

## The cutaneous loss of nitrogen compounds in African adults

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Loss of nitrogen compounds from the skin results from the activity of the sweat and other cutaneous glands, and from the shedding of epithelial structures. This loss may introduce significant errors into N-balance studies.

Concentrations of 23–141 mg N/100 ml sweat have been quoted in the literature. The sweat used in the analyses was collected directly from the skin, often in hot, humid conditions. The daily excretion of N in sweat from the whole body has been determined by washing the whole body and any clothes with large volumes of distilled water and analysing the washings. Values of 0.071–5.28 g N/24 h have been given (see reviews by Robinson & Robinson (1954), and by Cuthbertson & Guthrie (1934)). Mitchell (1949) carried out N-balance studies on twenty-four healthy American students for periods of from 156 to 212 days, and found an 'apparent nitrogen retention' of 1.379 g/24 h which was attributed partly to unmeasured cutaneous loss.

The work described here was undertaken primarily to ensure that N balances carried out under local tropical conditions were not invalidated by an underestimate of cutaneous N loss. An attempt has been made to measure the total N loss occurring in 24 h from all cutaneous structures, i.e. that in epithelial debris as well as in cutaneous secretions.

### EXPERIMENTAL

The subjects were all adult African males. Some were convalescent patients in the metabolic ward at Mwanza hospital, and others were laboratory assistants or ward dressers. They spent the whole experimental period at the ward. Their activities consisted of eating, sleeping, sitting or lying, and walking about the ward. At no time was any violent exercise taken. Maximum and minimum day and night temperatures and humidity were recorded.

At the beginning of the 24 h collection, the subject was well washed with soap and hot water, swabbed with acetone to remove all fatty secretions and then washed with several litres of distilled water. After being dried on a previously boiled, clean towel, he put on a 'sweat-suit' consisting of long-sleeved jacket, which fitted at the neck, and long trousers and socks, into which the bottom of the trousers were tucked. The suit and socks were made of best-quality Egyptian cotton, and had been boiled several times in distilled water, washed in acetone, and washed again in distilled water until the washings were free from chloride and gave a negative ninhydrin test, and a negative test for ammonia. This suit was worn for 24 h. As most African patients do not wear shoes, the socks were protected by a second layer of prepared cotton material, and

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then by another loose piece of material. The jacket and trousers were not covered with any protective layer which might have induced sweating, nor was anything worn on the head because it was thought important to reproduce the normal conditions of the African day as nearly as possible. At night, the pillow and upper part of the bed were covered with a layer of the same cotton material as the suit, previously treated in the same way.

A slight loss of nitrogenous compounds was unavoidable at each meal, as no African would eat without washing his fingers. It may also be contended that a loss occurred because no protective outer layer was worn when in bed, but at no time during any experiment was there any visible perspiration on the skin. Another obvious source of error would be contamination of the skin or suit during micturition and defaecation. To avoid it, the subject was instructed how to carry out these processes with care, and afterwards to clean the nearby skin with swabs moistened with distilled water.

At the end of 24 h, the subject stood in a clean plastic bath and removed the suit, which was turned inside out and placed with the pillow covering in a 5 l. beaker. The subject was washed in succession with acetone and several litres of distilled water, a square of the washed cotton material being used for this purpose, and was finally sprayed with about 2 l. of distilled water. The suit and cotton squares were washed twice in acetone and then repeatedly in distilled water until the washings were free from chloride and ammonia and gave a negative ninhydrin test. The operator wore rubber gloves for all these manipulations. All washings were at once acidified with sulphuric acid, and finally pooled. The total volume of washings was about 12 l. Sufficient fatty material was contained in the acetone washings to render the combined liquids turbid. The combined washings were filtered, the residue washed, and the filtrate evaporated under reduced pressure to a small bulk, and finally made up to 500 ml with distilled water.

The material which was filtered off was sticky. It contained formed elements (hair and epithelial scales), and presumably sebaceous secretions. It was very difficult to dry, and quite impossible to sample for analysis, and was therefore all incinerated with sulphuric acid, with copper sulphate and selenium dioxide as catalysts, and the nitrogen determined by Kjeldahl's method in a Markham apparatus. Portions of the concentrated filtrate were analysed for N by the same method, for urea by the method of Caraway & Fanger (1956), for ammonia by the method of King & Wootton (1956), and for creatinine by the method of Bonsnes & Taussky (1945). Lactic acid was determined by the method of Hullin & Noble (1953), and chlorides by the Volhard-Arnold method as quoted by Hawk, Oser & Summerson (1947).

#### RESULTS

Atmospheric temperature and humidity were very variable. Temperature ranged from a minimum night temperature of 14° to a maximum day temperature of 32°, and the humidity varied from 44 to 77%.

Table 1 gives the results for total N of the residue and filtrate, and for other constituents of the filtrate. There were fourteen determinations on twelve subjects.

Nos. 12 and 13 refer to two determinations on the same subject at an interval of 5 days. The temperature conditions were almost the same on these two occasions, and it will be seen that the results of the analyses show good agreement. Nos. 7 and 11 were also for two determinations on another subject at an interval of 1 month. Unfortunately, the atmospheric conditions for Expt 7 were only recorded as a 'hot day', whereas Expt 11 occurred on a 'cool day', the maximum temperature being 23° and the minimum temperature 19°. The maximum temperature on a 'hot day' could easily be 32°, and this difference in temperature may account for the fact that the total loss of N in Expt 7 was 273.9 mg, whereas in Expt 11 it was only 187.6 mg. The results showed a wide scatter with a mean daily cutaneous loss of 254 mg N, made up of

Table 1. *Daily cutaneous loss of nitrogen compounds, lactic acid and chloride in twelve African male adults (fourteen determinations)*

| De-termination no. | Weight of subject (kg) | Surface area* (m <sup>2</sup> ) | Total N (mg) | N in residue† (mg) | N in filtrate† (mg) | Urea N (mg) | Ammonia N (mg) | Creatinine N (mg) | Lactic acid (g) | Chloride (g) |
|--------------------|------------------------|---------------------------------|--------------|--------------------|---------------------|-------------|----------------|-------------------|-----------------|--------------|
| 1                  | 67.4                   | 1.91                            | 222.6        | 65.5               | 157.1               | 45.5        | 60.0           | 0.6               | 0.46            | 0.14         |
| 2                  | 58.0                   | 1.69                            | 299.6        | 61.9               | 237.7               | 66.3        | 103.8          | 1.3               | 0.76            | 0.20         |
| 3                  | 61.5                   | 1.71                            | 231.9        | 47.3               | 184.6               | 22.9        | 48.8           | 0.6               | 0.40            | 0.04         |
| 4                  | 48.1                   | 1.49                            | 224.2        | 81.5               | 142.7               | 12.4        | 51.3           | 0.8               | 0.51            | 0.30         |
| 5                  | 57.7                   | 1.64                            | 272.8        | 39.5               | 233.3               | 48.5        | 106.0          | 1.4               | 0.70            | 0.11         |
| 6                  | 51.2                   | 1.57                            | 189.1        | 53.3               | 135.8               | 22.6        | 59.8           | 0.7               | 0.44            | —            |
| 7‡                 | 56.4                   | 1.58                            | 273.9        | 32.7               | 241.2               | 54.1        | 136.7          | 1.6               | 1.19            | 0.83         |
| 8                  | 53.9                   | 1.58                            | 368.7        | 49.0               | 319.7               | 74.0        | 137.7          | 1.5               | 1.24            | 0.83         |
| 9                  | 66.4                   | 1.80                            | 147.0        | 32.8               | 114.2               | 12.6        | 35.2           | 0.7               | 0.30            | 0.41         |
| 10                 | 57.8                   | 1.64                            | 178.7        | 43.7               | 135.0               | 26.3        | 65.2           | 0.5               | 0.34            | 0.83         |
| 11‡                | 55.7                   | 1.58                            | 187.6        | 35.3               | 152.3               | 30.3        | 54.0           | 0.6               | 0.42            | 0.59         |
| 12§                | 67.1                   | 1.80                            | 234.3        | 40.8               | 193.5               | 37.3        | 89.4           | 1.3               | 0.57            | 0.42         |
| 13§                | 67.1                   | 1.80                            | 246.4        | 73.4               | 173.0               | 26.6        | 86.1           | 1.3               | 0.55            | 0.43         |
| 14                 | 67.7                   | 1.82                            | 479.7        | 85.3               | 394.4               | 126.0       | 149.6          | 0.8               | 1.20            | 0.20         |
| Mean               | 59.7                   | 1.69                            | 254.0        | 53.0               | 201.0               | 43.2        | 84.6           | 1.0               | 0.65            | 0.41         |
| S.E. of mean       | 1.8                    | 0.03                            | 22.9         | 4.9                | 21.1                | 8.1         | 9.9            | 0.1               | 0.09            | 0.07         |

\* Calculated from the formula of Du Bois & Du Bois (1916).

† See p. 116.

‡ Nos. 7 and 11 were made on the same subject at an interval of 1 month.

§ Nos. 12 and 13 were made on the same subject at an interval of 5 days.

53 mg of water-insoluble, and 201 mg of water-soluble, nitrogenous substances. Some correlation between cutaneous loss of N and body-weight or body surface area might be expected, but the calculated correlation coefficients do not show this, possibly because of the wide variation in atmospheric conditions.

The mean value for the 24 h cutaneous loss was for urea 44.8 mg N, and for ammonia 84.6 mg N. The high ammonia figures may well have been due to decomposition of the urea by bacteria on the skin during the 24 h period of investigation, and it would perhaps be more correct to sum urea and ammonia N (see Table 1). There was no quantitative relationship between 'urea + ammonia' N and filtrate N. The mean value for 24 h loss of creatinine was 7.8 mg, or 1.0 mg expressed as N, and the mean figure for lactic acid was 0.65 g. Robinson & Robinson (1954) state that

0.2 m-equiv./h of chloride, i.e. 0.15 g/24 h, are lost from the skin without visible sweat-gland activity. The mean value for chloride in this set of determinations was 0.28 g/24 h.

## DISCUSSION

Table 2 compares the results for N with those of other workers. Most workers analysed the filtrate from skin washings only. Zuntz (1902) and Freyberg & Grant (1937) included epidermal loss of nitrogenous substances with the soluble N compounds in sweat, and so obtained a figure for total cutaneous N loss under resting conditions, which is in agreement with the results presented here.

Table 2. *Comparison of estimations by various workers of daily loss of nitrogen in sweat under resting conditions*

| Reference                       | Experimental details   | Mean loss of N (mg/24 h) |
|---------------------------------|--|--------------------------|
| Zuntz (1902)                    | No. of subjects not stated; total cutaneous loss                                 | 460                      |
| Benedict (1905)                 | Five subjects; water-soluble nitrogenous substances only                         | 71                       |
| Schwenkenbecher & Spitta (1907) | Eight determinations; five subjects  | 330                      |
| Cuthbertson & Guthrie (1934)    | Fourteen determinations; six subjects; water-soluble nitrogenous substances only | 338                      |
| Freyberg & Grant (1937)         | Two subjects; total cutaneous loss   | 277                      |
| Mitchell & Hamilton (1949)      | Eight subjects; water-soluble nitrogenous substances only                        | 360                      |
| Darke (present paper)           | Fourteen determinations; twelve subjects; total cutaneous loss                   | 254<br>(188-480)         |

Since so much work has been done on this subject in the early part of the century, it is surprising that cutaneous losses have been so generally ignored in metabolic work. A N balance purporting to measure output more closely than to the nearest 400 mg, even in the absence of visible sweating, would be invalidated unless cutaneous losses were included.

## SUMMARY

1. Fourteen determinations of total cutaneous loss of nitrogen were carried out on twelve male adult African subjects by a technique in which the washings from the skin and a sweat-suit were analysed.
2. The mean total daily loss of N was 254.0 mg (S.E. of mean, 22.9).
3. The washings were analysed for urea, ammonia, creatinine, lactic acid, and chloride, and the mean total daily excretion of these substances is given.

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