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Research Article

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Solute and Water Secretion in Sweat *

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In man sweat is a hypotonic fluid. Except in rare instances (1) the ratio of sweat osmolality to serum osmolality is always less than one. In those studies where osmolality and the component solutes have been measured, the principal ions of extracellular fluid, sodium, and chloride are evidently the principal ions of sweat, and potassium, lactate, urea, and bicarbonate play a smaller osmotic role (1, 2).

There are few studies of osmolality in conditions of altered sweat physiology such as Addison's disease, cystic fibrosis of the pancreas, and acclimatization; but the electrolyte analyses in these conditions and in normal individuals of varying ages suggest the following approximations.

The acclimatized individual may have a sweat to serum osmolality ratio of about 0.1 (3). The ratio in normal unacclimatized preadolescent children may range from 0.1 to 0.3 (4), whereas the range in normal unacclimatized men and women may be from 0.1 to 0.5 (4). In Addison's disease the ratio would be about 0.7 (5), and in cystic fibrosis the expected range would be from 0.4 to 1.0 (6).

In contrast to the above is the sweat to serum ratio of urea concentrations, which is always greater than one. Schwartz, Thaysen, and Dole (7) reported an average ratio to be 1.8 with a range of 1.0 to 3.2, whereas Bulmer (8) noted a ratio that was somewhat lower, but still significantly greater than 1. Of further interest was Schwartz's observation that this ratio is independent of serum urea concentrations. Children affected with cystic fibrosis also have high sweat to serum urea ratios not dissimilar from normal (9). Both Schwartz and Bulmer postulated that the elevated urea in sweat is the result of a constant amount of water reabsorption in a distal site within the sweat gland.

Sweat secreted from the cat foot pad has been reported by Brusilow and Munger (10) to have an average sodium and potassium concentration of 175 and 20 mEq per L, respectively, suggesting a sweat to serum osmolality ratio greater than 1.

Because Schwartz and Thaysen (11) noted increasing sodium concentrations with increasing secretory rates, they suggested that the hypotonicity of sweat is the result of a predominance of electrolyte reabsorption over water reabsorption by the duct of the sweat gland. The data obtained from the cat provided further evidence for this theory in that light and electron microscopy revealed the chief difference between the sweat gland of man and cat to be the presence in the latter of a short duct poorly endowed with mitochondria (12). However, Schwartz' calculations indicated that the precursor fluid was hypotonic, whereas the sodium and potassium concentrations in cat sweat (if representative of precursor fluid unmodified by duct reabsorptive processes) suggested that precursor fluid was hypertonic.

The present investigations were undertaken to study *1*) precursor fluid osmolality of sweat glands, *2*) the relationship between osmolality and urea concentrations in externally delivered sweat, and *3*) the variations in osmolality among individual sweat glands.

Methods

Osmolality was determined on serum and externally delivered sweat by the Ramsay-Brown melting point method (13, 14) using volumes of 10^{-6} to 10^{-7} ml. The instrument was calibrated against standard sodium chlo-

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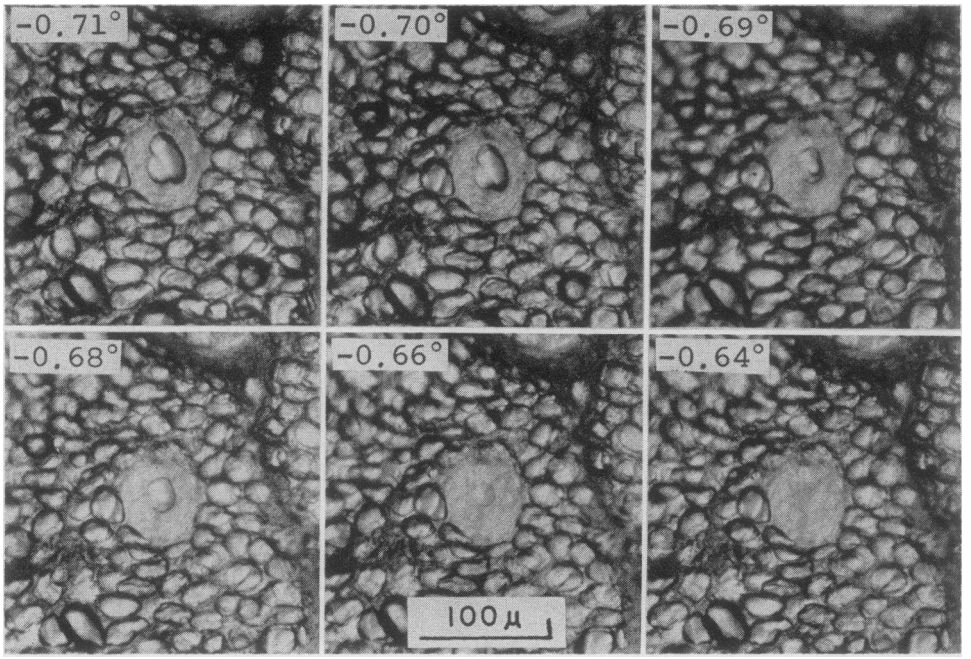

FIG. 1. SEQUENCE OF MELTING OF INTRALUMINAL ICE CRYSTALS IN THE CAT SWEAT GLAND. The single cross section is surrounded by subcutaneous fat, a characteristic of the proximal portion of the cat sweat gland. The number in the upper left hand corner of each frame represents degrees centigrade.

ride and urea solutions whose melting points were obtained from the International Critical Tables (15).

Urea determinations were made by the Chaney and Marbach modification of the indophenol reaction (16). Sodium, potassium, and chloride were determined as previously described (17).

Sweating in man was induced by the pilocarpine iontophoresis method of Gibson and Cooke (18) using the extensor surface of the forearm. With a low power dissecting microscope and a Zeiss micromanipulator, sweat was pipetted directly from the skin under a mineral oil vapor barrier in a chamber fixed to the arm. The chamber was made from a lucite ring 25 mm in diameter and 5 mm deep or a larger rubber chamber (inside dimensions, 50 mm × 30 mm) and fitted to the extensor surface of the forearm. After the pilocarpine iontophoresis was completed and the skin surface was rapidly washed five times with distilled water and dried, the well formed by the chamber was filled with mineral oil lightly stained with Sudan black. Sweat appearing from single glands was evident immediately. Quartz micropipettes containing mineral oil were used to draw up sweat from individual glands. Without exposing the specimen to air, a small amount of mineral oil was drawn up to seal the pipette. From these pipettes now filled with sweat bounded on each side by mineral oil, the small amount of sweat required for the melting point determination was drawn into similar micropipettes. After a sweating period of approximately 10 minutes had elapsed, all the remaining sweat in the chamber was drawn up anaerobically in a tuberculin syringe and placed in a 3-ml centrifuge tube. The droplets of sweat were separated from the oil by centrifuging gently for several minutes. In the same individual two collection periods of about 10 to 15 minutes duration were possible. Usually 30 to 50 μl of sweat was obtained during each period.

Sweat from the foot pad of the cat was also collected under a mineral oil vapor barrier, either by pipetting the sweat under mineral oil or by immersing the foot pad in a small, mineral oil-filled funnel with a closed stem. No more than 20 μl of cat sweat was obtained.

Precursor fluid osmolality in man and cat was investigated using a modification of the direct cryoscopy method described by Wirz, Hargitay, and Kuhn (19) in determining renal papillary osmolality. In five adult male volunteers sweating was induced in the interscapular area by pilocarpine iontophoresis (18). The foot pads of five cats were made to sweat by subcutaneous injection of methacholine chloride. The sweating skin was then biopsied using a high speed 5-mm punch (20), taking care to penetrate into the subcutaneous fat. The base of the core was cut, and the specimen was immediately immersed in liquid nitrogen. The biopsy was then frozen to a specimen block in an International-Harris cryostat and liberally coated with cold mineral oil. The knife blade was also coated with

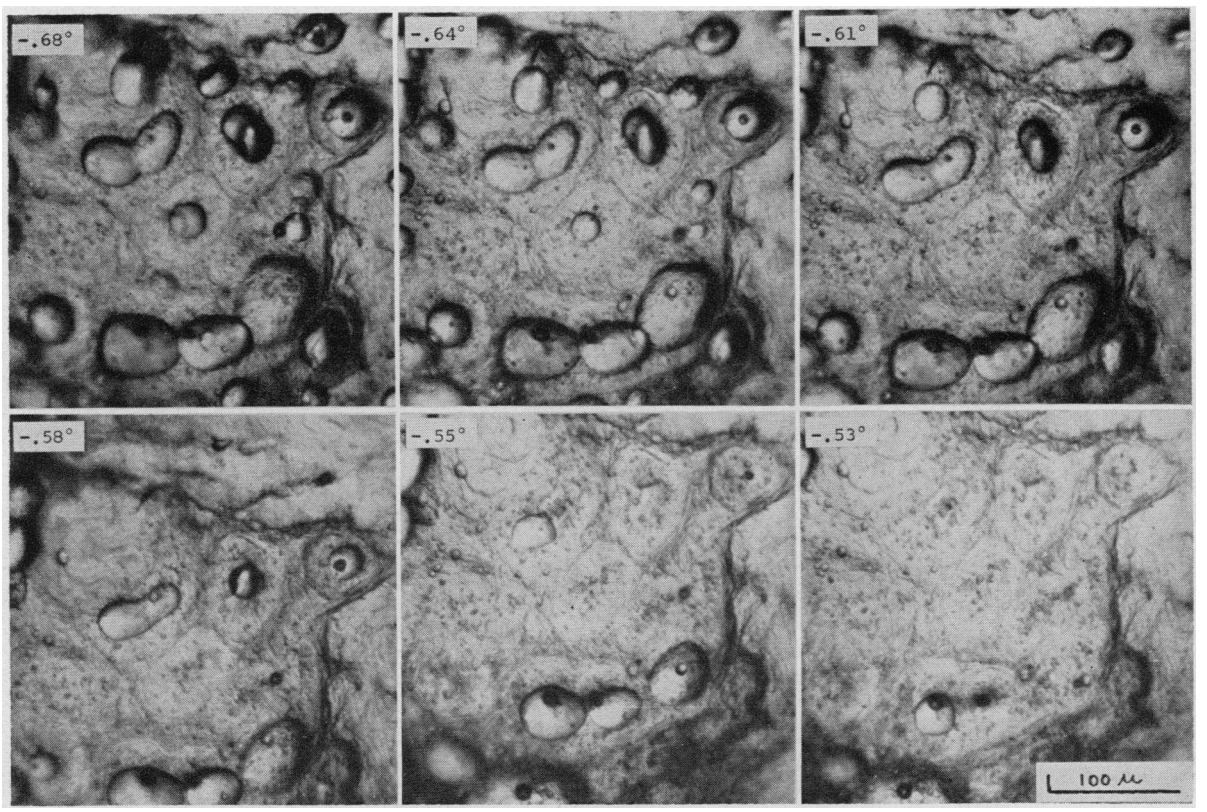

FIG. 2. SEQUENCE OF MELTING OF INTRALUMINAL ICE CRYSTALS IN THE HUMAN SECRETORY COIL. Attention should be placed only on those crystals that are clearly within cross sections of sweat glands.

mineral oil. With the cryostat maintained at −10° C, the skin was cut in sections 40 μ thick. Each section was mounted between two coverslips containing mineral oil, all at −10° C. The coverslips containing a section were placed in an adaptor and quickly inserted into the Ramsay-Brown melting point apparatus with a starting temperature of −0.8°. (Preliminary experiments had revealed that no crystals within the sweat gland would

TABLE I

*Results of analyses of sweat obtained from the cat foot pad**

	Serum					Sweat			
								Milliosmolality sw / Milliosmolality se	
Cat no.	Milliosmolality	Na	K	Cl	Urea	Milliosmolality	Urea		$\frac{U_{sw}}{U_{se}}$
	mOsm	*mmoles/L*				*mOsm*	*mmoles/L*		
611	365	181		143	7.7	388†	7.8	1.06	1.01
						385‡		1.05	
722	320	158	5.0	125	8.7	355	8.5	1.10	0.97
318	312	156	4.2	120	8.9	408	10.7	1.30	1.20
614	344	155§	8.4‖	124	6.7	350†	6.7	1.01	1.00
						355‡		1.03	
64	318	160	5.8	122	3.7	358	3.5	1.12	0.94
619	317	155	7.8	116	15.2	388	15.7	1.22	1.03

* Abbreviations: U_{sw}/U_{se} = ratio of sweat urea concentration to serum urea concentration.
† Left front paw.
‡ Right front paw.
§ Lipemic serum.
‖ Slight hemolysis.

visibly melt at lower temperatures.) As the temperature was slowly raised (0.01° C per minute), ice crystals within the lumen of a sweat gland decreased slowly in size. The temperature at which the crystal disappeared was recorded and converted to osmolality. Figures 1 and 2 show the sequence of melting in the cat and human gland. Whereas Wirz, in the direct cryoscopy studies on rat kidney, chose as his end point the disappearance of all crystals within an anatomic area (the almost circular papilla), in the present experiments the end point was the disappearance of crystals only within the lumen of a cross section of a sweat gland. Because there were usually many cross sections available in the human secretory coil, several melting points could be determined in a single slice, whereas in the cat studies, rarely would more than two melting points be possible in a single slice.

Similar direct cryoscopy studies were performed on rat liver, where melting point determinations were done on cross sections of bile ducts of a size similar to that of the sweat gland. Before removal of the liver, bile was obtained from the common duct. Aortic blood samples were also obtained.

Results

Table I shows the osmolality and urea concentrations of cat sweat compared to the simultaneously obtained serum levels. The sweat osmolality was never lower than serum osmolality, whereas with only one exception the urea concentration in sweat was about the same as in the serum.

In Table II are seen the results of studies of sweat in several normal adults as well as normal children and children with cystic fibrosis. In the collection period immediately following stimulation (Period 1), the osmolality and solute concentrations were always greater than in the second collection period, although the urea concentrations did not change.

Because the urea method used in these studies depends upon the liberation of ammonia by the

TABLE II

Results of analyses of externally

Patient Sex Age	Period 1								
	Milliosmolality of sweat from single glands			Pooled sweat					
				Milli-osmol-ality	Urea	$\frac{U_{sw}}{U_{se}}$	Na	K	Cl
years				*mOsm*	*mmoles/L*			*mmoles/L*	
R.S.	75	85	75	70	4.8	1.71	30.1	8.7	16.2
♂	73	90	73						
11	73	85	73						
D.K.*	245	245	240	240	7.5	1.25	110.1	15.0	102.6
♂	240	247	240						
6	247	240	245						
			240						
J.L.	48	50	55	48	8.0	1.66	20.3	8.2	10.0
♂	55	48	53						
12	48	55							
K.M.	50	50	48	53	8.0	1.70	22.1	4.8	12.0
♂	53	53	53						
24	53	50	48						
			48						
H.H.	65	65	65	67	14.2	1.73	27.6	6.7	15.0
♂	67	67	63						
33	70	70	70						
R.D.	53	53	53	55	8.0	1.86	21.3	8.0	10.8
♂	55	50	50						
30	48	48	55						
W.B.	55	60	60	60	7.7	1.63	25.1	6.0	20.1
♂	53	55	55						
10	55	60	60						
J.B.*	275	255	260	253	5.3	1.23	120.4	10.3	109.9
♀	255	255	257						
12	255	260	255						
L.K.	90	67	67	67	8.0	1.73	27.1	5.4	15.0
♀	70	75	70						
24	75	70							

* Cystic fibrosis.

action of urease, this determination is subject to the error that may be imposed by the presence of ammonia, which has been reported to vary in sweat from 1.7 to 5.6 mmoles per L (21). Nevertheless, when urea is determined by methods not depending on ammonia liberation, the high sweat to serum ratios are also found (7, 8).

The osmolalities of the single sweat glands showed a uniformity in the same person, which was particularly notable in the second collection period. The relatively low osmolalities obtained were probably the result of acclimatization; these studies were done during the months of July and August.

Figure 3 shows the cumulative results obtained in the direct cryoscopy experiments in four cats and five men. These data indicate that in both species proximal glandular fluid is hypertonic, although in man there is an increasing frequency of decreasing osmolalities, suggesting a pattern of decreasing osmolality within the secretory coil. On the other hand, the data obtained from direct cryoscopy of the cat sweat gland form an approximation of a normal curve, suggesting only minor modifications of sweat osmolality from formation to external delivery.

Table III shows the results of direct cryoscopy of rat liver compared with the determination of osmolality of bile obtained from the common duct and serum. These results show no tendency of the present method to give falsely high osmolalities.

Discussion

Apparent in the results of the direct cryoscopy studies is the failure to detect the hypotonic fluid that should be present in the lumen of the duct of the human sweat gland. The absence of such hy-

TABLE II

delivered human sweat

Period 2												
			Pooled sweat						Serum			
Milliosmolality of sweat from single glands			Milli-osmol-ality	Urea	$\frac{U_{sw}}{U_{se}}$	Na	K	Cl	Milli-osmol-ality	Urea	Na	K	Cl
			mOsm	*mmoles/L*		*mmoles/L*			*mOsm*	*mmoles/L*			
43	45	45	45	5.0	1.78				287	2.8	142	4.8	110
45	43	43											
45	43	45											
		45											
197	197	193	197	7.7	1.28	97.6	6.0	117.7	293	6.0	146	5.0	112
193	197												
190	193												
43	40		43	8.0	1.66				283	4.8	140	5.2	100
43	40												
40	40												
37	37	40	40	8.2	1.74	16.0	3.6	10.0	285	4.7	142	4.8	109
40	40	40											
40	37	40											
53	53	53	50	14.2	1.73	22.5	3.7	12.5	290	8.2	144	4.8	111
53	53	53											
50	53												
40	43	43	43	8.0	1.86	16.6	6.0	7.5	287	4.3	144	5.0	110
43	43	40											
40	43	43											
		43											
48	48	48	45	7.7	1.63	21.1	4.2	17.0	287	4.7	144	4.8	112
45	48	48											
45	45												
226	226	224	224	5.5	1.27	110.4	8.5	104.9	284	4.3	142	4.8	110
224	226	226											
224	228												
60	60	57	57	8.2	1.78	20.1	4.2	10.0	280	4.6	140	4.8	106
57	60	57											
60	57	60											
		60											

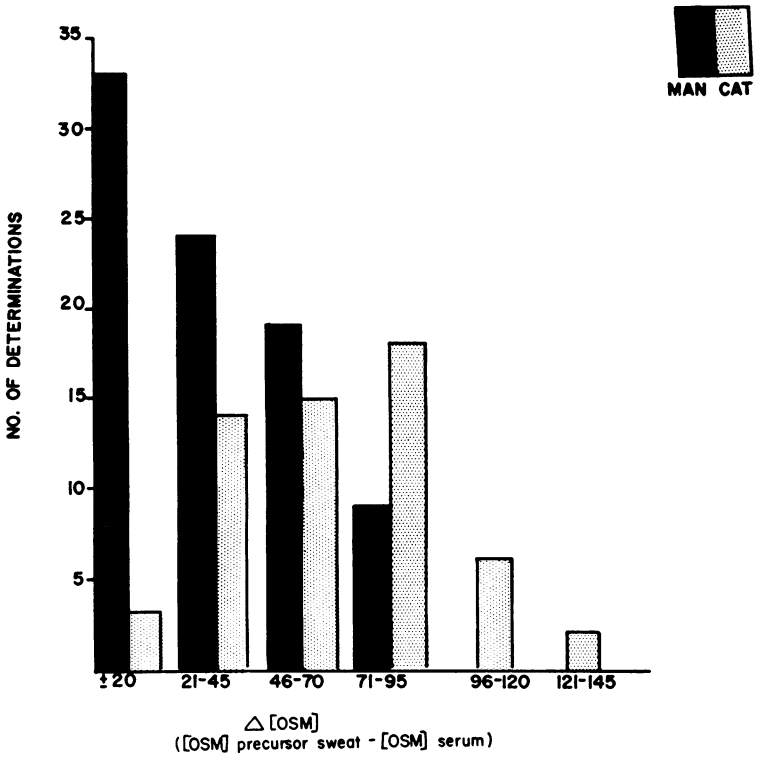

FIG. 3. CUMULATIVE DATA FROM THE DIRECT CRYOSCOPY STUDIES PERFORMED IN MAN AND CAT. The abscissa represents the difference between the sweat osmolality determined by direct cryoscopy and the osmolality of serum obtained at the time of biopsy.

potonic values may be due to a limitation of the method of direct cryoscopy. Wirz and colleagues (19) pointed out that *in situ* determinations of osmolality are subject to a methodologic error imposed by contaminating effects of melt derived from hypertonic fluid. For example, if two adjacent tubules containing fluid of different osmolalities are quickly frozen and slowly thawed, the osmolality of the melt derived from the crystals of both tubules will be the same at any given temperature as long as the temperature of the bath is below that of the melting point of the more hypertonic fluid. Once the melting point of the hypertonic fluid has been exceeded, the composition of the melts in the two tubules will be quite different as the temperature slowly rises. Assuming free diffusion of water and solute, the melt from the more hypertonic tubule can then artificially depress the melting point of any ice-melt systems with which it may come in contact.

TABLE III

Results of osmolality determined by direct cryoscopy of biliary ducts compared to osmolality of simultaneously obtained bile and blood in four rats

	mOsm			
Milliosmolality of serum	317	308	307	310
Milliosmolality of bile	317	310	312	315
Direct cryoscopy of	317	315	297	310
biliary ducts	320	300	297	315
	317	297	300	312
	312		297	317
	312			315

Thus in the data obtained from the human sweat glands where there are many adjacent channels, there would be a tendency for this methodologic error to skew the results toward higher osmolalities, although the highest osmolalities would be the most representative of the composition of the particular cross section studied. Concerning failure to detect hypotonic fluid by this method, the assumption may be made that after the melting point of interstitial fluid has been exceeded, crystals derived from hypotonic fluid could not be expected to exist in a solid state

for any appreciable period of time in a virtual sea of isosmotic melt. [This argument would also explain Wirz's (19) as well as the author's (22) failure to detect the distal tubular hypotonicity of the kidney by direct cryoscopy, although Bray (23) in similar direct cryoscopic experiments reported finding hypotonic cross sections.]

The anatomy of the human sweat gland (12, 24) may be divided into two chief parts corresponding to the nature of the epithelial lining: the duct composed of a double layer of mitochondrial-rich cells, the luminal margin of which is surmounted by a peculiar eosinophilic layer, and the secretory cells of which there are two types, clear cells and dark cells. The duct itself, however, may be further divided into three parts: the epidermal duct, the cutaneous duct, and the largest section, the coiled duct. It may be important that the coiled duct is intertwined with that part of the sweat gland composed of the secretory cells.

The sweat gland of the foot pad of the cat is similar to man's with certain exceptions. *1*) That part of the gland composed of secretory cells is not coiled at the junction of the skin and subcutaneous tissue but penetrates in a somewhat tortuous course into the subcutaneous fat, *2*) the duct therefore is short and not in contact with secretory cells, and *3*) the duct cells are poorly endowed with mitochondria (12).

Because the cat has a relatively rudimentary duct, its sweat may reasonably be assumed to be an approximation of precursor fluid, i.e., a slightly hypertonic fluid with a urea concentration the same as serum. The direct cryoscopy studies support this concept in that both man and cat had evidence of proximal fluid hypertonicity. Because sweat in man has sweat to serum osmolality ratios much less than 1 and urea ratios greater than 1, it follows that the well-developed duct in man may be responsible for these differences.

An active ionic transport system in the duct combined with a water-impermeable membrane is the most likely explanation of the hypotonicity. To conclude whether one duct segment is more responsible for such transport than another is difficult, but since duct epithelium and secretory epithelium are adjacent to each other in the secretory coil, there may be a countercurrent system for transport of ions from ductal fluid to precursor fluid. Such a system would have the benefit of reusing the reabsorbed ionic constituents for the secretion of precursor fluid.

Schwartz and associates (7) and Bulmer (8) have postulated that the high sweat to serum urea concentration is due to a constant amount of back diffusion of water with impermeability to urea. As sweat in man is a markedly hypotonic fluid, some limited back diffusion may occur in response to the established osmotic gradients, but the finding of a constant sweat urea concentration in the presence of decreasing sweat osmolality would suggest that there may be independent mechanisms for producing the elevated urea concentration and the marked hypotonicity.

Summary

1. An anaerobic method for collecting sweat is described.

2. The osmolality of sweat secreted by single glands is similar.

3. The osmolality of sweat obtained from the foot pad of the cat is slightly above serum osmolality, whereas the urea concentration of cat sweat is similar to that of the serum.

4. Direct cryoscopy showed that fluid within the secretory segment of the sweat gland of man and the cat is hypertonic.

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