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 2.8×10^{-3} moles of Mg^{2+} are bound to 1.7×10^{-2} g of a complete sonicate of bacteria, contained in a volume of 1 ml, when the free ion concentration of Mg^{2+} is 0.5×10^{-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 1.4×10^{-12} and 1.6×10^{-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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3 We are most indebted to Prof. H. G. Seiler (Institute of Analytical Inorganic Chemistry, University of Basel) for his extremely valuable help with atomic absorption spectrophotometry. In addition, H. Jütte and, particularly, F. Borle, have helped in the experiments which are only mentioned here and which will be published in extenso together with these authors in forthcoming papers.
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Midbrain regions involved in call production of Japanese quail[1]

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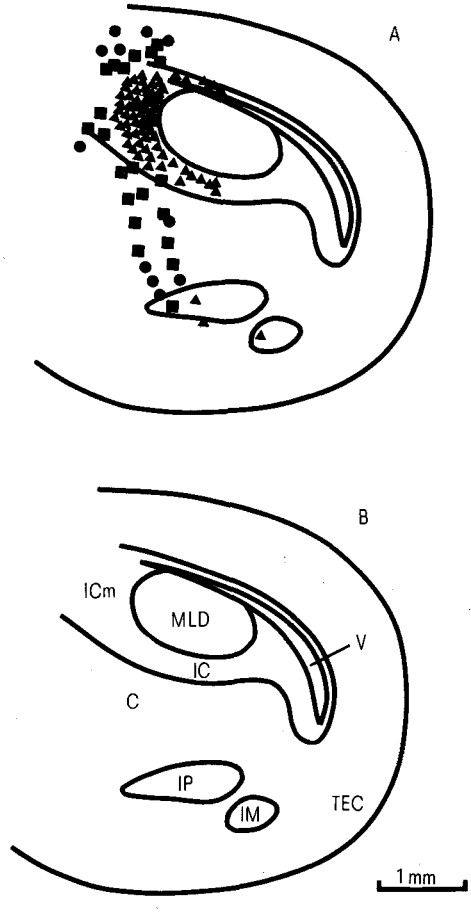
Summary. The midbrain areas that yielded calling with the smallest currents were within the nucleus intercollicularis and isthmi complex. Natural calls were evoked, but they could not be localized. Rather, some calls were more easily evoked than others.

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

agreement that the region of the midbrain requiring the lowest current levels to produce calls is in the nucleus intercollicularis, especially its medial part. This nucleus surrounds the nucleus mesencephalicus lateralis pars dorsalis (MLD), the avian homologue of the inferior colliculus in mammals[8]. Calling was evoked from more ventral parts of the midbrain as well, but slightly higher currents were required[3,5,7,9]. These sites are in an anatomically less well defined part of the midbrain, variously referred to as the nucleus mesencephalicus lateralis pars ventralis[9] and area C[10].

Acute experiments were performed in adult Japanese quail, of both sexes, anaesthetized with halothane. Details of the monopolar platinum electrodes and implantation techniques have been given previously[5]. The brain was stimulated with biphasic rectangular pulses, each phase 2 msec long, at 50 Hz and of preset current. 10-sec periods of stimulation were separated by 60 sec. Electrode tracts were run through the brain and threshold determinations made at 0.2 mm depth intervals. Calls elicited were recorded, analyzed on a Kay Elementrics Sona-Graph and compared with sample natural calls. Sites stimulated were identified histologically by reference to a quail atlas[11].



A Diagrammatic cross-section of the optic lobes, showing the positions of the 108 positive sites identified in the study. Thresholds grouped as: ▲ 0–50 μA; ■ 55–100 μA; ● 105–200 μA.
B Nomenclature of the main structures. Abbreviations: C, area C and nucleus mesencephalicus lateralis pars ventralis; IC, nucleus intercollicularis; ICm, medial nucleus intercollicularis; IP, nucleus isthmi pars parvocellularis; IM, nucleus isthmi pars magnocellularis; MLD, nucleus mesencephalicus lateralis pars dorsalis; TEC optic tectum; V, ventricle.

Calling was evoked from 108 sites in 28 animals, and these are summarized in figure A. 76 sites had thresholds of 50 μA or less, of these, 59 were within that part of the nucleus intercollicularis medial to the MLD. Many sites (35) had thresholds of less than 30 μA. At 14 sites, calling occurred without electrical stimulation, when apparently the mechanical disturbance was sufficient to elicit calls. Additional low threshold sites were found dorsal (7 sites) and ventral (6 sites) to the MLD, still within the nucleus intercollicularis, and ventrally (3 sites) in the nucleus isthmi pars parvocellularis and magnocellularis. Surrounding the low threshold sites of the medial intercollicularis, were those having somewhat higher thresholds (55–100 μA, 19 sites). Mostly these were outside the intercollicularis and in the area C. Highest threshold sites (105–200 μA, 12 sites) were peripheral to those already discussed. No positive site was found in the MLD.

The spontaneous calls recorded were grouped into 12 types, 3 less than recognized by Potash[6,7]. However, only 1 of his call types was not recorded in the present study. The calling elicited was stimulus bound and usually consisted of a rapid repetition of similar elements. Each of the elicited calls was similar to all or part of a natural call, as judged by listening and inspection of the sonagrams. In most animals only 1 call type was produced along an electrode tract and during 1 experiment. However, in 8 birds (29%) 2 different calls were produced. All the elicited calls were obtained from both sexes, even if the call was produced spontaneously in only 1 sex. The calls elicited most frequently were the chirrup or separation call of the female (13 animals, 46%), part of the crow call of the male (9 birds, 32%) and cheep or contact call (6 birds, 21%). 5 other calls were elicited, each in less than 5 birds. Comparisons between birds showed that it was not possible to localise a call type to a specific brain region.

These results confirm and extend those of Potash[6,7]. Lowest thresholds were found in the medial part of the nucleus intercollicularis, and a more ventral area, sensitive to slightly higher current levels, was delineated. This agrees with work in other species[3–5,10,12]. The new evidence of low threshold sites in the nucleus isthmi complex, that is known to be associated with vision[8] and hearing[13], supports the suggestion of an integrative function for this nucleus[5]. Call types were not associated with specific brain regions rather certain types (separation, crow and contact calls) were easier to elicit than others. The function of the midbrain in call production is still unclear. More than one efferent pathway must be present[10], indeed much of the midbrain seems to be involved. However, specific calls and possible pathways do not seem to be related.

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