

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.

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## 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



Fig. 1. Entire chick embryo subsequently treated with *p*-aminosalicylic acid not showing reversal.  $\times 24$ .



Fig. 2. Entire chick embryo subsequently treated with acetyl salicylic acid showing reversal to normal development; also shown by subsequent treatment with the remaining chemicals.  $\times 24$ .

**Results and discussion.** It is evident from the Table as well as the Figures that, except for *p*-amino-salicylic acid (Figure 1), all the other 4 substances tested, namely acetyl salicylic acid, *para*-hydroxy-benzoic acid, 2-methyl-1,4-naphtha quinone and DL- $\alpha$ -tocopherol, have effected reversal (Figure 2). Earlier observations regarding

the influence of phenyl lactic acid and alanine were also confirmed.

The reversal by *p*-hydroxy-benzoic acid and acetyl salicylic acid taken together with the earlier observations with phenylalanine, tyrosine, *O*-amino-benzoic acid and phenyl lactic acid, and the failure to effect reversal by *p*-amino-salicylic acid and *p*-amino-benzoate, suggest that a reversal by aromatic organic acid is possible provided *para* position is either free or hydroxylated.

It is likely that a substance derivable either from phenylalanine, tyrosine, *ortho*-amino-benzoic acid, *p*-hydroxy-benzoic acid or salicylic acid is the actual metabolite effective in the reversal of arrested morphogenesis.

2-methyl-1,4-naphtha quinone, a substance related to vitamin K, and DL- $\alpha$ -tocopherol (vitamin E), act in a comparable manner. The consideration of structural features of 2-methyl-1,4-naphtha quinone and vitamin E may give rise, either directly or indirectly, to a metabolite comparable to the one obtainable from effective aromatic compounds studied.

Chloramphenicol is known to inhibit protein biosynthesis either by inhibiting transfer of amino acids from S-RNA to ribosomes<sup>3</sup> and/or by interference with uridine coding at m-RNA level<sup>4,5</sup>. The toxic and/or chemotherapeutic effects of chloramphenicol may be on account of this influence.

In the present system, the reversal has however been effected by substances like acetyl salicylic acid, *p*-hydroxy-benzoic acid, 2-methyl-1,4-naphtha quinone and vitamin E which are not associated, at any rate directly, with amino acid or protein metabolism. As suggested in the earlier paper, the observed reversal by several substances is therefore unlikely to be on account of their influence on amino acid and/or protein metabolism.

Effective moiety appears to be one containing hydroxy aromatic residue and seems to be unrelated to amino acids or protein metabolism. This material is likely to serve as a hormone or inducer and triggers the process of differentiation. Di-hydroxy-phenyl alanine and several other dihydroxy aromatic substances like adrenaline, serotonin, etc., are known to act as hormones and take part in embryogenesis<sup>6</sup>.

The observations reported here show a distinct influence of 2-methyl-1,4-naphtha quinone, a substance related to vitamin K and vitamin E, which seems unrelated to their known role as vitamins.

**Zusammenfassung.** Acetylsalicylsäure, *p*-Hydroxybenzoesäure, 2-Methyl-1,4-Naphthachinon und Vitamin E heben die in einem Hühnerembryo durch Chloramphenicol bewirkte Hemmung der Morphogenese auf, während *p*-Aminobenzoessäure und *p*-Aminosalicylsäure keinen Einfluss auf diese Hemmung haben.

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<sup>5</sup> O. JARDETSKY and G. R. JULIAN, *Nature* **201**, 397 (1964).

<sup>6</sup> G. A. BUZNIKOV, I. V. CHUDAKOVA, L. V. BERDYSHNEVA and N. M. VYAZIMINA, *J. Embryol. exp. Morph.* **20**, 119 (1968).