CXCIII. THE EFFECT OF VARIATIONS IN PROTEIN AND SALT INTAKE ON THE NITROGEN AND CHLORIDE CONTENT OF SWEAT.

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PREVIOUS work on sweat and its composition has mainly been concerned with the quality and quantity of the various solutes secreted during short periods of profuse sweating induced by heat. Mosher [1933] has recently reviewed this aspect of metabolism.

Less numerous experiments have been made to determine the daily output of the sweat constituents at ordinary air temperatures, and, in particular, the effect of diet.

Most observers have determined the daily output of nitrogen and chloride by analysing extracts of the underclothing worn by their experimental subjects. With subjects at rest the average daily values for nitrogen were 0.071 g. [Benedict, 1905], 0.128 g. [Cramer, 1890], 0.286 g. [Graham and Poulton, 1912] and 0.33 g. [Schwenkenbecher and Spitta, 1907]. In tropical climates the nitrogen excretion is considerably increased [Eijkman, 1893]. When work is done the output of nitrogen may be as much as 0.22 g. per hour [Benedict, 1905].

Schwenkenbecher and Spitta [1907] recovered on an average 0.33 g. NaCl per day from the clothing of subjects at rest in bed. Cramer [1890] found a maximum of 2.27 g. per day secreted in the sweat of subjects actively employed.

The effect of variations in the amount of dietary protein on the composition of sweat was studied by Berry [1916], who induced sweating by cycling on an ergometer for periods of four hours. He found that the percentage composition of the sweat as regards nitrogen and chloride underwent no change on varying the protein content of the diet. The total amount of sweat produced was estimated from a calculation of his total water metabolism for the period of four hours. He found that the sweat-N corresponded to 2.0–5.6 % of the total N excreted in urine and sweat. Sweat-Cl corresponded to 13–30 % of total Cl excretion. This percentage increased both for N and Cl as the total protein metabolism decreased.

Bost and Borgstrom [1926] found that in two subjects doing office work and walking in the morning and evening, the average air temperature being 29.4° and the relative humidity 67 %, the excretions of nitrogen in the sweat during 24 hours were 0.900 g. and 0.613 g. The outputs per sq. m. body surface were respectively 0.522 g. and 0.353 g. per day. The nitrogen intakes were respectively 9.8 g. and 16.1 g. (61 and 101 g. protein). These writers concluded from other experiments lasting 2.5 to 3.75 hours that the protein content of the food eaten

in the twelve hours previous to the experiment did not seem to alter to any appreciable extent the amount of nitrogen excreted per sq. m. body surface per hour.

As for the effect of changes in the chloride content of the diet Kittsteiner [1911] asserted that systematic addition of salt to the diet increased the NaCl content of the sweat and *vice versa*, but that changes in diet of short duration had no effect.

The present investigation represents an attempt to determine the effect of variations in dietary protein and chloride on the composition of the sweat of resting subjects, care being taken that in each experiment nitrogen and, as far as possible, chloride equilibrium was first established. As has already been indicated, previous experiments have been concerned with the effect of variations in dietary protein of short duration and with the subjects not at rest. The writers thought that the extensive cutaneous excretion induced under these conditions might possibly mask such effects as could have been produced by diet alone. It was for this reason that the present experiments were conducted with the subjects lying in bed.

EXPERIMENTAL METHODS.

The duration of each period of sweat collection was 48 hours in all the normal subjects and in two of the abnormal cases. In five of the eight abnormal subjects the period was 72 hours. In one case (exophthalmic goitre) sweating was so profuse that for the subject's well-being 24 hours were thought sufficient.

The experimental subjects were divided into a normal group and an abnormal group.

(i) *Normal group.* Subjects I-IV.

These were four healthy young males aged from 17 to 26 years. Though they had all been unemployed for several months before the experiments, they were in good condition due to participation in some form of sport.

Subjects I and II were placed first on a high protein + normal salt diet (A), then on a low protein + normal salt diet (B) and finally on a low protein + low salt diet (C). Subjects III and IV were placed on the same types of diet but the order was reversed.

Diet A. High protein (175-187 g. per day). Normal salt (11-12 g. per day).

The excess protein in these otherwise ordinary diets was made up by giving 500 g. meat and 400 g. egg-white per day. All food was cooked without salt. Saltless bread and fresh butter were used. 5-6 g. NaCl were added to bring the salt intake to a normal level.

Diet B. Low protein (67-77 g. per day). Normal salt (11-12 g. per day).

The diet consisted of saltless bread, fresh butter, milk, eggs, bran, potatoes, carrots, peas, sugar and tea. No meat was given. As before salt was dispensed with in cooking and 5-6 g. added each day.

Diet C. Low protein (67-77 g. per day). Low salt (5-6 g. per day). This diet was identical with *B* except that the extra salt was omitted.

Subjects II and III had exactly similar diets throughout the experiments. Slight modifications were made in those of Subjects I and IV, according to appetite. Throughout each period the same diet was adhered to from day to day. A constant fluid intake was observed.

Daily estimations of the urinary output of total N and NaCl were made. When there was less than 1 g. of difference between the urinary N outputs on two successive days, and the urinary excretion of NaCl was at a relatively constant level, the collection of sweat was commenced. In Exp. 1 B, however, considerable

variations were found in the urinary output of chloride even at the end of 13 days with approximately the same chloride intake.

The durations of the various diets prior to collection of sweat were as follows:

Diet Subject	A days	B days	C days
I	5	4	6
II	5	5	11
III	5	5	10
IV	4	7	8

(ii) *Abnormal group.* Subjects V–XII.

This group consists of:

(a) 2 males recovering from injuries.

V—Fracture of tibia (3–5 weeks after accident).

VI—Lacerated ankle with severed tendo Achillis (1–2 weeks after accident).

(b) 2 female cases of muscular dystrophy (VII and VIII).

(c) 2 cases of exophthalmic goitre, 1 female (IX); 1 male (X).

(d) 2 female cases of myxoedema (XI and XII).

Diet. In all these abnormal subjects sweat collection was effected while on a diet of low or moderate protein content (53–96 g. per day). In only four cases was it possible to arrange for a second collection when on high protein diet (117–240 g. per day). No account was taken of the intake of salt, the patients salting their food according to their taste. The intake of sodium chloride may therefore be taken as “normal.” The subjects had the same diet for 4 to 7 days before sweat collections, except in Exps. V A, VI A and VII B where the periods were 21 days, 14 days and 3 months respectively.

Method of collection of sweat.

A roomy one-piece suit of calico was obtained with gloves and socks attached. All openings were fastened by tapes so arranged that the two sides overlapped. A loosely fitting jaconet suit constructed on somewhat similar lines was worn over this calico garment. While it is natural to suppose that such an arrangement must tend to prevent normal evaporation to a certain extent, yet some waterproof material was necessary to prevent loss of sweat on the bed linen.

A blank estimation was performed on control pieces of calico and jaconet of the same weight and quality as the suits. Each garment was boiled along with the control material in ordinary tap-water, to remove any loose matter and distribute evenly between the suit and control any traces of soluble substances still present. Both were washed out several times in the same changes of water and dried.

On the day the collection of sweat commenced, blood was withdrawn. The patient had no food in the morning prior to venepuncture. At 10 a.m. approximately, the subject had a bath, was sprayed down with distilled water and carefully dried. He donned the calico garment over which was worn the jaconet suit. The hands and feet were enclosed in both garments but the head and neck were left exposed. For two days (three in some of the abnormal cases) the subject remained in bed being covered only by a cotton sheet, a blanket and a counterpane.

During this time the urine was collected in 24-hourly lots. The greatest possible care was taken to avoid contamination of the garments with urine, the parts being washed after each passage. It was realised that a few ml. of urine of high nitrogen content voided on the clothing would lead to false results.

Wet and dry bulb hygrometer readings were taken every four hours, the instrument being attached to the head of the bed.

After 48 or 72 hours, as the case might be, the clothing was removed and the gain in weight noted. In most cases the clothing seemed quite dry except for the hands and feet. When the temperature was high or sweat secretion abnormally active, the calico garment was damp throughout and moisture was present on the inner surface of the jaconet suit.

The patient was washed down with 4 l. tap-water and dried with gauze towels which had been well boiled and rinsed. The clothing was placed in an enamelled bath containing the washings from the body and a further 8 l. tap-water added.

Control pieces of jaconet, calico and gauze towelling of the same weight and quality as those used for the subject were placed in a similar bath containing 12 l. tap-water. The contents of both baths were acidified with 5 ml. concentrated sulphuric acid.

As a control for evaporation 12 l. water were placed in a third similar bath.

After 24 hours' steeping, during which time the contents of the first two baths were stirred round from time to time, the clothing and control pieces of calico, jaconet and gauze were removed. Half the original volume, corrected for evaporation, was filtered in each case and concentrated to 1 l.

Analyses were then made on the extracts of (1) sweat and clothing, (2) clothing, and by subtraction values for the sweat were obtained.

Analytical methods.

Blood-plasma. Non-protein-N }
Urea } Folin and Wu's colorimetric methods.
Chlorides (as NaCl): Van Slyke's method.

Urine. Total N: Kjeldahl.

(Urea + ammonia)-N: Aeration with urease incubation.

Chlorides (as NaCl): Volhard-Arnold method.

Sweat. The same methods were employed as for urine, 100 ml. of concentrated sweat washings being used in each case.

Diet. The values for the protein content of the diet were calculated from the tables of Sherman [1924].

The chloride content was determined on 3 g. of dried diet by the method of Sunderman and Williams [1933].

Talbert [1922] showed that sweat produced by work or heat is invariably acid and that when sweat is secreted under the same environmental conditions except temperature the work- and heat-sweats have the same p_H (5.65 and 5.64 in his experiments). Sweat collected under rubber jackets is more acid, probably because there is not quite the same freedom for the escape of volatile organic acids. Mosher [1933] obtained p_H values of 5.02 to 5.71 and McSwiney [1934] 5.1-7.35 with an average of 6.14 in 14 normal males.

It might be supposed that sweat collected in clothing over a period of two or three days would exhibit a tendency for its reaction to swing to the alkaline side due to decomposition. The wash-water from the body and clothing of our subjects gave with one exception (Exp. VI A) a p_H of less than 6.8.

In the case where the sweat had become alkaline, the ward temperature was high (20.0°) and the subject's clothing was very moist. In this case there was no doubt that there was some escape of ammonia as the sweat (urea + ammonia)-N was only 20 % of the total N as against an average of 50 % in all the other

Table I.

All values for intake, urine and sweat are in g. per day. Figures for urine and sweat are average excretions for 24 hours. The results for blood-plasma are in mg. per 100 ml.

Condition		Normal												Fracture		Laceration		Musc. dystrophy			Exoph. goitre			Myxoedema	
Subject No.	Age	I			II			III			IV			V	VI	VII	VIII	IX	X	XI	XII				
		A	B	C	A	B	C	A	B	C	A	B	C									A	B	C	A
Sex		Male			Male			Male			Male			Male		Female		Fe- male		Fe- male					
Experiment		A	B	C	A	B	C	A	B	C	A	B	C	A	B	A	B	Fe- male	Fe- male	Fe- male	Fe- male				
Period of sweat collection (hrs.)		48	48	48	48	48	48	48	48	48	48	48	48	72	72	72	72	48	48	24	24				
Surface area (sq. m.)		1.52	1.52	1.52	1.78	1.82	1.82	1.63	1.65	1.63	1.79	1.75	1.76	1.44*	1.33	1.34	1.65	1.25	1.24	1.68	1.51				
Av. room temp. (° C.)		16.1	17.2	16.1	17.2	15.0	16.9	16.7	17.2	16.7	16.7	17.8	16.7	16.1	20.0	16.7	17.2	18.3	16.7	17.2	18.3				
<i>Nitrogen.</i>																									
Protein intake		175	67	67	187	75	75	187	75	75	77	187	240	59	177	96	75†	117	53†	53	61				
Urine—total N		24.32	10.92	11.48	24.36	9.80	10.78	23.52	10.36	9.94	25.20	26.43	11.53	22.54	12.72	11.06	14.14	8.40	6.30	17.78	24.50				
Sweat—total N		0.458	0.307	0.227	0.465	0.215	0.412	0.231	0.249	0.328	0.336	0.370	0.431	0.388	0.305	0.440†	0.262	0.221	0.224	0.270	1.019				
Sweat—total N/sq. m.		0.301	0.202	0.146	0.261	0.118	0.226	0.140	0.151	0.201	0.188	0.211	0.245	0.270	0.212	0.331†	0.196	0.133	0.179	0.218	0.606				
Plasma N-P-N		39	25	32	49	32	31	24	26	29	26	—	40	28	29	30	33	28	33	—	—				
<i>Chloride (as NaCl).</i>																									
Intake		11.42	11.21	5.21	12.17	12.27	5.77	11.77	12.17	5.63	11.29	11.17	—	—	—	—	—	—	—	—	—				
Urine		9.77	14.51	4.64	9.95	10.53	5.38	11.23	12.75	3.91	9.20	10.76	10.53	8.39	15.91	8.39	7.80	8.89	6.49	7.37	5.85				
Sweat—total		0.417	0.483	0.219	0.360	0.151	0.298	0.395	0.405	0.307	0.265	0.325	0.219	0.234	0.278	0.541	0.307	0.146	0.165	0.219	0.452				
Sweat—total/sq. m.		0.274	0.318	0.144	0.202	0.083	0.164	0.241	0.246	0.188	0.148	0.186	0.125	0.155	0.184	0.407	0.229	0.088	0.069	0.175	0.364				
Plasma		576	597	608	608	601	610	598	604	610	610	—	585	596	625	601	597	583	—	—	—				

* Allowance made for surface area covered by plaster of Paris.

† Minimum values—probably loss of ammonia due to alkalinity of sweat.

‡ In addition to this allowance, 15 g. glycine (=2.8 g. N) given daily.

experiments. In a later experiment on the same subject, when the average temperature was 16.7° the sweat washings were acid. The (urea + ammonia)-N was then 66 % of the total N.

McSwiney [1934] found that in a sample of freshly voided sweat kept in a sterile bottle at 37°, the ammonia-N increased gradually from 3.8 mg. to 37.58 mg. per 100 ml. at 66 hours. He proved that this decomposition was mainly accomplished by the bacteria normally present on the skin. These organisms attack the urea and then the epithelial débris. For these reasons we lay no stress on results obtained for the absolute values of ammonia and urea in sweat collected over a period of 48 to 72 hours.

DISCUSSION.

It will be seen from Tables I and II that, irrespective of the nature of the diet, the N and NaCl content of the sweat per square metre body surface of

Table II. *Range of values (g. per day) irrespective of temperature and diet.*

Condition	No. and Sex	Nitrogen		Chloride (as NaCl)		Basal metabolic rate
		Total	Per sq. m.	Total	Per sq. m.	
(a) Normal	4 M	0.215-0.465	0.118-0.301	0.151-0.483	0.083-0.318	—
(b) Healing injury	2 M	0.262-0.440 + *	0.196-0.331 + *	0.234-0.541	0.155-0.407	—
(a) + (b)	6 M	0.215-0.465	0.118-0.331 + *	0.151-0.541	0.083-0.407	—
(c) Muscular dystrophy	2 F	0.221-0.224	0.133-0.179	0.146-0.219	0.088-0.175	—
(d) Exophthalmic goitre	1 F	0.270-1.126	0.218-0.607	0.427-1.006	0.254-0.600	+ 37 to + 60%
(e) Myxoedema	1 M					
	2 F	0.059-0.062	0.039-0.041	0.043-0.092	0.028-0.060	- 33 to - 47 %

* Minimum values for Exp. VI A—some N probably lost owing to alkalinity of sweat.

Note. The surface areas of the subjects were calculated from their heights and weights by the formula of Du Bois and Du Bois [1916]. The values given for the output per sq. m. per day are lower than the actual values as the sweat from head and neck is not included. In Subject V (fracture of tibia) the area covered by plaster of Paris (0.069 sq. m.) was subtracted from the calculated total surface area (1.51 sq. m.) to give the value (1.44 sq. m.) in Table I.

Cases I-VI is 0.118-0.331 g. N and 0.083-0.407 g. NaCl per day at 15-20°. The upper figure 0.331 g. N (Exp. VI A) is, however, probably too low owing to loss of ammonia.

Increase in the weight of the clothing under the conditions of these experiments cannot be an accurate guide to the total weight of the sweat secreted. The greater part of the water of the sweat was found to evaporate and, in order to keep the experimental conditions as normal as possible, this evaporation was desirable.

The relative humidity obtaining in the ward during the experiments can be but a rough guide to the relative humidity of the air contained within the clothing, especially if sweating is profuse, owing to increased air temperature or pathological cause, *e.g.* exophthalmic goitre. We have therefore not included any figures for the relative humidity.

The following remarks apply only to the normal subjects I-IV where the conditions of experiment were comparable.

The effect of increased temperature when the diet is constant.

(i) *Protein.* Fig. 1 shows that with constant protein intake the excretion of total N per sq. m. per day rises with increase of air temperature.

(ii) *Chloride (as sodium chloride).* Fig. 2 indicates that the output of NaCl rises with increase of temperature if the salt intake is constant.

The effect of increase in intake when the temperature is constant.

(i) *Protein.* Exps. A and C, Subjects I-IV, were selected for comparison as the average temperatures during each pair of experiments were the same. This

Fig. 1.

Fig. 2.

Fig. 1. Total sweat-N per sq. m. per day at different room temperatures but at same protein intake.

Fig. 2. Sweat-chloride (NaCl) per sq. m. per day at different room temperatures but at same chloride intake.

Fig. 3.

Fig. 3. Total sweat-N per sq. m. per day during high and low protein diets but at same average room temperature.

Fig. 4.

Fig. 4. Sweat-chloride (NaCl) per sq. m. per day during normal and low chloride diets, but at same average room temperature.

Fig. 5. Total sweat-N per sq. m. per day during high and during low protein diets (in subjects I-IV average of two low protein diets) and irrespective of room temperature.

similarity in the temperatures was quite fortuitous. No attempt was made to keep the ward temperature at a definite level. It is seen that a rise in protein intake increases the output of N through the skin (Fig. 3).

(ii) *Chloride*. Increase of salt intake, at least within the limits obtaining in these experiments, has no constant effect on the excretion of chloride in the sweat. In the four normal cases where the temperatures were the same for both experiments in each subject, comparison of the sweat-NaCl on diets C (low salt) and A (normal salt) reveals that in two cases increase in the chloride intake was followed by a rise in sweat-NaCl and in the other two cases by a fall (Fig. 4). Kittsteiner [1911] stated that increase of salt intake raised the chloride content of the sweat but not in experiments of short duration. It is possible that a positive result might have been obtained in the present experiments if they had been prolonged.

The effect of variations in diet, the temperature being inconstant.

(i) *Protein*. It will be observed from Table I and Fig. 5 that within the limits of these experiments increment in dietary protein has a greater effect than changes in air temperature, though, as has already been pointed out, these have a definite effect.

(ii) *Chloride (as sodium chloride)*. In contrast to the preponderating effect of increases in dietary protein, increments in chloride intake do not possess this effect. As already indicated, temperature was the important factor in determining the output of chloride in these experiments.

The total values for (urea + ammonia)-N in Subjects I-VI while on high protein diets ranged from 0.232 to 0.260 g. per day, if we exclude Exp. VI A where the sweat was found to be alkaline. The (urea + ammonia)-N as a percentage of the total N gave an average of 60. On low protein diets in Subjects I-VI the limits were 0.106 and 0.209 g. per day and the average proportion of the total N as urea + ammonia was 51 %.

The fraction of total N in the urine present as urea + ammonia in these six subjects while on high protein diets averaged 83 %. The corresponding value for low protein was 84 %. Over this series of experiments the proportion of the total urinary N as urea + ammonia did not seem to have been affected by reducing the protein content of the diet. It is to be noted, however, that very low protein diets were not used, the smallest allowances in Subjects I-VI being 59 g. per day.

In the blood-plasma on high protein diets Subjects I-VI showed a variation in the non-protein-N from 29 to 49 mg. per 100 ml. with an average of 39 mg. per 100 ml. On low protein diets the range was from 24 to 32 mg. per 100 ml., the average being 28 mg. per 100 ml.

In Subjects II and IV the non-protein-N was definitely higher when on high protein diet. In Subjects I and III, however, the two values when on low protein diet showed variations practically as great as the differences between the higher figures for low protein and the values for high protein.

The urea-N of the blood as a percentage of the non-protein-N averaged 53 on high protein diet and 54 on low protein diet.

The daily output of sodium chloride in the urine was naturally higher when the salt intake was increased. The plasma-NaCl, as was to be expected, remained at a fixed level for the individual, being unaffected by diet. No conclusions can be drawn from a study of the non-protein-N and chloride contents of the plasma at one particular moment as to the presence of a correlation between these values and the excretion in the sweat of nitrogen and chloride over a period of 48 hours.

It was not found possible to investigate normal females. The values for the female cases of muscular dystrophy (both thin persons) fall within the normal

male limits, if these can be used for comparison. In comparing Exps. III C (normal male) and VII B (dystrophic female) where the temperature and protein intake were exactly the same, we find that the output of N per sq. m. per day was 0.140 g. in III C and 0.133 g. in VII B. The corresponding values for NaCl at the same temperature were, however, 0.241 g. and 0.088 g. despite the fact that in the former the salt intake was lower. In Exp. VII B the patient was receiving as treatment 15 g. glycine per day (2.8 g. N). This lessens considerably the difference in total N intake between Exps. B and A and may account in part for the similarity in sweat-N excretion.

There is a considerable divergence in the results for the two cases of exophthalmic goitre. The basal metabolic rates before the experiments commenced were +37 % for Subject IX and +60 % for Subject X. The basal metabolic rate in Subject IX after one month's subsequent treatment with iodine was still +44 %. Subject IX, who was a comparatively mild case of exophthalmic goitre, had excretions of N and NaCl by the skin comparable with the normal male cases though considerably above the upper limit for the female subjects with muscular dystrophy.

Subject X was a very severe case of exophthalmic goitre. In the two experiments the values for total N were 0.606 and 0.607 g. per sq. m. per day. On diets of normal salt content, the values for the sweat-Cl (as NaCl) were 0.600 g. and 0.254 g. per sq. m. per day. Owing to the extreme nervousness of the subject, the volume of sweat was much greater in the first experiment (B)—360 g. increase in weight of clothing as against 180 g. in Exp. A. This difference is reflected in the results for sweat-chloride. On account of this variable psychic factor, no conclusions can be drawn with regard to the effect of diet on the sweat output of nitrogen in this case.

Magnus-Levy [1907] stated "In myxoedema even when the body is exposed to great heat, no sweat appears; this feature is very characteristic. Of course a certain degree of invisible sweating may occur. It is interesting to notice that the appearance of sweat on the surface of the body is nearly always the first sign of improvement." We have shown that in cases with a reduction of basal metabolic rate of 33 % (XI) and 47 % (XII) there was a slight secretion of sweat while resting in bed even before any treatment was given. The output of N per sq. m. per day was practically the same in both cases, 0.039 g. (XI) and 0.041 g. (XII). In the sweat of Subject XII the value for NaCl was twice that in XI but was still well below the lowest normal result.

SUMMARY.

1. When the dry bulb temperature was constant, high protein intake (175–187 g. daily) was accompanied by an increased daily output of nitrogen in the sweat of normal subjects as compared with their output on low protein intake (67–77 g. daily).
2. If the intake of protein was the same, increase in air temperature of the order described apparently led to a higher daily excretion of nitrogen in the sweat.
3. The effect of alterations in protein intake was in all cases in excess of that due to temperature. The variations in protein intake were, however, of a much greater order than the changes in air temperature.
4. At the same temperatures, increase in salt intake of the order described had no constant effect on the output of chloride in the sweat, at least where the subjects had the same intake of chloride for 6–13 days beforehand.

5. The output of chloride in the sweat rose with increase of air temperature if the intake of chloride was constant.

6. In two cases of myxoedema there was a greatly reduced output both of nitrogen and chloride in the sweat.

7. In two cases of exophthalmic goitre the output of total nitrogen and chloride appeared to vary with the severity of the disease as shown by the basal metabolic rate.

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