anaemia or other hypoxic stress, the unusual β^{C}-like chain called $\beta^{C(na)}$. Other individuals of *A. lervia* synthesize a typical ovine-caprine β^{C} chain which differs by eight amino acids from *A. lervia* $\beta^{C(na)}$[10]. Otherwise, the evolution of C-type chains is relatively conservative, for *A. lervia* β^{C} differs by only 2 amino acids from *C. hircus* β^{C} and by only 1 amino acid from *O. aries* and *O. musimon* β^{C}[11]. Another remarkable feature of *A. lervia* $\beta^{C(na)}$ is that the middle of the chain, tryptic peptides T-9 and T-10, contain 4 amino acid substitutions not found in other β^{C} chains but shared in the sequence of the foetal chain, γ, of sheep and goats.

Accordingly, the haemoglobin data suggest that *A. lervia* is no closer to *Ovis* than to *Capra*; indeed, the nature of the α-chain duplication indicates that there is more in common between *A. lervia* and *C. hircus* than between *A. lervia* and *O. aries*. However, especially with the unusual $\beta^{C(na)}$ chain and the total of 39 differences between *A. lervia* β^{B} and other β-chains, it might be best to regard the aoudad as distinct from both *Ovis* and *Capra*, perhaps representing an early offshoot from the ovine-caprine stock which has retained some primitive characters but has also evolved some uniquely specialized ones.

Such a position would not be incompatible with the zoogeographic information, for *Ammotragus lervia* occurs in a restricted part of northern Africa, distinct from 'the great arc' of *Ovis* in Eurasia and North America[12]. The distinctness of *Ammotragus* is shown by its failure to produce viable hybrids with either domestic goats or domestic sheep, although development of *A. lervia* × *C. hircus* foetuses proceeds to term, whereas *A. lervia* × *O. aries* fails to be conceived[13].

In contrast to suggestions that *Ammotragus lervia* is close to progenitors of the domestic sheep, discussions of data for both biochemical[4] and chromosomal[14] characters suggest the mouflon, *Ovis musimon*, as a better candidate. As CLARK[15] says, 'the Barbary sheep (*Ammotragus*)..., for reasons it would be interesting to have restated, is excluded from any part in the ancestry of African domestic sheep'.

Summary. Data on haemoglobin do not support suggestions that the aoudad *Ammotragus lervia* is close to a hypothetical ancestor to the genus *Ovis* in general or to the domesticated sheep *Ovis aries* in particular. *Ammotragus* haemoglobin is more like that from the domestic goat *Capra hircus* than that from the domestic sheep *Ovis aries*, but also shows some unique characteristics, perhaps more specialized than primitive.

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PRO EXPERIMENTIS

A Method for Distinction Between RNA and DNA in Aldehyde and Osmiumtetroxide-fixed Electron Microscopic Autoradiographs[1]

Histochemical procedures in electron microscopy usually require a special fixation or other treatment of the tissue prior to embedding. If an investigation with tissue fixed and embedded according to a routine procedure is in progress and one wishes to perform a histochemical reaction, the fixation, embedding, and preceeding experiments have to be repeated. This is especially time-consuming with electron microscopic autoradiography, as the exposition time of the autoradiographs ranges from weeks to several months.

The present paper shows that the so-called 'regressive staining method' described by BERNARD[2] for the distinction of RNA and DNA on aldehyde-fixed tissue can also

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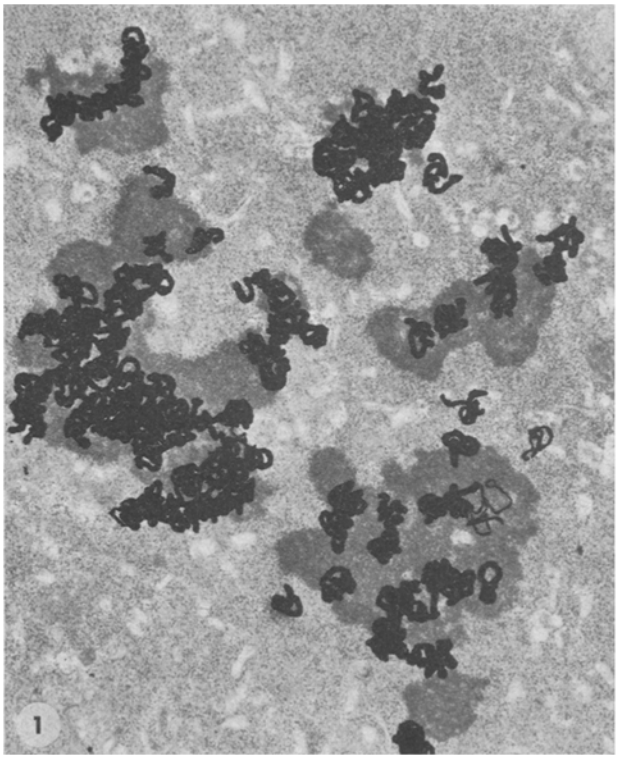
Processing and staining of autoradiographs for conventional contrast and RNA-DNA differentiation

Solution	Time of treatment for	
	Conventional contrast	RNA-DNA differentiation
Microdol-X- (Kodak)	5 min	5 min
Dist. water	a few sec	a few sec
Na-thiosulfate (3%)	no longer than 3 min	no longer than 3 min
Dist. water (room temp.)	3 × 10 min	3 × 10 min
Dist. water 37 °C	30 min	30 min
Acetic acid 37 °C	15 min 4%	up to 20 min, up to 8%
Dist. water (room temp.)	3 × 5 min	3 × 5 min
Uraniumacetate 2,5% in dist. water	6 min	none
Lead citrate[8]	3 min	3 min



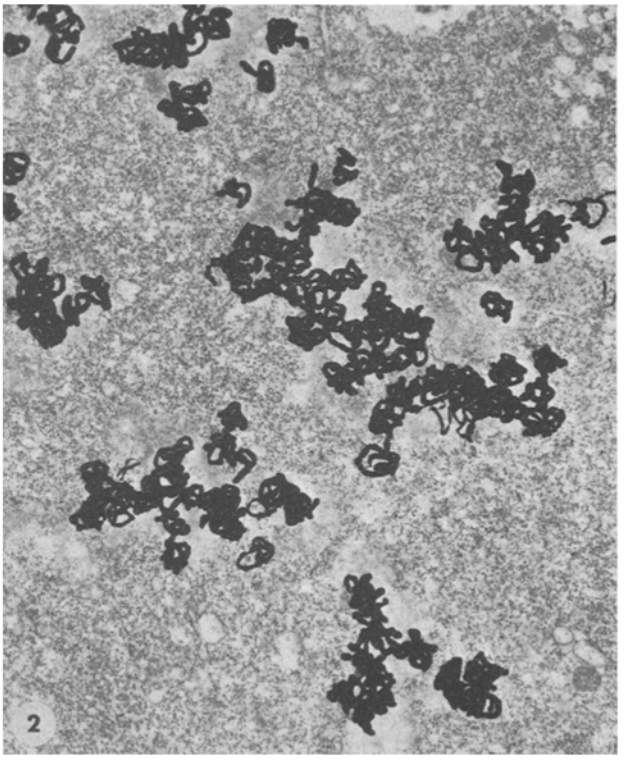


Fig. 1. Autoradiograph (^{3}H-thymidine) with conventional contrast: Silver grains are located over the dark stained DNA (chromosomes) of a mitotic HEp-2 cell. ×14,000.

Fig. 2. Autoradiograph (^{3}H-thymidine) with selectively destained DNA. The silver grains are only associated with the DNA-containing structures, thus proving the specificity of the destaining reaction. ×12,000.

be performed on conventionally (glutaraldehyde and osmiumtetroxide) fixed autoradiographs by simply changing the staining procedure of the exposed and developed specimens. The use of conventionally fixed tissue has the additional advantage that the ultrastructural preservation of cytoplasmic structures is better than in the original procedure.

Materials and methods. HEp-2 cells, obtained through the courtesy of Dr. D. A. WOLFF, Ohio State University, were grown in suspension on a gyrotory shaker in Joklik modified MEM (Gibco), supplemented with 10% calf serum. Labelling with radioactive precursor (^{3}H-thymidine, Radiochemical Centre, Amersham, GB) was carried out at a concentration of 10 μCi for 24 h. The cells were fixed in cold 6.5% glutaraldehyde in cacodylate buffer (0.125 *M*) for 90 min, rinsed for 48 h in several changes of cacodylate buffer to remove unincorporated label[3] and postfixed for 30 min in 2% OsO_4 in cacodylate buffer. Clumps of cells were dehydrated in increasing concentrations of acetone. For block staining, the first acetone solution contained 2% uranium acetate (Uac). Embedding in Epon was done according to LUFT[4]. Our autoradiographic procedures were described previously[5]; they were based on the techniques given by STEVENS[6]. The sections containing grids were coated by the loop method[7] with Ilford L-4 emulsion, and the grids exposed at 4 °C in a desiccator. No protective carbon layer was applied between section and emulsion. Developing, gelatine removal and staining procedures are given in the Table.

Results. The re-staining with Uac (Table) of the already 'block-stained' specimens is necessary to obtain conventionally stained sections (Figure 1), because processing of the autoradiographs tends to extract some of the Uac from the tissue. To obtain similar distinction between RNA- and DNA-containing structures as produced by the 'regressive staining method'[2], simply the re-staining step with Uac prior to lead staining is omitted (Figure 2 and 3 and Table). The final staining with lead citrate[8] enhances the contrast of the pre-existing Uac stain in combining preferentially with those structures (RNA) which already contain Uac.

Thus, as in BERNHARD's method[2], where chelating agents, such as EDTA, extract Uac selectively from DNA-containing structures of aldehyde fixed tissue, in our procedure the influence of the photographic processing is used to accomplish the selective extraction in aldehyde- and osmiumtetroxide-fixed cells.

The degree of 'bleaching' of the DNA-containing structure, however, unlike in BERNHARD's method[2], is not influenced by the section thickness or the age of the Epon block, but can be changed to a certain extent by the time and concentration of the acetic acid treatment during gelatine removal: increasing the time up to 20 min and the concentration up to 8% may be necessary for some specimens to get adequate destaining of the chromatine. Optimal results for a given tissue have to be found

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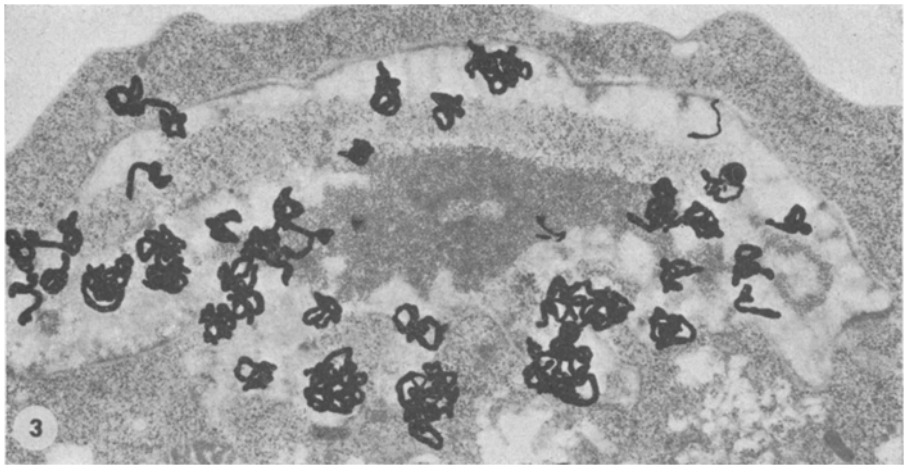

Fig. 3. Autoradiograph (^{3}H-thymidine) of a nucleus in a poliovirus infected cell, 6 h p.i. The silver grains are found over the destained, condensed chromatin. The RNA-containing structures are free of silver grains and retain their normal contrast. ×15,000.

and leaving all other parameters constant, because changing developing and fixation time or concentration might affect the ultrastructural preservation adversively.

In order to find the substance responsible for the destaining effect, we tested the developer (Microdol X), another developer (D 19), the fixer and the acetic acid alone or in different combinations. It was found that a destaining effect on sections not coated with emulsion only appeared if the sections were in contact with one of the developers. The fixer, the acetic acid alone, or both in combination, did not change the staining pattern.

Likewise, the high pH-values of the developers are not responsible for the destaining of DNA: distilled water, adjusted to the pH of the developers (7.9 for Microdol X and 10.7 for D 19) has no destaining effect. The two substances (Metol and sodium sulfite) common to both developers, were tested for their destaining action: only sodium sulfite (14%) showed the same Uac extracting property as the complete developer.

Thus, the method described can be used not only with autoradiographs but with every aldehyde-osmium fixed specimen by floating the block stained sections for 5 min empirically by changing the acetic acid treatment only on a 14% sodium sulfite solution and afterwards adjusting the degree of destaining by the acetic acid treatment prior to a lead citrate stain. This procedure might be useful if a distinction between RNA and DNA with already conventionally fixed and embedded material turns out to be necessary later in the course of an investigation.

Summary. A simple method for distinction between RNA- and DNA-containing structures in aldehyde- and osmiumtextroxide-fixed electron microscopic autoradiographs (or ordinary thin sections) is described: the developer and the acetic acid used for processing autoradiographs extract selectively uranium acetate from DNA containing-structures which, after staining with lead citrate, leads to a characteristically 'bleached' appearance of the DNA.

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Evaluation of a Conductometric Method to Determine the Volume Fraction of the Suspensions of Biomembrane-Bounded Particles

Although frequent need has been felt for a simple and rapid method to determine the volume concentration of cells or isolated organelles in suspension, there have so far been few methods applicable to routine biological work. In this communication we propose a new method which is based on simple conductometry and can be used as a substitute for the conventional microscopic and space-marker methods, provided the suspended particles are bounded by intact, limiting membranes and their electrical conductivity is several orders of magnitude smaller than that of the suspending medium.

Principle. According to theories[1,2], the relative conductivity of a suspension of non-conducting spheres is related to its volume fraction Φ as:

$$\varkappa/\varkappa_m = (1-\Phi)^{1.5} \qquad (1)$$

where $\varkappa$ and $\varkappa_m$ are the conductivities of suspension and medium, respectively. Remarkable points of Eq. 1 are: that since derived originally in an effort to extend the MAXWELL-WAGNER theory[3] on the conductivity behavior of dilute dispersions of spherical particles to more concentrated ones, this relation would hold for any values of Φ, and that a variety of experiments including suspensions of glass beads[1] and oil-in-water type emulsions[4,5] have given strong evidence for the usefulness of Eq. 1 in dealing not only with monodisperse but with moderately polydisperse systems. In addition, that some anomaly in particle shape, unless extreme in its extent, is allowable, has also been confirmed[1] by experiment. Hence it is reasonable to expect that we can determine the electrically effective volume fraction Φ_E of a given suspension

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