Table 1

Group No.	No. mice	Dose at each application (mg)	No. dead after: 1 month	4 months	8 months	12 months	No. tumours at site of painting: Papilloma	Carcinoma
1	24	5	0	0	2	2	1	0
2	24	10	0	4	6	7	0	0
3	24	20	0	0	2	2	0	0
4	24	40	0	0	4	6	0	0
5	24	80	0	1	9	10	1	0
6	24	160	10	12	16	17	0	1
7	24	20–80	0	0	6	8	0	2
8	24	20–80	0	3	7	9	0	0
9	24	10–80	0	0	4	6	0	0
10	24	Acetone-water	0	0	4	8	0	0
11	50	Control	0	1	6	9	0	0

amount of tar received over a longer period. The pattern of mortality in the other groups did not significantly differ from that in the control groups.

Histological examination of the tissues of the mice which died early revealed widespread hæmorrhage in a variety of tissues, the liver, spleen and lymph nodes, with atrophy of the lymphoid elements in the latter. There were benign and malignant tumours of the skin at the site of painting and there was an increase in the invasive character of these tumours as the total dose of tar at each application was increased.

The results therefore show that a dose of whole tobacco tar of up to 40 mg, though capable of inducing neoplastic response at the site of application, has no immediately toxic effect on this strain of mice. Toxic effect could be discerned at a dosage level of 80 mg per application and at 160 mg the mortality reached a level of about 50 per cent within the first months. The animals that survived the early months proved to be reasonably viable during the remainder of the experiment.

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Absence of Kynurenine in Hair of Patients with Scleroderma

One of the diseases in which there may be a defect of tryptophane metabolism is scleroderma. This belief is based on such experimental evidence as the increased excretion of the intermediate metabolites of tryptophane after oral administration of large doses of trytophane[1]. One of these metabolites, kynurenine, has been identified in water extracts of rat hair[2]. It seemed of interest, therefore, to determine whether or not kynurenine could be found in the hair of patients with scleroderma. Kynurenine has not been reported in previous analyses of normal human hair[3,4].

Hair clippings were obtained from five patients with scleroderma of the progressive systemic sclerosis type. Hair clippings were also obtained from three normal subjects. All the specimens were handled identically. The hair was pulverized in a jar mill with stainless steel balls, and the resulting powder was then defatted by refluxing with ether for five hours. The powder was then extracte with distilled water at 50° C overnight, evaporated *in vacuo* to concentrate the extract, and then spotted on 'W atman No. 1' chromatograph paper.

Dc ending chromatograms were run, using commercially obtained kynurenine as well as rat hair extracts as controls. The solvent used consisted of *n*-butanol, methanol, benzene and water in proportions of 2 : 4 : 2 : 2 (ref. 2). Kynurenine was detected by fluorescence under long-wave ultra-violet light, by ultra-violet absorption spectra after elution from the paper, and by spectrophotofluorometry after elution.

No kynurenine could be detected in normal human hair or in the hair from patients with scleroderma, although it was found that amounts as low as 0·05 γ of pure kynurenine could be detected on chromatograms by visible fluorescence.

It appears, therefore, that patients with scleroderma, in spite of an apparent metabolic defect which may result in the presence of increased amounts of kynurenine in the urine, do not deposit this amino-acid in the hair.

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Cell Differentiation and the Primary Lesion in Mouse Mammary Carcinogenesis

In high cancer strains of mice carrying the milk agent[1], the formation of hyperplastic alveolar nodules, the preneoplastic nature of which was demonstrated by De Ome *et al.*[2], is at present considered as the earliest sign of carcinogenesis. These nodules appear in adult mice, usually after one or several pregnancies, and therefore much later than the initial malignant transformation of the original mammary cell. Experimental data gathered from tissue culture tests now appear to locate this initial lesion in some of the alveolar cells which differentiate from the elementary duct system of the virgin mouse.

When mouse mammary fragments are explanted as organ cultures on a nutritive medium, the morphology and to some extent the functional activity of the glandular tissues can be maintained only in the presence of very specific hormones[3,4]. These hormones normally control mammary differentiation in the animal and their combination varies according to the stage of pregnancy[5]. In early pregnancy, the prevailing hormones are ovarian (œstradiol; progesterone) and hypophyseal (growth hormone; prolactin). In late pregnancy and lactation, prolactin and adreno-cortical hormones are principally involved. Whereas the first combination induces lobulo-alveolar differentiation and active cell replication, the second mostly stimulates the secretory function[6,7].

When attempts are made *in vitro* to reverse the physiology of the mammary cell, for example, by placing lactating explants in the presence of hormones which stimulate growth, extensive necrosis of the glandular tissues occurs. If, however, explants of early pregnancy are exposed to adrenal hormones and prolactin, cell multiplication stops and vesicular structures are formed[8]. In other words, the death of the mammary tissues which results from attempts to return the cells to an earlier stage of differentiation does not necessarily occur when the differentiation process is accelerated rather than reversed. These findings suggest a physiological irreversibility in the gradual differentiation of the mammary cell towards maturity and functional activity.

Evidence for this hypothesis of irreversibility was recently obtained from monolayer cultures of normal mouse mammary epithelium. As opposed to the organotypic cultures mentioned in the preceding paragraphs, where fragments of organs explanted *in vitro* as a whole grew in an organized fashion, the monolayer cultures were derived from a total enzymatic dissociation of the pooled glands from one mouse. The glands were minced in a solution of collagenase (1 mg/ml. of balanced salt solution) and dissociated at 37° C in about 45 min with moderate agitation. A mild centrifugation then eliminated most of