

Fig. 2. Columnar epithelium of brain of 12-day-old embryos after treatment with 5-azacytidine. (a) Control. (b, c, d) 4, 8 and 12 h after administration of 5-azacytidine (4 mg/kg).

(Figure 2c) remnants of pycnotic nuclei and mitotic cells with irregular arrangements of chromosomes were still present in the same region. Similar picture, however, could be observed also in the mantle zone. At 12 h (Figure 2d) pycnotic nuclei accumulated in the marginal zone, whereas in the ependymal region normal dividing cells began to appear. At 24 h pycnotic nuclei diminished in size and were present prevalently in the marginal region.

The ability of low doses of 5-azacytidine (1.5–4 mg/kg) to destroy or damage embryonic tissue at doses non-toxic to pregnant mice ( $LD_{50}$  115 mg/kg<sup>8</sup>) led us to investigate its incorporation into embryonal organs. After the i.p. administration of 5-azacytidine-4-<sup>14</sup>C to pregnant mice (1  $\mu$ Ci/0.25  $\mu$ mol) the drug was incorporated in an amount of 0.49  $\mu$ mol and 0.14  $\mu$ mol/mg nucleic acids into foetal liver and central nervous system respectively. It is known that this compound is readily incorporated into different types of nucleic acids<sup>9,10</sup>.

**Zusammenfassung.** 5-Azacytidin wurde trächtigen Mäusen am 4.–6. und 11.–13. Tag verabreicht. Bei der ersten Gruppe zeigte sich eine völlige Resorption der Fetusse, während in der zweiten Gruppe hauptsächlich die Leber und das Nervengewebe geschädigt waren. In der ependymalen Zone werden die in Teilung befindlichen Zellen in der Metaphase gestoppt und unterliegen einer pyknotischen Degeneration.

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## Agglutination of Cultured Cells by Wheat Germ Lipase; Effect of Prednisolone

Increased electrophoretic mobility of malignant cells seems to be related to their invasiveness<sup>1</sup>. Since chemical groups susceptible to the action of neuraminidase constitute major charge determining components of the cell surface, elevated levels of sialic acids may be related to the neoplastic properties of such cells. This was shown experimentally by FORRESTER, AMBROSE and STOKER<sup>2</sup> in hamster kidney fibroblasts transformed with the oncogenic polyoma virus. Moreover, malignant cells contain specific sialic acid-rich agglutinogens which are reactive with agglutinins present in preparations from wheat-germ lipase<sup>3,4</sup>.

The effect of certain steroid hormones, particularly those of the adrenocorticoid group seems to be associated with changes in the surface properties of treated cells<sup>5,6</sup>, possibly involving sialoproteins. Recently CARUBELLI and GRIFFIN<sup>7</sup> have reported that hydrocortisone increases intracellular levels of sialic acid in HeLa cells. It seems that prednisolone stimulates synthesis of sialic acids and does not merely prevent the release of this compound into the culture medium.

In this communication we are presenting evidence to show that agglutinability of cultured cells by wheat germ lipase is increased by exposure to prednisolone during growth. The effect is abolished if prednisolone grown cells are treated with neuraminidase.

HeLa S3 and Henle embryonal intestinal lines were grown for 4 days in glass bottles in the presence of 0.5  $\mu$ g/ml of prednisolone. The cell monolayers were washed with balanced salt solution and the cells were scraped from glass with perforated cellophane strips. They were then

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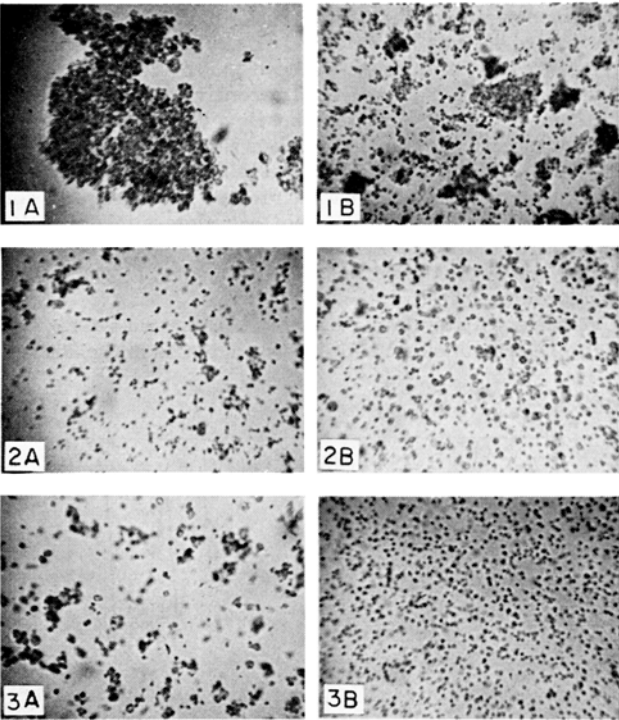
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Agglutination of prednisolone treated cells by wheat germ agglutinin

Agglutinin concentration (ml/ml cell suspension)	Henle		HeLa S3	
	Agglutination reaction			
	Prednisolone	Control	Prednisolone	Control
0.100	++++	+++	++++	+++
0.075	+++	+++	+++	++
0.050	+++	+++	+++	++
0.025	++	++	++	+
0.010	++	++	+	0
0.005	+	0	+	0
0.000	0	0	0	0



Effects of neuraminidase on the agglutination of HeLa S3 cells by wheat germ lipase. Photographs 1A, 2A and 3A cells grown in the presence of prednisolone; 1B, 2B and 3B cells grown without prednisolone; 1, wheat germ agglutination (0.025 ml/10<sup>6</sup> cell suspension), 2 same as 1 but pretreated with 5 units of neuraminidase, 3 control suspension.

suspended in saline to a density of 1 × 10<sup>6</sup> cells/ml. Wheat germ lipase purchased from Sigma Chemical Company was treated according to the procedure described by BURGER and GOLDBERG<sup>4</sup>. The homogenized enzyme preparation was heated at 63°C for 15 min and centrifuged. The clear supernate was used as the source of agglutinin. No further purification of the preparation was attempted. The agglutination assay was performed according to the procedure of AUB, TIESLAU and LANKESTER<sup>3</sup> and scored as described in their paper.

Results of one such experiment are shown in the Table. They indicate that pretreatment with prednisolone resulted in a qualitative and quantitative change in the agglutination pattern. In both cell lines agglutination in steroid treated cells was considerably stronger, moreover it was detectable at higher dilutions of the wheat germ agglutinin. In order to check the effect of neuraminidase on cell agglutination, the cells were treated before addition of agglutinin for 1 h at 37°C with purified neuraminidase from *Clostridium perfringens* (5 units/10<sup>6</sup> cells). As shown in the Figure the agglutination of both steroid-treated and control cells was abolished by the treatment with neuraminidase.

At the present time it is too early to speculate upon the significance of these results. As suggested by KRAEMER<sup>8</sup> the increased sialic acid content reflects an increase in the surface area of the cells which is also one of the results of steroid treatment. On the other hand, prednisolone could modify the structure of the cells in such a way as to expose more sialic acid groups to the action of agglutinin<sup>9</sup>.

*Zusammenfassung.* In menschlichen Zellkulturen mit Zugabe von Prednisolon wird die Agglutinierbarkeit der Zellen durch den Einfluss der Weizenkeimlipase gesteigert. Die Vorbehandlung mit Sialidase (N-Acetylneuraminidase) verhindert die Agglutination, was auf eine Hormonwirkung an der Zellmembran hinweist.

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Effect of Erythropoietin on Red Cell Differentiation Binding of Erythropoietin to DNA

It is now well established that erythropoietin is involved in red cell differentiation<sup>1</sup>. Although its mechanism of action is not fully understood, the outstanding work of KRANTZ et al.<sup>2</sup> suggests that its mode of action can be explained through the genetic activation hypothesis postulated by MONOD and JACOB<sup>3</sup>. Such a hypothesis implies, (1) identical structure of the DNA in all the cells from different tissues within an organism, (2) permanent repression of a given region of the DNA through the different stages of the development of such organism, and (3)

removal of the repressor in a particular cell by the differentiation factor or corepressor while the same region in the rest of the cells remains unaffected. In the case of

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