Table 3. Effect of acidic deoxyribonucleoproteins on the inhibition produced by histones on DNA-synthesis in vitro

	Specific activity (percent of control)	Range (%)
DNA-polymerase + DNA	100	
DNA-polymerase + histones + DNA	38	32– 45
DNA-polymerase + (histones + DNA) + acidic	80	71– 90
DNA-polymerase + DNA + (histones + acidic)	100	91–108
(DNA-polymerase + histones) + DNA + acidic	143	125–160

The source of DNA-polymerase was the nucleoplasm of isolated brain nuclei, 100 µg of protein per reaction tube. Histones were 25 µg and acidic deoxyribonucleoprotein 96 µg per reaction tube when indicated in the table. Substances between brackets were allowed to interact at 20 °C for 5 min before they were mixed with the other components of the DNA polymerase assay. Native DNA from salmon sperm was 20 µg per assay. Spec. act. at 100% 189,400 dpm/mg of enzyme protein ± 20,433. The results are the average of 4 experiments.

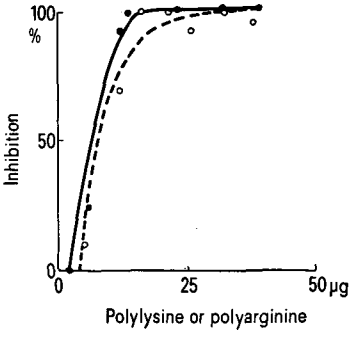

Fig. 2. Effect of polylysine and polyarginine on DNA-synthesis in vitro using native DNA as template. The reaction mixture and assay contitions were as in figure 1. ●—●, Polylysine; ○—○, polyarginine. The results are the average of 3 experiments.

polyglutamic acid, and maximal (100%) when the interaction was between polyglutamic acid and histones. A tube was added in which the DNA-polymerase was allowed to interact with the histones before the addition of DNA or polyglutamic acid, to see if the possible formation of a complex between the enzyme and histones could result in an inhibition of its activity. The activity was 100% of the control, and so no inhibition was observed. In all the other tubes, the enzyme was added just before the beginning of the incubation.

In table 2 the effects of polyglutamic acid on the inhibition produced by polylysine or polyarginine are shown. The conditions are the same as in table 1, except for the histones that are replaced by polylysine or polyarginine. As before, the polyglutamic acid reversed the inhibition produced by polylysine or polyarginine, more effectively in this case than with the histones. As before too, the reversal was maximal when polyglutamic acid was allowed to interact with the basic polypeptides and minimal when the polypeptides and DNA were allowed to interact.

In another set of experiments (table 3) the effects of acidic deoxyribonucleoproteins on the inhibition produced by histones on DNA-synthesis were investigated. The inhibition is partially reversed in the case where the histones were previously allowed to interact with the DNA. In the case where the acidic proteins are interacted with histones, the reversal is completed and in the case where the DNA-polymerase is allowed to interact with the histones a stimulation of activity is observed, this was to be expected because acidic proteins are known to act on the template increasing the synthesis directed by DNA-polymerase.

The results of these experiments demonstrate that histones from brain, polylysine and polyarginine, inhibit the replication of DNA in vitro. The reversal of inhibition by polyglutamic acid or acidic proteins is completed in all cases except when the DNA is previously complexed with histones, polyarginine or polylysine. This suggest that histones masking of DNA towards the polymerases involves electrostatic forces.

A mutant of the antibiotic resistance factor R124 with altered copy number

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Summary. A mutation conferring increased antibiotic resistance on *Salmonella typhimurium* strain 11G carrying R124 was plasmid determined; strains harbouring the mutant plasmid contained more DNA as ccc plasmid than those harbouring R124. The increased copy number was manifested at all growth rates tested.

In spite of a number of studies on the regulation of plasmid replication, the mechanisms governing plasmid number and the timing of plasmid replication in the cell cycle are unclear[2,3]. To obtain information on the control of plasmid replication, we are examining the replication of several stringent plasmids with different copy numbers and also isolating plasmid copy mutants. The properties of these are of interest because the positive and negative theories proposed to explain plasmid regulation can be used to predict the occurrence of copy mutants with specific changes in, for example, plasmid incompatibility[4]. A further reason for our interest in copy mutants follows from a recent study of plasmid instability in a temperature-sensitive *dnaC* mutant of *Salmonella typhimurium*. F*lac* replication was aberrant at permissive temperatures in this strain (strain 11G) in the presence of pLT2 or F-like plasmids such as R1, R136, R124 and ColB-K98; F*lac* was stably maintained in this strain in the absence of other plasmids[5,6]. The availability of copy mutants would make it possible to follow any changes in F*lac* stability in the *dnaC* strain with changing copy number of

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Table 1. The copy number of R124 and R124-J1 in strains of *Salmonella typhimurium*

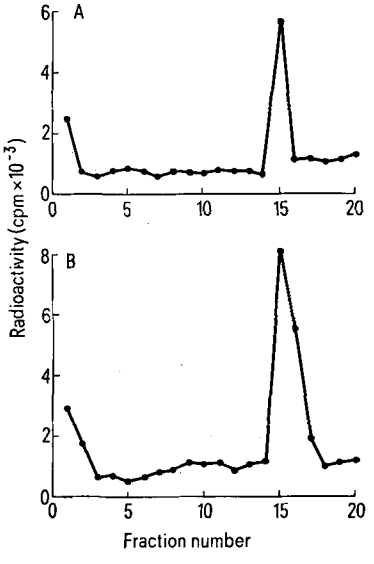
Strain	Percent of total cell DNA as ccc plasmid	R-factor copies per genome equivalent
M827 pLT2⁻ R124	1.4	0.75
M827 pLT2⁻ R124-J1	2.8	1.50
11G pLT2⁻ R124	1.3	0.70
11G pLT2⁻ R124-J1	2.7	1.45

To organisms growing exponentially at 30 °C in glucose minimal medium + 1.5 mM uridine, was added ^{3}H-thymidine (10 μc/2 μg ml) and incubation was continued for 5 h at 30 °C. The plasmid content was then analyzed as described in 'materials and methods'.

Table 2. The growth rates of strains carrying R124 and R124-J1

Growth medium	Growth rate (doublings/h)		
	Strain M827 pLT2⁻	Strain M827 pLT2⁻ R124	Strain M827 pLT2⁻ R124-J1
Glucose minimal medium	1.05	0.98	0.92
Casamino acids minimal medium	1.38	1.33	1.27
Nutrient broth	1.62	1.51	1.44
LB broth	1.79	1.66	1.53

Organisms were grown with shaking from single colonies in the appropriate medium at 37 °C overnight. Cultures were then diluted and growth continued until exponential. The growth rate was then followed by measuring the OD of the cultures.



Alkaline sucrose gradient centrifugation of R124 and R124-J1. Organisms of strains M827 pLT2⁻R124 and M827 pLT2⁻ R124-J1 grown overnight at 30 °C in glucose minimal medium + 1.5 mM uridine, were grown to exponential phase in the same medium at 30 °C and after addition of ^{3}H-thymidine (10 μc/2 μg ml) incubated for 5 h at 30 °C. Organisms were then lyzed and the plasmid DNA separated on alkaline sucrose gradients[8]. The graphs indicate the radioactivity in fractions collected from such gradients. The bottom of the tubes is to the left. *A* Plasmid in M827 pLT2⁻ R124 (ccc plasmid is ca. 1.4% of total DNA); *B* plasmid in M827 pLT2⁻ R124-J1 (ccc plasmid is ca. 2.8% of total DNA).

the destabilizing plasmid. We describe here the isolation of a plasmid copy mutant (R124-J1) deriving from the antibiotic resistance factor R124 of incompatibility group F_{IV}[7].

Materials and methods. The strains of *Salmonella typhimurium* used were M827 pLT2⁻, 11G *dnaC* pLT2⁻ *Flac* and 11G *dnaC* pLT2⁻R124. The characteristics of these have been described previously[8]. Stock cultures were maintained on slopes of Oxoid nutrient agar. The liquid growth media used were minimal medium[8] containing glucose (0.2%) and in some cases casamino acids (0.2%), Oxoid nutrient broth No. 2, and LB broth[9]. Where appropriate these were solidified with 2% Difco Bacto Agar. To measure the percentage of Lac⁺ and Lac⁻ organisms in a culture, suitable dilutions were plated on lactose deoxycholate agar[10]; after overnight growth at 30 °C, Lac⁺ colonies were red, Lac⁻ colonies white.

For isolation of the mutant with increased resistance to tetracycline, 2×10^{7} cells of strain 11G *dnaC* pLT2⁻ R124 were plated on NA containing 100 μg/ml tetracycline. After 48 h incubation at 30 °C, colonies were purified and then tested for resistance to ampicillin and tetracycline by plating on a series of NA plates with tetracycline concentrations from 0 to 150 μg/ml or ampicillin concentrations from 0 to 30 μg/ml.

For growth experiments, organisms were grown with vigorous shaking and used in the exponential phase. Plasmids were transferred by conjugation as described previously[8]. The techniques used for separation and mol. wt determinations of plasmid DNA on alkaline sucrose gradients have also been described previously[8].

Results and discussion. The resistance factor R124 confers a low level of resistance to tetracycline and ampicillin. In the strains of *S. typhimurium* used here, the presence of R124 led to minimum inhibitory concentration (m.i.c.) values of ca. 25 μg/ml for tetracycline and 10–12.5 μg/ml for ampicillin. As stated in 'materials and methods', derivatives of strain 11G *dnaC* pLT2⁻ R124 with increased resistance to tetracycline were sought by plating the strain on NA plates containing tetracycline. One such spontaneous mutant was more resistant to both tetracycline and ampicillin (m.i.c. values for strain 11G *dnaC* pLT2⁻ R124 were 25 μg/ml for tetracycline and 12.5 μg/ml for ampicillin; for the mutant the m.i.c. values were 55 and 22.5 respectively).

To test whether the mutation(s) causing increased antibiotic resistance was plasmid-determined or not, the resistance factor was transferred from the mutant to *S. typhimurium* M827 pLT2⁻ and the resistance pattern of the resulting strain compared to that of strain M827 pLT2⁻ R124. The resistance factor transferred from the mutant conferred increased resistance compared to that in strain M827 pLT2⁻ R124 (m.i.c. values for M827 pLT2⁻ R124 were 25 μg/ml with tetracycline and 10 μg/ml with ampicillin; for M827 pLT2⁻ carrying the R124 derivative from the mutant, the m.i.c. values were 50 μg/ml for tetracycline and 20 μg/ml for ampicillin). The increased resistance in the original mutant was evidently plasmid determined. We have termed the mutant plas-

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mid R124-J1. Since the mutation(s) conferring increased antibiotic resistance is plasmid determined and affects both the resistances carried by R124, we examined the possibility that R124-J1 might be a copy mutant. To examine copy number, we measured the amount of plasmid DNA and its approximate molecular size in strains M827 pLT2$^-$ R124-J1 and 11G pLT2$^-$ R124-J1. Table 1 shows that strains carrying the mutant plasmid had about twice as much DNA in the ccc plasmid form as strains harbouring the parental R124. Measurements of the sedimentation of the mutant plasmid on alkaline sucrose gradients showed a mol. wt for R124-J1 of ca. 65 md with the mutant plasmid running very similarly to the parental R124 (figure). Such gradients showed no indication of the presence of a larger plasmid in strains carrying R124-J1. The copy number of R124-J1 is therefore greater than that of R124 in strains 11G pLT2$^-$ and M827 pLT2$^-$ (table 1).

R124 behaves like R1[4] in that the number of copies per chromosome equivalent increased with decreasing growth rate. The same is true for the copy mutant R124-J1 and in strain 11G *dnaC* pLT2$^-$ over a range of growth rates the ratio of R124-J1 copy number/R124 copy number remained at about 2. The copy number of R124 in this strain at 30 °C increased from 0.45 in nutrient broth to 0.70 in glucose minimal medium while that of R124-J1 increased from 0.93 in nutrient broth to 1.45 in glucose minimal medium. Strains harbouring copy mutants of R1*drd*19 have a decreased growth rate compared to those harbouring R1*drd*19 itself or free of plasmid[11]. The same proved to be the case for strains harbouring R124-J1. They grew at a slightly reduced rate in all the media tested (table 2).

We have tested the effect of R124-J1 on the maintenance of F*lac* in the *dnaC* mutant. R124-J1 was more effective than R124 in causing F*lac* instability. With strain 11G *dnaC* pLT2$^-$ R124 F*lac*, overnight growth in broth at 30 °C gave rise to 22.4% of Lac$^-$ organisms; under the same conditions 81.3% of organisms from strain 11G *dnaC* pLT2$^-$ R124-J1 F*lac* lost the F*lac* plasmid. Interestingly a copy mutant of R1*drd*19 (mutant R1*drd*19-B42) also destabilizes F*lac* more than R1*drd*19 does in strain 11G *dnaC* whereas another copy mutant of R1*drd*19 (mutant R1*drd*19-B2) allows relatively stable maintenance of F*lac*[12]. R1*drd*19-B42 apparently has an altered replication repressor[4] and therefore the increased copy number of R124-J1 may be consequent upon an abnormality of this kind.

The replication behaviour, incompatibility properties and other characteristics of R124-J1 will be further compared to those of R124. The mol. wt studies suggest that substantial amounts of DNA have not been added to or deleted from R124 in the formation of R124-J1. These 2 plasmids are, however, being further tested for structural differences using restriction endonucleases.

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Regulation of ammonia uptake in *Aspergillus nidulans*[1]

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Summary. The ammonia uptake in *A. nidulans* was found to be linear for about 20 min, and was proportional up to 1.5 mg/ml dry cell density. The transport of ammonia does not involve energy. Normal and biotin deficient *A. nidulans* showed an identical K_m-values of 10.26×10^{-5} M ammonia for uptake. The uptake of ammonium ion has been shown to be regulated by the intracellular concentration of ammonia.

Earlier work from this laboratory indicated that biotin deficiency in *Aspergillus nidulans* causes significant increase in the cellular synthesis when ammonium nitrate was used as a sole nitrogen source[2,3]. Further, it was demonstrated that the increase in cellular synthesis is associated with the marked increase in the protein content with the concomitant decrease in the lipid content of the mold[2]. Biotin deficiency in this culture showed significant change in the permeability properties of the cell[4]. The glucose[5–7] and phosphorus[8] uptake systems have already been studied in *A. nidulans* and now we report the characteristics and the regulatory aspect of its ammonia uptake system.

Materials and methods. The strain, composition of the basal medium and cultural conditions are the same as described earlier[3,5]. Cultures grown in the presence of 5 units of avidin (General Biochemicals, Ohio, USA) were 65% lower in their fatty acid content and were referred as biotin deficient [3–7]. The method of Brown and Romano[9] previously modified[4,5] was followed for the uptake studies. Ammonia was determined by the method of Fawcett and Scott[10]; 24-h-old cells grown on basal medium were collected, washed and about 100 mg wet cells were added to the fresh medium containing the indicated amount of ammonia. Cells were further incubated on a rotary shaker (200 rpm) at 30 °C for about 24 h and collected. The intracellular concentration of ammonium ion was calculated on the basis of 4 µl water/mg dry cell weight[5,11].

Results and discussion. It has been found that when cells were grown on KNO_3 as a sole nitrogen source, the uptake

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