Mitogenic Fractions of Human Skin.* (31324)

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It has been shown(1) that when peripheral lymphocytes from eczema patients are cultured in the presence of autologous or homologous skin extract they are transformed into large lymphocytes, plasma and blast cells and may undergo mitosis. This was taken as evidence of autosensitization to skin. In the present communication it is shown that lymphocytes from normal individuals will undergo such cell transformation but only in the presence of homologous skin. Furthermore, the transforming agent in human skin resides for the most part in particular RNA fractions of the skin.

Experimental. Skin biopsies 4 × 4 mm were obtained under strictly aseptic conditions from 4 children undergoing surgical operations. These biopsy specimens were finely minced and the RNA extracted in the following manner: 3 ml of Perry's acetate buffer(2) of pH 5.0 containing 0.5% of sodium dodecyl sulphate and magnesium chloride were added and allowed to stand for at least one hour. The resulting suspension was then extracted 3 times with equal parts of water saturated phenol. The phenol layer was discarded each time and the aqueous layers containing the interface were combined and were further extracted with an equal volume of ether, the ether being then blown off by bubbling nitrogen through. The residual aqueous phase containing the interface material was then dialyzed for 22 hours against Perry's solution containing 5% sucrose but without sodium dodecyl sulphate. After the dialysis a portion of the material within the sac was tested for cell transforming effect as described below. The remainder was layered on 4 ml of a sucrose gradient (5 to 20%) in lusteroid tubes and centrifuged in a model L Spinco ultracentrifuge for 10 hours at 35,000 rpm. Five resulting RNA fractions (I-V) were collected from each gradient tube by puncturing its bottom. The optical density was determined for each fraction at 260 mμ and the fractions were then dialyzed against medium 199 and added to separate aliquots of homologous lymphocyte cultures set up so as to contain 4:6 million cells per 8:10 ml in medium 199 containing 15% fetal calf serum and antibiotics. The cultures were incubated at 37°C for 5 days. They were scored for mitosis and cell transformation by counting 1000 cells. Transformed cells including mitotic cells in excess of 3% were considered significant results.

TABLE I. Range of Transformation (%) in Homologous Lymphocytes Cultures by Skin RNA Fractions.

Skin RNA fraction	Range of transformation (%)
I	25-40
II	20-30
III	0- 8
IV	0- 5
V	0- 5
Whole skin RNA	10-35

Results. In Table I are shown the results of cell transformation in the cultures of the 5 fractions obtained as described above.

Total skin RNA (phenol extracted) as well as sucrose (5-20%) gradient fractions I and II persistently induced significant mitogenic and transforming activity in homologous lymphocytes cultures. Similar transforming effects were obtainable by ribosomal fractions of homologous lymphocytes(3) as well as by RNA of autologous lymphocytes challenged with specific antigens(4). Ribonuclease treatment of total skin RNA or fractions I and II however did not abolish their mitogenic activity completely. Whether this could be attributed to the presence of other mitogenic non-RNA skin fractions remains to be elucidated.

Work is also in progress to compare the mitogenic effect of these skin RNA fractions with similarly isolated RNA fractions of homologous lymphocytes.

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Summary. Unlike the lymphocytes from eczematous subjects which are stimulated to mitosis and cell transformation by skin extract of either autologous or homologous skin, lymphocytes from normal individuals are stimulated only by extracts of homologous skin. The mitogenic property of skin appears to be closely associated with certain RNA fractions.

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Amphetamine: Augmentation of Pressor Effects of Tyramine in Rats. (31325)

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We have found(1) that intravenous amphetamine potentiates the pressor response to intravenous tyramine in the dog. This potentiation of tyramine by amphetamine in the dog is comparable to the potentiation of tyramine by monoamine oxidase inhibitors reported by Goldberg and Sjoerdsma(2)

Tedeschi and Fellows(3) and others(4,5) have demonstrated the potentiation of the pressor responses to tyramine by monoamine oxidase inhibitors in other species and have suggested the possible dangers associated with the ingestion of cheeses containing high concentrations of tyramine by patients on monoamine oxidase therapy. This is a report of results which show that amphetamine potentiates intraduodenally administered tyramine in the rat.

Methods and materials. Adult male albino rats (Harlan Industries) weighing 400-550 g were anesthetized with pentobarbital (45 mg/kg) intraperitoneally. The trachea was cannulated with a short piece of polyethylene tubing. Blood pressure from the carotid artery was measured with a Statham transducer (Model P-23-AC) and all recordings were made on a Honeywell Visicorder (Model 1508). A jugular vein was catheterized for the intravenous injections. Intraduodenal administration of drugs was achieved by needle puncture following exposure of the duodenum by a midline abdominal incision. (In early experiments tyramine was given by gastric intubation, but it was found that the pressor responses to tyramine were highly variable.)

Results and discussion. The intraduodenal tyramine was given at least 30 minutes after anesthesia and the rats were not given more than one dose of tyramine. In all cases the blood pressure was allowed to return to base levels after the intravenous doses of amphetamine (5-10 minutes) before tyramine was given. The intraduodenal injection of amphetamine was given one hour before the tyramine. The results are summarized in Table I.

The data clearly demonstrate that amphetamine, over a wide dose range, can markedly potentiate the pressor response to intraduodenally administered tyramine. In the absence of amphetamine, 4 to 10 times as much tyramine is required to evoke a comparable pressor response. The potentiation of tyramine was not seen with the highest dose of amphetamine.

It has been shown in the dog that amphetamine interferes with the inactivation of circulating tyramine(1). Since amphetamine is at best only a very weak inhibitor(6) of monoamine oxidase, it may interfere with uptake of tyramine rather than with its oxidation. It is suggested that a similar mechanism may account for the results reported here in the rat.

Summary. Amphetamine markedly enhanced the pressor potency of intraduodenally