

to the 2 major toxins of this mushroom: the  $\alpha$ -amanitine and the phalloidine.

In a recent paper, which we have seen after our experiments were performed, IKEKAWA et al.<sup>5</sup> were able to show an antitumour activity of an aqueous extract of edible mushrooms. The active antitumoral material was suggested by chemical analysis to be a polysaccharide. At this moment we do not know if the antitumoral fraction of the *Amanita phalloides* is also a polysaccharide.

**Riassunto.** L'estratto totale acquoso di *Amanita phalloides* Fr., se somministrato in dose non letale, inibisce il trapianto del tumore ascite di Yoshida AH130 nel ratto. Tale attività non dipende nè dalla  $\alpha$ -amanitina

nè dalla falloidina. I ratti così trattati sono immunizzati contro altre inoculazioni di ascite. L'attività inibitoria della *Amanita phalloides* è confermata con ricerche in vitro.

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<sup>5</sup> T. IKEKAWA, N. UEHARA, Y. MAEDA, M. NAKANISHI and F. FUKUOKA, Cancer Res. 29, 734 (1969).

### Comparative Study by Scanning Electron Microscopy of Synovial Surfaces of Four Mammalian Species

Recent evidence<sup>1</sup> obtained by scanning electron microscopy has shown that the adipose and areolar synovial tissue of man displays a surface pattern in which large numbers of blunt processes of approximately 50  $\mu$ m diameter cover the macroscopic synovial villi (Figure 1). The shape of these processes, which resemble the tips of a cluster of fingers or the *rubus idaeus* surface, is apparently determined by the underlying fat cells; and dissected adipose tissue (Figure 6) displays a pattern which closely resembles that of the synovial tissue. By contrast, the surfaces of the articular hyaline and fibrocartilages of synovial joints in man and other mammals are covered by numerous shallow pits of approximately 20–40  $\mu$ m diameter<sup>2–8</sup>.

The evidence presented in this paper demonstrates that a surface pattern of blunt synovial processes similar to that identified in man can be seen in at least 3 other mammals: pig, rabbit and rat. The pattern is therefore

presumed to represent a relationship between structure and function common to most mammalian synovial joints.

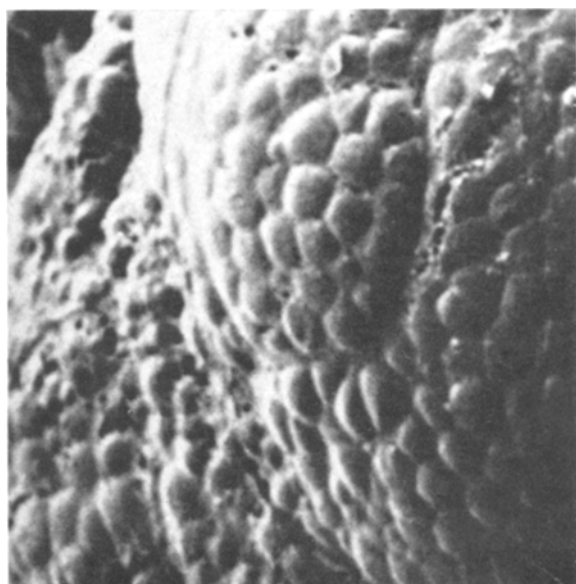


Fig. 1. Human synovial tissue. Broad convex surfaces of synovial villi covered by flat, finger-tip processes of pentagonal shape each measuring approximately 50  $\mu$ m in diameter. Compare with Figures 3, 4 and 5.  $\times 140$ .

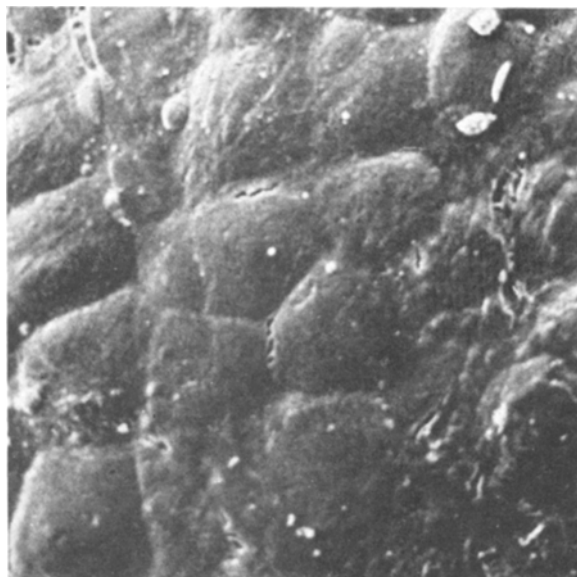


Fig. 2. Human synovial tissue. Field from same specimen seen at higher magnification. Occasional, discrete cells, possibly lymphocytes, seen on synovial surface at upper right.  $\times 1020$ .

<sup>1</sup> A. GRYFE, D. H. WOODWARD and D. L. GARDNER, Lancet, in 2, 156 (1969).

<sup>2</sup> P. S. WALKER, D. DOWSON, M. D. LONGFIELD and V. WRIGHT, Ann. rheum. Dis. 27, 512 (1968); (Proc. Heberden Society, Nov. 1968).

<sup>3</sup> D. L. GARDNER and D. H. WOODWARD, Ann. rheum. Dis., 28, 470 (1969); (Proc. Heberden Society, Nov. 1969).

<sup>4</sup> J. G. McCALL, Lancet 2, 1194 (1968).

<sup>5</sup> D. L. GARDNER and D. H. WOODWARD, Lancet 2, 1264 (1968).

<sup>6</sup> P. S. WALKER, J. SIKORSKI, D. DOWSON, M. D. LONGFIELD, Ann. rheum. Dis. 28, 1 (1969).

<sup>7</sup> D. L. GARDNER and D. H. WOODWARD, Ann. rheum. Dis., 28, 379 (1969).

<sup>8</sup> D. L. GARDNER, A. GRYFE and D. H. WOODWARD, in preparation (1969).

**Methods.** Hip and knee joints from freshly killed rabbits and rats were opened and dissected with the aid of a binocular light microscope. Material from pig joints was obtained 6–12 h after slaughter. The exposed synovial surfaces were identified by incident light microscopy, orientated, removed from the joints and plunged into ice-cold buffered osmium tetroxide at pH 7.3. After fixation, the specimens, which varied in size from 4 to 5 mm<sup>3</sup> to approximately 8–9 mm<sup>3</sup>, were dehydrated by progression through graded strengths of alcohol, coated with carbon and with gold-palladium alloy and examined in a Cambridge Instrument Company 'Stereoscan' scanning electron microscope at magnifications ranging from  $\times 20$  to  $\times 10,000$ .

**Results.** Clearly defined patterns of blunt processes were recognized on the synovial villous surfaces of the knee joints of pig, rabbit and rat (Figures 3–5). The processes ranged in diameter from 50–60  $\mu\text{m}$  in the freshly preserved rabbit and rat tissue, to 110–125  $\mu\text{m}$  in the pig. Under incident light microscopy an impression was gained that small vascular channels ran in an orderly manner between the prominences at the base of the shallow channels which demonstrated their outlines. A superficial resemblance to the exposed, dissected surface of adipose tissue (Figure 6) was noted. The synovial tissue examined was mainly of adipose type and underlying fat presumably influenced the surface pattern. The synovial processes closely resembled those recognized in

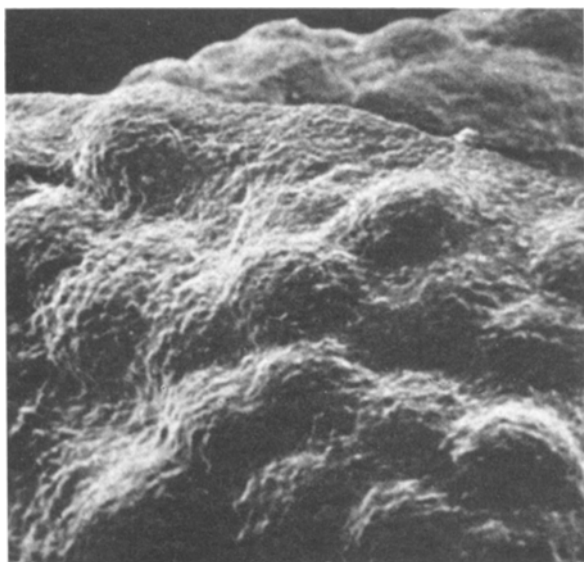


Fig. 3. Pig synovial tissue. Regular pattern of prominences of finger-tip shape shown on surface. Tissue, unlike human (Figures 1, 2) was not collected immediately at death and finer pattern of irregularities may be artefactual.  $\times 170$ .

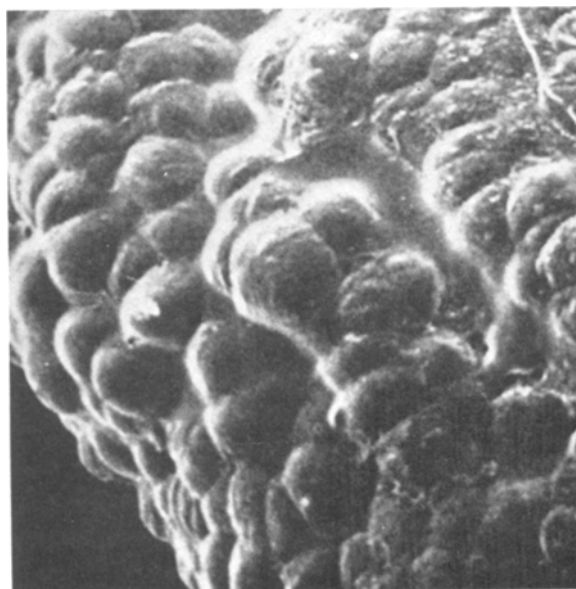


Fig. 5. Rat synovial tissue. General pattern of processes on synovial surface is extremely similar to that found in man (Figures 1, 2), pig (Figures 3, 4) and rabbit.  $\times 215$ .

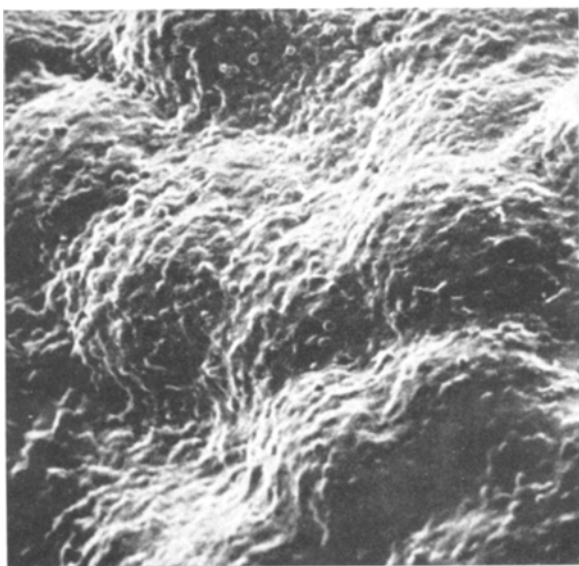


Fig. 4. Pig synovial tissue. Higher power view of tissue displayed in Figure 3. Many dimpled pits, resembling red blood cells, mark surface of shrunken synovium.  $\times 260$ .

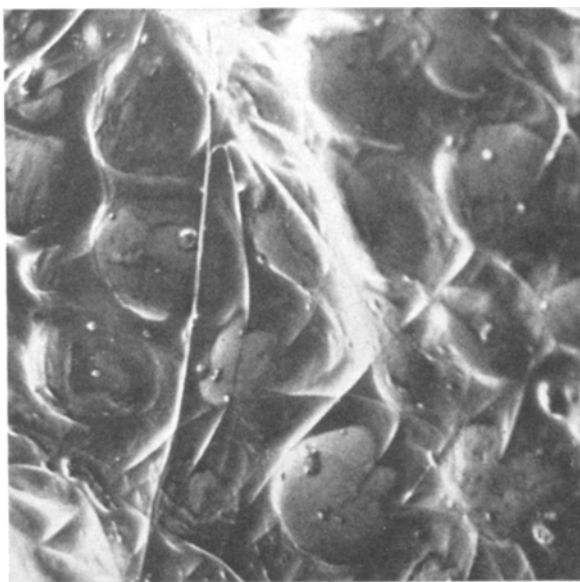


Fig. 6. Rabbit adipose tissue. For comparison with synovial surface, to demonstrate that main factor responsible for surface pattern of synovial finger-tip processes could be arrangement of underlying fatty tissue.  $\times 110$ .

comparable situations in the human knee joint<sup>1</sup>. Superimposed on this pattern were scattered particles of cellular debris, occasional intact red blood cells, the presence of which served to confirm that fixation and drying had not caused excess artefactual distortion, and irregular aggregates of what was assumed to be denatured and depolymerized synovial fluid.

*Discussion.* The synthetic, secretory and phagocytic activities of synovial cells are well-known<sup>9</sup>. The secretion of synovial fluid, the release from synovial blood vessels of the metabolites essential for the survival of the avascular articular cartilage and the need to adapt spatially to changes in the internal surface structure of the joint during movement are believed to be 3 of the main reasons why the adipose and areolar synovial surfaces examined are disposed in coarse folds covered with the fine, blunt villi described in this paper. Previous investigations have shown that the main articular cartilaginous surfaces of man, pig, guinea-pig, rat and rabbit are all covered quite uniformly by shallow hollows which are believed to play an important role in retaining lubricating synovial fluid in pools during joint movement<sup>6</sup>. It now appears that the synovial joints of

these animals share a surface arrangement of the synovial processes<sup>10</sup>.

*Zusammenfassung.* Die Oberflächen der Gelenke von Mensch, Kaninchen, Schwein und Ratte zeigen elektronenmikroskopisch feine zottenartige Bildungen. Daneben finden sich auch leichte Vertiefungen. Diese Bildungen sind für eine gleichmässige Gelenkbewegung von Wichtigkeit.

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<sup>9</sup> D. V. DAVIES, in *Textbook of the Rheumatic Diseases* (Ed. W. S. C. COPEMAN; E. and S. Livingstone, Edinburgh 1969), p. 56.

<sup>10</sup> The facilities for this study were provided by Dr. B. V. KINGSLEY SMITH of the Department of Microbiology, St. George's Hospital Medical School, to whom we are deeply indebted for his courteous help. The work described in this report was supported by the Arthritis and Rheumatism Council for Research.

## Further Observations on the Mode of Action of Chloramphenicol on the Chick Morphogenesis

In our earlier studies<sup>1</sup> on the reversal of the inhibitory influence of chloramphenicol on the morphogenesis of chick embryo, it was observed that certain aromatic substances bring about the reversal. In continuation of these observations and to probe further into the mechanism of action of chloramphenicol, the possible reversal of the arrested morphogenesis of chick embryo, with additional substances was investigated.

*Materials and methods.* Fertilized eggs of white leghorn hens were incubated at 37.5°C to obtain a definitive primitive streak stage as in the previous work. Routine precautions of sterilization of the glassware and autoclaving of the solutions used in the culturing of the embryos were taken. The procedure as described previously<sup>2</sup> was followed for explanting the chick embryos, and the same concentration of chloramphenicol (0.2 mg/ml) was used. After 6 h of incubation with chloramphenicol, the embryos were washed free of this substance and

subsequently treated with Pannet Compton Saline (PC Saline), mounted and further incubated for 20–22 h. These served as controls.

Embryoes subsequently treated with the following chemicals served as experimental subjects: (1) Acetyl salicylic acid; (2) *para*-amino-salicylic acid; (3) *para*-hydroxy-benzoic acid; (4) 2-methyl-1,4-naphtha quinone and (5) DL- $\alpha$ -tocopherol (vitamin E). With the exception of the last chemical, all the chemicals had a concentration of 0.3 mg/ml. The last chemical, DL- $\alpha$ -tocopherol had a concentration of 0.45 mg/ml.

<sup>1</sup> LEELA MULHERKAR, P. N. JOSHI and B. A. DIWAN, *Experientia* 23, 901 (1967).

<sup>2</sup> D. A. T. NEW, *J. Embryol. exp. Morph.* 3, 326 (1955).

Percentage abnormalities caused by chloramphenicol and their subsequent reversal with chemicals

Set No.	No. of embryos treated with chloramphenicol	% of abnormal embryos	No. of chloramphenicol-treated embryos subsequently treated with:	% of embryos showing normal development (reversal effect)
1	26	80	Acetyl salicylic acid – 28	82
2	21	80	<i>Para</i> -amino-salicylic acid – 19	16
3	24	100	<i>Para</i> -hydroxy-benzoic acid – 24	91.6
4	24	79.1	2-methyl-1,4-naphtha-quinone – 22	86
5	24	100	DL- $\alpha$ -tocopherol – 23	78.7