CXXXVI. ON THE BIOCHEMISTRY OF SKIN, AND THE CHEMICAL BASIS OF SKIN SWELLING.

By MADGE KAYE and DOROTHY JORDAN LLOYD.

(*Received July 21st, 1924.*)

I. On the Biochemistry of Skin.

In previous investigations by the authors [1924] it has been shown that the swelling of a fibrous tissue, such as skin, depends on both structural and chemical factors. The chemical factors considered were those due to variation in the external swelling medium. There remain those contributed by the skin itself. The influence of these is well shown by comparing the swelling of fresh goat skin with that of commercial sun-dried goat skin. The drying of the skin at a tropical temperature has caused a chemical change in its proteins which is made visible by the firm cementing together of the fibrils of the fibres. Skin is composed chiefly of proteins, though it contains in addition fats, sterols, and sterol esters. Rosenthal [1916] gives the following analysis of calf skin dried *in vacuo* at 55–60°.

Fresh calf skin	Butt	Shoulder
Albumin and globulin	4·14	5·16
Mucoids	4·81	2·29
Elastin	12·31	16·74
Collagen	58·83	39·66
Keratin, etc.	19·91	36·15
Total dry weight	100·00	100·00

The different proteins are distributed in the skin as follows: the keratin in the hair and epidermis; the elastin in the elastin fibre network of the grain; the collagen in the skin fibres, and the albumin and globulin, as will be shown later, although possibly present to some extent in the skin fibres, chiefly in the interfibrillary matrix. The evidence for the localisation of the last two is indirect, and is derived mainly from the parallel behaviour exhibited by plasma proteins (albumin and globulins) and by "interfibrillary substance" under the same external conditions. Albumins and globulins differ from the other classes of skin protein by being irreversibly coagulated by heat. Heat coagulated serum albumin is insoluble in water, and dried serum always contains an insoluble residue if the temperature of drying be allowed to rise above 37° [Michaelis and Rona, 1910; Hartley, Eagleton and Okell, 1923]. Eitner [1880] has shown that skins dried at a series of temperatures become increasingly difficult to soften in water in proportion to the temperature at which they are dried.

In the paper previously quoted [1924] it has been shown that in sun-dried skins the fibrils are cemented together by some non-elastic material. This condition is not found in fresh skin, and the material can be removed by ammonia, trypsin, and strong solutions of sodium chloride. In these properties, therefore, it behaves like a coagulated albumin.

Additional evidence bearing on the nature of the interfibrillary substance is obtained by a further comparison of the behaviour of fresh and sun-dried skins. The swelling curve of fresh goat skin over a wide range of reaction is shown in Fig. I (*a*). It is of the same type as that for sun-dried skin shown

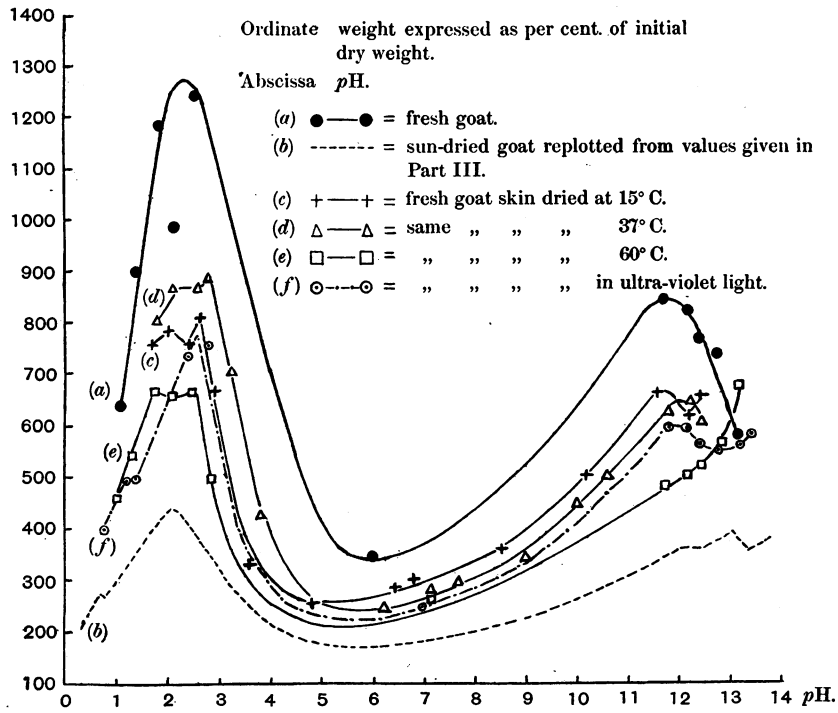

Fig. 1. The effect of albumin-coagulation on skin swelling.

in the same figure, curve (*b*), but differs from it in its much higher level throughout, particularly at the acid maximum. In the dried skin the acid and alkali maxima lie at about the same level; in fresh skin the acid maximum lies, as in swollen gelatin, well above that attained with alkali. Besides this difference in the quantitative behaviour, certain qualitative differences are also found between fresh and dried skin; for instance, in the lower concentrations of the sodium hydroxide the fresh skin pieces are thick, white, and flaccid, and a microscopical examination shows that extensive splitting of the fibres has taken place. This condition has never been found in sun-dried skin treated with similar solutions. Tables I and II, which give the external and internal condition of soaked skin at most of the experimental points recorded on

curves (*a*) and (*b*) in Fig. 1, are given below. It can be seen that in the fresh skin splitting of the fibres is found throughout the series whilst in the dried skin there is no splitting except in the strongly acid solutions. Experiments made on sun-dried goat skin by treating it with trypsin, showed that by this means the skin could be brought nearer to the fresh condition. The swelling curve for a skin so treated is shown in Fig. 2, curve (*c*) and its structural condition described in Table III. It will be seen that the fibrils have been separated by the trypsin and the samples absorb water more freely throughout the series. At the lower concentrations of alkali there occurred the separated condition of the fibrils approaching that of fresh skin at these points.

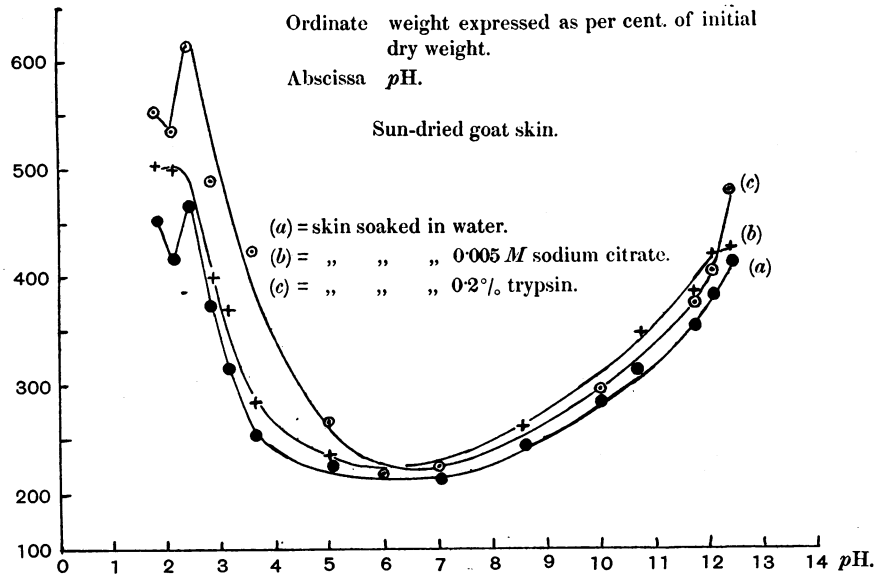

Fig. 2. The effect of dispersing coagulated albumin on skin swelling.

In the fresh skin the material between the fibrils must be in a fluid condition, since it offers no resistance either to the swelling or to the separation of the fibrils. In the dried skin, apparently this material between the fibres has passed into an insoluble condition, cements the fibrils together and causes mechanical interference with swelling. These facts are consistent with the view that the fibrils are surrounded by interfibrillary proteins of the same nature as the plasma proteins.

Albumins are irreversibly coagulated not only by heat but also by light [Young, 1922], though Harris [1923] states that this only occurs "after prolonged exposure." Coagulation also occurs when albumins are dried by means of alcohol at room temperature, but not at zero centigrade [Hardy and Gardiner, 1910]. In order therefore to prove that coagulating agents acting on a skin during drying lead to a cementing together of the fibrils, the following experiments were tried: the skin of a freshly killed goat was taken and cut

into small pieces (about 3 × 4 inches). The samples were dried in various ways (see Table IV) and were soaked for three days, (*a*) in water, or (*b*) in a 0·2 % solution of commercial trypsin.

Table I. *Fresh goat skin. Swelling in acid and alkali.*

No. of sample	p_H at equilibrium	Macroscopical examination	Microscopical examination
1	1·8	Thick, swollen, semi-transparent and rubbery	Fibrils separated and very well swollen; a few encircling bands remain
2	2·4	Thick, swollen, but not so transparent and rubbery as before	Fibrils separated and well swollen. Only a few bands remain
3	2·7	Thick, swollen, and fairly rubbery but opaque	Fibrils swollen. Many bands remain. Where bands are broken the fibrils are separated
4	4·4	Fairly thick and swollen but rather soft and white	Fibrils fairly swollen but mostly confined into bundles. Some bands are ruptured
5	7·6	Thin, flaccid and opaque	Fibrils thin, unswollen and wavy; no encircling bands evident
6	9·0	A little thicker but soft, flaccid and opaque	Fibrils unswollen but well separated
7	10·1	As thick as No. 6. Quite soft, flaccid and opaque	Fibrils similar to No. 6, but slightly thicker
8	12·0	Much thicker and more swollen and rather rubbery. Still rather soft and white	Fibrils a little more swollen and well separated
9	12·3	Thick, swollen, semi-transparent and rubbery	Fibrils much thicker and fairly well separated. No encircling bands

Table II. *Sun-dried goat skin. Swelling in acid and alkali.*

No. of sample	p_H at equilibrium	Macroscopical examination	Microscopical examination
1	1·9	Thick, swollen and rubbery	Fibres swollen but not separated into fibrils
2	2·3	Very thick, swollen and rubbery	Fibres swollen but not separated
3	3·4	Thin, only slightly swollen	Fibres only slightly swollen
4	5·9	Thin, not swollen	Fibres neither swollen nor separated
5	6·9	Thin, not swollen	Fibres thin; neither swollen nor separated
6	9·2	A little thicker but very soft	Fibres thin but slightly separated
7	11·0	Thin and soft	Fibres thin and only slightly separated
8	11·4	Thicker and partially swollen but still soft	Fibres partially swollen and slightly separated
9	12·2	Swollen, fairly thick and stiff; rubbery	Fibres swollen but not separated
10	12·5	Fairly thick; swollen and stiff; rubbery	Fibres swollen but not separated
11	12·8	Thick, swollen, very stiff and rubbery	Fibres swollen but not separated

Table III. *Sun-dried goat skin soaked in pancreatin. Swelling in acid and alkali.*

No. of sample	p_H at equilibrium	Macroscopical examination	Microscopical examination
1	2·3	Very swollen, thick and rubbery	Fibrils well swollen and separated
2	2·6	Very swollen, thick and rubbery	Fibrils swollen and separated
3	3·2	Fairly thick, swollen and rubbery but rather opaque	Fibrils only partially swollen but very well separated
4	4·9	Thin, not swollen, but opaque	Fibrils partially separated
5	7·0	Thin, not swollen but white, opaque and flaccid	Fibrils separated but not swollen
6	8·3	Thin, flaccid and not swollen	Fibrils only partially separated; not swollen
7	9·2	Thin, flaccid and not swollen	Fibrils thin and partially separated
8	10·5	A little thicker but soft and flaccid	Fibrils slightly swollen and partially separated
9	12·0	Fairly swollen but still rather opaque	Fibrils a little more swollen and separated
10	12·3	Fairly thick and swollen. Rubbery	Fibrils fairly swollen and separated

Table IV. *Results.*

		Series A. Water soak		Series B. 0·2 % Trypsin soak	
No.	Experimental conditions	Macroscopical examination	Microscopical examination	Macroscopical examination	Microscopical examination
1	Fresh goat. Undried control	Very supple and thick	Well split into fine fibrils	Very supple and thick	Rather more split than 1 A. Transparent
2	Dried at 15° C in dull light	Supple but slight feeling of hardness	—	Very soft but not quite so thick as B 1	—
3	Dried at 40° C. in dull light	Slightly harder than 2 A	Fibres not split into fibrils	„	Fibres well split into fibrils
4	Dried at 60° C. in dull light	Marked hardness; very thin	„	„	„
5	Dried at 80° C. in dull light	Horny and contracted	—	Contracted and rotten	—
6	Dipped for 30 secs. in boiling water	Contracted and rubbery	Not split into fibrils. Fibres are shrunken	Contracted. Slimy on the flesh side	Fibres swollen, transparent and broken into short pieces
7	Dried under mercury arc	Very hard	No separation into fibrils	Soft	Well separated into fibrils
8	Dried in absolute alcohol at 15° C.	Rather hard	Fair splitting into fibrils	Very soft	Well split
9	Dried in absolute alcohol at 0° C.	Very soft	Well split	Very soft	Well split

Note. The B series throughout is whiter than the A series, showing that there is a greater separation into fibrils.

It can be seen that in every experimental condition which is such as would cause irreversible coagulation of serum albumin, the skin becomes difficult to soak, and the hardness is proved by microscopical examination to be due to the non-separation of the fibrils. The absolute identity of the conditions producing hardness with those causing irreversible coagulation shows that the interfibrillary substance must be of the nature of protein and that the cement-

ing of the fibrils found in some dried skins must be due to its coagulation. The effect of trypsin in removing the interfibrillary cement confirms this view. Conditions of extreme coagulation lead to destruction of the fibrils, suggesting that albumin may also be present to some extent in the fibrils themselves.

It is to be expected that any reagents which destroy or dissolve coagulated albumin should lead to a separation of the fibrils and to an increased power of water absorption by the skin as a whole. Among such substances are: trypsin, neutral solutions of salts, acids, and alkalies.

1. *Trypsin* rapidly digests coagulated albumin. Its effectiveness as an agent for separating fibrils, and its influence on water absorption has been previously described [Kaye and Jordan Lloyd, 1924].

2. *Neutral solutions of salts* disperse coagulated serum albumin, the efficiency of the salt varying with the basicity of the acid radicle, as shown by Chick and Martin [1910, 1911, 1912, 1, 2]. The most efficient salts are those which form ter- or quadri-valent negative ions in solution, among which Chick and Martin specially mention sodium citrate. Experiments were made to test the effect of sodium citrate, sodium pyrophosphate, and sodium ferrocyanide on sun-dried skins. The results are given in Table V. It can be seen that in every case the salts have the power of removing interfibrillary substances at the same concentration as would disperse coagulated albumin.

Table V.

No.	Soak-liquor	Macroscopical examination	Microscopical examination
1	Distilled water	Rather horny	Only coarse fibres showing
2	Sodium citrate 0·005 *M*	Softer than 1	Well separated into fibrils
3	Sodium ferrocyanide 0·005 *M*	About like 2	,, ,,
4	Sodium pyrophosphate 0·005 *M*	Soft and soaked	,, ,,
5	Trypsin 0·2 % solution	Very soft and white	Split into fine fibrils

3. *Acids and alkalies.* Both these have the power of dispersing heat-coagulated albumin, but they also have the property of causing swelling of the fibrils. For this reason, when sun-dried skin is soaked in dilute solutions of hydrochloric acid or sodium hydroxide, the fibrils distend very rapidly and close up the interfibrillary spaces, thus checking any chemical action which might take place in these spaces. Acids and alkalies in general, therefore, although they disperse coagulated albumin, do not loosen the skin structure except under a few special circumstances.

Ammonia stands out from most alkalies as being one of the most effective substances known for separating the fibrils. This is probably connected with its weak ionisation and with its special power of penetrating cell walls.

The analogy between the behaviour of serum albumin and "interfibrillary substance" is thus seen to be a very complete one; both are rendered insoluble (coagulated) by the same conditions, and both can be again taken back into solution by the same means. The influence of coagulating factors (heat, ultra-

violet light and alcohol) acting during the drying of skin, on subsequent water absorption, is shown graphically in Figs. 1 and 4. In every case the water absorption has been reduced below the level of that of fresh skin. The influence of dispersing agents, such as trypsin or sodium citrate, on skins in which coagulated albumin may now be presumed to occur, is shown in Figs. 2, 3 and 4. In Fig. 2 it can be seen that the water absorption of a commercial dried skin can be increased by previously soaking for three days with 0·005 *M* sodium citrate or 0·2 % trypsin. In Fig. 3 is shown the influence of heat in reducing the power of water absorption, the influence of trypsin in restoring

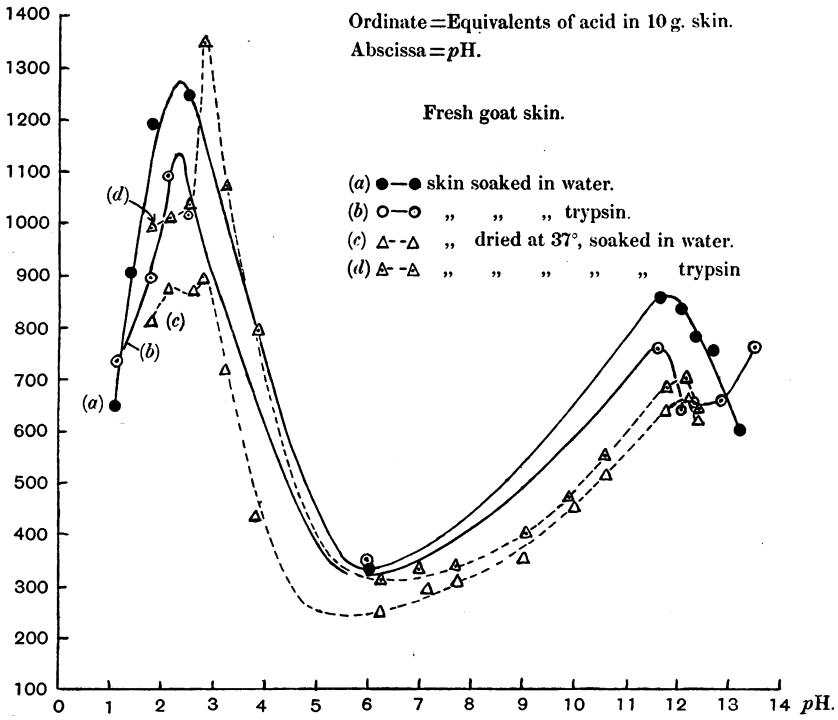

Fig. 3.

it. It must be borne in mind that drying *per se* has an influence on the subsequent behaviour of the skin. Maclaughlin and Theis [1923] have shown that mere withdrawal of water from a skin has, in itself, an action on the skin which checks re-absorption. They showed that samples of skin (ox) dried to varying extents (up to half the total water) at a constant temperature of 33°, when immersed in either water or saturated lime water (p_H 12·4) re-absorbed water in proportion to the water remaining in the partially dried skin. The temperature at which they dried their material should have precluded the possibility of heat coagulation. It can also be seen from Fig. 1 that drying a skin at a temperature as low as 15°, lowers the capacity for absorbing water at all reactions. The skin so dried and then soaked in water is not so supple as

fresh skin. The crucial test as to the influence of drying as apart from coagulation is found in the experiments on drying with alcohol at 0°. This method was used by Hardy and Gardiner [1910] to prepare pure serum proteins in a chemically unchanged condition. Skins dried by this method become as soft as fresh skins when soaked in water, but in spite of this their swelling power has been reduced. This is shown graphically in Fig. 4, curve (*c*). The microscopical condition of the fibres has also been altered, though not so much as with other methods of drying. The appearance of these skins after soaking in water, hydrochloric acid and sodium hydroxide respectively is described in Table VI. Treatment of the skins with trypsin restores the swelling power (curves (*b*) and (*d*), Fig. 4), a fact which suggests that the drying affects not the collagen fibres themselves, but rather the accessory structures in the skin.

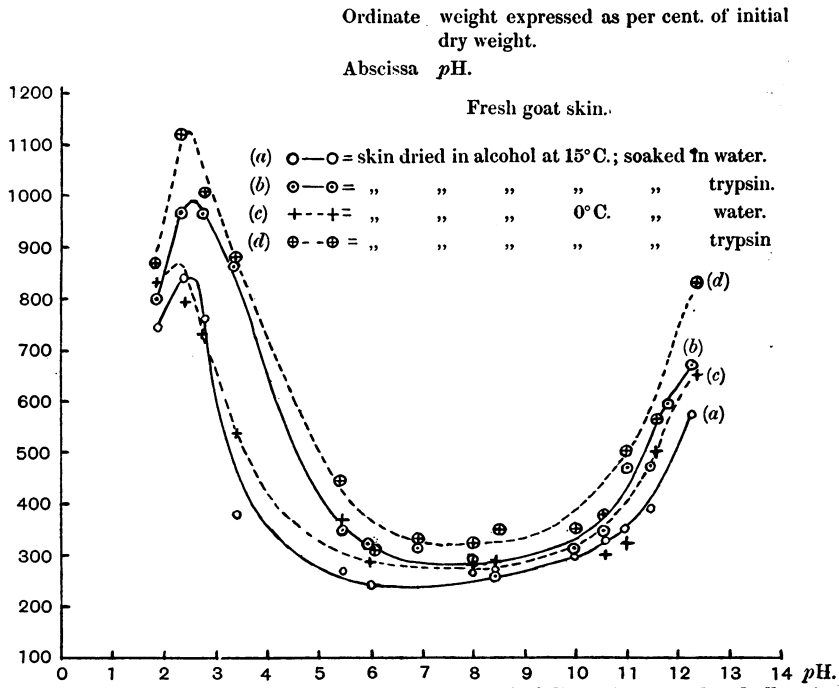

Fig. 4. The effect of drying at low temperatures and of dispersing coagulated albumin in skins so dried.

Comparative experiments were made with skin dehydrated with alcohol at 15°. The quantitative differences between drying at 0° and 15° are not great (Fig. 4, curves (*a*) and (*c*)), but the qualitative differences are marked, as shown in Table VI, and particularly in Table IV.

Up to the present no conditions have been found under which a skin once dried can subsequently be restored exactly to the fresh condition. Even under conditions chosen to produce the least chemical disturbance of the skin proteins (alcohol at 0°) it can be seen (Table VI (*a*)) that the freedom of the fibrils has largely been lost, with the accompanying reduction of the power

Table VI. *Fresh goat skin dried out in alcohol at 0° and 15°. Swelling in acid, water and alkali with and without treatment with trypsin. Microscopical examination.*

p_H at equilibrium	Dried in alcohol at 0°		Dried in alcohol at 15°		Fresh goat. Undried. Control
	Water soaked (*a*)	Trypsin soaked (*b*)	Water soaked (*c*)	Trypsin soaked (*d*)	
2·4	All fibres swollen, Split well at the ends into fibrils and shew fibril striation down their lengths	Fibres swollen. Well split at ends and along the lengths of the fibres into fibrils	Fibres not swollen as much as (*a*) or (*b*) but show signs of splitting along the fibres and also at the ends	Fibres swollen, partially split along the lengths and well split at the ends	Fibres fully split into fibrils and well swollen. Encircling bands numerous
8	Fibres not swollen; not confined into bundles and are not split into fibrils but show striations. On swelling with acid the fibres split more into fibrils, but show no encircling bands	Fibres well split into fibrils; these are well separated—much more than in the water-soaked skin. Fibrils not swollen. Swell and split further in acid solution but show no encircling bands	Fibres split into fibrils in many places. All bundles are ruptured. Fibrils not swollen; swell and split more in acid solution, but do not swell to the same extent as (*a*) and (*b*)	Fibres well split into fibrils. All bundles are ruptured. Fibrils not swollen. Swell and split more in acid solution, but do not swell to the same extent as in (*a*) and (*b*), and show no encircling bands	Fibres thin, unswollen and wavy; confined into bundles; individual fibrils clearly visible. On swelling with acid the encircling bands appear.
11·8	Fibres swollen but not so much as in acid. Fairly well split into fibrils but these are not separated	Fibres swollen but not so much as in acid. Split and separated into fibrils	Fibres separated and swollen but not as much as in acid. Split and well separated into fibrils	Fibres swollen but not so much as in acid. Split and well separated into fibrils	Fibres split into fibrils and swollen but not as much as in acid. Some encircling bands present

All fibres, when teased out of the skin, swell more in the equilibrium solutions. This extra swelling is greater in acids than in alkalies. It does not, of course, occur in water.

The waviness of the fresh fibril has been lost in all the dried fibrils, and the encircling bands characteristic of the swollen fresh fibre-bundles have also been destroyed.

of colloidal swelling (Fig. 4, curve (*c*)). Both the loss of freedom of the fibrils and also the swelling power can be restored by the action of trypsin (Table VI (*b*) and Fig. 4, curve (*d*)), but the action of the enzymes must have been to remove some of the constituents of the fresh skin. Other changes caused by drying, such as the loss of wavy outline of the fresh fibril, and the rupturing of the encircling bands of the fibre bundles, are also permanent.

II. The Chemical Basis of Skin-Swelling.

Microscopical investigations described in a previous paper [1924] show that the swelling of skin in strong acids or alkalies, is due entirely to the colloidal swelling of the collagen fibrils. Neither the keratins of the epidermis nor the elastin of the elastin fibre network becomes swollen under conditions which cause marked swelling of the collagen fibres. The absorption of water by the latter is a process parallel in many ways to its absorption by gelatin, but the fibrous structure present in skin has an important influence on the quantity absorbed.

Both gelatin and skin, when swelling in an acid or an alkaline liquid, absorb not only water but also either acid or alkali, as the case may be. An investigation has been made on the quantity of acid (or alkali) absorbed by skin when the fibrils are in various degrees of mechanical freedom, and the results have been compared with results previously obtained for gelatin. Fig. 5 shows the curve of absorption of hydrochloric acid by goat skin. The number of equivalents absorbed by 10 g. of dry skin is taken as ordinate and the reaction of the external solution as abscissa. Experimental points are shown for the absorption of acid by (i) fresh goat skin, (ii) sun-dried goat skin, (iii) sun-dried skin which had previously been treated with ammonia, or (iv) with salt solution. It has previously been shown that the water absorption of skin in these four different conditions varies considerably. Their absorption of hydrochloric acid is the same within the limit of experimental error, i.e. the mechanical freedom of the fibrils does not affect their relations towards the hydrochloric acid molecule. The curve for absorption of hydrochloric acid by gelatin (plotted from values given by Procter [1914]) is also given in Fig. 5. The resemblance between the two curves suggests that the chemical basis of the absorption of the acid is the same in the two cases. The acid absorbed is not all *chemically* combined; part is present as free acid. Fig. 5 also gives the curve of combined acid for gelatin, the values being again taken from Procter's paper. The curves of *absorbed* and *combined* acid are coincident on the scale of ordinate used in Fig. 5 up to a reaction of about p_H 2·7. After that they become increasingly divergent. Absorbed acid in a distended skin must therefore not be assumed to be all combined, except at acid reactions below p_H 2·5, where the establishment of a membrane equilibrium would result in the expulsion of most of the free acid from the system. At more acid reactions the membrane equilibrium would persist, but increasing external concentration would drive acid into the system.

The absorption of alkalies by skins follows a similar course to the absorption of acid, but in the absence of the corresponding data for gelatin, a detailed analysis of the experimental values has not been made. The absorption of alkali is not affected by the structural condition of the skin.

The actual quantity of acid (or base) combined with the skin proteins at any reaction cannot be calculated from any data at present available. In view of the mixed composition of the skin any definite value, even if obtained, would have little meaning.

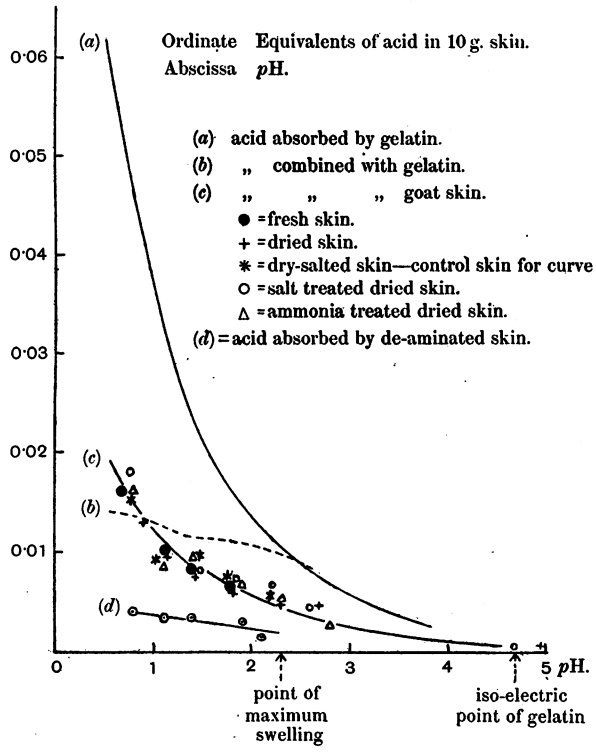

Fig. 5. The absorption of acid by gelatin and skin.

The swelling of gelatin gels which occurs both in acid and in alkaline solutions in excess of that which would occur in water only, can be attributed to the osmotic pressure of the ionised salts formed by the gelatin with the acid or alkali. Procter [1914] first explored the mathematical basis of this theory, and gave equations which are in good agreement with experimental facts. On this theory the free-NH_2 groups of the gelatin molecule serve as the points of attachment for the acid (see Jordan Lloyd, 1920, and Jordan Lloyd and Mayes, 1922).

If the swelling of gelatin in acid solutions is due to salt formation by addition at the $-NH_2$ groups, a crucial test would be to remove these and use the de-aminated gelatin as experimental material. A quantity of gelatin

was de-aminated by the method of Blasel and Matula [1914]. After dialysis for three weeks against distilled water the precipitate was dissolved in a small volume of warm water, re-precipitated with alcohol, dried with alcohol and ether and stored in a vacuum desiccator. This de-aminated gelatin in powder form was submitted to the full range of acid and alkaline reaction. The volume occupied by unit weight of the powder was measured and compared with that occupied by unit weight of gelatin powder of the same average diameter. De-aminated gelatin is very soluble in dilute alkalies, but in all the alkaline solutions the volume occupied by both the de-aminated and the control powders was the same within the limit of the experimental error. This shows that de-amination has not affected swelling in alkali. In the acid solutions, the swelling of the de-aminated gelatin was about half that of the control. The measurement of swelling by the volume changes of powders is not very accurate and all that can be safely deduced from this experiment is that de-amination reduces the power of swelling in an acid solution.

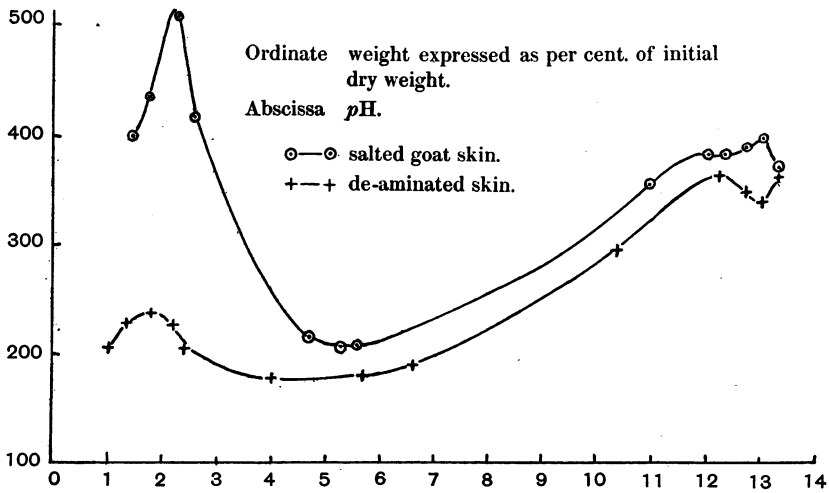

Fig. 6. The effect of de-amination on skin swelling.

It has been shown that the curves of increase of weight plotted against reaction are closely parallel in gelatin and in skin. A piece of dried goat skin[1] was therefore treated by the same method used to prepare de-aminated gelatin. It was dried and the swelling curve was taken for both acid and alkaline solutions. This is shown graphically in Fig. 6, together with the curve for the untreated control. It will be seen that in the de-aminated skin there is very little swelling in acid solutions. This is not due to any alteration in the physical state of the skin fibres, since the two curves of alkaline swelling lie very close together. It is impossible at the moment to say whether the slight swelling that occurs between p_H 3 and p_H 1 is due to incomplete de-amination or whether it is due to an entirely separate swelling mechanism. Separate fibres were

[1] This material was kindly prepared for us by Dr E. W. Merry.

teased out from the edge of the skin and the change in volume examined under the microscope, using distilled water, sodium hydroxide solutions (0·05 *N* and 0·02 *N*) and hydrochloric acid solutions (0·01 *N* and 0·002 *N*). The results are given in Table VII, together with figures for dried goat fibres as controls. It can be seen that in the alkaline solutions the swelling of both kinds of fibre is of a similar order, whereas in acid solutions the swelling of the de-aminated fibres is not markedly in excess of that obtained in the distilled water.

Table VII. *Swelling figures for fibres.*

	(A) From de-aminated dried goat skin			(B) From dried goat skin		
Solution	Change in length from 100 to	Change in breadth from 100 to	Change in volume from 100 to	Change in length from 100 to	Change in breadth from 100 to	Change in volume from 100 to
Distilled water	102	138	194	116	115	153
NaOH 0·05 *N*	109	211	485	90	182	298
NaOH 0·02 *N*	97	201	392	75	246	454
HCl 0·01 *N*	101	162	265	70	298	624
HCl 0·002 *N*	104	140	204	67	416	1160

The curve of absorption of hydrochloric acid by de-aminated skin is given in Fig. 5, curve (*d*). It lies considerably nearer to the horizontal axis than that of the unde-aminated control (*c*). This fact proves that some of the acid is combined at those free amino-groups which can be removed by nitrous acid. The curve of absorption of alkali is the same in the de-aminated material as the control, within the experimental error of the method of measurement. Recently Hitchcock (1923) has shown that de-amination by nitrous acid reduces the power of gelatin to combine with acid by about one-half. This corresponds closely with the results obtained for skin, which are summarised in curves (*c*) and (*d*) in Fig. 5. At p_H 2 the absorption of acid by the skin is reduced by de-amination to about a half. At more acid reactions the reduction is still greater, but if the curves (*a*) and (*b*) for acid absorbed and combined by gelatin are compared with (*c*) the curve of acid absorbed by skin, and (*d*) the curve of acid absorbed by de-aminated skin, it seems most likely that the rise in curve (*c*) at reactions more acid than p_H 2 is due to acid which is absorbed only but not combined. Curve (*d*) does not show this later rapid rise, and therefore the relations of the two curves at or near p_H 2 would seem to indicate the true relations of the combined acid in the two systems. At this reaction, judging from curves (*a*) and (*b*) the greater part of the absorbed acid is combined with the protein, and the value for the de-aminated skin is about half that for the unde-aminated.

The experiments given above show that the absorption of water, acid and alkali follows similar lines in a system such as a gelatin gel and a tissue of collagen fibres such as skin. The colloidal swelling in both cases is due to the combination of an ampholyte with either acid or base, with the production of ionisable salts which draw water into the system by an osmotic force. With

both gelatin and collagen, combination with acid, and acid swelling are shown to depend largely (but not entirely) on the presence of free amino-groups which can be removed by means of nitrous acid. The combination of the protein with the acid (or base) is independent of the histological structure, if any, of the system, i.e. the chemical equilibrium is independent of histological structure within the error of the experiment. On the other hand, the absorption of water as the result of osmotically active protein salts is undoubtedly largely influenced by the mechanical condition of the system.

We should like to take this opportunity of thanking the Director and Council of the British Leather Manufacturers' Research Association for permission to publish this work.

Summary.

1. The fibrils and fibres of skin are embedded in a matrix, the interfibrillary fluid, the properties of which closely resemble those of the plasma proteins.

2. In skins which have been allowed to dry under conditions which lead to the coagulation of albumin, such as at a temperature above blood heat, in a strong light, in the presence of alcohol at room temperature, the interfibrillary proteins form a non-swelling, insoluble, non-elastic deposit round the fibres and the water absorbing power of the skins is reduced. Such skins do not soften when soaked in water.

Substances which disperse or destroy denaturated albumin, such as sodium citrate or trypsin, remove this coagulated interfibrillary matter, and skins so treated have a greater capacity for water absorption, though this is not restored to the level found in fresh skins.

3. A fresh skin has a greater capacity for absorbing water than a skin which has been dried even when dehydration has been carried out by means of alchohol at 0°.

The water absorbing power of skins which have been dried at 37° or less, can be brought near to the level of that of fresh skins by treatment with trypsin. The drying therefore has not affected the fibrils themselves so much as the interfibrillary proteins.

4. The colloidal swelling of skins is due to the network of skin-fibres. These contain all the collagen found in the skin. The swelling of skin in acid and in alkaline solutions is due to the formation of ionisable salts of collagen.

The absorption of acid or alkali by skins is not influenced by the structure.

5. Swelling in acid solutions is shown to be due to the presence of free amino-groups in the skin. Removal of these groups reduces the amount of acid both absorbed and combined. Near the reaction of maximum swelling, where the absorbed acid is almost all combined, the reduction in the amount of acid absorbed brought about by de-amination is about one-half.

REFERENCES.

Blasel and Matula (1914). *Biochem. Z.* **58**, 417.
Chick and Martin (1910). *J. Physiol.* **40**, 404.
—— —— (1911). *J. Physiol.* **43**, 1.
—— —— (1912, 1). *J. Physiol.* **45**, 61.
—— —— (1912, 2). *J. Physiol.* **45**, 261.
Eitner (1880). *Der Gerber*, 112.
Hardy and Gardiner (1910). *J. Physiol.* **40**, *Proc.* lxviii.
Harris (1923). *Proc. Roy. Soc. London*, A, **94**, 246.
Hartley, Eagleton and Okell (1923). *J. Path. Bact.* **26**, 53.
Hitchcock (1923). *J. Gen. Physiol.* **6**, 95.
Kaye and Jordan Lloyd (1924). *Proc. Roy. Soc. London*, B, **96**, 293.
Jordan Lloyd (1920). *Biochem. J.* **14**, 147.
Jordan Lloyd and Mayes (1922). *Proc. Roy. Soc. London*, B, **93**, 69.
Mclaughlin and Theis (1923). *J. Amer. Chem. Ass.* **18**, 324.
Michaelis and Rona (1910). *Biochem. Z.* **27**, 38.
Procter (1914). *J. Chem. Soc.* **105**, 313.
Rosenthal (1916). *J. Amer. Leather Chem. Ass.* **11**, 463.
Young (1922). *Proc. Roy. Soc. London*, B, **93**, 235.