ethylsuccinate, josamycin, midecamycin, spiramycin or an equivalent amount of saline orally twice a day, over 3 days. 2 h after the last antibiotic administration, mice received an i.p. injection of pentobarbital (60 mg/kg). In a 2nd series, triacetyloleandomycin-and josamycin-treated mice were injected with sodium barbital (350 mg/kg, i.p.) and in a 3rd series, mice received 1 single oral dose of triacetyloleandomycin followed by i.p. pentobarbital after a varying time interval. Student's t-test was used for statistical analysis of results.

Results. Pentobarbital-induced sleeping time was found to be significantly increased after triacetyloleandomycin or josamycin pretreatment whatever the dose. Pretreatment with any of the other macrolide antibiotics proved ineffective. Barbital-induced sleeping time was not affected by either triacetyloleandomycin or josamycin. Finally, a single oral dose of 25 mg/kg triacetyloleandomycin 12 h before pentobarbital increased sleeping time significantly.

Effects of six macrolide antibiotics on barbiturate sleeping time

Tested drug	Oral dose	Sleeping time (mean ± SEM)
3 days pretreatment, last administration 2 h before pentobarbital		
Controls	–	122.0 ± 6.5
Erythromiycin ethylsuccinate	12.5 mg/kg	116.1 ± 4.9 (a)
	25 mg/kg	125.4 ± 4.9 (a)
Erythromycine propionate	12.5 mg/kg	120.8 ± 4.9 (a)
	25 mg/kg	126.6 ± 4.9 (a)
Josamycin	12.5 mg/kg	184.2 ± 9.2 (b)
	25 mg/kg	233.6 ± 6.1 (c)
Midecamycin	12.5 mg/kg	124.3 ± 4.5 (a)
	25 mg/kg	128.3 ± 3.2 (a)
Spiramycin	12.5 mg/kg	118.3 ± 4.0 (a)
	25 mg/kg	113.2 ± 4.2 (a)
Triacetyloleandomycin	12.5 mg/kg	188.0 ± 4.0 (b)
	25 mg/kg	188.9 ± 7.0 (c)
3 days pretreatment, last administration 2 h before barbital		
Controls	–	240.8 ± 10.4
Josamycin	12.5 mg/kg	225.4 ± 17.1 (a)
	25 mg/kg	217.7 ± 25.3 (a)
Triacetyloleandomycin	12.5 mg/kg	254.7 ± 18.2 (a)
	25 mg/kg	267.7 ± 23.4 (a)
1 single administration before pentobarbital		
Controls	–	122.0 ± 6.5
Triacetyloleandomycin		
2 h before pentobarbital	12.5 mg/kg	114.9 ± 6.0 (a)
	25 mg/kg	126.4 ± 5.0 (a)
12 h before pentobarbital	12.5 mg/kg	112.7 ± 5.2 (a)
	25 mg/kg	168.5 ± 6.1 (b)

Significance in comparison with control animals is expressed as (a) for $p > 0.05$, (b) for $p < 0.01$ and (c) for $p < 0.001$.

Discussion. Among the 6 macrolide derivatives studied, only triacetyloleandomycin and josamycin were found capable of increasing pentobarbital-induced sleeping time. That they did not influence barbital-induced sleeping time is in agreement with the proposed mechanism of an inhibition of drug metabolizing enzymes, as pentobarbital is transformed to an inactive metabolite while barbital is excreted mostly unchanged[3]. Our results are consistent with those of Lavarenne et al.[4] and Azria et al.[5] who did not find any pharmacokinetic interaction involving midecamycin and spiramycin respectively. Current data are more controversial with erythromycin derivatives[6,7] and josamycin[8,9]. It is apparent that triacetyloleandomycin-induced enzymatic inhibition develops very quickly, as previously suggested by clinical findings[1]; furthermore, the fact that triacetyloleandomycin given 2 h before pentobarbital does not influence sleeping time, although it increases it when given 12 h before, is in agreement with Pessayre et al.[2] who showed that triacetylolandomycin binds to cytochrome P 450, thus inactivating it. Finally, barbiturate sleeping time appears to be a valuable tool for predicting drug interactions mediated by liver enzymes, owing to the good correlation between our experimental findings and clinical data.

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On the complementarity of long repeated sequences in DNA to hnRNA[1]

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Summary. Long repeated sequences containing up to 18,000 base pairs were found in a human DNA fraction isolated with the nuclease S1-dioxane method. Hybridization studies showed that the long repeats contained a greater proportion of sequences complementary to hnRNA than short repeats. They also exhibited homology to the latter, as shown by cross-hybridization experiments.

In eukaryotic cells the lengths of the primary transcripts of DNA exceed those of mRNA by several fold, and deletion of intervening sequences appears to be necessary to the formation of many mRNAs[2,3]. Several studies have indicated the sequence heterogeneity of introns. Members of the *Alu* family and other short repeated sequences have been found in the regions flanking some genes[4]. Regions of the genome that do not contain repeated or foldback sequences have been identified in other introns[5]. These observations have not, however, accounted for the considerable length that must be possessed by some introns, if the size discrepancies between hnRNA and mRNA are to be explained.

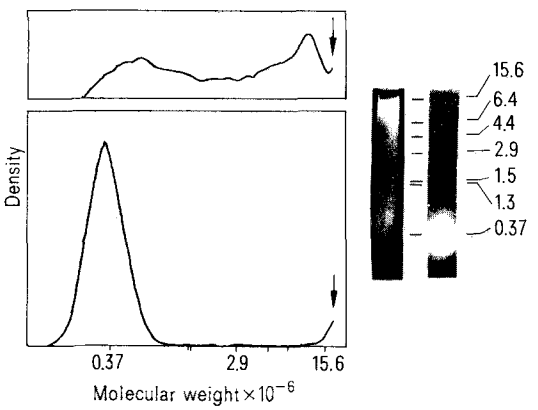
This report describes the finding of repeated sequences that contain up to 18,000 base pairs. The long repeats were found because the molecular weight of the starting material was much larger than those of DNA preparations usually employed for the study of repeated sequences[6–8].

Nuclear DNA was isolated from human placental nuclei and its mol.wt was determined by viscometry[9,10]. The DNA was denatured with alkali and reassociated to Cot 10 in 0.2 M sodium phosphate[11]. Subsequently, the nuclease S1-dioxane procedure for isolating the duplex fraction was carried out in entirety[10]. Single-copy sequences were excluded from the duplex fraction by the choice of the Cot value and the isolation method. The amount of duplex DNA formed at Cot 10 represented 10% of the total DNA. Using the techniques of gel electrophoresis and densitometry that are described under the figure, it was found that 70% of this fraction had a modal mol.wt of 300,000, corresponding closely to the fraction which contained the *Alu* family[13]. The remainder of the Cot 10 duplex fraction consisted of longer fragments covering a wide range of mol.wt values up to 12×10^6.

The Cot 10 duplex fraction was capable of annealing with homologous hnRNA (table 1). It was notable that the longer repeated sequences, like the shorter repeats, contained sequences complementary to hnRNA. In order to compare the hybridization behavior of the different sizes of repeats, 'long' and 'short' subfractions were prepared by means of column chromatography[19], and their size distributions are shown in the figure.

The relative efficiency of long and short repeats in hybridization with hnRNA was studied (table 2). Under the experimental conditions of large DNA excess over hnRNA, and little change in the level of unbound hnRNA, uptake of RNA was proportional to DNA concentration[20]. Since the mass of DNA per filter was the same, the amounts of hnRNA hybridized simply reflected the degree of complementarity that each DNA preparation possessed. Short repeats took up the least amount of hnRNA. Twice as much RNA was hybridized to long repeats; thus, a larger fraction of the latter was complementary to hnRNA. Unfractionated DNA, which in addition to repeats contained single-copy sequences, took up three times as much hnRNA as short repeats. This finding would be consistent with the view that single-copy transcripts were continuous with transcripts of repeated sequences. The relatively rapid annealing of hnRNA regions to repeated DNA sequences would accelerate the formation of hybrids to single-copy regions.

Cross-hybridization experiments showed that short and long repeats shared common sequences. In these studies, 2 cycles of hybridization were carried out. Each 25-mm filter, loaded with short repeats, was incubated with ^{3}H-labeled hnRNA for 18–21 h at 65 °C (input: 203,000 dpm in 0.5 ml SC). After washing and RNAse treatment[21], the RNA-DNA hybrids were denatured during 2 cycles of heating in 0.1 SC-2% sodium dodecyl sulfate (15 min, 70 °C). The eluates were pooled, made up to full strength SC, and aliquots of this solution (0.5 ml, containing 1600 dpm) were incubated with fresh filters to which 7 µg of long or short repeats were fixed. Blank filters lacking DNA were likewise incubated for 21–23 h at 65 °C. At the end of the 2nd cycle of hybridization, the filters were washed, treated with RNAse A and assayed for radioactivity. The uptake, expressed as percent of the input, was 0.4 ± 0.5 (mean ± SD) in the blank filters, 5.5 ± 1.3 in the short repeats and 9.5 ± 1.5 in the long repeats. Long repeats,



Size distribution of long and short repeats separated on hydroxylapatite columns. The Cot 10 duplex fraction was applied to the column in 0.05 M potassium phosphate and eluted with 0.178 M buffer (fraction I) and then with 0.24 M buffer (fraction II). Fraction II, in 0.05 M buffer, was applied to a fresh column that was eluted with 0.181 M and 0.24 M buffer. The DNA appearing in the latter subfraction was designated long repeats and its size distribution is shown in the upper panel. Fraction I was rechromatographed in a second cycle identical to the first, yielding short repeats with the 0.178 M eluent (lower panel). Long and short repeats represented 15 and 63% of the original sample, respectively. Samples of the 2 subfractions were electrophoresed in 0.7% agarose slabs, together with lambda DNA (*Hind* III) markers[12]. The densitometric scans were made on photographic negatives obtained on ethidium-stained gels. The mol.wt ($\times 10^{-6}$) of the individual markers are given on the right. Arrow indicates the origin.

Table 1. Length of repeated sequences that hybridize to (^{3}H)-hnRNA*

Length of DNA (mol. wt $\times 10^{-6}$)	(^{3}H)-hnRNA hybridized (dpm/segment)
Above 6.2	384 ± 88**
2.7–6.2	370 ± 138
1.0–2.7	326 ± 91
0.4–1.0	441 ± 36
Below 0.4	221 ± 79

*Samples of Cot 10 duplex fraction were electrophoresed in 0.7% agarose[12] and blotted on to nitrocellulose membranes (type HA, from Millipore Corp., Bedford, MA, USA)[14]. ^{3}H-labeled hnRNA was prepared as follows: about 60×10^6 human cells (strain Raji) were pre-incubated with 0.05 µg/ml actinomycin-D. Following a 75-min incubation with 5 mCi (^{3}H)-uridine (30 Ci/mmole) in 13 ml medium, hnRNA was extracted with hot phenol[17] and subjected to sucrose gradient ultracentrifugation. Fractions >45S were pooled, heat-denatured and filtered through nitrocellulose membranes[18]. The blots (containing 1–2 µg DNA) were incubated with (^{3}H)-hnRNA in SC (0.3 M NaCl-0.03 M sodium citrate, pH 7.4) for 21 h at 65 °C (input, 99,900 dpm). After hybridization, the washed, RNAse A-treated strips were cut into 5 equal segments with positions corresponding to the given mol. wt ranges for double-stranded DNA. The radioactivity in each segment was assayed and corrected for ^{3}H found in blank segments, that represented about 7% of the radioactivity in the blots. **Mean ± SD.

Table 2. Hybridization of long and short repeats to (^{3}H)-hnRNA*

Immobilized DNA	(^{3}H)-hnRNA hybridized (dpm/filter) Incubation period	
	18 h	27 h
Short repeats	6,332 ± 811 (4.0)**	5,988 ± 133 (3.8)
Long repeats	12,513 ± 965 (7.9)	12,884 ± 1,624 (8.1)
Unfractionated DNA	20,242 ± 4,106 (13)	18,863 ± 1,666 (12)

*Exactly 9 µg of DNA was fixed to 25-mm nitrocellulose filters (Schleicher & Schuell, Keene, NH, USA)[21]. The DNA content was checked by analysis[22]. Each filter was incubated with (^{3}H)-hnRNA at 65 °C (input, 158,900 dpm in 0.5 ml SC). After the specified incubation periods, the membranes were cooled, washed, treated with RNAse A and assayed for radioactivity[21]. **Mean ± SD (% input).

then, contained transcribed sequences that also occurred within the short repeats subfraction. The frequency of these transcribed sequences was significantly greater in the long repeats. The chances that hybridization of long repeats to hnRNA was due entirely to a few single-copy sequences that might be present in the DNA subfraction were, accordingly, diminished.

The findings in this study introduce the possibility that long reiterated sequences are transcribed into hnRNA. The value of the hypothesis is that it may account for some of the observed size differences between hnRNA and mRNA. If long reiterated sequences are transcribed at all, they must be transcribed in entirety, since introns and exons within a single transcription unit are physically continuous[2]. In this connection, it may be noted that the sizes of the longest repeats were only slightly less than that of the longest primary transcripts found in human cells, about 20,000 nucleotides long[23].

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Babesial antigens in a saline eluate of sucrose washed bovine erythrocytes infected with *Babesia bovis*

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Summary. A saline eluate from sucrose washed bovine erythrocytes infected with *Babesia bovis* contains at least 4 babesial antigens. The antigens are located not only in the parasite but also in the internal matrix of the infected erythrocyte.

Bovine erythrophylic proteins constitute a group of partly characterized proteins obtained by washing freshly collected bovine erythrocytes several times with an isotonic sucrose solution and then eluting bound proteins from the erythrocytes with physiological saline[1]. Both quantitative and qualitative changes occur to these proteins during acute infection of cattle with the intra-erythrocytic protozoan parasite *Babesia bovis*. Such changes contribute markedly to the pathophysiology of infection, namely the hypotensive shock syndrome and the sludging of infected erythrocytes within the microcirculation[2,3]. Although it seemed reasonable to assume that a saline eluate from infected erythrocytes might also contain babesial antigens, we were unable to detect any antigens in initial studies using established immunological assays such as hemagglutination and immunodiffusion. The present studies, however, demonstrate that the eluate does contain babesial antigens which may be detected by electrophoresis and nitrocellulose transfer and are located in situ by immunofluorescent staining.

Blood (9 vols) was collected into sterile 3.13% trisodium citrate (1 vol.) from cattle experimentally infected with *B. bovis* by blood passage from carrier animals infected with the Samford strain[4]. The blood, containing ~ 10% infected erythrocytes, was centrifuged at 250 × g for 10 min and the supernatant platelet-rich plasma removed. The blood was washed 3 times in 10 vols of 0.27 M sucrose in 0.0004 M phosphate buffer pH 7.2 at 1500 × g for 10 min at 4 °C. After each wash the top layers of residual leucocytes and platelets were removed. The sediment of erythrocytes was mixed with 10 vols of saline (0.85% aqueous NaCl) and centrifuged at 10,000 × g for 30 min. The supernatant saline eluate was concentrated by ultrafiltration, using a membrane of 10×10^3 exclusion, to the original volume of blood and stored at −20 °C until required. For control purposes, a similar preparation was obtained from pooled blood of five normal cattle.

A rabbit antiserum to the saline eluate was prepared by a series of i.m. and i.v. injections as described by Coombs and Gell[5]. The rabbit antiserum and a commercially prepared goat antirabbit IgG coupled to fluorescein isothiocyanate (FITC) were used to stain thin films of acetone-fixed *B. bovis* blood as described by Goodger[6]. Both infected and non-infected erythrocytes stained avidly. However absorption of the rabbit antiserum with a suspension of normal bovine lysate and stroma abolished the staining of the non-infected erythrocytes. Infected erythrocytes stained moderately while the parasite stained avidly (fig.). No staining occurred in films fixed in either methanol or