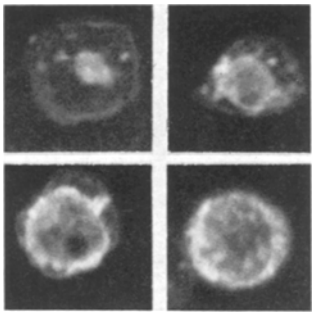

Fig. 2. Stages in the asexual cycle of *P. falciparum* from the small trophozoite (top left) to the schizont (bottom right). The cytoplasm of the parasite and 'stippling' in the cytoplasm of the infected erythrocyte are stained with the IF technique. × 2000.

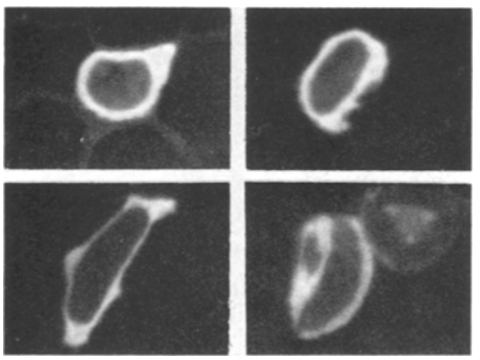

Fig. 3. Stages in the development of gametocytes of *P. falciparum* from the earlier stages (top left) to the mature gametocyte (bottom right). The parasites are unstained and the IF staining is restricted to the cytoplasm of infected erythrocytes. × 2000.

that the gametocytes stain brightly[1]. Since immature gametocytes are found in peripheral blood very rarely and then only in small numbers, we studied these forms in the parasitized haemorrhagic ascitic fluid of an unique patient[7]. The parasites were shown to be viable on in-vitro culture: furthermore the maturation of gametocytes in vivo was followed in samples of ascitic fluid removed at intervals to relieve abdominal distension[7]. It is therefore highly probable that our observations on the antigenicity of the immature gametocyte are valid, particularly since our findings in mature gametocytes in the ascitic fluid were confirmed by similar observations on scanty mature gametocytes in the peripheral blood of children convalescent from acute *P. falciparum* malaria. This antigenic difference, although previously unrecorded, is hardly surprizing in view of the other obvious changes in morphology and metabolism that the organism undergoes during gametogenesis[9]. This, or some similar antigenic difference, might explain the susceptibility of asexual parasites and the resistance of gametocytes in malarious children to passive transfer of malaria immunity by transfusion of γ globulin from hyperimmune adults[10].

The stippled IF staining of the cytoplasm of erythrocytes infected with asexual parasites has not yet been adequately explained, but it corresponds closely with the MAURER's clefts seen in ROMANOVSKY-stained preparations[4]. The cytoplasm of erythrocytes parasitized with *P. falciparum* gametocytes never contains MAURER's clefts. Single, much larger, elongated and somewhat tortuous clefts, known as 'GARNHAM's bodies' are seen on the concave side of gametocytes – these would appear to correspond to the areas lacking the IF antigen[11].

Résumé. Des études effectuées par l'immunofluorescence en utilisant les sérums de malades qui ont subi plusieurs infections de paludisme (*P. falciparum*) ont montré que l'antigène correspondant peut être détecté dans le cytoplasme de toutes les formes asexuelles du plasmodium, mais non dans les gamétocytes. Par contre, l'antigène est moins abondant dans le cytoplasme des globules rouges contenant les formes asexuelles que dans celui des cellules ayant des gamétocytes.

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The Effect of L-Asparaginase on the Synthesis and Processing of Ribosomal Precursor RNA in PHA-Stimulated Lymphocytes

The mechanism through which L-asparaginase affects growth of L-asparaginase-sensitive cells is still unclear. Amino acid incorporation into the microsomal fraction of 3 sensitive tumors is markedly inhibited[1] and this effect seems to be preceded by a pronounced inhibition of the incorporation of uridine into 18S and 28S ribosomal RNA subunits[2]. However, the relationship between the action of L-asparaginase and the decrease in ribosomal RNA synthesis awaits further explanation.

Evidence has been presented that the metabolic action of L-asparaginase is not limited to certain lymphoid tumors, but it is exerted on normal lymphoid cells as well[1,3]. Since phytohemagglutinin (PHA)-stimulated lymphocytes are characterized by a high rate of synthesis of ribosomal RNA[4], a group of experiments were performed in order to investigate the effect of L-asparaginase on macromolecular metabolism of ribosomal RNA in PHA-stimulated lymphocytes, and some of our results are here preliminarily reported.

Materials and methods. Heparinized blood from normal donors was sedimented by gravity at 37 °C in the presence of 0.6% dextran, and the supernatant, diluted with 1 volume of minimal essential medium (MEM), was filtered through a column of nylon fibers (Leukopak, Fenwal Lab.)

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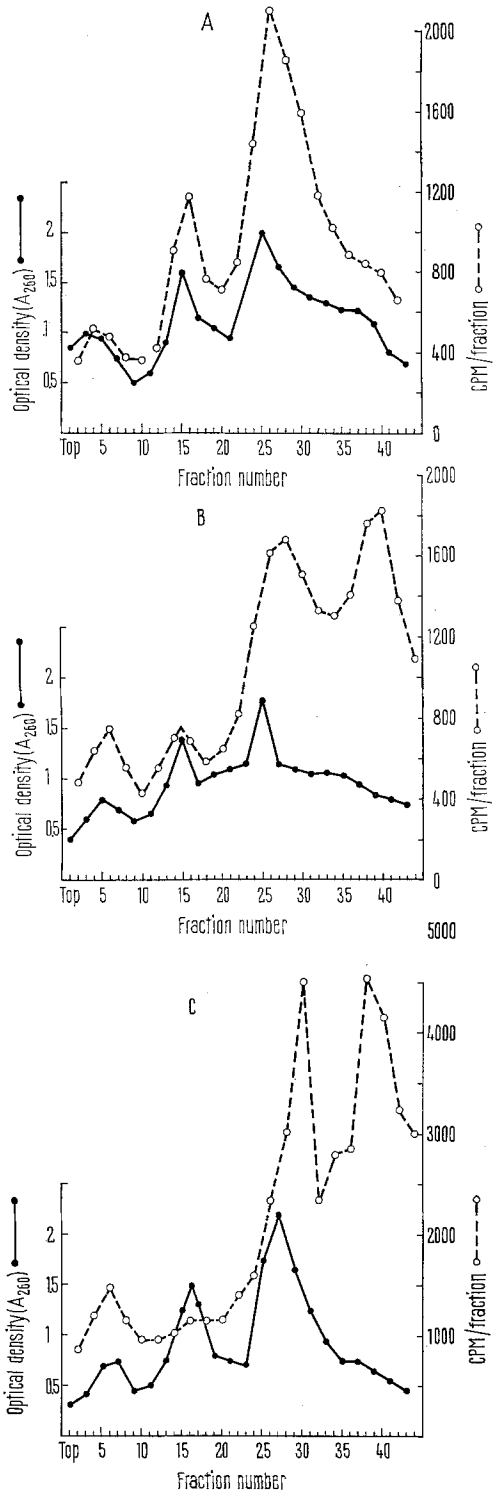

Fig. 1. Sedimentation patterns of labelled RNA from PHA-stimulated lymphocytes incubated with uridine-^{3}H (10 μc/ml for 3 h). A) Cells were incubated with the labelled precursor without any previous treatment. B) Cells were incubated with uridine-^{3}H after 30 min of treatment with L-asparaginase (5 U/ml). C) Cells were incubated with uridine-^{3}H following 60 min of treatment with L-asparaginase. Since in all sedimentation studies labelled lymphocyte RNA and unlabelled KB cell RNA were extracted simultaneously, the optical density profile in this and in the following figure represents mainly the unlabelled KB cell RNA. Different amounts of labelled RNA were put on each gradient. Gradients were centrifuged at 16,000g for 16 h at 4 °C.

to separate granulocytes. The final cell suspension, containing lymphocytes, platelets and a small number of erythrocytes, was centrifuged at 1000g for 10 min, and the cell pellet was diluted with MEM and 20% autologous plasma to a concentration of 4–6 × 10^6 lymphocytes per ml. 10 ml cultures were set up in 25 ml screw stoppered containers, and added with 0.2 ml of PHA solution (Borrough Wellcome). All operations were performed under sterile conditions.

All treatments were started following at least 24 h of PHA stimulation. In time course studies, uridine-5-^{3}H (Radiochemical Centre, specific activity 19 C/mM) and L-asparaginase (*E. coli* L-asparaginase, Worthington Bioch. Corp.) or uridine-^{3}H alone were added to the cul-

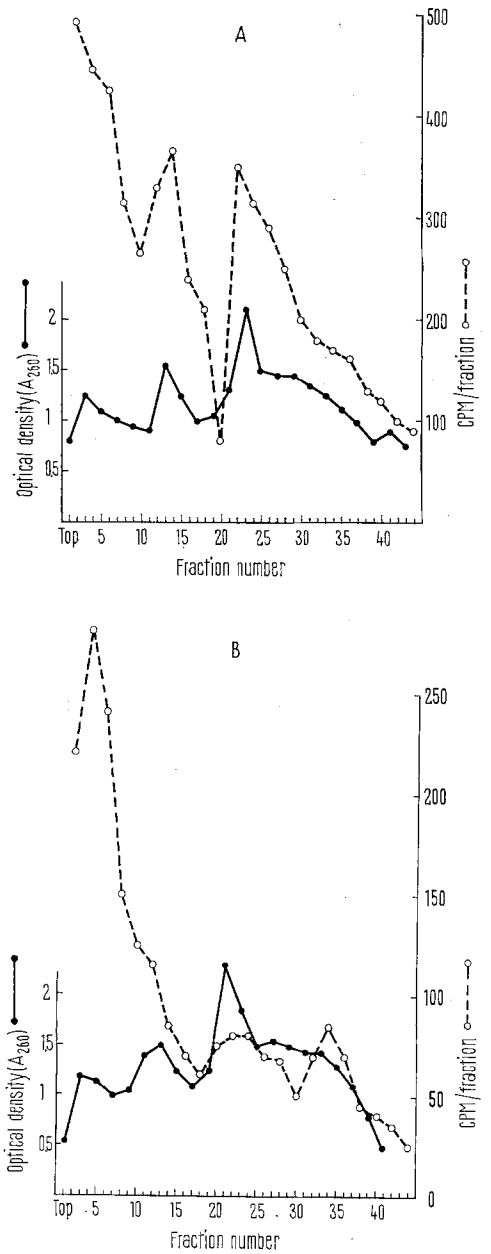

Fig. 2. Sedimentation patterns of labelled RNA from PHA-stimulated lymphocytes incubated with H^3-methyl-methionine (10 μc/ml) for 3 h. A) Cells were incubated with the labelled precursor without any previous treatment. B) Cells were incubated with H^3-methyl-methionine after 60 min of treatment with L-asparaginase (5 U/ml).

tures, at the concentration respectively of 10 μc and 5 units per ml. Duplicate 2 ml aliquots were taken at 30 min intervals. The cells were harvested by chilling on frozen saline and centrifugation at 1200*g* and washed once with cold saline.

For RNA sedimentation studies, L-asparaginase was added to the cultures at the concentration of 5 units/ml. At intervals varying from 30 min to 3 h, uridine-^{3}H was added to the cultures at the concentration of 10 μc/ml and 3 h later the cells were harvested by chilling on frozen saline and centrifugation. In all sedimentation studies, because of the low number of lymphocytes available for each experiment, unlabelled RNA from KB cells was used as carrier. These cells were grown in monolayer in Roux bottles with Eagle's medium supplemented with 5% horse serum and glutamine. $2–8 \times 10^7$ lymphocytes were extracted together with $1–2 \times 10^8$ KB cells in each experiment.

For kinetics experiments, RNA radioactivity was assayed by the modification of the SCHNEIDER[5] procedure already described[4].

For sucrose gradient analysis the RNA was extracted by the modification of the methods of SCHERRER and DARNELL[6] and KIRBY[7] already described[4]. Centrifugation was carried out for 16 h on 5–20% sucrose gradients in 0.01 *M tris* buffer, pH 7.4, with 0.1 *M* NaCl, at 16,000*g* in the SW 25.1 rotor of a Spinco L ultracentrifuge. 45 fractions were obtained with a gradient analyzer (ISCO). Radioactivity determinations were performed as previously described[4].

Results. Our time course experiments showed that the addition of L-asparaginase to the medium reduced, already after 30 min, the uptake of tritiated uridine in acid insoluble material. After 1 h of incubation the rate of RNA synthesis was about 50% of the controls, while from the second hour onward no further percent decrease was observed (Table).

Figure 1, A shows the distribution of radioactivity of whole cell RNA extracted from PHA-stimulated lymphocytes incubated with uridine-^{3}H for 3 h. 2 major peaks of radioactivity are evident in coincidence with the optical density peaks of the 2 ribosomal RNA subunits, 18S and 28S RNA.

A markedly different distribution of radioactivity in total cell RNA is observed when incubation with uridine-^{3}H is preceded by a 30 min period of treatment with L-asparaginase (Figure 1, B). A major peak of radioactivity appears at 45S and the peak in the middle region of the gradient becomes larger, constantly showing its highest point at 32S. 2 further minor peaks are observed in the 18S and 4S region of the gradient.

When the 3-h incubation with uridine-^{3}H was preceded by 1 h of treatment with L-asparaginase, the pattern was quite similar, except for the constant lack of the radioactive peak associated with 18S RNA (Figure 1, C).

Figure 2, A shows the sedimentation profile of radioactive RNA extracted from PHA-stimulated lymphocytes incubated with ^{3}H-methyl-methionine for 3 h. Definite labelling of the 2 ribosomal subunits is evident, besides the high level of radioactivity in the 4S region, presumably representing methylated soluble RNA and methioninyl-S-RNA. A fairly different pattern is obtained when 3 h of incubation with ^{3}H-methyl-methionine are preceded by a 1 h treatment with L-asparaginase (Figure 2, B). A major peak at 45S is observed, and the peak in the middle region of the gradient is lowered and enlarged. Furthermore, no definite radioactive peak is seen at 18S.

Discussion. The results of our kinetics studies show that uridine-^{3}H incorporation in PHA-stimulated lymphocytes is markedly inhibited by L-asparaginase, and confirm

Effect of L-asparaginase on incorporation of uridine-^{3}H into acid-insoluble material by human lymphocytes incubated with PHA for 24 h

Incubation with L-asparaginase and/or uridine-^{3}H (min)	CPM per 9×10^6 cells	
	Control	Enzyme-treated
30	184	125
60	420	245
90	770	335
120	1370	550
150	2010	850
180	3170	1360

Results of one experiment.

that this enzyme causes in normal lymphoid cells the same metabolic effect described in L-asparaginase sensitive tumors by STEVENS et al.[2]. However, the sedimentation analysis gives a further insight into the mechanism through which the metabolic effect of L-asparaginase is determined. In fact, our observations indicate that, following treatment with L-asparaginase, a relevant proportion of radioactivity is associated with the 45S RNA even after 3 h of incubation with the labelled precursor. This clearly indicates that the decrease in ribosomal RNA synthesis is paralleled by a decrease in the rate of cleavage of the 45S ribosomal RNA precursor. Inhibition of the synthesis of ribosomal RNA precursor has been observed in several conditions of reduced cell growth[8,9]. However, as for the mechanism through which L-asparaginase causes the metabolic changes described above, it must be pointed out that rapid fall of RNA synthesis, parallel to impaired processing of 45S RNA, has been observed in HeLa cells as a consequence of protein synthesis inhibition by cycloeximide[10].

It therefore seems conceivable that, because of the marked sensitivity of nucleolar function to inhibition of protein synthesis, one of the earliest effects of L-asparaginase in sensitive cells is the impairment in synthesis and processing of ribosomal precursor RNA.

Riassunto. Gli AA. hanno studiato l'effetto della L-asparaginasi sulla sintesi dell'ARN nei linfociti coltivati in vitro con PHA. L'enzima provoca una marcata riduzione della velocità di sintesi dell'ARN e l'analisi sedimentimetrica dell'ARN marcato suggerisce che l'enzima rallenta la velocità di elaborazione del «precursore» ribosomiale.

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