basis. The osmotic pressure of all solutions was 300 mosmol ± 1%, the pH 7.15.

Results. Even a slight increase in the potassium concentration reduced the pressure of the facial arteries (vasodilatation). A maximum dilatation was reached with 10 mmole/l potassium chloride (average decrease in pressure of about 50 mm Hg, Figure a). A further increase in the potassium concentration to about 35 mmole/l still produced vasodilatation, but the extent of vasodilatation was diminished with increasing concentrations. Concentrations of more than 50 mmole/l potassium chloride invariably led to contracture (Figure b). A decrease in the potassium concentration below the normal level always increased the pressure, and the increased pressure was maintained for a long period of time (observed up to 60 min) (Figure c).

Increase in the potassium concentration also induced vasodilatation in the coronary arteries (Figure d). But this effect could be elicited only with higher concentrations of potassium and the extent of vasodilatation was much smaller compared with that of the facial artery.

Decrease in the potassium concentration and a concentration of more than 50 mmole/l produced vasoconstriction in the same way as in the facial artery, but the force of contraction was much smaller in the coronary artery (Figure e).

Discussion. There seems little doubt that potassium ions play an essential role in regulating the skeletal muscle blood flow during muscular exercise[1-7,13,14]. The results obtained on resistance vessels in perfused limb preparations were complemented by our experiments on isolated arteries, demonstrating that potassium is able to influence vascular tone from the outside of the arteries or from the interstitial space. The increase in the potassium concentration found in human subjects during exercise decreased the pressure in our experiments at an average of about 30 mm Hg. But one can assume that the local concentration of potassium in the interstitial fluid during muscular activity reaches higher levels than in the venous blood[3].

Principally the same reaction of vasodilatation, although smaller, could be observed in the coronary artery, but the results of these experiments seem not to support the hypothesis that potassium plays the same essential role in regulating coronary blood flow as it does in skeletal muscle blood flow.

The increase in pressure with decreasing potassium concentration observed in the facial and coronary artery might be partially compared with the results obtained on *Taenia coli*[15,16], where decreasing potassium concentrations decreased the membrane potential and increased spike activity. The increase in pressure with a potassium content of more than 50 mmole/l is probably due to a direct depolarizing action.

The site of action of potassium on vascular smooth muscle as regards vasodilatation and vasoconstriction (low or zero potassium) has not been established. Some evidence points to a direct action and it might be possible that there exists a Ca^{++}-K^{+}-antagonism[4,13,14,17]. But further studies are necessary to investigate the different potassium effects[18].

Zusammenfassung. Die vasodilatatorische Wirkung der K^{+}-Ionen wird bei Applikation von aussen auf Segmente der Art. facialis des Rindes, die in einer feuchten Kammer unter Druck gesetzt wurden, nachgewiesen. Die Befunde werden im Hinblick auf die Bedeutung des Kaliums für die Durchblutungsregulation im Muskel diskutiert.

P. KONOLD, G. GEBERT
and K. BRECHT

Physiologisches Institut der Universität Tübingen (Germany), 13 September 1967.

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Uptake of ^{35}S-Sulphate in Dimethylbenzanthracene Painted Mouse Skin

Previous studies using radioactive sulphur, ^{35}S, as a tracer have shown that sulphated compounds exist throughout the body, being especially concentrated in the connective tissue. The acid mucopolysaccharides of the ground substance of dermal connective tissue play an important role in the responses of the dermis to injury. Two days after peritoneal injection, practically all the ^{35}S-sulphate is found in sulphomucopolysaccharides in various tissues, among others the skin (BOSTRÖM and GARDELL[1]). In epidermal carcinogenesis, these substances have been thoroughly studied. The results of analyses for mucopolysaccharides and collagen in the skin of dimethylbenzanthracene painted mice were described by KETKAR[2]. By incorporating ^{35}S-sulphate in skin connective tissue, it is possible to obtain information about the metabolic activity of the sulphomucopolysaccharides. The aim of the present study was to investigate the uptake of ^{35}S-sulphate in mouse skin painted with 0.5% 9,10-dimethyl-1,2-benzanthracene in benzene once a week for 4, 8 and 12 weeks.

Materials and methods. Forty female ST/Eh mice aged 6 weeks and weighing about 20 g each, were maintained on a laboratory diet with free access to water. The animals were divided into 3 experimental and 2 control groups, so that initial mean body weights were as close as possible. Group 1 consisted of 8 mice serving as untreated controls. Group 2: 8 animals were painted on the abdominal skin with 0.05 ml thiophene-free benzene once a week for 6 weeks. Group 3, 4 and 5: 24 mice were painted on the abdominal skin with 0.05 ml of 0.5% 9,10-dimethyl-1,2-benzanthracene in benzene once a week for

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4, 8 and 12 weeks. Forty-eight hours before sacrifice, each animal received 0.2 ml of the radioactive solution i.p. Carrier free ^{35}S-sulphate in aqueous solution with an activity of 10 mc/ml was obtained from the Radiochemical Centre, Amersham, England. Suitable volumes of 0.04% sodium sulphate were added to a final activity of 1 mc/ml. The mice were killed by ether anaesthesia. The skin was carefully dissected off the underlying tissue, fascia, and muscle. The fur was removed with an electric clipper and then with a sharp knife. From the painted area, rectangular skin samples of about 2×1 cm in size were removed and weighed immediately. Twenty to forty mg of dry, defatted skin was homogenized in 25 ml of 0.5*N* NaOH. The uptake of ^{35}S-sulphate was recorded according to the procedure of MOLTKE[3] with modifications as suggested by MARCKMANN[4]. After wet ashing of 2.5 ml of homogenate with fuming nitric acid in a sand bath, the radioactive sulphate was precipitated as $BaSO_4$. The radioactivity of the samples was measured at infinite layer thickness with a Geiger-Müller tube, counting to a statistical error of 3%. The radioactivity was calculated in counts/min/10 mg of dried, defatted tissue, after correction for background activity, physical decay of the isotope and for variations in body weights of the mice. The results were statistically evaluated according to Student's *t*-test.

Results. The experimental animals showed a loss in body weight as compared with controls. The benzene painted mice showed degranulation of mast cells superficially while the untreated controls revealed no visible changes. The mice painted with carcinogen showed erythema, edema, epidermal hyperplasia and alopecia. The carcinogen painted groups developed papillomas after about 6 weeks of painting. Malignant tumours became evident after 9 weeks in 4 mice.

It appears from the Table that the uptake of ^{35}S-sulphate is higher in benzene and carcinogen painted mouse skin than in the control mouse skin. The difference was significant in all the groups. This increase in uptake of ^{35}S-sulphate was most pronounced in the 4 weeks dimethylbenzanthracene painted group, but reduced gradually in the 8 and 12 week groups.

Discussion. BOSTRÖM[5] demonstrated that the dermis is capable of taking up ^{35}S-sulphate in smaller quantities than cartilage and bone, incorporating it almost entirely into the chondroitin sulphuric acid of the ground substance. A negligible proportion is found in inorganic sulphate (BOSTRÖM[6]) or cystine (DZIEWIATKOWSKI[7]). The turnover rate of the sulphomucopolysaccharide molecule is the same as that of the sulphate group taken alone (SCHILLER[8] et al.). Therefore the radioactivity is believed to represent the synthesis of sulphomucopolysaccharides (MARCKMANN[4]).

Since the initial reaction after topical paintings on the abdominal skin involves formation of inflammatory tissue, it was anticipated that the rate of mucopolysaccharide synthesis in the tissue would be enhanced. The increased water content, an elevation of hexosamine and uronic acid, together with the findings of metachromasia of the ground substance, and the increased uptake of ^{35}S-sulphate, suggest an accumulation of acid mucopolysaccharides (KETKAR[2]).

The increased uptake of ^{35}S-sulphate by injured tissue indicates a stimulated synthesis of sulphomucopolysaccharides in granulation tissue (BERENSON and DALFERS[9]). The alterations observed might accordingly be interpreted as a repair process elicited by injury to the skin. Traumatizing and chemically irritating action of a carcinogen elicits fibroplasia, elevated mucopolysaccharide content and other healing reactions. This is characterized by high radioactive sulphate uptake, it being a part of connective tissue response to injury.

The production of acid mucopolysaccharides by mast cells has been the subject of considerable interest. In this study, considering the overwhelming accumulation of mast cells of varying granularity in precancerous skin, it must be considered highly probable that sulphomucopolysaccharides present in the mast cells are responsible for the radioactive sulphate uptake.

In papillomas ultimately developed in a certain % of the animals, there was comparatively high uptake of ^{35}S-sulphate. These results are in agreement with the earlier studies by DANISHEFSKY et al.[10].

There are 2 possible mechanisms by which radioactive sulphate may be incorporated into tissue. One of these is an enzymatic transsulphation process involving the movement of radioactive sulphate into sulphated compounds that are already formed. The other mechanism involves the utilization of the injected radiosulphur in the synthesis of new sulphated compounds (DENKO and PRIEST[11]). The findings are suggestive of changes in the mucopolysaccharides of precancerous tissue.

Zusammenfassung. Die Synthese von Sulfomucopolysacchariden wurde durch den Einbau von ^{35}S-Sulfat in die Haut weiblicher Mäuse nach DMBA-Behandlung verfolgt. Es wurde eine signifikante Steigerung der Aufnahme von ^{35}S-Sulfat in den karzinogen behandelten Tieren beobachtet.

M. B. KETKAR

University of Copenhagen, Department of Dermatology with Connective Tissue Research Laboratories, Rigshospital, Köbenhavn (Denmark), 23 October 1967.

Uptake of ^{35}S-sulphate in DMBA painted and control skin of ST/Eh female mice

Group	^{35}S-sulphate uptake
Control (8)	85 ± 7
Benzene (7)	158[a] ± 26
Carcinogen 4 weeks (7)	495[b] ± 61
Carcinogen 8 weeks (8)	338[b] ± 41
Carcinogen 12 weeks (7)	336[b] ± 28

Figures in brackets indicate the number of mice. Mean values and standard error of mean are indicated. Radioactivity is given in counts/min/10 mg dry, fat-free tissue. Significantly different from controls at the [a] = 5% and [b] = 0.1% level of probability.

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