*vulpis, T. suis;* trematodes: *F. hepatica, Paragonimus westermani, P. miyazakii;* cestodes: *Dipylidium caninum, D. erinacei* (plerocercoid). Figure 2 shows the effects of Avermectin B1a and other anthelminthics on the motility of some parasitic helminths. Avermectin B1a at $3.6 \times 10^{-9}$ M showed little effect on the motility of *A. caninum, T. canis* and *P. westermani.* On the other hand, hexylresorcinol at $10^{-5} - 10^{-4}$ M, and bithionol at $3 \times 10^{-6} - 3 \times 10^{-5}$ M showed paralyzing effects on *A. caninum* and *P. westermani,* respectively.

Thus, under our experimental conditions, Avermectin B1a was especially effective against worms which belong to the Metastrongylidae. The ineffective result of this compound against *D. immitis* agreed with that reported in the in vivo experiment[9]. Though the action of Avermectin B1a against *A. caninum* was reported in the in vivo experiment[9], this compound showed little effect in our in vitro experiment in which the observation was made only for a shorter period.

We have obtained results suggesting that Avermectin B1a elicits paralyzing effects through a neuropharmacological mechanism including $\gamma$-aminobutyric acid (GABA) and acetylcholine (ACh) in *A. cantonensis*[11,12]. The nervous system found in *A. cantonensis* is similar to that described in *A. suum*[4,5,13]. Further experiments with higher concentrations of Avermectin B1a and/or longer periods of treatment may show the effectiveness of this compound against other nematodes such as *A. caninum* and *A. suum.*

It has been reported that the anthelminthic effects of many drugs are due to indirect paralytic or spastic action rather than to direct vermicidal action[14,15]. And their effects may also be due to host functions such as the peristalsis of the digestive tract, and trapping in the tissues which leads to killing through tissue reactions. Therefore, the anthelminthic effects of Avermectin B1a against *A. cantonensis* recently reported in rats[16] may be caused through the paralyzing action of this compound observed in the in vitro experiment.


1 S. Rebello and J.T. Rico, C.r. Soc. Biol. *94,* 915 (1926).
2 M.R.A. Chance and T.E. Mansour, Br. J. Pharmac. *4,* 7 (1949).
3 E. Baldwin, Parasitology *35,* 89 (1943).
4 I.L. Natoff, Br. J. Pharmac. *37,* 251 (1969).
5 E. Hayashi, A. Haruno, T. Shimizu and M. Terada, Folia pharmac. jap. *76,* 15 (1980).
6 G.R. Hillman and A.W. Senft, J. Pharmac. exp. Ther. *185,* 177 (1973).
7 R.H. Fetterer, R.A. Pax and J.L. Bennett, Expl Parasit. *43,* 286 (1977).
8 M. Sano, M. Terada, A.I. Ishii, H. Kino and M. Hayashi, Jap. J. Parasit., in press (1981).
9 L.S. Blair and W.C. Campbell, J. Helminth. *52,* 305 (1978).
10 J.R. Egerton, D.A. Ostlind, L.S. Blair, C.H. Eary, D. Suhayda, S. Cifelli, R.F. Riek and W.C. Campbell, Antimicrob. Agents Chemother. *15,* 372 (1979).
11 M. Sano, M. Terada, A.I. Ishii and H. Kino, Jap. J. Pharmac., in press (1981).
12 M. Sano, M. Terada, A.I. Ishii, H. Kino and M. Hayashi, Jap. J. Parasit., in press (1981).
13 J. del Castillo and T. Morales, in: Physiology and Biochemistry, vol. 2, p. 209. Academic Press, New York 1969.
14 T.E. Mansour, Adv. Pharmac. *3,* 129 (1964).
15 I.M. Rollo, in: The Pharmacological Basis of Therapeutics, p. 1018. Macmillan Publishing, New York 1975.
16 M. Sano, M. Terada, A.I. Ishii, H. Kino and M. Hayashi, Jap. J. Parasit., in press (1981).


## High magnesium content of *Escherichia coli* B[1]


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*Summary.* We have found that the intracellular concentration of magnesium in exponentially growing *Escherichia coli* B is much higher than has been previously assumed; it is about 100 mM. Results of equilibrium dialysis suggest that nearly all of this Mg is bound, probably most of it to nucleic acids. These findings could have important consequences for the study of protein-DNA interactions and the in vitro simulation of protein biosynthesis.


Knowledge of the intracellular concentrations of ions and other small molecular components is of prime importance for understanding protein-nucleic acid and protein-protein interactions which occur in nuclear material, and during the morphogenesis of viruses. An extensive study covering Mg, K, Na, ATP and polyamines will be reported in detail elsewhere. In the course of this work we discovered that the Mg content of *E. coli* B cells is 2.5–5 times higher than has generally been assumed[4].

It is well known that metabolic disturbances in cells which are packed in a centrifugation pellet (starvation of oxygen and nutrients) lead to massive leakages of ions; we therefore used a filtration method similar to that introduced by Epstein and Schultz[5]. During the deposition of cells from an exponentially growing culture (1 ml of a $2 \times 10^8$ bacteria/ml suspension) on a membrane filter (Millipore GSWP, 0.2 µm) under suction, the cells were maintained in the presence of $O_2$ and small amounts of nutrients. These conditions of continued metabolism were also maintained during washing. Control experiments showed that washing lead to plateaux of the internal ionic content, which were maintained through at least 2 cycles of washing, before the content of the most sensitive component, K, decreased to reach that of stationary phase cells. The washed deposit of cells on the membrane filter was then extracted with 1 M $HNO_3$, and the solution was analyzed by atomic absorption spectrophotometry.

A large size derivative of *E. coli* strain B (Hermann Epstein, $B^E$) was grown in 1% tryptone with 0.2% glucose and 0.1 M salt (NaCl or KCl) added, and harvested at $2 \times 10^8$ bacteria/ml. Tryptone was chosen as the basis of our growth medium because it has good buffering capacity, provides the possibility of achieving low ionic strengths, and has a low Mg-content (0.09 mM). We found the following internal contents and concentrations in cells grown in this way:

These contents are dependent on growth conditions; we confirmed Epstein and Schultz's[5] observations that $K^+$ is mainly used for compensating the outside osmolality of the growth medium. We found in addition that the Mg content is 55–50 and 170–140 mM, when the total molarity of the growth medium is 50 mM or 400 mM. Our values for the Mg content are about 4 times those reported by Lusk et al.[6]. The details of the procedures, in particular the new method

for calculating the cellular volume by determining dry density, wet density, cell number and dry weight, will be given in detail in forthcoming papers. In our determination of volume an uncertainty remains with respect to dry weight and dry density; we have not yet any definite, precise measurements for evaluating a) the anions and cations retained bound to the macromolecular components and b) the nature of the anions which are possibly *not* leaking out of the cells together with the cations. During washing with water the lost cations might, indeed, simply be replaced by protons. The 2 values in the last column of the table are thus based on upper and lower estimates of these remaining anions. Work is being continued in order to gather additional data in this respect.

Studies by equilibrium dialysis on free and bound ions will be described later in detail, as will be the extension of the observations to other bacterial species and other *E. coli* strains. As an example, we found that $2.8 \times 10^{-3}$ moles of $Mg^{2+}$ are bound to $1.7 \times 10^{-2}$ g of a complete sonicate of bacteria, contained in a volume of 1 ml, when the free ion concentration of $Mg^{2+}$ is $0.5 \times 10^{-3}$ M. At equilibrium, this corresponds to the possibility of binding about 600 µg of Mg/g of dry weight of the salt-free extract. This value for bound Mg corresponds with that determined by Damadian[7]. The first extensive estimates of polyamines (putrescine and spermidine) show that the intracellular, molar concentration of both together is about half of that of Mg. Our competitive binding studies confirm the general view that polyamines have a still stronger affinity for the bacterial extract than Mg. Thus, it seems very likely that within the cell, part of the DNA is neutralized by polyamines. The great importance of Mg is in agreement with the observation that a mutant of *E. coli*, that is unable to synthesize polyamines, still grows in the absence of endogenous polyamines although at a very reduced rate[8]. The available results strongly suggest that the majority of the acidic groups of DNA might be neutralized by $Mg^{2+}$. As has frequently been considered, the complexity of the hydrated magnesium ion and of its nature of binding to DNA[9] does not even preclude a charge reversal of DNA with Mg as partner. The amount of Mg bound to ribosomes is under investigation.

These new findings have obvious and important consequences concerning the postulated existence and nature of basic histone-like proteins which would be structurally-involved partners of DNA in prokaryotic cells, mitochondria and chloroplasts. These partners would have to be extremely basic in order to be bound at the high concentration of 650 mM $K^+$ which we found in cells growing in media of high osmolality. The state of metabolically active DNA in eukaryotes, and of developing viruses, has to be explored in this respect also, as must the process of DNA packaging in viruses. In eukaryotic cells the chromosomes undergo a cycle of condensation-decondensation; transcription and replication of DNA occurs only during defined periods of the cycle. In contrast to this, non-eukaryotic nuclear material, as a rule, does not need cyclic variations of DNA compaction: under favourable conditions (exponentially growing cells in a rich medium), prokaryotes replicate DNA and produce RNA without a cyclic interruption.

The implications of this new finding for *in vitro* experiments related to replication, transcription and translation (ribosomes) should be seriously considered, particularly since with current methods for isolation of nuclei and nucleoids, $Mg^{2+}$ is removed.

Intracellular contents of potassium, sodium and magnesium of *E. coli* B

| Component | g/g of dry weight of the salt-free cells | Concentrations calculated with cellular volumes of $1.4 \times 10^{-12}$ and $1.6 \times 10^{-12}$ ml |
|---|---|---|
| K | $4.3 \pm 0.3 \times 10^{-2}$ | 340-300 mM |
| Na | $3.4 \pm 0.6 \times 10^{-3}$ | 50- 45 mM |
| Mg | $7.9 \pm 0.9 \times 10^{-3}$ | 110- 90 mM |

Each value is the average of at least 8 independent experiments. For the contents of K, Na and Mg the standard deviation is indicated. The problems encountered in determining the intracellular concentrations are mentioned in the text.

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4 S. Silver, in: Bacterial Transport, p. 221. Ed. B. P. Rosen. Marcel Dekker, New York 1978.
5 W. Epstein and S. G. Schultz, J. gen. Physiol. *49*, 221 (1965).
6 J. E. Lusk, R. J. P. Williams and E. P. Kennedy, J. biol. Chem. *243*, 2618 (1968).
7 R. Damadian, Biophys. J. *11*, 739 (1971).
8 E. W. Hafner, C. White-Tabor and H. Tabor, J. biol. Chem. *254*, 12419 (1979).
9 D. Murk Rose, M. L. Bleam, M. T. Record, Jr, and R. G. Bryant, Proc. natl Acad. Sci. USA *77*, 6289 (1980).

## Midbrain regions involved in call production of Japanese quail[1]

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*Summary.* The midbrain areas that yielded calling with the smallest currents were within the nucleus intercollicularis and isthmi complex. Natural calls were evoked, but they could not be localized. Rather, some calls were more easily evoked than others.

Calling has been elicited with small currents from the midbrain of a variety of birds such as redwinged blackbirds *Agelaius phoeniceus*[3], chickens[4], Java sparrows *Padda oryzivora*[5] and in Japanese quail *Coturnix coturnix japonica*[6,7]. However, a detailed threshold survey of the midbrain of the latter species is lacking. To date, there is general