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THE FRACTIONATION OF SWEAT FROM ATOPIC AND NORMAL SUBJECTS

D. W. K. Cotton, A. H. M. Sutorius and E. J. M. Urselmann

From the Department of Dermatology, Catholic University, Nijmegen, The Netherlands

Abstract. The fractionation of sweat as a function of molecular weight from atopic and normal subjects is reported. The contents of the various fractions were examined by a number of chemical spot tests and by optical measurements in the UV and IR regions. No sialo-mucins or glycosaminoglycans were found in any sweat samples. No differences were found between atopics and normals.

A number of experimental findings indicate an abnormality in eccrine sweat gland function in the atopic individual. It is well known that atopics sweat minimally on thermal stimulation (7), and Warndorff (5) has recently shown that the sweat response to local injections of acetylcholine is abnormal in these subjects. They can, however, be "trained" to sweat in the dry sauna by repeated exposure, and this generally results in a reduction of the pruritus and an improvement in the dermatitis.

One of the difficulties that exercises the dermatologist dealing with atopic dermatitis is the absence of any reliable and objective diagnostic parameter for the disease.

These two considerations led us to examine the sweat of normal and atopic subjects.

MATERIALS AND METHODS

Patients were attending the outpatient or inpatient departments of this clinic, and only those with a clear diagnosis were used as subjects in this investigation. The diagnosis was based on the personal and family history, clinical picture and the HAL test (3). Normal subjects were members of the staff of the clinic.

The chemicals used were "Merck" pro analysi grade.

Collection of sweat. Subjects were seated, naked, in a "Swiss Infra-Sauna" (Ch. Rossi and Co. AG, Kusnacht ZH). They were instructed to collect the sweat in a test tube from the trunk and arms and to avoid the axillar areas. The total time in the sauna was about 20 min and the time taken to collect approximately 2 ml of sweat was generally about 10 min. The temperature reached was about 45°C.

The sweat was filtered through Millipore filters (AP 2001000 and SSWP 01300) and frozen until used.

Fractionation of sweat. The filtered sweat was placed on a Sephadex G 50 column, length 55 cm, diameter 0.9 cm and eluted with distilled water. 30 fractions were collected with a 2 ml siphon using an LKB fraction collector and these were stored at 4°C until assayed.

The column was standardised with glucose and with blue dextran 2000 (Pharmacia).

Preliminary experiments. UV absorption spectra were plotted for all fractions using a Unicam SP 8000 spectrophotometer to determine the λ max for various molecular weight species.

Fractions which showed high absorptions at any wavelength were subsequently examined for infra-red spectra using a Perkin-Elmer 21 infra-red spectrophotometer.

A number of spot tests were applied; Ninhydrin for amino acids, hydroxylamine for the esters, biuret for protein (2), carbazole for uronic acids (1), resorcinol for sialic acids (8), and cetylpyridinium chloride for anionic polymers.

Subsequent routine experiments. From the results of the preliminary experiments it was decided to examine routinely the UV absorption at 220, 260 and 280 nm and to apply the resorcinol and carbazole tests to all fractions obtained from all sweat samples.

In one normal subject the sweat was collected in the manner described on each of 5 consecutive days. It was then examined according to the routine described except that complete UV absorption spectra were plotted for each fraction on each day.

RESULTS

In the high molecular weight fractions, the first to come from the column, the solutions occasionally showed a marked birefringence which correlated with a 220 nm absorption but not with any of the chemical spot tests that we applied.

UV absorption spectra. Fig. 1 shows typical spectra from 5 molecular weight regions A, B, C, D, and E, from a normal subject, representing

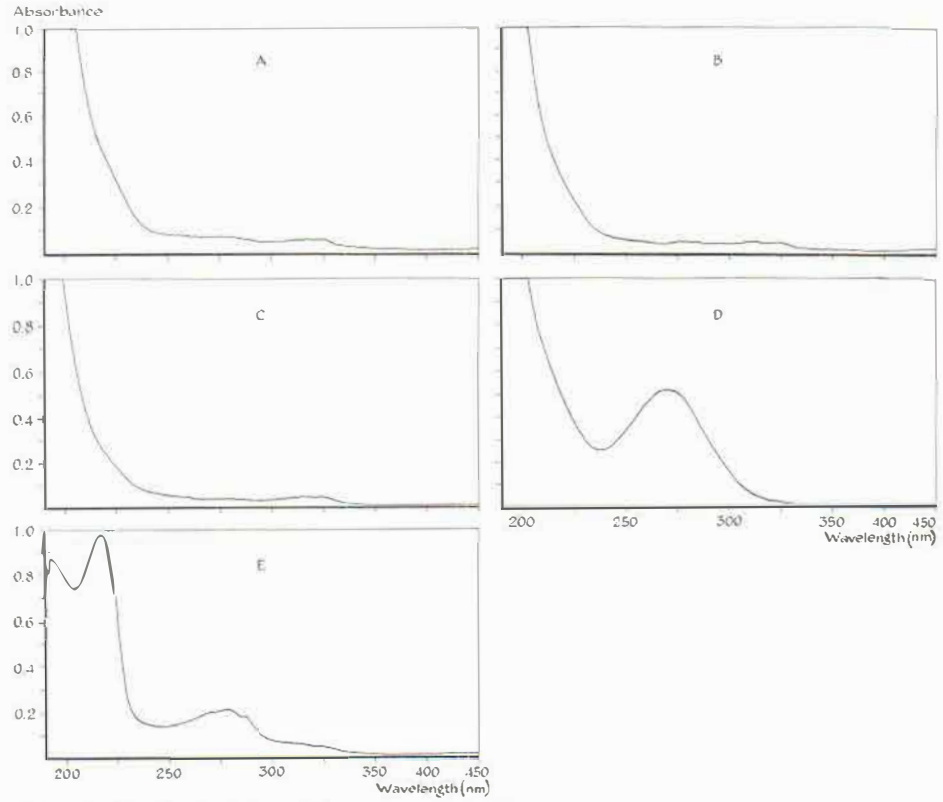

Fig. 1. UV absorption spectra of five regions of a molecular weight fractionation of normal sweat. For explanation see text.

molecular weights of approximately 55 000, 10 000, 500, 200 and 100, respectively. From these it can be seen that there are peak absorptions at 220, 260 and 280 nm primarily.

Chemical spot tests. All spot tests, except the carbazole reaction, gave negative results. Some non-specific colours occasionally developed in the low molecular weight region D. The high molecular weight regions A and B gave occasional positive results with carbazole and resorcinol but not with the absorption specific to uronic and sialic acids. The carbazole reaction was generally positive in region D but produced a yellow-brown colour indicative of lactic or pyruvic acid rather than uronic acid.

Routine experiments. Fig. 2 shows a typical 220, 260 and 280 nm absorption pattern of a molecular weight fractionation of sweat from a normal subject.

Fig. 3 shows the distribution of carbazole- and resorcinol-positive reactions in arbitrary optical density units.

Comparison of normal subjects and dermatological patients. The ratios of the absorptions at 220, 260 and 280 nm were calculated taking region C as unity. Region D was not used in this calculation since it represents the non-specific end products of general intermediary metabolism. The results of these calculations for 220, 260 and 280 nm are shown in Figs. 4, 5 and 6 respectively.

Daily fractionation of sweat. The fluctuations in the sweat of one normal individual examined on 5 successive days were the same as in the range found for normal subjects and dermatological patients (Figs. 4, 5 and 6).

DISCUSSION

The technique for collecting the sweat is open to criticism due to the possibility of concentration differences caused by evaporation. For this reason we have made no absolute measurements such as "rate of sweating", "volume of sweat",

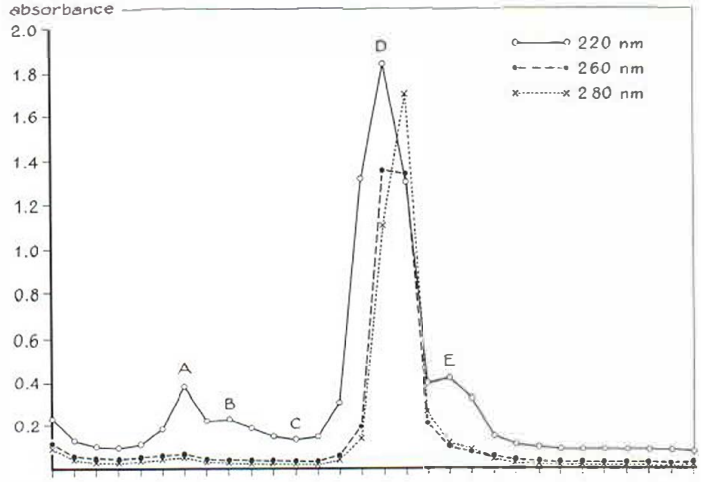

Fig. 2. The absorption at 220, 260 and 280 nm of a molecular weight fractionation of normal sweat.

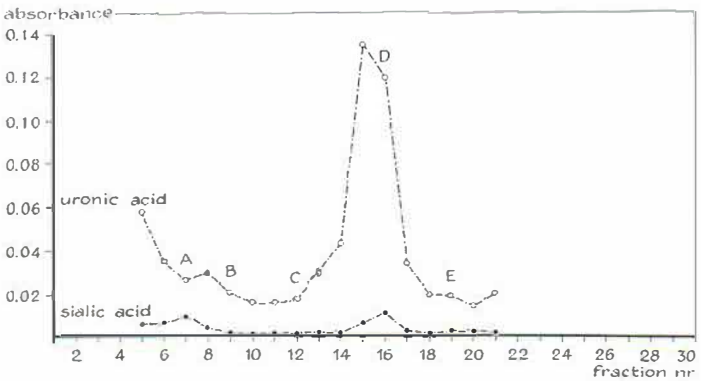

Fig. 3. Carbazole- and resorcinol-positive reactions in a molecular weight fractionation of normal sweat.

"absolute concentrations" etc., but have restricted ourselves to internal, relative measurements, e.g. the ratio of UV absorbances of different molecular weight fractions from the same individual. Such ratios can then be compared between different subjects and are independent of the overall vagaries of the collecting method. It is pertinent to observe that *all* current methods of collecting sweat have been extensively criticised.

No qualitative differences were seen between any of the subjects studied according to any of the physical or chemical parameters used. The quantitative differences observed are small and unreliable. It may be possible to render them sta-

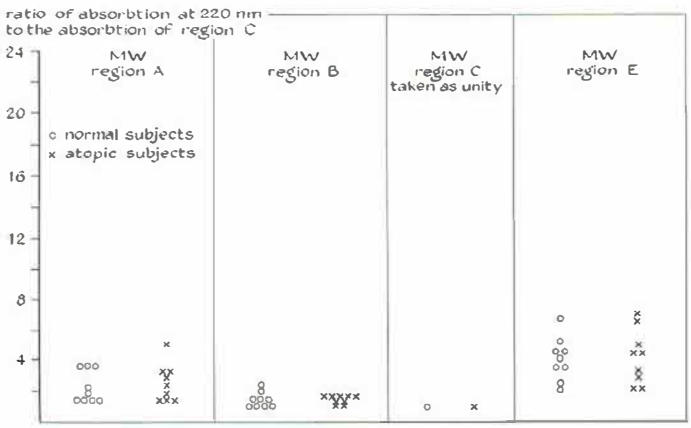

Fig. 4. The ratio of absorbances at 220 nm of molecular weight fractionations of normal and atopic sweat taking region C as unity.

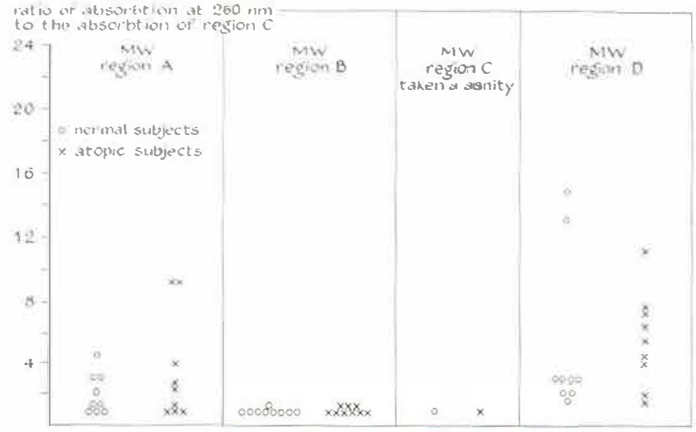

Fig. 5. The ratio of absorbances at 260 nm of molecular weight fractionations of normal and atopic sweat taking region C as unity.

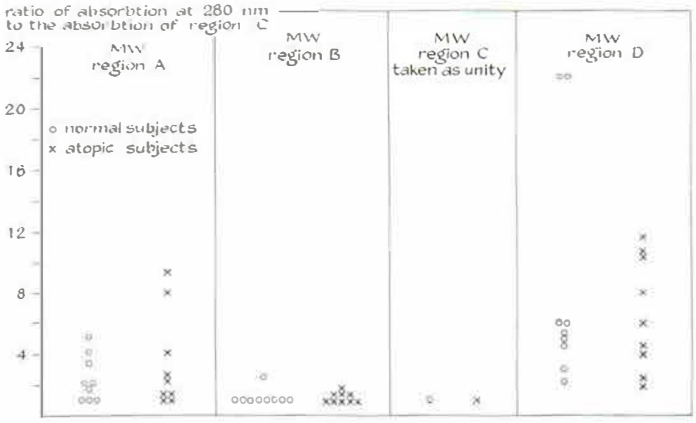

Fig. 6. The ratio of absorbances at 280 nm of molecular weight fractionations of normal and atopic sweat taking region C as unity.

tistically significant by a more extended study, but they are certainly of no diagnostic value.

One interesting qualitative result of these experiments is the absence of significant quantity of uronic and sialic acids from sweat. There is, therefore, less than 10 μg/ml hyaluronic acid and chondroitin sulphate and less than 10 μg/ml sialomucin in sweat.

The chemical nature and the function of the high molecular weight fraction of sweat is a problem that may repay further attention. Seutter (4) has shown that this fraction has some catalytic activity that may be enzymatic, and it is often described in the literature as "slime" or "mucin" (4). But whilst we could detect its presence by 220 nm absorption we did not find any by corresponding chemical test, which is curious in a substance with a molecular weight of more than 55 000.

The technique, although not of use diagnostically in atopy, could easily be scaled down and might well find application in the study of different pharmacologic activators of sweating (6).

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D. W. K. Cotton, M.D.
Department of Dermatology
Catholic University
Javastraat 104
Nijmegen
The Netherlands