2. Alkaline sucrase was present in the ungerminated seed. β-Fructofuranosidase was absent from the ungerminated seed but appeared in the embryo on germination, reaching a maximum (under etiolated conditions) after 9–10 days. Much of the activity appeared in the roots.

3. The alkaline sucrase could be purified only twofold, whereas the β-fructofuranosidase was purified 110-fold.

4. The K_m for the β-fructofuranosidase was 2·4 mM on sucrose and 14 mM on raffinose. Alkaline sucrase had K_m 8·9 mM on sucrose.

5. Both enzymes were inhibited by Ag^+ ions (0·1 mM), though iodoacetamide (10 mM) had little effect. Alkaline sucrase was strongly inhibited by tris. Transferase activity was observed with the β-fructofuranosidase but not with alkaline sucrase.

6. The function of the sucrases *in vivo* and their relation to the metabolism of raffinose-type oligosaccharides during germination is discussed.

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The Effect of Sex and Site on the Composition of Skin in the Rat and Mouse

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It is necessary to observe a number of precautions if consistent values are to be obtained for the composition of skin (Rothman, 1954). The samples of skin must be taken from specified sites, and from animals of similar age and sex. It is also known that the composition of skin is affected by the nature of the diet (Haldi, Giddings & Wynn, 1941–42), by the state of hydration of the animal (Skelton, 1926; Hamilton & Schwartz, 1935; Flemister, 1941–42), by undernutrition (Widdowson, Dickerson & McCance, 1960; Dickerson & McCance, 1964) and by a low-protein diet (Hark-

ness, Harkness & James, 1958; Dickerson & Cabak, 1962). Further, skin contains variable quantities of fat, and consistent values can be obtained only if the composition is expressed on a fat-free basis (Eichelberger, Eisele & Wertzler, 1943; Eisele & Eichelberger, 1945).

In a review of the literature on the composition of skin (Widdowson & Dickerson, 1964) it was noted that there was a large discrepancy in the values reported by different workers for some aspects of the composition of skin, for example the concentration of potassium in human and in rat skin. Workers who had analysed the entire skin of either of these species reported values that were about double those reported by other workers who analysed small pieces. This indicated that areas of skin not usually taken for analysis must contain a high concentration of potassium.

Moreover, in the mouse the entire skin of the female contains a considerably higher concentration of phosphorus and lower concentration of collagen than that of the male of the same age (E. M. Widdowson & S. A. Barnett, unpublished work). The question whether this difference was due to the mammary glands in the skin of the female or to a variation in the structure of the skin as a whole was investigated in the rat and mouse. Total nitrogen, collagen, potassium and phosphorus were determined and the results are presented in this paper. The concentration of collagen gives a measure of the size of the dermis, and the concentrations of potassium and phosphorus a measure of cellularity.

EXPERIMENTAL

Animals. The animals used were black-and-white hooded rats from the laboratory colony. They were 18–19 weeks of age and had been fed from weaning on diet 41B (Bruce, 1958). Seven animals of each sex were used, four in the first experiment and three in the second.

The mice were outbred animals, of about 17 weeks of age, from a closed colony kept in the Zoology Department, University of Glasgow (Barnett, 1961). Eight males and seven females were used.

Methods. All the animals were killed with ether and weighed, and the skin was removed, shaved and divided into the required number of samples, which were placed in weighed tubes and corked.

In the first experiment on rats, the skin was removed after a ventral incision in the mid-line. After being shaved, the skin of each of the four animals of each sex was divided into three parts: head skin, a rectangular strip from the back, and the whole of the remainder treated as one sample and described as 'remainder'. Each of these three pieces from each of the eight animals was then analysed separately.

In the second experiment the skin of the three rats of each sex was removed after cutting it along the left side of the animal. The skins were shaved as before, and each skin was divided into the following seven pieces: nose, ears, remainder of head skin, rectangular strip from the back, rectangular strip from the abdomen (which included the mammary glands), the tail skin, and the 'remainder' of the body skin. Similar pieces from each of the three animals were pooled for analysis.

The skins of the mice were removed, shaved and divided in the same way as those of the rats in the second experiment.

The samples were dried to constant weight at 95°, and the fat was extracted at room temperature from the dry samples by repeated changes of light petroleum (b.p. 40–60°). When the extraction was complete the residual light petroleum was evaporated off, first at room temperature and then overnight at 95°. The concentrations of total N, collagen, K and P were determined in the dry fat-free solids by methods described by Widdowson & Dickerson (1960).

Pieces of back, abdominal, nose and tail skin from a rat and mouse of each sex and of similar age were shaved, fixed in formalin–0·9% NaCl (1:9, v/v) and sectioned for histological examination.

RESULTS

All the skins lost moisture by evaporation during the shaving process, and this loss was more rapid from some parts (e.g. the abdominal skin) than it was from others. Therefore the composition has been expressed on a dry fat-free solid basis.

The upper part of Table 1 shows the results of the first investigation (Expt. 1) on rat skin, and the lower part shows those for the corresponding pieces in the second investigation (Expt. 2). The values shown for the head skin and 'remainder' in Expt. 2 were calculated from the weights and composition of the three pieces into which these two parts were later subdivided. In all but a few instances the results of Expt. 2 are in good agreement with those of Expt. 1, and the statistically significant differences may be assumed to hold. Table 2 sets out the values obtained for the mouse skins. The values shown for the head skin and 'remainder' were calculated from the results of the more detailed study as described above for Expt. 2 on the rat. Since only pooled samples were analysed it has not been possible to test any of the differences in the mouse statistically.

In the entire skin of the rat the amount of collagen/kg. was similar in the two sexes. In the entire skin of the mouse, however, the concentration was higher in the male than in the female, and was lower in both sexes than in the rat. In both species the concentrations of potassium and phosphorus in the entire skin were higher in the female (in the rat, $P < 0{\cdot}01$ for both elements). In both sexes of the mice the concentration of phosphorus was higher than it was in the rats.

In both species the sex differences between the remainder samples in the amounts of potassium

and phosphorus were similar to those in the entire skin. In the mouse these differences were also found in the skin from the back and head.

In neither of the two species was there any real variation with respect to sex, or site, in the amount of total nitrogen/kg. Comparison of the values for the other constituents in skin from the back and in that from the head showed that in the rat the concentration of collagen was higher in the back, and the concentrations of potassium and phosphorus were lower ($P < 0{\cdot}01$ for each constituent and sex). Similar differences were found in the mouse. In the female rat a comparison of the values for skin from the back and those for the 'remainder' sample showed that the concentration of collagen was higher in the back, and that the concentrations of potassium and phosphorus were lower ($P < 0{\cdot}01$ for each constituent). In the male rat there was probably no real difference in the results for these two samples ($P \geqslant 0{\cdot}05$).

Table 3 sets out in the two species the composition of the three parts into which the original head skin and 'remainder' sample were subdivided in the second experiment. The results further emphasize the variability in composition from one area to another, between the two sexes and between the two species. Since pooled samples were analysed the significance of the differences could not be tested statistically. However, the following general points emerge from Table 3: The nose and ears of male rats and mice had a lower concentration of collagen than the skin from the remainder of the head. Similar differences were found in the female rat, but not in the female mouse. In both sexes of both species the concentrations of potassium and phosphorus were higher in the nose than they were in the ears or remainder of head skin. In both species the concentration of potassium was higher in the nose of the female than in that of the male. Of the three parts into which the original 'remainder' was subdivided, the tail skin of both sexes of both species had the lowest concentration of collagen. In both species the skin of the female contained more potassium/kg. than that of the male.

Table 1. *Composition of rat skin*

In Expt. 1 the values are means (±S.D.) of samples from four animals of each sex and are expressed/kg. of dry fat-free solids. In Expt. 2 the values were obtained on pooled samples from three animals of each sex.

	Head		Back		Remainder		Entire skin	
	Male	Female	Male	Female	Male	Female	Male	Female
Expt. 1								
Total N (g.)	150 (±4·11)	147 (±5·2)	164 (±6·53)	168 (±5·2)	156 (±8·57)	157 (±6·1)	156 (±6·0)	157 (±5·9)
Collagen (g.)	470 (±24·0)	417 (±61·0)	693 (±81·1)	760 (±15·5)	576 (±25·3)	519 (±60·5)	563 (±51·8)	539 (±52·8)
K (m-equiv.)	133 (±11·4)	150 (±12·7)	84·3 (±4·98)	77·6 (±8·04)	107 (±14·8)	166 (±16·5)	106 (±10·3)	152 (±11·8)
P (m-moles)	144 (±12·5)	139 (±12·1)	73·3 (±14·7)	65·4 (±10·4)	85 (±8·3)	109 (±4·04)	87 (±5·3)	107 (±5·0)
Collagen N × 100 / Total N	56·5 (±2·5)	51·2 (±7·65)	76·0 (±7·8)	81·4 (±3·41)	66·7 (±3·7)	59·6 (±5·3)	64·8 (±6·24)	61·9 (±4·56)
Expt. 2								
Total N (g.)	161	162	175	170	160	163	163	165
Collagen (g.)	477	501	778	735	579	509	593	574
K (m-equiv.)	134	143	70·5	85·0	102	170	103	147
P (m-moles)	127	134	58·1	66·2	86·0	103	88·4	100
Collagen N × 100 / Total N	53·5	55·9	80·0	77·9	65·1	56·2	65·5	62·6

Table 2. *Composition of mouse skin*

The values are expressed/kg. of dry fat-free skin and were obtained on pooled samples of tissue from eight males and seven females respectively.

	Head		Back		Remainder		Entire skin	
	Male	Female	Male	Female	Male	Female	Male	Female
Total N (g.)	143	144	156	158	155	159	154	155
Collagen (g.)	344	229	579	434	487	345	480	328
K (m-equiv.)	138	180	92·4	164	108	170	110	172
P (m-moles)	185	205	93·5	161	127	172	130	178
Collagen N × 100 / Total N	43·3	28·7	66·8	49·5	56·6	39·0	56·1	38·0

Table 3. *Composition of three different parts of the head skin and 'remainder' of rat and mouse skin shown in Tables 1 and 2*

The values are expressed/kg. of dry fat-free solids and were obtained on pooled samples.

	Head skin					
	Nose		Ears		Remainder of head skin	
	Male	Female	Male	Female	Male	Female
Rat						
Total N (g.)	163	175	135	162	165	163
Collagen (g.)	208	384	249	368	567	515
K (m-equiv.)	154	195	127	137	132	135
P (m-moles)	162	190	135	133	119	124
$\frac{\text{Collagen N} \times 100}{\text{Total N}}$	23·0	39·5	33·3	40·9	61·9	57·0
Mouse						
Total N (g.)	156	151	122	131	160	171
Collagen (g.)	301	256	284	223	423	243
K (m-equiv.)	185	230	126	169	133	155
P (m-moles)	296	283	160	161	171	197
$\frac{\text{Collagen N} \times 100}{\text{Total N}}$	34·7	30·5	42·0	30·6	47·6	25·6

	Remainder					
	Abdomen		Tail skin		Remainder	
	Male	Female	Male	Female	Male	Female
Rat						
Total N (g.)	165	163	179	155	166	165
Collagen (g.)	606	719	332	317	646	603
K (m-equiv.)	124	181	137	165	90·3	171
P (m-moles)	108	103	87·0	120	85·0	96·4
$\frac{\text{Collagen N} \times 100}{\text{Total N}}$	66·1	79·6	33·4	36·7	70·2	65·9
Mouse						
Total N (g.)	163	166	174	171	152	154
Collagen (g.)	574	460	411	318	487	388
K (m-equiv.)	94	201	104	127	110	178
P (m-moles)	115	183	134	128	127	183
$\frac{\text{Collagen N} \times 100}{\text{Total N}}$	63·8	50·0	42·5	33·6	57·8	45·4

DISCUSSION

These results show that the composition of the skin from different sites of the same animals, and from the same site in the two sexes, varies. Similar results were obtained by Bottoms & Shuster (1963) and Shuster & Bottoms (1963) in the rat and man respectively. The composition of the entire skin of an animal is not that of a homogeneous structure, for the skin from different areas of the body does not contribute the same proportion of tissue. The findings on the mice are in agreement with those of E. M. Widdowson & S. A. Barnett (unpublished work) in that there is a higher concentration of phosphorus and a lower concentration of collagen in the entire skin of the female mouse. A similar sex difference in the concentration of phosphorus, but not in that of collagen, was found in the rat. These sex differences in the composition of the entire skin were not due to any one particular area, such as that of the abdomen, for they were also present in the 'remainder', and this sample accounted for about 40 % of the dry fat-free weight.

The composition of skin samples will be affected by the thickness of the epidermis relative to the dermis, by the density of hair follicles and glands, and by the presence of other structures such as muscle fibres. These structural features vary from one site to another, between the sexes and between different species (Montagna, 1962).

Of the four pieces of rat and mouse skin examined histologically tail skin had the greatest thickness of epidermis relative to dermis, and this is in agreement with the findings that the skin of the tail contained a lower concentration of collagen than that of the back or abdomen. Nose skin was more heterogeneous than any of the other samples and the amount of true skin (i.e. epidermis and dermis) in the sample was quite small. Much of the sample consisted of the follicles of the vibrissae,

and muscle fibres, both of which would contribute to the high potassium concentrations.

In the female mouse, the abdominal skin contained a lower concentration of collagen and higher concentrations of potassium and phosphorus than that of the male. The presence of mammary glands and the thickened epithelium around the nipples (Fekete, 1941) may in part be responsible for the higher concentrations of potassium and phosphorus, but a more important factor was probably the higher density of the hair follicles in the female mouse.

In man, the skin is thicker on the dorsal surface than on the ventral, and is thicker in men than women (Montagna, 1962). The skin of the back in both sexes of the rat and the mouse was thicker histologically than that of the abdomen, and in both species the back skin of the male was thicker than that of the female. A similar sex difference in skin thickness was found in the abdominal skin of the mouse, but not in that of the rat. Over the general body surface, differences in skin thickness are largely accounted for by the relative thickness of the dermis. Dermal thickness may thus be one factor contributing to the higher collagen content of the abdominal skin of the male mouse as compared with the female, and may also account for the higher collagen content of four of the pieces of rat skin compared with that of similar pieces in the mouse. The influence of the thickness of the skin on its composition tends to be masked when the values are expressed per unit weight (Shuster & Bottoms, 1963). If our results had been expressed in absolute terms per unit area the various differences would probably have been more pronounced and more might have been found.

In man variation in skin composition and structure may account for the proneness of certain areas of skin to certain diseases. It might also help to the understanding of why in a generalized disease, such as kwashiorkor, some areas of the skin are affected more than others.

SUMMARY

1. The shaved skin of mature rats and mice of both sexes was divided into nose, ears, remainder of head skin, back, abdomen, tail and the 'remainder' of the pelt, and the samples were analysed chemically.

2. The values for collagen, potassium and phosphorus varied considerably from one site to another.

3. The entire dry fat-free skin of the female of both species contained higher concentrations of potassium and phosphorus than that of the male. In the female mouse, but not in the female rat, the concentration of collagen in the entire skin was lower than in the male.

4. The sex differences in composition of the entire skin were not due to large differences in composition of skin from any particular site.

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