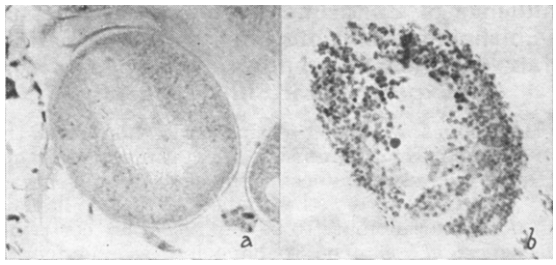

Fig. 2. Section through the Malpighian tubes of *Culex pipiens* treated with phosphorus-32. (*a*) 4th instar larvæ; (*b*) three-day old pupæ. Toluidine-blue stain

comparable with the granules in the Malpighian tubes of blowflies, which were analysed in detail by Waterhouse[3].

The phosphorus-32 reaches the Malpighian tubes by way of the blood. This can be concluded from experiments on animals in which the gut has ceased to show radioactivity. In spite of this absence of radioactivity in the gut, accumulation of phosphorus-32 occurs in the Malpighian cells; hence the phosphorus-32 could not have originated from the gut content, but must have been taken up from the blood.

Table 1. AMOUNTS OF PHOSPHORUS-32 (AS A PERCENTAGE) WHICH CAN BE EXTRACTED FROM ENTIRE ANIMALS (*Culex pipiens*) BY VARIOUS SOLVENTS

The animals lived in a solution containing phosphorus-32 for 24 hr. and in normal tap water for 24 hr. before the extractions were made

	Alcohol-ether	Perchloric acid (2%)	Perchloric acid (10%)	Residue
Early 4th instar larvæ	35·4	22·4	28·8	13·4
Late 4th instar larvæ	29·4	41·1	24·1	5·4
Young pupæ	25·3	46·1	22·2	6·4
Old pupæ	24·7	45·3	21·6	8·4

The amount of acid-soluble phosphorus-32 in the animals increases during metamorphosis (Table 1). This is in accordance with the quantitative determination of phosphate[4]. We have shown (unpublished experiments) that it originates from the histolysed organs (especially the gut) in which the synthesis of phosphorus-containing compounds rapidly declined and the breakdown of these substances was accelerated. It seems that this increased level of inorganic phosphates in the blood is partially compensated for by the Malpighian tubes, which take up and store the phosphate. The conclusion is justified that the Malpighian tubes are active in the regulation of phosphate balance in the animal, and take part in the processes of histolysis and histogenesis.

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Synthesis of (L) α-2,4-Dinitrophenylhistidine

THE reaction of histidine with 1 : 2 : 4-fluorodinitrobenzene (FDNB) could yield a di-substituted histidine and two mono-substituted histidines. Didinitrophenylhistidine has been prepared in pure form[1–4], and although the isolation of a monodinitrophenylhistidine has been claimed[1], neither physical constants nor proof of structure were given. Later workers have not been able to prepare either of the mono-substituted compounds in pure form[4,5]. Efforts to synthesize α-dinitrophenylhistidine were recently reported to have resulted in mixtures containing equal amounts of imidazole dinitrophenylhistidine and α-dinitrophenylhistidine[5].

The present synthesis of α-dinitrophenylhistidine was conceived as a result of chromatographic analysis (Fig. 1) of the products formed in the reaction between histidine and 1 : 2 : 4-fluorodinitrobenzene when the ratio of reagent to amino-acid was varied within wide limits. Examination of the chromatogram suggested that, with 0·5 mole of the fluorodinitrobenzene and 1·0 mole of histidine, only a mono-substituted compound was formed. With larger amounts of reagent this was transformed to the di-substituted compound; the transformation was complete when more than two moles of the reagent were present. The spot obtained with the lower concentration of fluorodinitrobenzene was ninhydrin-negative and reacted strongly positive to the Pauly diazo test. This indicated that the product was α-dinitrophenylhistidine, and suggested reaction conditions for its preparation.

Pure α-dinitrophenylhistidine was prepared as follows: 1·917 gm. (0·01 mole) of L-histidine monohydrochloride and 8·4 gm. of sodium bicarbonate were dissolved in 200 ml. of water, and 0·453 gm. (0·0025 mole) of 1 : 2 : 4-fluorodinitrobenzene in 25 ml. of ethanol was added with stirring. After 1 hr. at room temperature, the volume was reduced *in vacuo* to 50 ml., the *p*H adjusted to 6·5 by

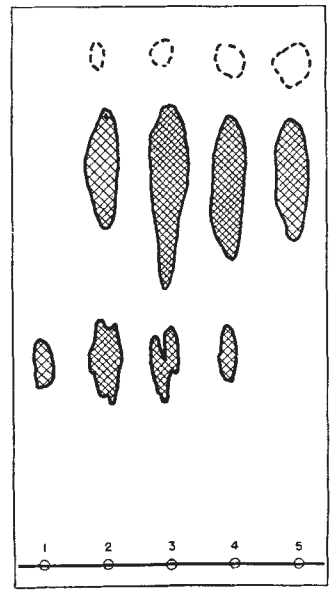

Fig. 1. Chromatogram of the yellow products resulting from the dinitrophenylation of 1 mole histidine for 3 hr. at room temperature with the following amounts of 1 : 2 : 4-fluorodinitrobenzene: (1) 0·5 mole; (2) 1·0 mole; (3) 1·5 mole; (4) 2·0 mole; (5) 2·5 mole

cautious addition of concentrated hydrochloric acid and the solution chilled to 5–10° C. The precipitate was removed by filtration and recrystallized from a minimum volume of dilute alcohol in water. The shiny yellow crystals obtained after 18 hr. in the cold were collected and dried *in vacuo* at 60° C.

Yield: 0·15 gm.
Melting point (uncorr.): 278–280° C. (decomp.).
Specific rotation: $[\alpha]_D^{24}$ − 100·9° (0·154 per cent, 4 *N* hydrochloric acid).
Analysis: found, C, 44·94; H, 3·405; N, 21·59 per cent; calculated for $C_{12}H_{11}O_4N_5$, C, 44·86; H, 3·45; N, 21·79 per cent.

The compound is ninhydrin-negative, indicating substitution of the α-amino group. The Pauly test[6] for the unsubstituted imidazole ring is strongly positive and, mole for mole, is of approximately the same intensity as given by histidine (Fig. 2). The absorption maximum for α-dinitrophenylhistidine in 92 per cent acetic acid is at 342·0 mμ, while that for the disubstituted compound is at 352·0 mμ. The material gives a single spot on paper chromatograms, with R_F = 0·32 (in water-saturated butanol) and 0·43 (in butanol/formic acid/water; 75 : 15 : 10).

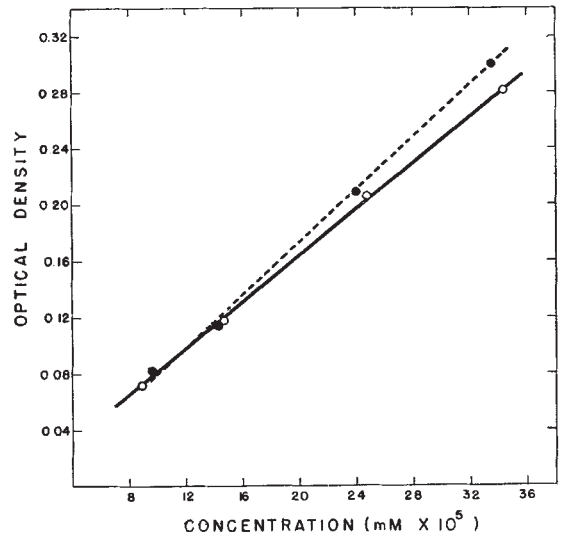

Fig. 2. Intensity of Pauly test for histidine and α-dinitrophenylhistidine. O—O, Histidine; ●—●—, α-dinitrophenylhistidine

We are indebted to Mr. J. A. Baignee for the carbon and hydrogen determinations.

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Influence of Cortisone on Connective Tissue—Epithelial Relations in Wound Healing, Hair Regeneration and the Pathogenesis of Experimental Skin Cancers

Most investigators agree that cortisone retards the development and diminishes the ultimate incidence of experimentally induced skin cancers[1]. Boutwell and Rusch[2] reported the induction of papillomata but not of carcinomata to be diminished by cortisone. Piccagli *et al.*[3] claimed to have increased induction of tumours with methylcholanthrene by cortisone. These differences in opinion may perhaps be attributable to variations in dosage, route and time of administration of cortisone relative to the application of carcinogens. This seems likely, since Baker[4], and we too, have shown that cortisone suppresses hair growth, while Andreasen and Engelbreth-Holm[5] demonstrated that tumour incidence depends on the phase of the hair-cycle at the time of application of the carcinogen.

We have studied the effects of cortisone on the histogenesis of wound healing in rabbits and on hair regeneration after plucking, and the pathogenesis of tumours in mice treated with methylcholanthrene, special attention being devoted to epithelial – connective tissue relations. We have found, *inter alia*, that cortisone affects wound healing in rabbits primarily by depressing the activity of the connective tissue. Whereas, both in rabbits and in mice, cortisone produces marked thinning of both dermis and epidermis, wounding in rabbits and plucking of hair and painting with methylcholanthrene in mice induce marked localized epithelial hyperplasia and regeneration even though connective tissue responses are still suppressed. However, epithelial 'invasions' of the new connective tissue (ultimately formed despite the action of cortisone) and 'pseudo-peg' formation, described by us as a constant reaction in healing wounds[6], are suppressed by cortisone.

Local application of cortisone in mice, to an area previously depilated by plucking, completely inhibits hair regeneration in the treated area. This seems to be due to suppression of invasion of dermis by new epidermal *Anlagen* as well as to the apparent inability of cortisone-suppressed connective tissue to form new hair papillæ, despite the presence of normal epidermal hyperplasia in the plucked areas.

Cortisone injections fail to suppress epithelial thickening and keratinization localized to the area painted with methylcholanthrene in mice. However, dermal atrophy and loss of subcutaneous fat are marked in mice receiving cortisone injections, and invasion of dermis by healing epidermal spurs is distinctly suppressed. Macroscopically, hair regeneration and the incidence of papillomata and carcinomas are significantly depressed, although on microscopic examination we found that malignant change occurs within the epidermis of cortisone-treated mice. Consequently, numerous carcinomata *in situ*, comparable with Bowen's disease in man, were encountered microscopically in cortisone-treated mice, even when neoplasms were not detectable macroscopically. This accounts for the discrepancy between the macro- and microscopically determined incidence of tumours, and for the apparent delay, but not suppression, of carcinogenesis by cortisone.

Thus, in two separate and slightly differently designed experiments, papillomas occurred in seven-