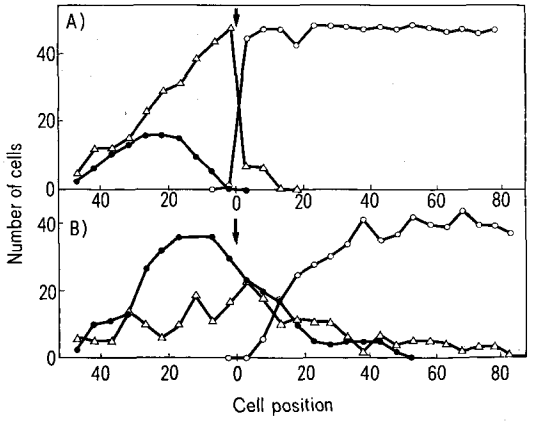
mitosis during the first 6 days are added to an already existing cell population. Villi from the intestines of 8-day-old pigs are, however, similar in size to those seen in the 6-day-old animals. This also applies to the 10-day-old animal (crypt + villus height 211 ± 7.1 cells). If labelled cells continue to migrate at the same rate, one can predict that they will reach the tips of the now nongrowing villi by day 10. It is clear though that any loss of physiological function seen to take place up to 6 days after birth must be explained on the basis of an already existing cell population changing its properties. This includes the ability to change sodium transport as well as to react with immunoglobulins in such a way as to stimulate oxygen consumption.



Cell migration during early postnatal development in the pig small intestine. Measurements taken *A* 2 h and *B* 48 h after the injection of [^{3}H] thymidine. Cell position 0 (↓) corresponds to the junction between crypt and villus, cell positions to the left and right referring to distance into crypts or onto villus respectively. The number of cells at positions greater than 30 in crypts is small since few crypts have depths greater than this value. 3 types of cell are considered: those labelled with thymidine which never become vacuolated (–●–); unlabelled cells which never become vacuolated (–△–) and unlabelled vacuolated cells (–○–). Values give the means of 10 observations.

Experiments showing the relative distribution of thymidine labelled and vacuolated epithelial cells on the villi of 2 and 48 h old piglets are illustrated in the figure. Vacuolation, caused by the endocytosis of immunoglobulins within 2 h of ingesting colostrum, is confined to cells on the villus (figure, A). Labelled cells first appear towards the base of the crypts. Trapped between labelled cells and vacuolated cells on the villus lies a population of nonlabelled crypt cells. The way these 3 populations redistribute themselves in the succeeding 46 h is shown in the figure, B. The total number of nonlabelled vacuolated and nonlabelled unvacuolated cells remains constant while the population of labelled cells increases by a factor of 4. Considerable cell mixing takes place during this period of development. After 48 h one can find some labelled cells ahead of nonlabelled unvacuolated cells and some of both of these populations ahead of some of the vacuolated cells. This leads to a rather inefficient displacement of the original cell population. Some of the originally vacuolated cells will, nevertheless, have been displaced by day 10. This is the time at which the small intestine ceases to respond to a period of starvation by increasing its transport of sodium[2].

The fact that little or no cell replacement takes place up to day 6 allows to conclude that deficiencies in function arising within this time period must occur within the original population of cells. Functional changes occurring with a time course greater than 6 days, probably arise from the physical displacement of some of the original population of cells from nongrowing villi.

Effect of histones from brain on DNA-synthesis in vitro[1]

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Summary. The results of these experiments demonstrate that histones from brain inhibit the replication of DNA in vitro. A similar effect is observed with polylysine or polyarginine. The reversion 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.

Several studies have shown that histones inhibit various DNA-polymerase systems in vitro[2–4]. Lysine-rich histones were the most effective and arginine-rich histones the least effective inhibitors of DNA-synthesis[4]. The histones can cover the DNA-strand or change its conformation in such a way as to render it unrecognizable by the polymerases.

In our laboratory we are interested in the role of nuclear proteins in the regulation of DNA-replication in the brain. We demonstrated in our previous experiments a close relationship between the rate of DNA-replication and the synthesis of chromatin proteins[5]. Lately, we have demonstrated the presence of DNA-polymerase and endonuclease activity within the nonhistone proteins of the brain chromatin[6,7].

As histones have a high positive charge, their interaction with DNA could be due, at least in part, to electrostatic forces between the positive charges of the histones and the negative charges of the phosphoric acid groups in DNA. In this way, polypeptides of high positive charge such as polyarginine and polylysine can be used as histone analogs.

In the present study we investigated the effects of unfractionated brain histones, polyarginine and polylysine on DNA-synthesis in vitro. We also studied the effect of polyglutamic acid, a highly anionic polypeptide and acidic proteins, that could compete with DNA in the binding of the basic molecules of polyarginine, polylysine or histones. This will provide evidence on the degree of affinity of the histones, polyarginine and polylysine for the DNA. We can also see if the inhibition of the DNA-polymerase activity produced by these can be reversed with polyglutamic and acidic proteins.

Material and methods. 8-day-old rats of either sex from a highly inbred Wistar strain were used. They were killed by decapitation and unfractionated histones and acidic de-

Table 1. Effect of polyglutamic acid on the inhibition produced by histones on DNA-synthesis in vitro

	Specific activity (percent of control)	Range (%)
DNA-polymerase + DNA	100	
DNA-polymerase + polyG	100	89–110
DNA-polymerase + histones	23	20– 25
DNA-polymerase + (DNA + histones) + polyG	53	49– 58
DNA-polymerase + (polyG + histones) + DNA	101	93–109
DNA-polymerase + (DNA + polyG) + histones	111	100–122
(DNA-polymerase + histones) + DNA + polyG	100	91–108

The source of DNA-polymerase was the nucleoplasm of isolated brain nuclei, 100 µg of protein per reaction tube. Histones were 25 µg and polyglutamic acid (polyG) 25 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% 112,000 dpm/mg of enzyme protein ± 12,328. The results are the average of 4 experiments.

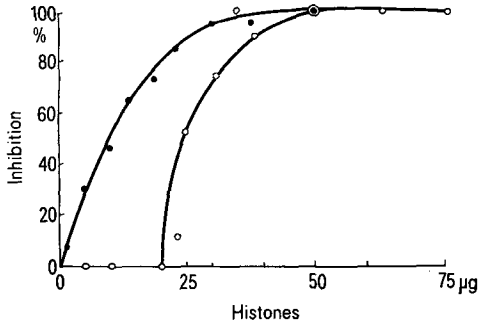

Fig. 1. Effect of histones on DNA-synthesis in vitro using chromatin or native DNA as template. Each assay contained 100 µg of enzyme protein and 20 µg of chromatin DNA or native DNA from salmon sperm. The reaction mixture and assay conditions were as mentioned in the text. ●—●, Chromatin; ○—○, native DNA. The results are the average of 3 experiments.

Table 2. Effect of polyglutamic acid on the inhibition produced by polylysine and polyarginine on DNA-synthesis in vitro

	Specific activity (percent of control)	Range (%)
DNA-polymerase + DNA	100	
DNA-polymerase + polyL	6	5–7
DNA-polymerase + polyG	92	81–101
DNA-polymerase + (DNA + polyL) + PolyG	70	64– 77
DNA-polymerase + (DNA + polyG) + polyL	86	76– 95
DNA-polymerase + (polyL + polyG) + DNA	108	89–120
DNA-polymerase + polyA	20	17– 22
DNA-polymerase ± (DNA + polyA) + polyG	79	72– 87
DNA-polymerase + (DNA + polyG) + polyA	106	94–119
DNA-polymerase + (polyA + polyG) + DNA	109	98–121

The source of DNA-polymerase was the nucleoplasm of isolated brain nuclei, 100 µg of protein per reaction tube. Polysine (polyL) 12.5 µg/tube; polyarginine (polyA) 12.5 µg; polyglutamic acid (polyG) 25 µg/tube and native DNA from salmon sperm 20 µg/tube. 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. Spec. act. at 100% 174,774 dpm/mg of enzyme protein ± 16,666. The results are the average of 4 experiments.

oxyribonucleoproteins prepared from the brain by the method previously described[5]. Poly L-Arginine HCl, type IIb, poly L-lysine HBr, type VIIb, and polyglutamic acid, sodium salt, type IIb were purchased from Sigma Chemical Co.

The assay for DNA-polymerase activity measures the incorporation of (Me-^{3}H) deoxythymidine-5-triphosphate, tetrasodium salt (spec.act. 53.7 Ci/mmole) into an acid-insoluble product by precipitation with trichloroacetic acid. The reaction conditions and further processing were described previously[7]. The source of DNA-polymerase was the nucleoplasm of isolated nuclei from rat brain cells[7].

Results and discussion. The incorporation of ^{3}H-TTP into DNA is inhibited by histones added to the incubation media, either with DNA or chromatin as a primer; as can be seen in figure 1. The inhibition is greater when chromatin is used as a primer instead of native DNA. This difference would represent the histones already present in the chromatin while the native DNA is naked.

The incorporation of ^{3}H-TTP into DNA is inhibited too when polysyne or polyarginine are added to the incubation media in place of histones (figure 2). The inhibition is proportional to the concentration of the polypeptides. The minimum quantity of the polypeptides giving maximal inhibition was determined giving 12.5 µg for polylysine and 25 µg for polyarginine in each assay tube. The polylysine was slightly more effective than polyarginine.

We tested the influence of polyglutamic acid on the activity of DNA-polymerase. It was found that high quantities of polyglutamic acid (more than 50 µg) inhibited the incorporation of ^{3}H-TTP. We therefore used the highest quantity that can be added to the media without producing significative inhibition (25 µg).

Once the optimum quantities of histones, polylysine, polyarginine and polyglutamic acid were determined, the activity of DNA-polymerase was assayed in the presence of these substances, as can be seen in tables 1 and 2. In table 1 we can see the effect of polyglutamic acid on the inhibition produced by histones of DNA-synthesis in vitro. As can be seen in the table, any 2 of DNA, histones or polyglutamic acid were added first and allowed to interact before adding the 3rd number. In this way, we could see if the previous formation of a complex between histones and DNA or polyglutamic acid could affect the activity of the DNA-polymerase. The polyglutamic acid reversed in all cases the inhibition produced by histones. This reversal was minimal (50%) when the histones were allowed to interact with DNA previous to the addition of

1 These studies were supported by the Consejo Nacional de Investigaciones Científicas y Técnicas and the Instituto Nacional de Farmacología y Bromatología, Argentina.

2 Abbreviations. DNA-polymerase: deoxynucleoside triphosphate: DNA deoxynucleotidyl transferase (E.C. 2.7.7.7.).

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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.

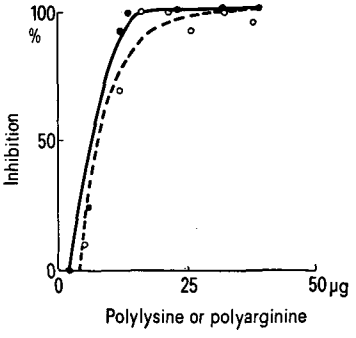

Fig. 2. Effect of polylysine and polyarginine on DNA-synthesis in vitro using native DNA as template. The reaction mixture and assay contitions were as in figure 1. ●—●, Polylysine; ○—○, polyarginine. The results are the average of 3 experiments.

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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23 May 1977

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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