4 monoclonal antibodies make it possible to discriminate MT-1 and MT-2 of Cd^r-CHL and of mouse liver. Thus, these antibodies represent a set of reagents with high specificity for use in biochemical and biological studies of MTs.

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Side effects of *Ricinus* lectin (RCA 120) on nucleic acid synthesis in chick embryo fibroblasts

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Summary. When fibroblasts from chick embryos were treated with *Ricinus* lectin, the effects observed depended on the stage of development of the embryo from which the cells were prepared. Thus, in 16-day fibroblasts, which have a weak proliferative capacity, nucleic acid synthesis was less sensitive to the effect of this lectin than that in 8-day fibroblasts, whose proliferative capacity is high.

Ricinus lectin (RCA 120) has been found to have a toxic effect on protein synthesis[3-6]. Only a few reports, however, have described its side effects on nucleic acid synthesis[7,8]. We previously have shown in chick embryo fibroblasts that the toxic effect of *Ricinus* lectin, as estimated by leucine incorporation, was differential during the embryo development[9]; thus, cells from young embryos were less sensitive than those from older ones, and the younger cells proliferated faster. It was therefore of interest to investigate the possible differential effect of Ricinus lectin on nucleic acid synthesis.

The present study describes this lectin's effects on such synthesis in chick embryo fibroblasts from 2 stages of embryonic development.

Materials and methods. Cells. Fibroblasts were obtained from 8- and 16-day chick embryos as previously described[10]. *Ricinus* lectin, the tetrameric form of *Ricinus communis* lectin (mol.wt 120,000) was purified by the procedure of Nicolson and Blaustein[11]. The lectin solution was prepared at a final concentration of 100 µg/ml in 0.15 M NaCl and was added to cell cultures at final concentrations ranging from 0.001 to 10 µg/ml. As to cell cultures, primary monolayers were made in 16 mm diameter wells using 0.5 ml of Eagle's medium (MEM, Flow) supplemented with 1% glutamine, 1% antibiotics (penicillin and streptomycin) and 10% foetal calf serum. The initial seeding concentration was 10^6 cells/ml. Cultures were grown in humidified air with 5% CO_2. A sample of cells was counted in a hemocytometer. Each measurement refers only to viable cells and represents the mean of 4 samples. For estimating nucleic acid synthesis, cell cultures were incubated for 1 h at 37 °C with 0.5 µCi of tritiated thymidine or uridine per well (Radiochemical Centre Amersham, sp. act.: 26 and 25 Ci/mmole, respectively). The cells were then removed from the wells by trypsin treatment as previously described[12]. The labeled cellular material was then allowed to precipitate overnight in 2 ml of trichloroacetic acid (TCA) 10% (v/v). The precipitate was washed twice with 5% TCA (v/v), dissolved in 3 ml ACS (Searle, Amersham) and radioactivity was then measured using a liquid scintillation spectrometer (Intertechnique SL 30).

Results and discussion. The fibroblasts from 8- and 16-day embryos reached subconfluency after 48 and 96 h respectively. *Ricinus* lectin was added to subconfluent cultures and its effects were noted after various incubation times. The number of cells ($0H.95 \pm 0.07 \times 10^6$ cells/well) and cellular protein content (147 ± 25 µg/10^6 cells) estimated by the method of Lowry et al.[13], remained unchanged in both control and *Ricinus* lectin-treated cultures from older and younger embryos.

As figures 1,a and b show, the optimum concentration of *Ricinus* lectin was 1.5 µg/well in cells from both types of embryo. This concentration was therefore used to study the time course of the effect of lectin (table). After 2 h incubation with *Ricinus* lectin, ^{3}H-thymidine incorporation was inhibited by 64 and 34% in 8- and 16-day cells respectively. The same incorporation was totally inhibited after 4 h of incubation in 8-day cells, but only partly

Effects of *Ricinus* lectin on tritiated thymidine and uridine incorporation in fibroblasts from 8- and 16-day chick embryos. Experimental procedures are described in 'Materials and methods'. Each value is the mean of the values obtained from 4 separate experiments conducted with 5×10^7 to 10^8 cells

		cpm/10^6 cells Thymidine 8-day cells	16-day cells	Uridine 8-day cells	16-day cells
2 h	Control	103± 7	290±51	75±1	14±1
	Ricinus lectin	38± 1	190±21	40±3	10±1
	Ricinus lectin + lactose	107± 4	291± 5	73±8	14±1
4 h	Control	150±12	295±60	58±2	11±2
	Ricinus lectin	7± 1	150± 6	25±3	8±2
	Ricinus lectin + lactose	160± 1	263± 2	57±2	12±3
8 h	Control	140± 7	287±27	20±1	12±1
	Ricinus lectin	7± 1	132±24	9±2	7±1
	Ricinus lectin + lactose	150± 1	237± 6	21±1	10±2

inhibited in 16-day cells. After 8 h of incubation, no further effect on cell of either age was observed. ^{3}H-uridine incorporation was less sensitive to lectin in both cell types (table). The effect of *Ricinus* lectin were specific, because they were totally blocked by 0.1 M lactose (table).

Thus, the differential sensitivity to *Ricinus* lectin of nucleic acid synthesis might be related to the stage of embryo development, since the younger cells were the most affected. Further, RNA synthesis was less sensitive to lectin than DNA synthesis. These results should be compared with those reported by Kornfeld et al.[8] for mouse leukemic lymphoblasts.

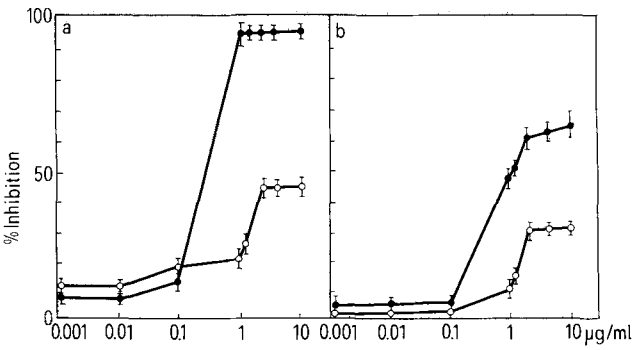

Figure 1. Dose-response of *Ricinus* lectin for the incorporation of ^{3}H-thymidine (a) and ^{3}H-uridine (b) in fibroblasts from 8-day (●) and 16-day (○) chick embryos after 4 h of incubation. Each point is the mean of the values for 4 separate experiments.

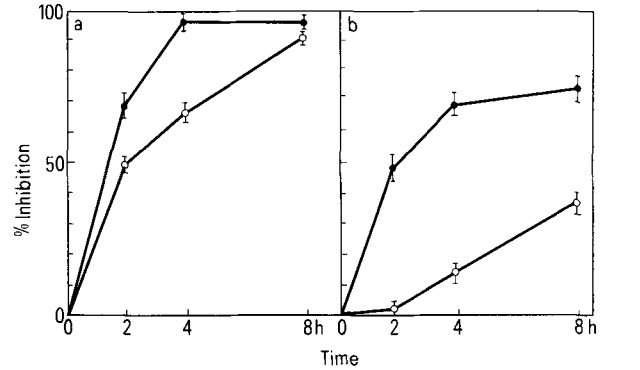

Figure 2. Effect of cycloheximide on the incorporation of ^{3}H-thymidine (●) and ^{3}H-uridine (○) in fibroblasts from 8-day (a) and 16-day (b) chick embryos. Each point is the mean of the values for 4 separate experiments.

As *Ricinus* lectin is known to be a potent inhibitor of protein synthesis in chick embryo fibroblasts[9], the reduction of DNA synthesis by this lectin observed have might be a result of this inhibition. Consequently, diminished protein synthesis may affect the turnover of key enzymes in the respective metabolisms of DNA and RNA. This hypothesis is supported by the fact that cycloheximide, a specific inhibitor of protein synthesis[14,15], also inhibited nucleic acid synthesis in embryo cells from both stages (fig. 2, a and b), the 8-day cells being the most sensitive. Alternatively, the differential inhibition of ^{3}H-thymidine incorporation might be due to the difference between the proliferative capacities of fibroblasts from young and old embryos[10]. Thus, 16-day fibroblasts, which exhibited a weak proliferative capacity, were less sensitive than 8-day fibroblasts to the inhibitory effect of *Ricinus* lectin. The differential inhibition of ^{3}H-uridine incorporation might be due to factor affecting RNA stability and turnover, which may change during embryo development[16]. To sum up, the differential effect of *Ricinus* lectin on nucleic acid synthesis in chick embryo fibroblasts seems to be related to the proliferative capacity of the cells, and thus to their state of differentiation.

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