Folgerungen. (1) Es hängt von der Kohledosis ab, ob nach GKB eine Stimulation oder eine Depression der Phagozytoseaktivität im RES gemessen werden kann. Durch die beiden verschieden hohen Kohledosen werden also 2 verschiedene Eigenschaften der Phagozyten erfasst. (2) Mit 8–16 mg Kohle wird die Geschwindigkeit der Aufnahme von Kohle in die Phagozyten gemessen. Die Abhängigkeit der Phagozytosestimulation von der Strahlendosis und dem Alter der Mäuse sowie ihr zeitlicher Verlauf (Beginn, Maximum) weisen auf eine ursächliche Beziehung zur Strahlenkrankheit hin. Vermutlich treten infolge Darmschädigung bakterielle Endotoxine in das Blut über; solche Endotoxine haben eine starke phagozytosesteigernde Wirkung[5]. (3) Mit 45 mg Kohle wird die Kapazität der Phagozyten für Kohle gemessen. Die schon vor Beginn der Strahlenkrankheit als Phagozytoseherabsetzung nachweisbare Leistungsminderung der Phagozyten ist die Folge einer Strahlenschädigung der Zellen[6].

Phagozytose-Anstieg bei röntgenbestrahlten Mäusen

Tage nach Bestrahlung	Phagozytose-Index K	α
2	0,043	6,26
4	0,068[a]	8,85[a]
5	0,088[b]	8,58[a]
7	0,077	7,96
9	0,057	7,22
Kontrolle unbestrahlt	0,034	5,40

Ganzkörperbestrahlung, 750 R. «carbon clearance», 8 mg Kohle/100 g Körpergewicht Varianzanalyse. Mittelwerte. s_x für K = ± 0,007, für α = ± 0,36. [a] $P < 0,05$; [b] $P < 0,01$.

Summary. The effect of X-irradiation on the phagocytic activity of the RES depends on the carbon dose. Using small doses a stimulation was found; this indicates an increased velocity of carbon uptake by the phagocytes. Using high doses a depression was found indicating a decreased phagocytic capacity of the cells.

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Biochemical Changes in 9,10-Dimethyl-1,2-Benzanthracene Painted Mouse Skin

Marked changes in the acid mucopolysaccharide pattern have been observed in dermal connective tissue submitted to painting with the carcinogen 9,10-dimethyl-1,2-benzanthracene (DMBA)[1,2]. Using methylcholantrene topically, others[3] found no significant changes in dermal hydroxyproline, tyrosine, hexoses, hexosamine, and uronic acid of mice and rabbits throughout the entire course of their carcinogenesis studies.

The present paper deals with biochemical changes after DMBA painting of the skin of ST/Eh female mice, specifically in the content of water, hydroxyproline, hexosamine and uronic acid.

Materials and methods. Forty young female mice aged 6 weeks and weighing about 20 g each were divided into 3 experimental and 2 control groups. 8 mice served as untreated controls (group I). 8 animals were painted on the abdominal skin with 0.05 ml thiophene-free benzene once a week for 6 weeks (group II). 24 mice (groups III, IV and V) were painted on the abdominal skin with 0.05 ml of 0.5% DMBA in benzene once a week for 4, 8 and 12 weeks. 48 h before sacrifice, each mouse received 0.2 mC/0.2 ml of ^{35}S in sterile water i.p. After sacrifice the skin was dissected off the underlying tissue. Samples of about 2 × 1 cm were removed from the painted area and weighed immediately. The water content was determined by the difference in weights before and after drying. The samples were defatted by acetone and ether.

20–40 mg of dried, defatted skin was homogenized in 25 ml of 0.5*N* NaOH in a 'VirTis 45' homogenizer for 14 min in an ice bath. Acid mucopolysaccharides were extracted at 4 °C for 24 h and isolated as described by BOLLET et al.[4]. Determinations of uronic acid were carried out by the carbazole[5] and orcinol methods[6] converted to a microscale according to MARCKMANN[7]. The total content of mucopolysaccharides were estimated by determination of the content of hexosamine on the original tissue homogenate, as described by KIRK and DYRBYE[8] and DYRBYE[9], using the ELSON and MORGAN procedure modified by BOAS. The content of hydroxyproline, representing the amount of collagen, was determined on the original tissue homogenate by the method of NEUMAN and LOGAN as modified by MARTIN and AXELROD[10]. All biochemical analyses were performed in duplicate, and simultaneously on control and experimental skin samples. Reproducibility experiments gave a recovery of 81.9% with the carbazole, 95.4% with the orcinol, and 98.1% with the hydroxyproline method.

Electrophoretic separation and identification of acid mucopolysaccharides in alkaline extracts of powdered

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skin samples of each group were carried out according to BRUNISH and ASBOE-HANSEN[11]. Alcian blue was used for staining acid mucopolysaccharides. Some strips were stained for protein with amido black (0.5% in 7.5% acetic acid) and rinsed with distilled water. Autoradiography using Kodirex X-ray films (exposure time 6–8 weeks) demonstrated the presence of radiosulphate.

Results. The skin of the experimental animals developed erythema, edema, epidermal thickening with hyperkeratotic lesions, and alopecia. Papillomas appeared after about 6 weeks of painting. 4 mice developed squamous cell carcinomas after 9 weeks. From the twentieth day after painting a considerable accumulation of mast cells was noted in the dermis. In case of malignancy they disappeared. The results of the biochemical analyses appear from Tables I and II. The values of the 3 different experimental groups were compared with the controls according to Student's *t*-test.

Both normal and carcinogen painted skin samples gave distinct electrophoretic bands of hyaluronic acid, chondroitin sulphate, and heparin[12] (Figure). One additional band, probably representing heparitin sulphate, was observed between the chondroitin sulphate and hyaluronic acid bands. No trace of protein was found corresponding to the bands of the migrating acid mucopolysaccharides. All bands except the one with a mobility like the hyaluronate standard contained radioactive sulphur.

Discussion. The mice had multiple paintings, and thus the role of the hair cycle[13] was considered to be negligible.

Carcinogen painting brought about a pronounced rise in the hexosamine values along with a significant increase in the water content of the skin. This accords well with the water-binding property of hyaluronic acid. The definite increase in the excess hexosamine, unrelated to

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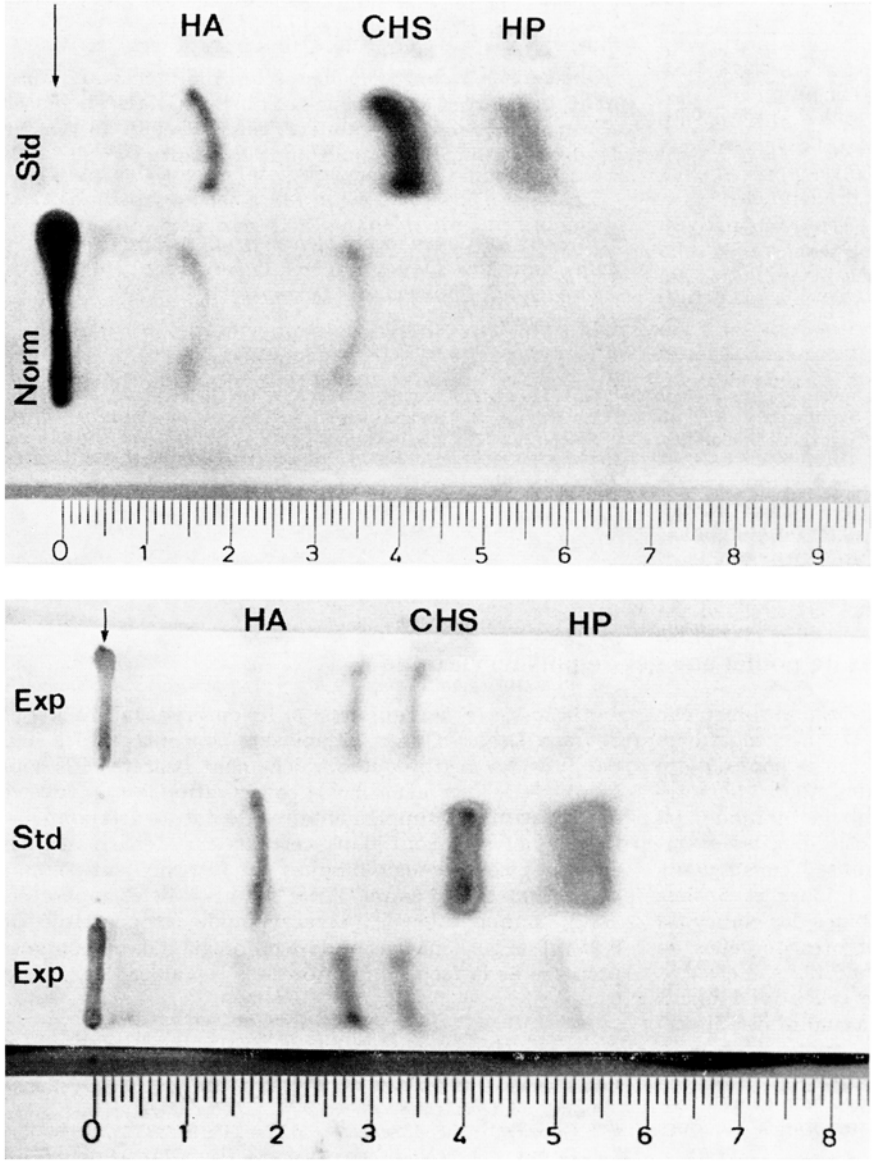

(A) Electrophoretic pattern of acid mucopolysaccharides in normal skin of mouse. Arrow indicates starting line. HA, hyaluronic acid; CHS, chondroitin sulphate; HP, heparin; STD, reference standards. Norm, normal skin of mouse. Staining: Alcian blue pH 3. (B) Electrophoretic pattern of acid mucopolysaccharides in carcinogen painted mouse skin. Symbols same as in (A). Exp, carcinogen painted skin of mouse. Staining: Alcian blue pH 3. One distinct band is seen between CHS and HA.

Table I. Water, hydroxyproline, hexosamine, uronic acid and excess hexosamine in control and DMBA painted mouse skin

Group	Water, % of wet weight	Water, % of dried and defatted weight	Hydroxyproline	Hexosamine	Uronic acid Carbazole	Orcinol	Excess hexosamine
Control (8)	57.9 ± 2.9	237 ± 14	23.9 ± 2.0	3.40 ± 0.21	0.97 ± 0.04	1.03 ± 0.07	2.45 ± 0.15
Benzene (8)	54.7 ± 2.2	233 ± 12	23.7 ± 1.4	3.70 ± 0.24	0.99 ± 0.03	1.11 ± 0.02	2.67 ± 0.23
Carcinogen 4 weeks (7)	68.5 ± 1.9[b]	368 ± 19[c]	27.2 ± 1.8	5.60 ± 0.14[c]	1.23 ± 0.01[c]	1.15 ± 0.07	4.54 ± 0.14[c]
Carcinogen 8 weeks (8)	68.9 ± 1.1[b]	304 ± 7[c]	31.8 ± 2.2[a]	4.50 ± 0.12[c]	0.97 ± 0.04	1.12 ± 0.06	3.47 ± 0.15[c]
Carcinogen 12 weeks (7)	68.2 ± 1.1[b]	295 ± 8[b]	24.3 ± 1.6	4.30 ± 0.20[b]	0.85 ± 0.07	1.25 ± 0.05	3.15 ± 0.24[a]

Figures in brackets indicate the number of mice. Values in g/mg dried, defatted tissue, in the last 6 columns. Mean values and standard error of mean are indicated. Significantly different from controls at the 5%[a], 1%[b] and 0.1%[c] level of probability.

Table II. Hexosamine to hydroxyproline, uronic acid to hydroxyproline, and carbazole to orcinol ratios in control and in DMBA painted mouse skin

Group	Hexosamine / Hydroxyproline	Uronic acid (orcinol) / Hydroxyproline	Carbazole / Orcinol
Control (8)	0.144 ± 0.008	0.045 ± 0.002	0.95 ± 0.03
Benzene (8)	0.160 ± 0.009	0.048 ± 0.001	0.89 ± 0.04
Carcinogen 4 weeks (7)	0.212 ± 0.017[b]	0.040 ± 0.003	1.09 ± 0.07
Carcinogen 8 weeks (8)	0.144 ± 0.009	0.036 ± 0.003[a]	0.88 ± 0.05
Carcinogen 12 weeks (7)	0.181 ± 0.012[a]	0.051 ± 0.004	0.67 ± 0.03[c]

Figures in brackets indicate the number of mice. Figures include mean values and standard error of mean. Significantly different from control values at the 5%[a], 1%[b] and 0.1%[c] level of probability.

uronic acid, indicates an increase in neutral mucopolysaccharides.

The increase in acid mucopolysaccharides found in this study probably originates from the accumulation of dermal mast cells[14].

The different ratios (Tables I and II) suggested qualitative changes in the composition of the connective-tissue ground substance of mouse skin[4,15]. These observations are supported by the electrophoretic results.

Zusammenfassung. Biochemische Analysen von Mäusehaut, die mit der karzinogenen Substanz DMBA bepinselt worden war, zeigten Veränderungen im Gehalt an Wasser, Hydroxyprolin, Hexosamin und Uronsäure.

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Electrophorèse des hémoglobines de poulet sur gel de polyacrylamide

L'électrophorèse sur gel de polyacrylamide lorsqu'elle est pratiquée selon la technique de DAVIS[1], sépare les hémoglobines de l'embryon de 5 jours et de la poule adulte en 2 constituants dont l'un paraît commun aux 2 hémolysats (Figure A). Or, l'électrophorèse sur gel d'amidon[2-4] et la chromatographie sur CM-cellulose[5] paraissent indiquer d'une part qu'il existe au moins 3 constituants hémoglobiniques chez l'embryon de 5 jours et 5 chez l'adulte, d'autre part que les hémoglobines des embryons les plus jeunes sont totalement différentes de celles de l'adulte. Il apparaît donc que les conditions d'électrophorèse décrites par DAVIS[1] ne sont pas celles qui donnent pour les hémoglobines de poulet le maximum de séparation possible.

Cette situation nous a conduits à modifier divers aspects de la technique de DAVIS et à appliquer la méthode obtenue à l'étude de l'évolution des hémoglobines au cours du développement du poulet.

Méthodes. (1) Les animaux et les embryons utilisés sont de race Leghorn. Les hémolysats sont préparés à 4 °C par lyse des érythrocytes fraîchement collectés. Ils sont employés le jour même car la conservation fait apparaître des constituants supplémentaires de nature artefactuelle. Les hémolysats sont dans certains cas débarrassés des protéines non hémoglobiniques par filtration sur colonne de Sephadex G75 avant d'être soumis à l'électrophorèse.

(2) Chaque tube de polyacrylamide est constitué de 0,25 ml de gel espaceur et de 1 ml de gel d'électrophorèse préparés de la façon présentée dans le Tableau.

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