suppression of migration was obtained at concentrations similar to those used in the papers cited. We reported a similar result previously in guinea-pigs[10].

GC in vitro also affect many biochemical functions of macrophages. However, the protein synthesis[11] and enzyme secretion[12] were inhibited with physiological or smaller concentrations of drug, indicating a different sensitivity of various cell functions to GC action. The appearance of resistance to prednisolone inhibitory action in the PPD-group is in accordance with ideas postulated by Claman[13] and Baxter and Harris[14]. They consider that lymphoid cells may be either steroid sensitive and resistant, according to their stage of activation in the course of the immune response. All the above-mentioned data about GC effects on migration of various cell types in cortisone-sensitive (rabbit, rat) and cortisone-resistant species (man, guinea-pig) may indicate interference with the basic mechanism involved in cell migration. At the same time, the GC-induced defects in cell movement may contribute to reduced cell defense during GC therapy.

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Incorporation of 5-bromodeoxyuridine in the total and ribosomal DNA of synchronously dividing chick embryo fibroblasts[1]

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Summary. The pattern of 5-bromodeoxyuridine incorporation into ribosomal DNA is quantitatively different from that for total DNA. It is concluded that 5-bromodeoxyuridine incorporation along the DNA chain is not a random process.

5-Bromodeoxyuridine (BrdUrd) is a nucleotide analogue of thymidine (dThd) which is easily incorporated into DNA in place of dThd. This analogue has been widely used in studies on DNA replication: its incorporation into DNA produces an increase of the buoyant density in cesium chloride gradients which allows the easy separation of replicated DNA from non-replicated DNA. There is an abundant literature dealing with the effects of BrdUrd on cellular metabolism during the differentiation process (for review see Rutter et al.[4]). Moreover, it was shown that some families of moderately repeated DNA from different organisms were preferentially substituted by BrdUrd when low concentrations of the analogue were used[5-8]: the substitution of dThd by BrdUrd was not a random process. For these reasons, we set out to make a comparative study of the extent of BrdUrd incorporation, depending on the concentration of the analogue in culture medium, into chick total DNA and ribosomal DNA (rDNA), the latter representing a well-defined, moderately repeated fraction of DNA. Nevertheless, as chick rDNA content is only 0.02–0.12% of bulk cellular DNA[9,10], i.e. about 0.25 to 1.5% of repeated and intermediate DNA sequences[11], our results could not be extended to the overall repeated and intermediate DNA. However, the extent of rDNA substitution, compared to bulk DNA, might represent an experimental approach to demonstrate a non-random incorporation process of the analogue along the DNA chain.

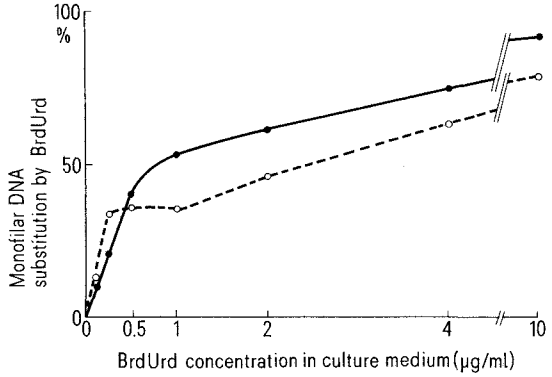
This paper describes the experimental strategy used to measure the extent of BrdUrd incorporation, depending on its concentration in the culture medium, and shows that the pattern of rDNA substitution differs notably from that of bulk DNA.

Materials and methods. Cell cultures. Primary cultures of chick embryo fibroblasts were prepared as described by Temin and Rubin[12]. The synchronization procedure was as described by Goldé et al.[13]. For the determination of total DNA substitution, labeled BrdUrd (5 µCi/plate) was added, at the time of release of mitosis, at concentrations ranging from 0 to 10 µg/ml. For the determination of rDNA substitution, unlabeled BrdUrd was added in the same concentration range. For the preparation of (^{3}H)rRNA, cultures were supplemented with ($^3H_5$)-uridine (500 µCi/plate) during 1 cell cycle and chased for 4 h with unlabeled uridine 100 times more concentrated than the labeled nucleotide.

DNA preparation. After 1 cell cycle, cells were killed by addition of 1.4×10^{-3} M NaF, and scraped into phosphate-buffered saline buffer. DNA was extracted according to Hughes et al.[14] and subjected to neutral CsCl density gradient centrifugation (refractive index adjusted to 1.4010, 28,000 rev/min for 95–100 h at 20 °C, rotor Beckman 60 Ti). After centrifugation, gradients were harvested into 22–24 fractions. For the determination of total DNA substitution, the radioactivity and the absorbance at 260 nm (A_{260}) of each fraction were assayed. Fractions corresponding to substituted DNA (selected on the basis of the radioactive profile) were pooled. For the determination of rDNA substitution, each fraction was assayed for A_{260} and was further processed as described under DNA-^{3}H-rRNA hybridization'.

^{3}H-rRNA preparation. Labeled chick rRNA was prepared as previously described[15].

DNA-^{3}H-rRNA hybridization. An aliquot (100 µl) from the fractions of each gradient was denatured and fixed on a



BrdUrd substitution patterns of total DNA (●——●) and rDNA (○ – – – ○), according to the analogue concentration in culture medium.

cellulose nitrate membrane filter (Schleicher and Schüell, BA 85). Hybridization was performed essentially according to Gillespie and Spiegelman[16]. All the filters of the same gradient were hybridized in the same vial. After hybridization (6 h at 60 °C) and RNAse treatments, filters were dried and counted in an Intertechnique spectrometer.

Analytical ultracentrifugations. For the determination of the buoyant density of substituted total DNA, an aliquot (about 2 μg) of each pooled substituted DNA was subjected to analytical ultracentrifugation (Beckman ultracentrifuge, Model E, 44,000 rev/min, 20 h, 25 °C) using phage E ($\rho = 1.742$ g/ml) or phage psi ($\rho = 1.715$ g/ml) DNA as density markers. For the determination of the buoyant density of substituted rDNA, an aliquot (about 2 μg) of the DNA fraction corresponding to the maximum of the DNA-rRNA hybridization peak was processed as described above. Densities were calculated according to Schildkraut et al.[17].

Determination of the substitution rate of total DNA and rDNA. Luk and Bick[18] have established an equation which allows a valid determination of the substitution rate of a given DNA if the buoyant density, in a neutral cesium chloride gradient, of the non-substituted DNA and that of the same substituted DNA are known. We have adapted this equation for a direct calculation of the rate of unifilar substitution in so far as, under our experimental conditions, DNA is duplicated only once.

$$\text{Rate of dThd substitution by BrdUrd (\%)} = \frac{\rho_s - \rho_{ns}}{T/50 \times 0.173} \times 200$$

where ρ_s = buoyant density of the unifilarly substituted DNA,
ρ_{ns} = buoyant density of the corresponding non-substituted DNA,
T = thymine content of non-substituted DNA, expressed as % of total base composition. This value may be deducted from the equation of Schildkraut et al.[17]:
$\rho_{ns} = 1.660 + 0.098\ (G + C)$

In our biological system, the values for ρ_{ns} are respectively 1.699 g/ml for total DNA and 1.724 g/ml[15] for rDNA.

An indirect method of determination of the DNA substitution rate was used because of the very low rDNA content which did not allow the use of a direct method of measurement, such as chromatographic analysis of base composition.

Results. The table shows the buoyant density values obtained from the differently BrdUrd-substituted total DNA and rDNA extracted from fibroblasts obtained from the same primary culture. The corresponding unifilar substitution rates, expressed in terms of percent of the dThd content of DNA, were then calculated from the modified Luk and Bick's equation and plotted against BrdUrd concentration in the culture medium (fig.). Other similar series of experiments, each derived from the same primary culture, have been carried out for total and ribosomal DNA (2 and 1 respectively). In these experiments, the patterns of BrdUrd substitution rate vs BrdUrd concentration presented the same characteristics as those described in this paper.

Buoyant densities, in neutral cesium chloride gradients, of chick total and ribosomal DNA prepared from cells grown in the presence of varying concentrations of BrdUrd

BrdUrd concentration (μg/ml)	Buoyant density (g/ml) Total DNA	rDNA
0	1.699	1.724
0.1	1.704	1.727_5
0.25	1.710	1.734
0.5	1.720	1.734_5
1	1.727	1.734_5
2	1.731	1.738
4	1.738	1.742_8
10	1.747	1.747_5

For total DNA (fig., a), the extent of substitution was directly proportional to BrdUrd concentration up to 0.5 μg/ml: a low BrdUrd concentration probably acts as a limiting factor for BrdUrd incorporation. From 0.5 μg/ml, the rate of dThd substitution tended asymptotically towards 100%. A substitution rate of 92–100% was generally attained for a BrdUrd concentration of 10 μg/ml in culture medium.

The pattern of substitution was notably different for rDNA (fig., b). The linear relationship between BrdUrd concentration and BrdUrd incorporation rate continued up to 0.25 μg/ml. At this concentration, about 33% of the dThd moiety was substituted (compare with 20%, at the same concentration, for total DNA). Then, between 0.25 and 1 μg/ml, the substitution rate did not increase significantly. As a consequence, the rDNA substitution rate was only 35% at a concentration of 1 μg/ml (compare with 54%, at the same concentration, for total DNA). Finally, the asymptotical evolution of the BrdUrd substitution rate started from about 1 μg/ml. At a concentration of 10 μg/ml, only 79% of the dThd moiety of rDNA was substituted by the analogue (compare with more than 92%, at the same concentration, for total DNA).

Discussion. The comparison of the BrdUrd incorporation patterns of chick total and ribosomal DNA, depending on the BrdUrd concentration in the culture medium, leads to the conclusion that the analogue is not randomly incorporated into whole chick DNA. Indeed, the pattern of incorporation into rDNA presents some distinctive features, when compared with total DNA. At low concentrations (up to 0.25 μg/ml), the substitution rate is more important for rDNA than for total DNA. That may reflect a preferential incorporation of the analogue into intermediate DNA sequences such as rDNA. A similar conclusion has been put forward, for moderately repeated DNA sequences of rat embryo fibroblasts, by Schwartz and Kirsten[5]. rDNA has been shown to replicate during early S phase[19–21], late S phase[22–24] or during the overall S phase[25–27]. The time course of chick rDNA replication follows that of bulk DNA[28]: it is then not possible, at first sight, to ascribe the preferential BrdUrd incorporation process into chick rDNA to a greater avaibility of the analogue during early S phase. Nevertheless, reassociation kinetics of total DNA substituted at various rates during an entire cell cycle has shown a preferential incorporation, at low concentrations, into DNA sequences reassociating between Cot 10^{-2} and Cot 10^2, i.e. some repeated and intermediate DNA sequences

(article in preparation). Moreover, the substitution of total DNA was essentially higher, into DNA samples reassociated at Cot 10^2, for DNA sequences duplicated during the first half of S phase (article in preparation). This finding is consistant with the fact that, at low BrdUrd concentrations, the buoyant density value of DNA synthesized during the first half of S phase is always higher than that of DNA synthesized later (Hartmann and Rode[29] and not published results). An explanation lies in the fact that the synchronization procedure used - including a 4-day cell starvation - generates some major metabolic disturbances, such as an unbalanced nucleotidic pool or quantitative variations of some of the enzymes involved in DNA synthesis, which would favor a preferential incorporation of BrdUrd during a short period (several hours) following release of mitosis. From another point of view, Kuebbing and Werner[30] have shown that exogenous dThd added to Hela cells grown in thymidine-free medium is incorporated into DNA almost immediately at full specific activity, blocking any further incorporation of de novo synthesized thymidine nucleotides. These experiments suggest a compartmentation of intracellular salvage and de novo thymidine nucleotide pools. Furthermore, Mattern and Painter[31] have found that, when moderately repeated sequences were replicated, exogenous dThd was incorporated at a faster initial rate - particularly during early S phase - than when highly repeated and unique sequences were replicated. As, in our system, exogenous BrdUrd can be only incorporated by the salvage pathway, the over-substitution of rDNA, relative to total DNA, observed at low concentration in culture medium, may then be explained by the association of the 2 above-mentioned metabolic processes[30,31]. In these conditions, the under-substitution of rDNA observed with higher BrdUrd concentrations is far from clear. The low A+T content of rDNA could be involved in this phenomenon, as well as a feedback control of the synthesis of the BrdUrd triphosphate pool, induced by a much greater concentration into the salvage nucleotidic pool. Finally, even if our results are concerned with an intermediate and well-defined DNA sequence, it is quite obvious that they cannot be directly extended to the whole of the intermediate cellular DNA.

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A semi-empirical formula for the polarizability of the naturally-occurring amino acids based on their side chain packing volumes

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Summary. A linear relationship between the polarizability of the side chains of the naturally-occurring amino acids and their side-chain packing volumes in protein crystals is demonstrated.

The polarizability of atoms and molecules is important in the analysis of a wide variety of physical and chemical problems, for example, in light scattering, electric dipole moment calculations, and the evaluation of van der Waals forces. The polarizability is usually determined from refractive index measurements by means of the Lorentz-Lorenz equation, and for atoms and simple small molecules it is of the order of the molecular volume. A similar consideration