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MICROCALORIMETRIC INVESTIGATIONS ON THE ACTION OF CERTAIN HEAVY METAL IONS TO NONGROWING E.COLI CELLS

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ABSTRACT:

Addition of 10mg/l of the haevy metal ions Zn^{++}, Cu^{++}, Pb^{++}, Cd^{++} and Cr^{++} to nongrowing E.coli cells shows a decrease in heat production by aerobic and anaerobic glycolysis up to 8o %.
The heat production of cells incubated without glucose was decreased drastically too, after addition of 10mg/l of these heavy metal ions.

The pattern of heat released during active transport of α MG was influenced by these haevy metal ions in a way, that there was a decrease in maximum heat flow, whereas the total heat production seemed to be constant.

After increasing the metal ion concentration up to 100mg/l no endogenous heat production could be detected. The inhibition of heat production after addition of glucose was higher than 9o % in this case and there was a strong inhibition in α MG uptake.

The results of these investigations indicate that the basic action of all tested metal ions is the energy metabolism and not the transport as stated earlier.

INTRODUCTION:

There are a great number of investigations dealing with the action of haevy metal ions on special enzymes, but only few investigations on the action on whole bacterial cells.

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Investigations on whole bacterial cells were carried out either by means of a Warburg apparatus, or by measurements of growth inhibition on plates substituted with haevy metals. All of these studies were carried out under aerobic conditions.

With increasing interest in anaerobic processes, the inhibitory effects of haevy metals on these processes could be of increasing importance.

Microcalorimetric measurements are very well suitable for investibations on anaerobic bacterial metabolism (Redl 1981). Therefore, together with the well known fact that haevy metal ions are potential inhibitors of enzymes envolved in energy metabolism, it was decided to use microcalorimetric methods for the present investigation.

Another advantage of microcalorimetric measurements is the possibility to observe heat production of cells incubated without substrates. So it could be possible to differenciate between metabolic effects and e.g. effects of transport.
In the last time there were some indications that bacterial transport could be inhibited by heavy metal ions (Harold 197o). So we made some investigations about the inhibitory action of heavy metal ions to the heat released by active transport of the nonmetabolized sugar α MG (Long 1975).
The heavy metal ions used in this investigations were Zn^{++}, Cu^{++}, Pb^{++}, Cr^{++} and Cd^{++}.

MATERIALS AND METHODS:

CULTIVATION OF ORGANISMS: Cells of Escherichia coli strain ATTC 113o3 were cultivated in Columbia Broth (Difco) at 37°C, Cells were harvested at a OD_{66o} of 1,O., washed twice with O,15 M NaCl or Tris buffer pH 7,5. and finally suspended in the same buffer.

MICROCALORIMETRY: Flow microcalorimeter LKB 2107-121 equipped with a flow through cell or a flow ampoule.
For aerobic conditions O_2 was pumped through the system by a second peristaltic pump.
Drop ampoules microcalorimeter LKB 2107-123, equipped with 5 ml ampoules (Wadsö 1974).
All runs were done at 30°C. Pump speed was 60 ml/h.

REAGENTS: All heavy metal ions were used as Cl_2 salts.
α MG (α Methyl-D-Glucoside) from Sigma.
labelled α MG from NEN Chemicals

OTHER METHODS: according to Anraku et al. (1975)

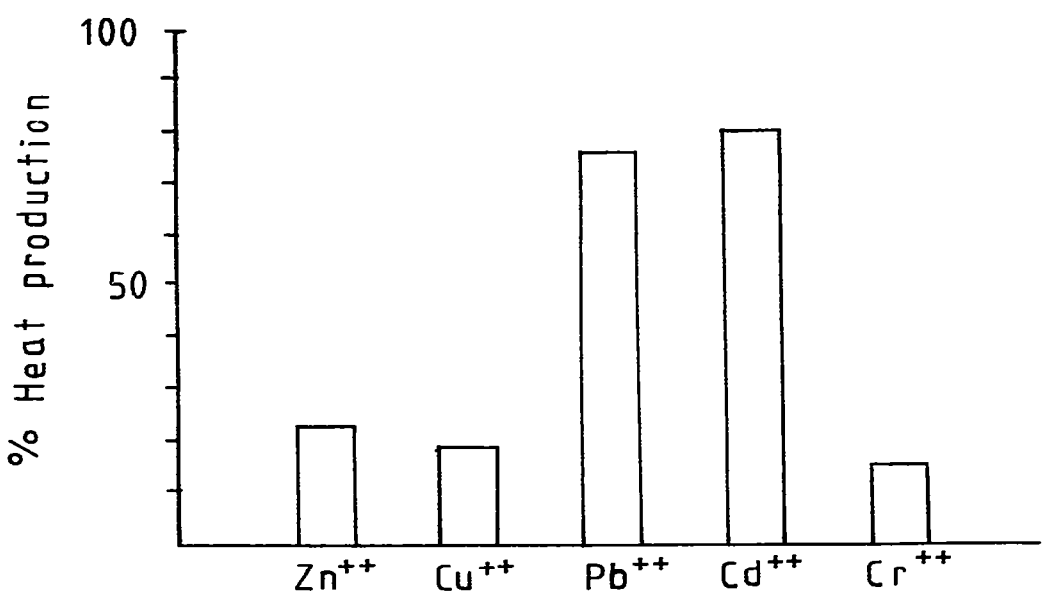

Fig. 1: Influence of 10 mg/l heavy metal ions on heat production by aerobic cells incubated with glucose. Results are expressed as percentage of the control activity without metal ions.

RESULTS

a) INFLUENCE OF HEAVY METAL IONS ON THE HEAT PRODUCTION BY GLYCOLYSIS AND ON THE ENDOGENOUS HEAT PRODUCTION.

Fig.1 shows the inhibition of 10 mg/l of the indicated metal ions to aerobic cells, incubated with glucose.
The results are expressed as percentage inhibition of the control activity without metal ions, 20 min. after addition of inhibitors and are related to mg protein.
This investigations ware done in a flow microcalorimeter equipped with a flow ampoule. Air was bubbled through the system by a second peristatic pump to avoid O_2 deficiency.
In this case the lowest inhibition was found with Cd^{++} (22 %) and Pb^{++} (25 %). The strongest inhibitory effect was seen with Cr^{++} (83 %), Cu^{++} (81 %) and with Zn^{++} (77 %).
The ATP pools of the cells decreased e.g. for Cu^{++} from 2,7 nmoles ATP/mg protein to 1,75 nmoles ATP/mg protein.
On addition of Cr^{++} there was a special heat effect shown in Fig.2.

Using the flow ampoule a short heat burst could be observed after addition of Cr^{++}, followed by a large heat production. Using the flow through cell the power-time curve shown in Fig. 3 was observed. There was the same heat burst after addition of Cr^{++} as in Fig. 2. But here it was followed by a decrease in heat production. After some time there was a large heat production again.

Using the ampoule calorimeter a decrease in heat production occured and then the heat production was constant. The first short heat burst could not be observed here, because of the long calibration time.

Using electron microscopy it was seen that the first little heat burst was due to an agglutination of cells. This would also be an explanation for the large heat production observed after this short heat burst with the flow microcalorimeter. It was found that the cells did accumulate in the flow ampoule.

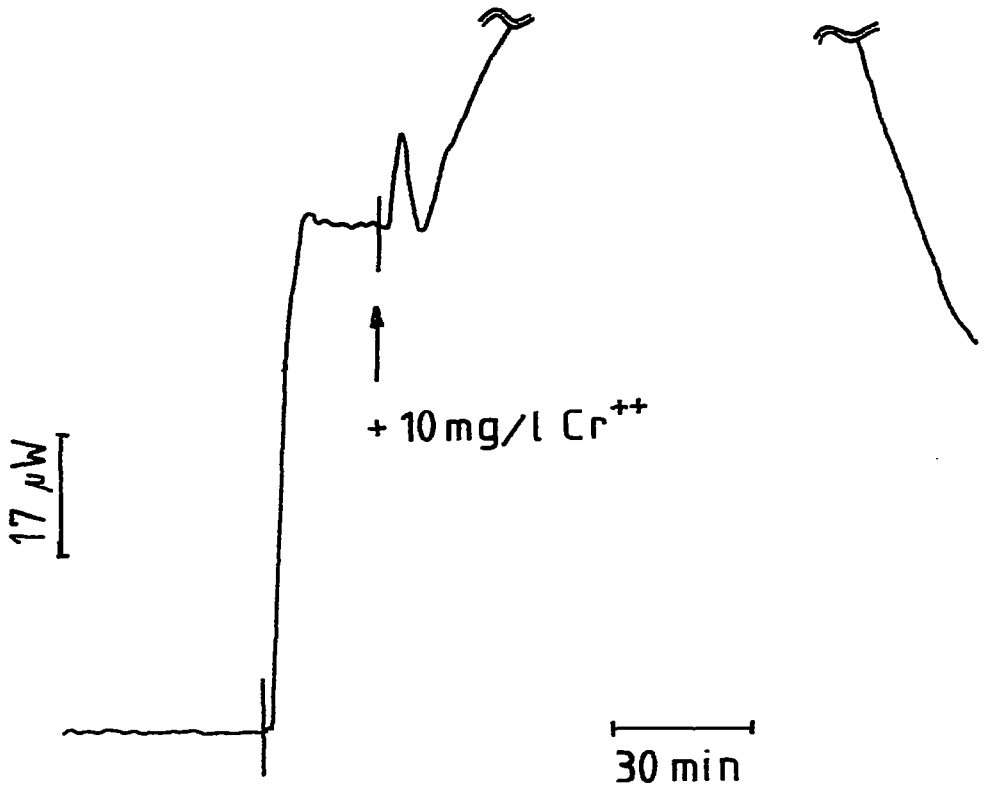

Fig. 2: Heat flow after addition of Cr^{++} using a flow microcalorimeter equipped with a flow ampoule.

Fig. 3: Heat flow after addition of Cr^{++} using a flow microcalorimeter equipped with a flow through cell.

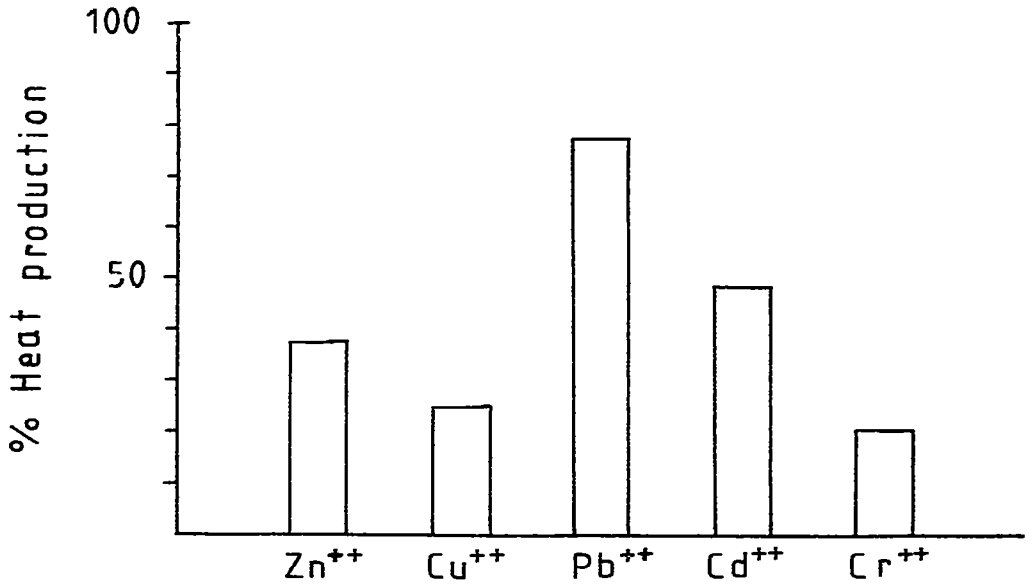

Fig. 4: Influence of 10 mg/l heavy metal ions on heat production by anaerobic cells incubated with glucose. Results are expressed as percentage of the control activity without metal ions.

Tests showed however that the cells were still alive. Fig. 4 shows the inhibition of haevy metal ions to anaerobic cells, with glucose incubated. The order of action was almost the same than in Fig. 1. Only Cd^{++} showed stronger inhibitory effect than with aerobic cells. Anaerobic conditions were established by flushing the system with N_2.

Fig. 5 shows the results with cells incubated without any substrate. Therefore only the endogenous metabolism was recorded. This investigations were done in an ampoule microcalorimeter.

The inhibitory effects on Zn^{++} and Cu^{++} were in the same order as in the tests described above. Pb^{++} and Cd^{++} showed a stronger inhibitory action than to cells incubated with glucoes. Cr^{++} showed a less inhibitory effect, but the action of Cr^{++} should be investigated further.

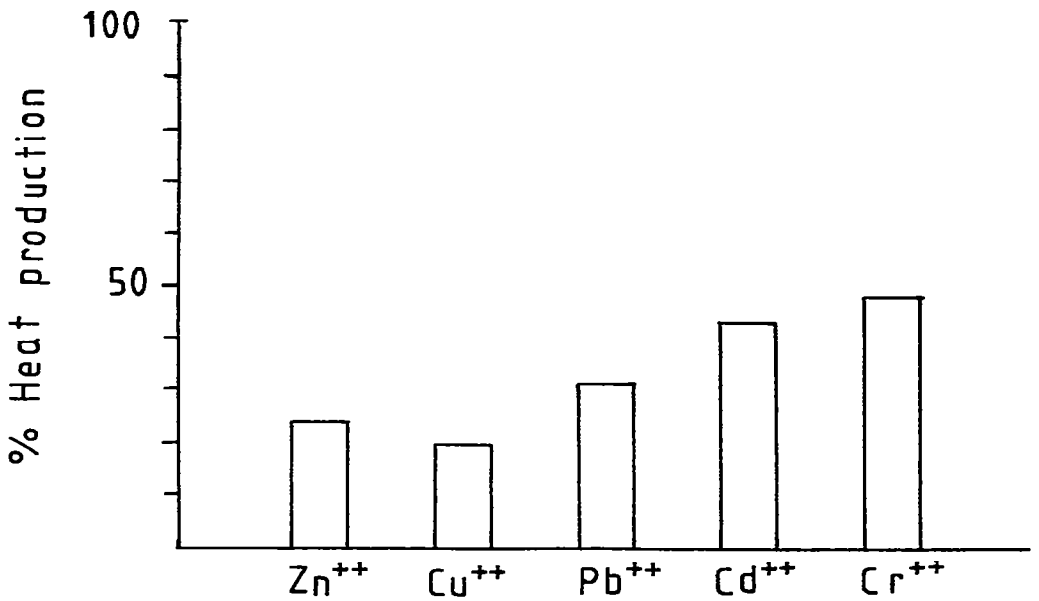

Fig. 5: Influence of 10 mg/l heavy metal ions on the endogenous heat production. Results are expressed as percentage of the control activity without metal ions.

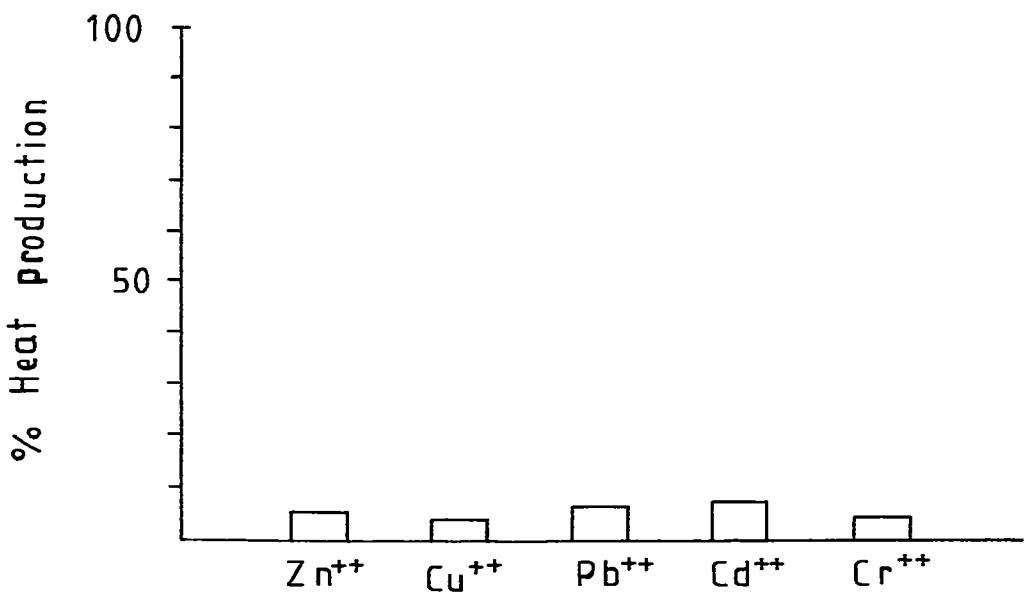

Fig. 6: Influence of 100 mg/l heavy metal ions on heat production by aerobic cells incubated with glucose.

The ATP pools of cells incubated without glucose were decreased drastically. For Cu^{++} a decrease from 2,04 nmoles ATP/mg protein to 1,19 nmoles/mg protein was found.

No endogenous heat production could be detected after increasing the concentration of heavy metal ions up to 100 mg/l. The inhibition of aerobic glycolysis was over 90 % in each case. This is shown in Fig. 6.

b) INFLUENCE OF HEAVY METAL IONS ON THE HEAT RELEASED BY ACTIVE TRANSPORT OF α MG

This effect was found by LONG et al (1975). In contrast to these authors, who used an ampoule calorimeter equipped with a direct injection ampoule, a flow microcalorimeter was used in this study. Using this instrument there was no heat effect produced by the injection of the substance.
Fig. 7 shows the power-time curve after addition of 1 mg/ml of α Methylglucoside, a sugar which could not be metabolized by E.coli cells. This sugar is transported via the glucose transport system (Christensen 1975).

The small second peak seen on the power-time curve is typical for the heat flow induced by α MG.

Fig. 8 shows the sample after treating the cells with 10 mg/l Cd^{++}. The typical pattern of heat flow could still be seen. But there is a decrease of the maximum heat flow.

Fig. 9 shows the sample after treatment with 10 mg/l Cu^{++}. The maximum heat flow is not seen any more.
Samples treated with other heavy metal ions showed patterns similar to these in Fig. 8 and 9.

Attention should be paid to the fact that the total heat released by this process seemed to be constant after treatment with heavy metal ions.

Fig. 10 shows the uptake kinetic of labelled α MG for cells treated with 10 mg/l Cu^{++}.

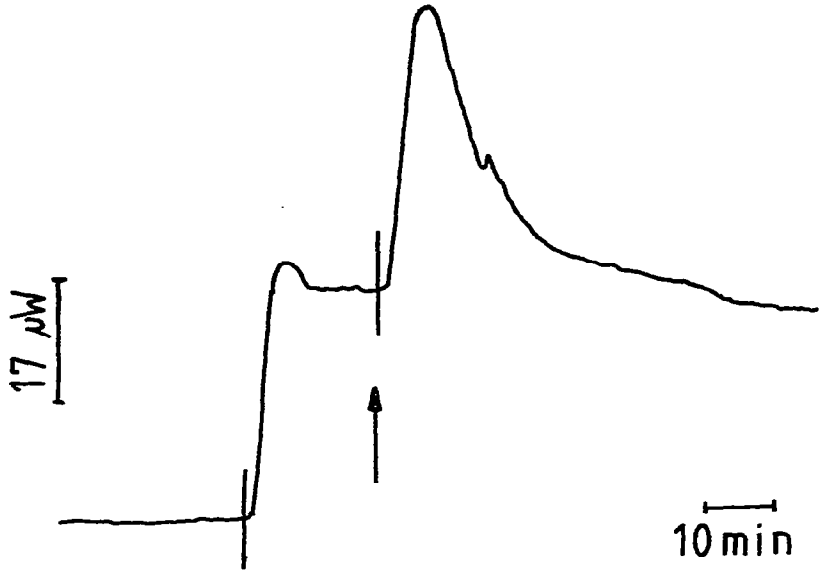

Fig. 7: Heat flow following addition of 1 mg/ml α MG. nontreated cells.

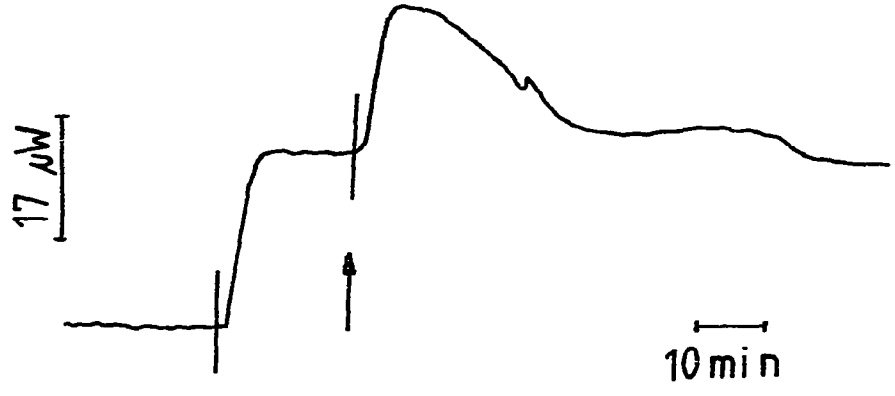

Fig. 8: Heat flow following addition of α MG. Cells were pretreated with 10 mg/l Cd^{++}.

Fig. 9: Heat flow following addition of ∝ MG. Cells were pretreated with 10 mg/l Cu^{++}.

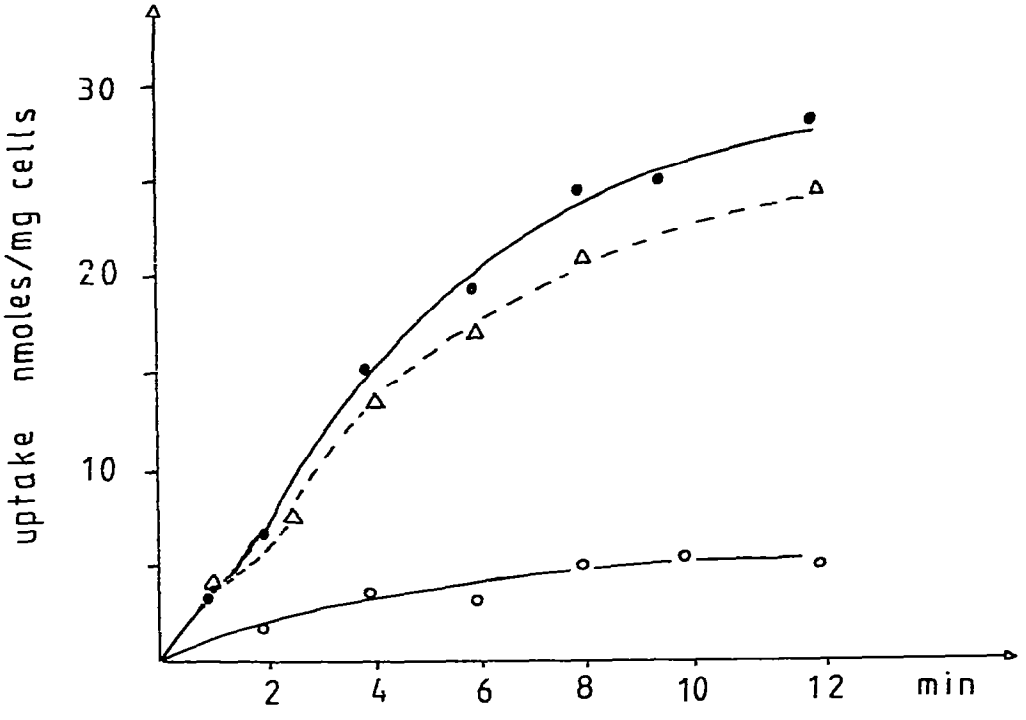

Fig. 10: Uptake of ^{14}C- MG in the absence (•), and in the resence of 10 mg/l (Δ) and 100 mg/l (o) Cu^{++}.

There is almost no inhibition of uptake at concentrations of 10 mg/l. The uptake of cells treated with 100 mg/l Cu^{++} was inhibited almost totally. And there was not detectable heat production at this high concentration.

DISCUSSION:

In this investigation it turned out that the inhibitory effects of heavy metal ions under aerobic conditions were in the same magnitude as under anaerobic conditions. This is in contrast to earlier investigations (De Walle 1979) which suppose anaerobic processes to be more sensitive to heavy metals than aerobic processes. By microcalorimetric methods it was possible to measure the effects of metal ions on the endogeous metabolism. The inhibitory effects of Cu^{++} and Zn^{++} on starved cells were in the same magnitude as with cells incubated with glucose, whereas Pb^{++} and Cd^{++} were more inhibitory to starved cells.

The effect observed with Cr^{++} was not described before. There was found a short heat burst which was due to agglutination of cells. The mechanism of this effect is not known yet.
It should be a subject of furhter investigation.
In general all of these results show a strong inhibitory effect of heavy metal ions to metabolism.
This is in good agreement with earlier investigations on the action of Zn^{++} (Anraku 1975).

The results of measurements on transport of α MG showed that the total heat released by cells treated with 10 mg/l metal ions was constant.

Only the maximum heat flow was decreased. This can be explained in that way, that the cells can produce enough energy to make active transport although there is a partialinhibition of metabolism. This is in agreement with the results of the uptake kinetics.

The decrease in maximum heat flow is due to a decrease in process intensity as a result of the inhibition of metabolism. No heat production could be observed after increasing the metal concentration up to 100 mg/l.

This means that there is an almost total inhibition of metabolism. And therefore a inhibition of active transport. So it is concluded that the primary mode of action of all tested metal ions is the energy metabolism and not the transport as stated earlier (Eagon 1969).

These investigations show that microcalorimetric studies are well suited for physiological measurements especially under anaerobic conditions and for investigations on resting bacerial cells. And that it is possible to detect new effects as it was demonstrated here with Cr^{++}.

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