for the structure of the compound and it might be developed for the synthesis of new compounds of the same type and/or derivatives of them. In addition, it opens up a possible new approach to the biosynthesis of isosepiapterin and sepiapterin and the related naturally occurring compounds such as biopterin (to which sepiapterin can be converted by chemical means). We are at present investigating the possible biochemical significance of this synthetic pathway.

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Removal and Regeneration of the Cell Coating in Tumour Cells

SOME ascitic tumour cells are coated with a mucopolysaccharide stainable by the Hale reaction¹. Experiments were carried out to characterize more specifically the chemical nature of this cell coating and to study the dynamics of cell coat formation. Cells of ascitic mammary carcinoma T43 were used *in vitro*. Experiments were carried out to determine whether some proteases and mucinases remove the cell coating, and, after removal, regeneration of the cell coating occurs.

Ascites cells were washed with and resuspended in Eagle's medium spinner, unless calcium was needed for enzyme activity, and then incubated at 37° C, with slow stirring, in the presence of one of the following enzymes or mixture of enzymes: pancreatin, crystalline trypsin, crude papain, crystalline papain, crystalline pepsin, testis hyaluronidase, and receptor-destroying enzyme (Behringwerke, Ag., Marburg-Lahn). The enzyme concentration was 1 or 2 mg/ml. of culture medium, except in the experiment with the receptor-destroying enzyme, in which case 4 drops of the buffer-enzyme solution per ml. were used. After 30–60 min of incubation* cells were washed in fresh Eagle's medium. Cells exposed to trypsin were incubated for 5 min at 37° C or overnight at 4° C. Treated cells were fixed in Love solution², and sections or smears were stained with Hale³ or periodic acid-Schiff methods.

The cell coating, as stained with the Hale reagents, was removed partially or totally by pancreatin, trypsin, pepsin, and receptor-destroying enzyme. In contrast, crude papain, crystalline papain, and testis hyaluronidase were completely ineffective. Receptor-destroying enzyme had no action on the periodic acid-Schiff-positive component of the cell coat, and indeed this component may show even better after the enzymatic treatment. Trypsin, however, eliminated both the Hale and the periodic acid-Schiff positive material of the coat.

(Other experiments were performed to see if enzymes would remove the Hale-positive coating from fixed dead cells attached to a glass surface. Receptor-destroying enzyme was very effective in this respect after incubation of the cells with the enzyme for 5 h at 37° C. Pure neuramidase also very actively removed the cell coating. Pepsin and trypsin, in shorter incubation, were ineffective, and, in some instances, the coating stained more intensively with pepsin.)

To study the regeneration of the cell coating, washed T43 cells were treated with receptor-destroying enzyme. After being uncoated by this procedure, they were cultured in Leighton tubes (Eagle's medium plus 10 per cent bovine foetal serum). The cells were withdrawn 1, 2, 3, 4, 5, and 22 h after incubation (37° C), fixed in Love, and stained with Hale. It was observed, in these circumstances, that the cell coating reappeared in cells after a minimum of 1 h of incubation. However, the coats were less spiny than in the normal control cells.

The action of receptor-destroying enzyme and neuramidase on the cell coat indicates that sialic acid (neuraminic acid) is one of the components of the coating. This is in agreement with the indirect proofs provided by Weiss⁴. Since testis hyaluronidase is inactive, mucopolysaccharides attacked by this enzyme did not co-exist with sialic acid at the cell surface.

These experiments suggest that the Hale stain depends on the contiguity of the galactosamine-neuraminic acid bond at the cell surface. Neuramidase breaks this bond, causing a release of neuraminic acid, and then the Hale staining becomes negative. In contrast, the periodic acid-Schiff stain acts on galactosamine, as this stain is unaffected or even increased by previous neuramidase treatment.

The sialic acid must be bound also, directly or indirectly, to proteins because some proteases remove the Hale-positive coating from live cells *in vitro*. However, destruction of the coat does not occur when dead, fixed cells are exposed to proteases. Under these conditions, the Hale-positive coat may stain intensely, probably because masking proteins are removed⁵.

The experiment on cell-coating regeneration shows that T43 cells start to restore their coats of sialomucin within 1–2 h. It seems likely that cells acquire their sialomucin coat from products made in the Golgi zone⁶. The Golgi zone also has Hale-positive material, and this substance may be removed from sections by the receptor-destroying enzyme or sialidase.

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* It was longer for RDE: 2–4 h.

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