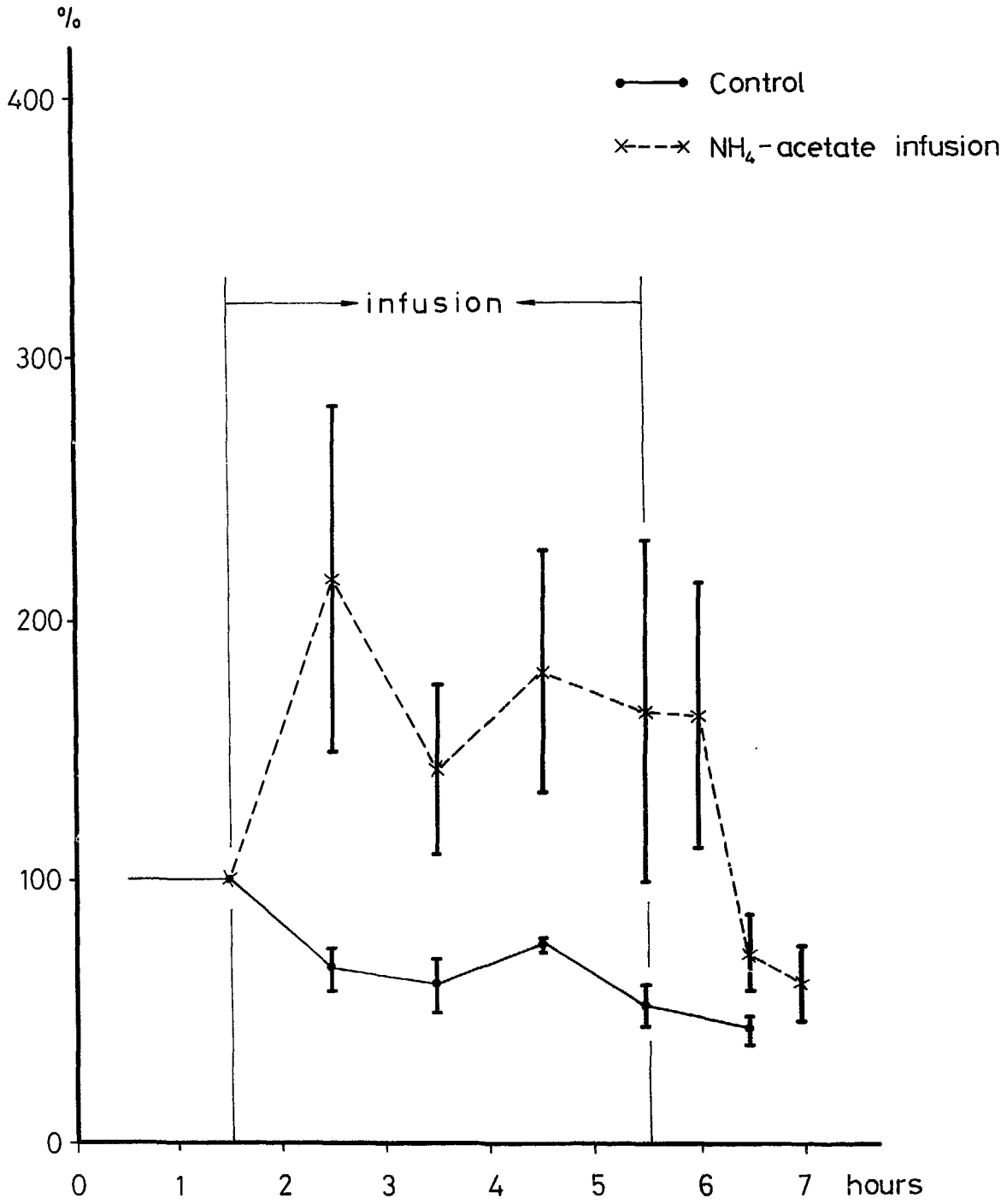
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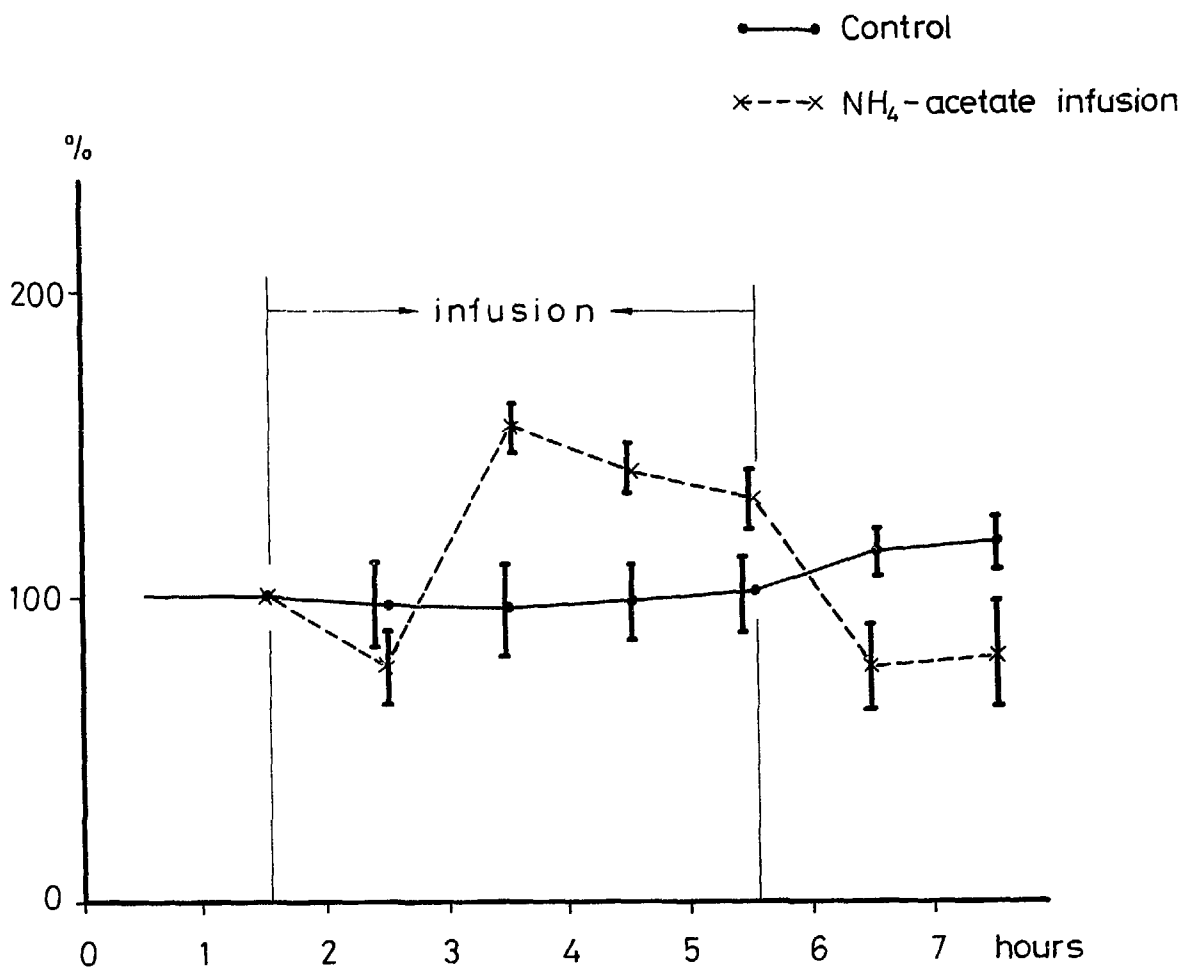
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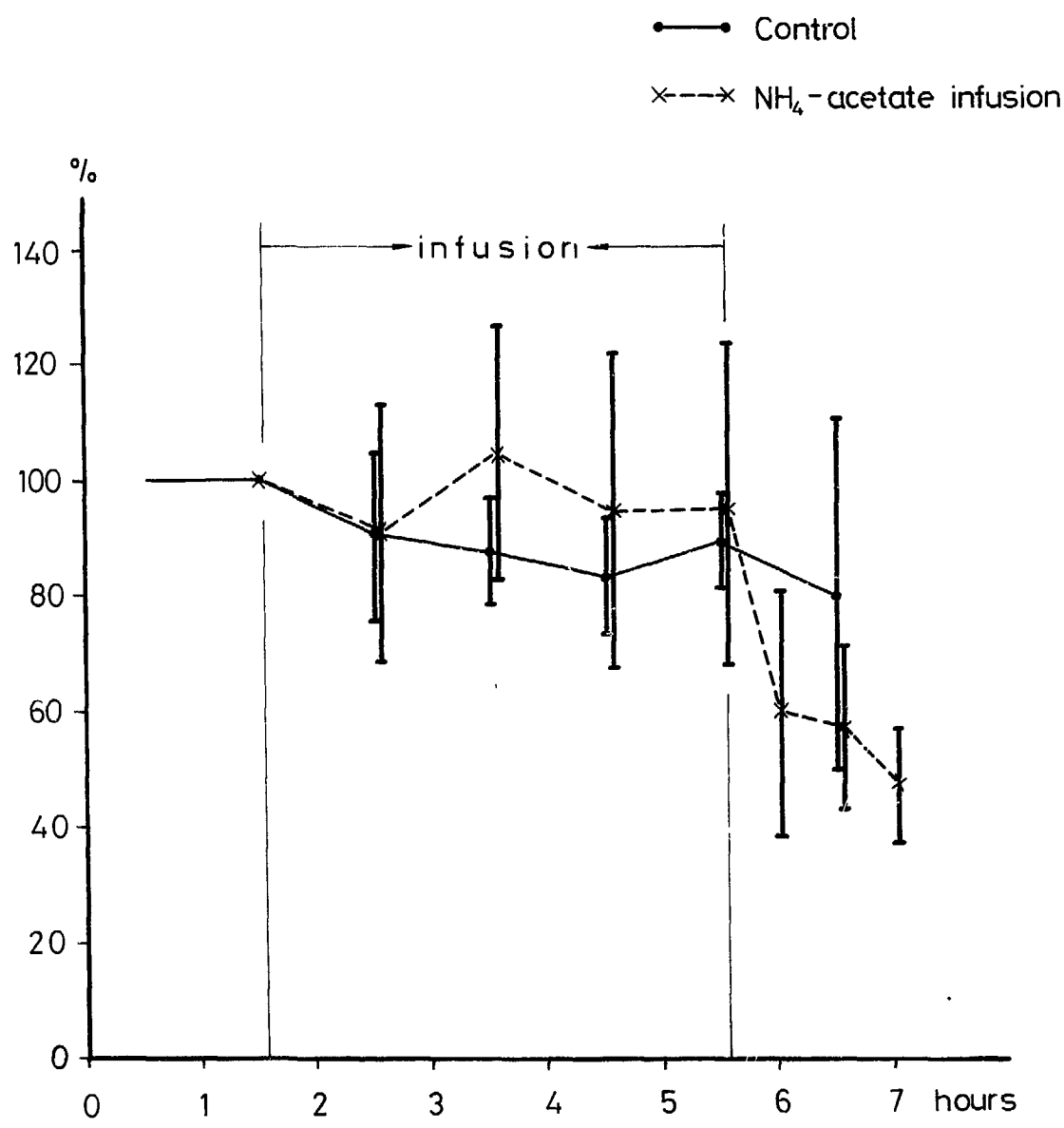
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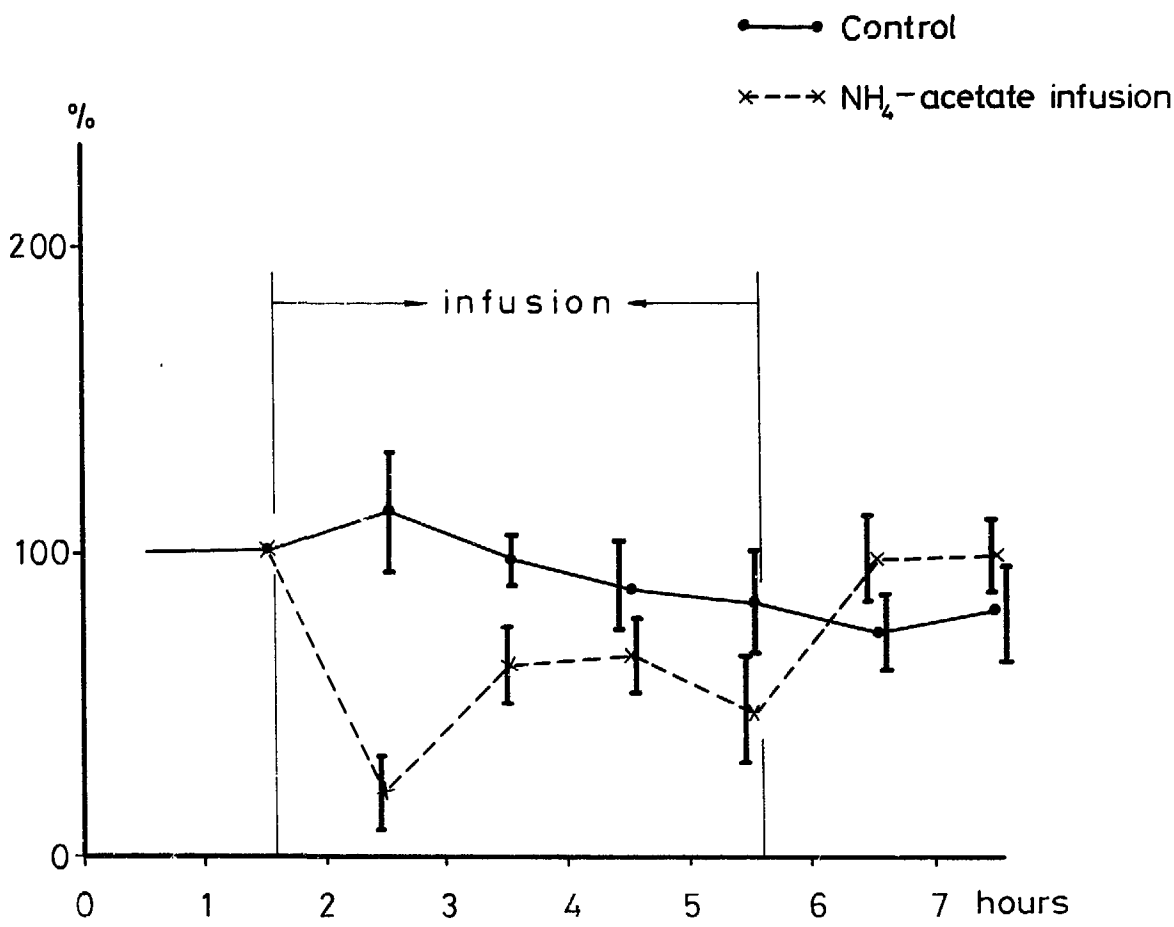
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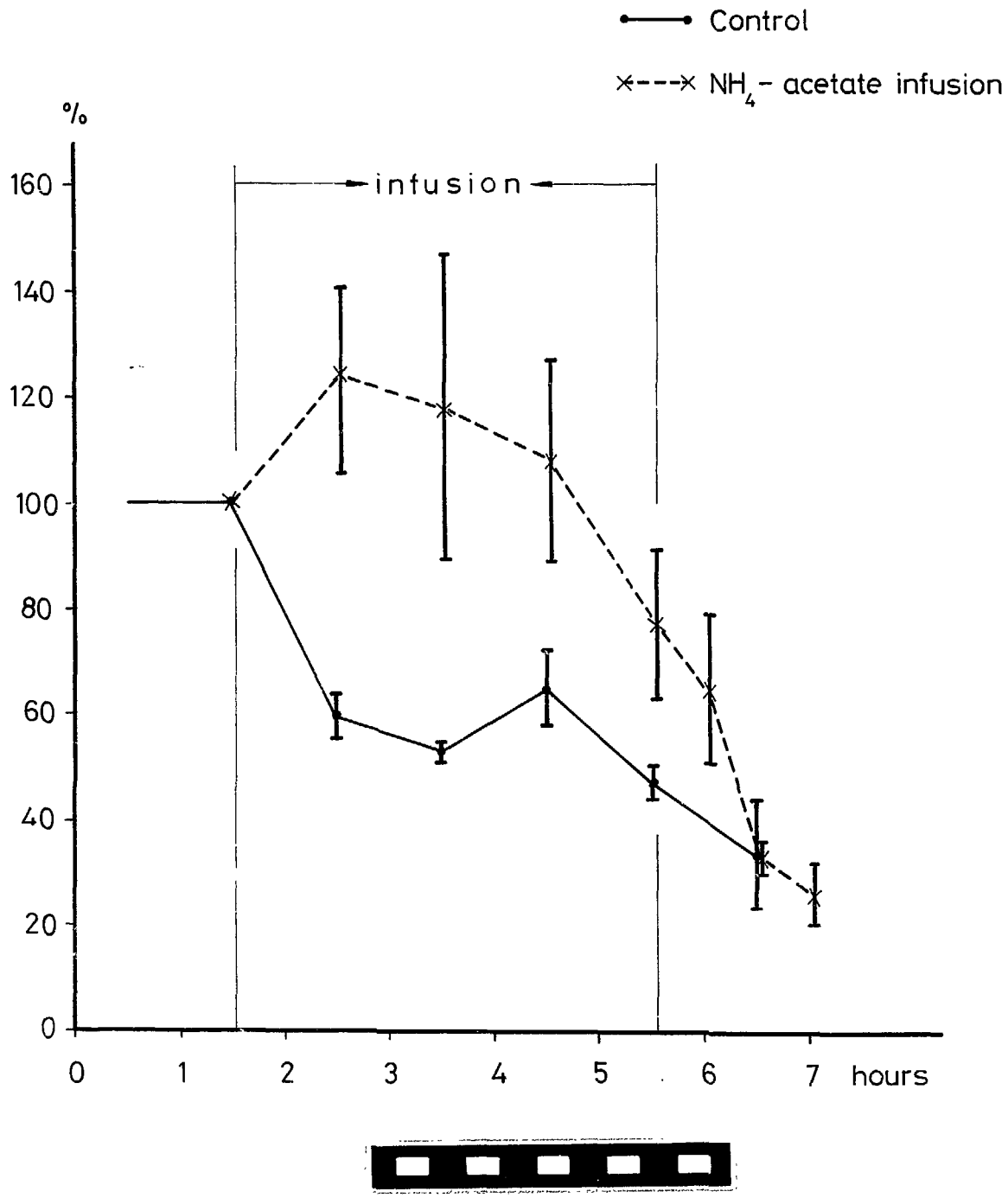
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