when fibrin lying on the surface of the synovial intima is apposed to the cell a dense filamentous coat may develop on the cytoplasmic aspect of the plasma membrane (figure 2). Such a phenomenon is at times a prelude to the formation of partially coated vacuoles by which synovial cells endocytose fibrin from the joint space[8].

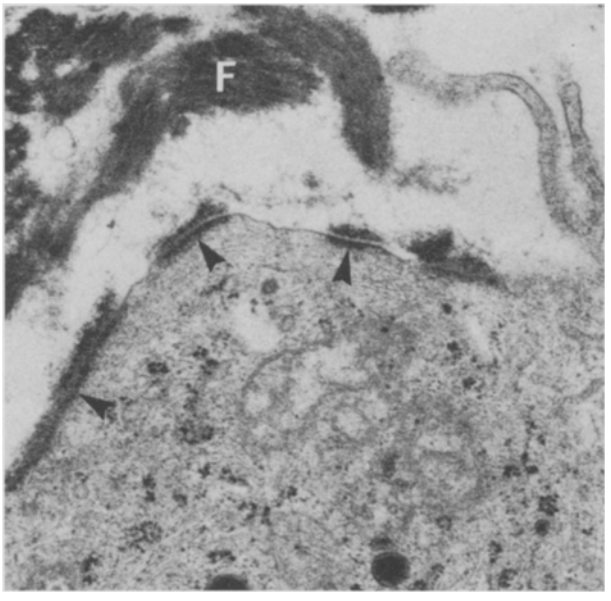


Fig. 2. Synovial membrane from a case of rheumatoid arthritis showing fibrin (F) deposits in the joint space, and focal plaques (arrowheads) that have developed on the surface of a synovial cell. The material forming the external coat of the plaque is similar in appearance to the fibrin in the joint space. Note also the dense coat on the cytoplasmic surface of the plasmamembrane and the lucid interval between the 2 dense structures comprising the plaque. ×29,000.

Yet another fact that supports our hypothesis is that haemorrhage and/or fibrin deposition occur in the pathological states (mentioned above) where desmosomes or desmosome-like structures have been found between human synovial intimal cells. It seems to us that if fibrin is trapped between 2 synovial cells and a filamentous coat develops (as in figure 2) on the cytoplasmic sides of the adjacent plasma membranes then an appearance minicking a desmosome will be created (as in figure 1).

It would appear that besides the synovial membrane there may be other tissues where also desmosome-like structures may develop in the manner described here. For example structures resembling intermediate junctions and desmosomes (remarkably similar to those seen in figure 1) have been reported in 3-methylcholanthrene-induced mouse sarcoma[9] and one may speculate that haemorrhage may have occurred in the tumour and led to the production of such structures.

Our thesis regarding the formation of desmosome-like structures in certain pathological synovial membranes of man leaves unexplained why desmosomes or desmosome-like structures occur in the normal synovial membrane of some animal species and not others. It seems to us that more than one mechanism may be involved and that if a close association of synovial cells occurs this may trigger some hitherto unknown mechanism(s) of junction formation.

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## Effects of exogenous thyroxine on the surface morphology of the developing chick anterior corneal epithelium[1]

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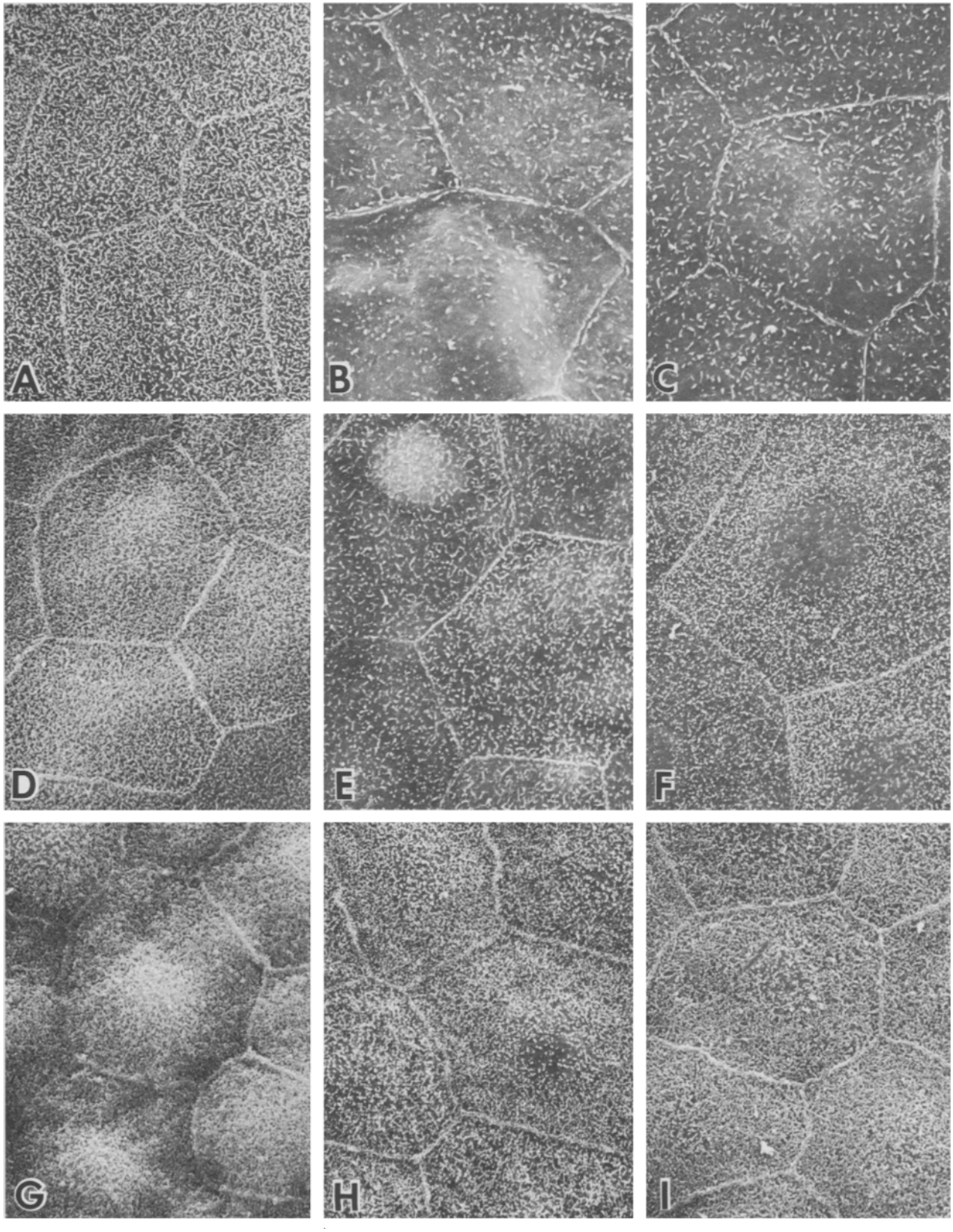
*Summary.* The production of microvilli on the developing chick anterior corneal epithelium is drastically accelerated by the administration of exogenous thyroxine and the effects are seen as early as 2 days after injection. Thiouracil administration slightly retards the production of microvilli though its effects are not so pronounced.

The cornea of the chick undergoes a dehydration and becomes transparent between the 14th and 19th day of incubation and it has been shown that these phenomena are under the influence of thyroxine[2,3]. It has also been shown that the administration of exogenous thyroxine to the chick at 10 or 12 days of incubation results in a decreased corneal thickness and a precocious development of intercellular complexes responsible for the barrier function of the epithelium and endothelium[4,5]. More recently scanning electron microscopy has shown that up to about the 15th day of incubation the anterior corneal epithelium is characterized by an ever increasing number of microvilli[6,7]. It was of interest, therefore, to see if this developmental parameter was influenced by exogenous thyroxine.

Fertile chicken eggs were incubated at 37.5 °C and 82% relative humidity in a circulating air incubator. Thyroxine (DL-thyroxine; Sigma) was dissolved in 0.1 N NaOH (prepared with 0.9% saline) and the pH was adjusted to 7.8–8.2 with 1 N HCl. Thiouracil (2-thiouracil; Sigma) was prepared as a suspension in 0.9% saline. After 9 days of incubation, a window was made through the shell and shell membranes and 1 µg thyroxine, 10 mg thiouracil, or an equal volume (0.5 ml) of the drug vehicle was injected directly onto the chorioallantois. Unoperated animals served as normal controls. The animals were sacrificed at 2, 4, or 6 days after drug treatment and the corneas were processed for scanning electron microscopy. The tissues were fixed according to the method of Hirsch and Fedor-




Scanning electron micrographs of the developing chick cornea (×3500). *A* 2 days after thyroxine treatment at 9 days incubation; *B* 2 days after thiouracil treatment at 9 days incubation; *C* 2 days after drug vehicle treatment at 9 days incubation; *D* 4 days after thyroxine treatment; *E* 4 days after thiouracil treatment; *F* 13 days normal; *G* 6 days after thyroxine treatment; *H* 6 days after thiouracil treatment; *I* 15-day normal.

ko[8], washed in buffer and dehydrated in ethanol. The dehydrated tissues were critical point dried using $CO_2$ as a transitional fluid. The dried corneas were mounted on aluminium stubs, coated with gold in a Hummer sputtering system and viewed in an ETEC autoscan scanning electron microscope.

The figure illustrates the results that were obtained. It can be seen, as early as 2 days after injection, that the thyroxine had a dramatic effect on the number of microvilli on the anterior corneal surface when compared to the normals or controls (normal and control groups were pooled because no differences could be detected). The precocious production of microvilli by the administration of exogenous thyroxine carries through the 4- and 6-day post-injection results. At the same time there is a slight decrease in the number of microvilli found on the anterior corneal surface of the thiouracil treated animals when compared to the control animals, indicating an inhibitory effect by this antithyroid agent.

The data from the thyroxine treated animals is in accordance with previous works[3-5] and the enhanced proliferation of microvilli is just as one would expect, if the density of microvilli is a true developmental parameter of the cornea epithelial cells. However, the data from the thiouracil treated animals did not give the clear results found when other parameters of corneal development were measured[3-5]. This could be the result of too low a dosage or the low solubility of the thiouracil.

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## Tissue distribution and nucleic acid binding of chlorambucil-$^3$H in tumor-bearing rats

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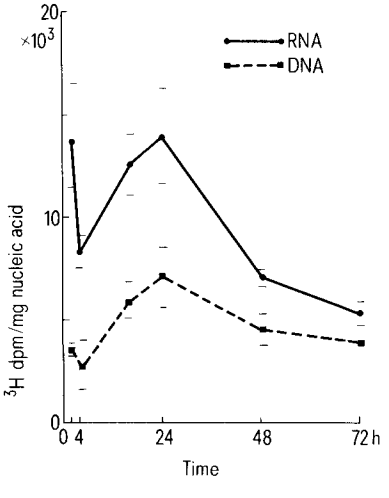
*Summary.* In tumour and normal rat tissues prolonged alkylation of DNA and RNA by chlorambucil-$^3$H occurs over periods of 24 h. It is suggested that this may indicate the slow release of an alkylating moiety from an intracellular drug-macromolecule complex.

The mechanism of action of chlorambucil is incompletely understood and its cytotoxic effects have been ascribed to both nucleic acid crosslinking[2-4] and covalent binding to nuclear protein[5]. The aim of the studies reported here was to compare the tissue distribution and nucleic acid alkylation patterns produced by chlorambucil-$^3$H with those previously reported for cyclophosphamide-$^{32}$P in the same animal system[6,7].

*Materials and methods.* The animals used were 5-6-month-old inbred female August rats bearing s.c. implants of the nonmetastasizing A-15 tumour, derived originally from a spontaneous anaplastic renal carcinoma. Animals were treated 10 days after transplantation when the tumour weighed approximately 1 g. Chlorambucil-$^3$H (4-4-di-(2-chloroethyl)-amino-3,5-$^3$H-phenyl butyric acid) (586 mCi/mmole) was supplied by Dr M. Jarman. The compound was dissolved in ethanol containing 1% HCl and diluted with propylene glycol and 0.5 cm$^3$ solution (100 µCi $^3$H) was administered i.p. to ether anaesthetised rats at a total dose of 12 mg chlorambucil/kg b.wt. Tissue samples were prepared and analysed as described previously[7].

*Results and discussion.* In the tumour, liver, spleen, kidney and jejunal mucosa the concentration of $^3$H from chlorambucil was greatest at 2 h post injection when the relative concentrations in these tissues were 1; 4.2, 0.9; 4.6 and 15.0 respectively. All these tissues showed a similar multicomponent clearance pattern over 72 h, with initial half-times of less than 2 h and final compartments, containing 25-50% of the 2-h $^3$H-concentration, with half times about 60-70 h. There was no evidence of selective tumour uptake or retention of the drug. The time course of the association of $^3$H from chlorambucil with DNA and RNA in the tumours is shown in the figure. This association, which survives the hot perchloric acid extraction in the Schmidt Thannhauser procedure, is considered to represent chemical binding of an alkylating $^3$H-moiety derived from chlorambucil to the nucleic acid bases[8,9], since direct incorporation of $^3$H released from drug metabolites into DNA is unlikely. A similar pattern of association occurred in liver, spleen and kidney, and in all the tissues studied the RNA-associated $^3$H was greater than that of DNA. The pattern




The time course of nucleic acid alkylation in the A.15 rat tumour following administration of chlorambucil-$^3$H (100 µCi) by i.p. injection at a dose of 12 mg/kg b.wt. Each point represents the mean of at least 4 animals ±1 SD. Abscissa represents time after drug administration and the ordinate drug-nucleic acid association in dpm/mg DNA or RNA.