OBSERVATIONS ON UREA-EXTRACTABLE SUBSTANCE OF THE HUMAN ABDOMINAL EPIDERMIS*

A. GEDEON MATOLTSY,† M.D. AND FRANZ S. M. HERBST, M.D.

I. INTRODUCTION

Rudall (1) reported that primary solution of epidermal proteins of the epidermis of the cow's nose is readily achieved by urea solutions. Some dispersal of the proteins took place in 1 M urea, but really effective solution was obtained only in the stronger concentrations up to 6 M urea. By the use of 6 M urea Rudall extracted from the epidermis of the cow's nose a fibrous and a non-fibrous protein. The fibrous protein was called "epidermin" and characterized as a protein of the keratin-myosin-fibrinogen group. It was precipitated at pH 5.5, found to contain 0.8 to 1.08 per cent sulfur, but no phosphorus. Films of epidermin showed an α-type of x-ray diffraction pattern. The non-fibrous protein was precipitated at pH 4.5, found to contain 1.12 to 2.86 per cent sulfur, and exhibited a β-type of x-ray diffraction pattern. Rudall found that both these proteins were present in the outer, middle, and inner portions of the malpighian layer. In a recent publication Carruthers, Woernley, Baumler, and Kress (2) reported that Rudall's proteins readily combine with detergents and form several complexes, indicating that these urea-extractable proteins are not simple structures.

In a previous work (3) we found that about 25 per cent of the proteins of the human abdominal epidermis is readily extractable with common solvents of proteins, such as (1) neutral phosphate buffer, (2) slightly alkaline acetate buffer or (3) sodium chloride in neutral phosphate buffer. Paper-electrophoresis studies indicated the presence of two proteins in such extracts. These observations indicate that some protein components of the human abdominal epidermis are different from the proteins of the epidermis of the cow's nose because they are soluble in simple solvents that are much less drastic in action than 6 M urea.

At this time it is not known if the human epidermis also contains some specific urea-extractable protein in addition to the two soluble proteins described above. Accordingly the human abdominal epidermis has been extracted with 6 M urea, and the nature of the urea-extractable material was studied and compared with those materials which were extracted by means of the more commonly used solvents of proteins.

II. SITE AND MODE OF ACTION OF 6 M UREA ON THE HUMAN ABDOMINAL EPIDERMIS

Two specimens of human abdominal skin were obtained 4 to 6 hours after death. The ages of the donors were 22 and 54 years respectively. Small pieces

* From the Department of Dermatology, Harvard Medical School, and the Dermatological Research Laboratories, Massachusetts General Hospital, Boston 14, Massachusetts.

This investigation was supported by a research grant, RG-3921(C2), from the National Institutes of Health, Public Health Service.

Received for publication July 2, 1956.

† Present address: The Rockefeller Institute for Medical Research, New York, N. Y.

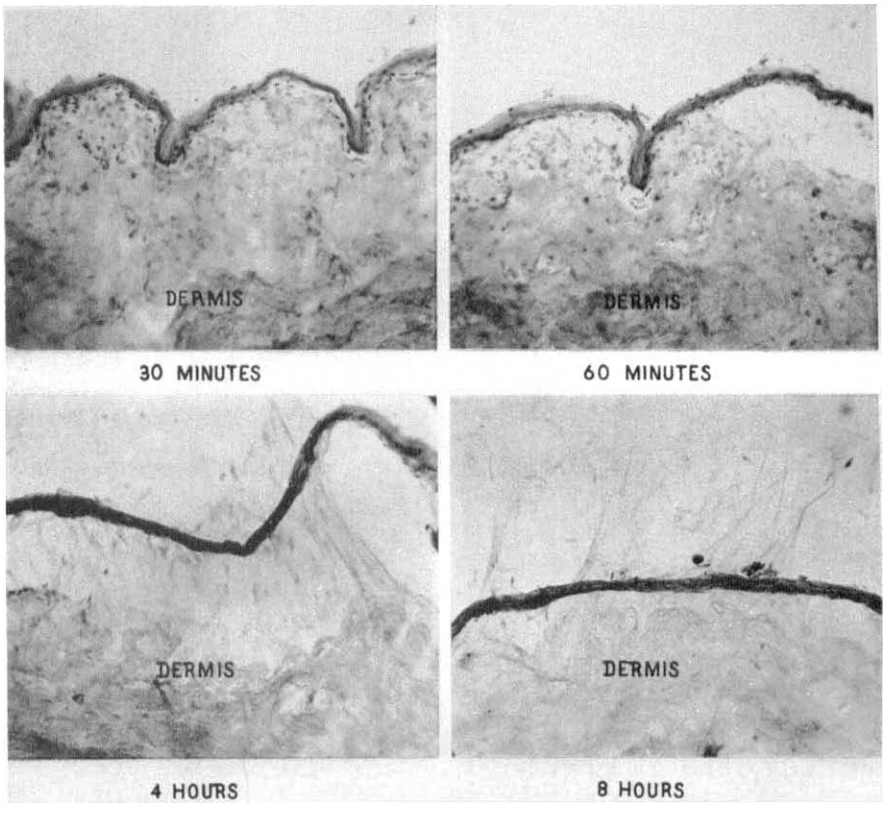

FIG. 1. Demonstration of progressive dissociation and dissolution of the malpighian layer of the human abdominal epidermis after immersion in 6 M urea solution.

of the autopsy skin were frozen at −20°C. and mounted on blocks. Cross sections about 20 to 30 μ thick were cut in the Linderström-Lang freezing cabinet. The sections were immersed in 6 M urea. After 30-minute, 60-minute, 4-hour, and 8-hour immersion periods the sections were washed in distilled water, fixed in 4 per cent formalin, and subsequently stained with hematoxylin-eosin.

The results are demonstrated in Fig. 1. Urea seemed to affect mainly the malpighian layer of the epidermis. The cells of this layer were dissociated and their contents flowed away, forming a fibrous precipitate on the slide. The cornified layer was intensely swollen but did not dissociate. Six M urea thus demonstrated its drastic action which affected chiefly the malpighian cells. Since these cells were seen to be solubilized, it is likely that it is mainly their constituents which are extracted from the epidermis by urea.

III. METHOD OF EXTRACTION OF THE EPIDERMIS

Abdominal skin from donors varying in age from 24 to 68 years was obtained 4 to 6 hours after death. The skin was stretched and attached to the surface of a

large Petri dish and incubated at 58°C. for about 10 minutes. The epidermis was then gently scraped away from the dermis, cut with scissors into small pieces, and about 100 mg. (in dry weight) was suspended in 10 ml. of each of the following solvents: (1) 6 M urea, (2) 1/15 M phosphate buffer of pH 7.2, (3) 0.1 M acetate buffer of pH 8.3, and (4) 1.0 M sodium chloride in 1/15 M phosphate buffer of pH 7.2. The epidermis was then homogenized for about 20 minutes in a glass homogenizer at about 0°C. and subsequently transferred into a 10 ml. centrifuge tube containing a few glass beads. The suspension was gently agitated by slow rotation of the tube for 48 hours in the cold room (+5°C.) and then centrifuged for 1 hour at 3,500 r.p.m. in the refrigerated centrifuge. The supernatant, containing the extracted material, was decanted.

IV. PROPERTIES OF EPIDERMAL EXTRACTS

Phosphate buffer and acetate buffer extracts of the epidermis appeared as slightly turbid solutions. On dialysis against distilled water or on acidification a precipitate did not occur. A fine globular precipitate settled on full saturation with ammonium sulfate.

Those extracts which were obtained by buffered sodium chloride or 6 M urea appeared very turbid and readily formed large flocculent precipitates upon dialysis against running distilled water as well as on full saturation with ammonium sulfate.

The precipitate of each extract could be redissolved in the same solution which was used for extraction. The precipitate of 6 M urea extract also could be redissolved in 1.0 M urea, a solution of much lower concentration. It was also noted that all extracts are very unstable and spontaneously form insoluble precipitates upon standing at room temperature or upon mechanical agitation.

V. COMPONENTS OF EPIDERMAL EXTRACTS

The components of the extracts were identified by paper electrophoresis according to the method of Grassmann and Hannig (4). The extracts were concentrated from 10 ml. volume to 1 ml. volume by evaporation in a collodion bag. The 6 M urea extract was dialyzed against 1 M urea in 0.1 M acetate buffer of pH 8.3; the other extracts were dialyzed against 1 liter of buffer solutions which were identical with those used for the extraction. Electrophoresis was carried out for 12 hours at room temperature at 110 volt E.M.F. in an Elphor apparatus using buffer solutions for each sample which were identical to those used for its dialysis. The filter paper strips were dried at 37°C. and stained with Amido Black 10 B.

The electrophoretic pattern of the urea extract is shown in Fig. 2. It can be seen that this extract contains a single component which appears in the form of a widely spread band on the filter paper. The electrophoretic pattern of the phosphate buffer, acetate buffer, and buffered sodium chloride extract shows two distinct components (Fig. 3) as has previously been described in a preliminary report (3).

These results show that whereas two components can be extracted from the

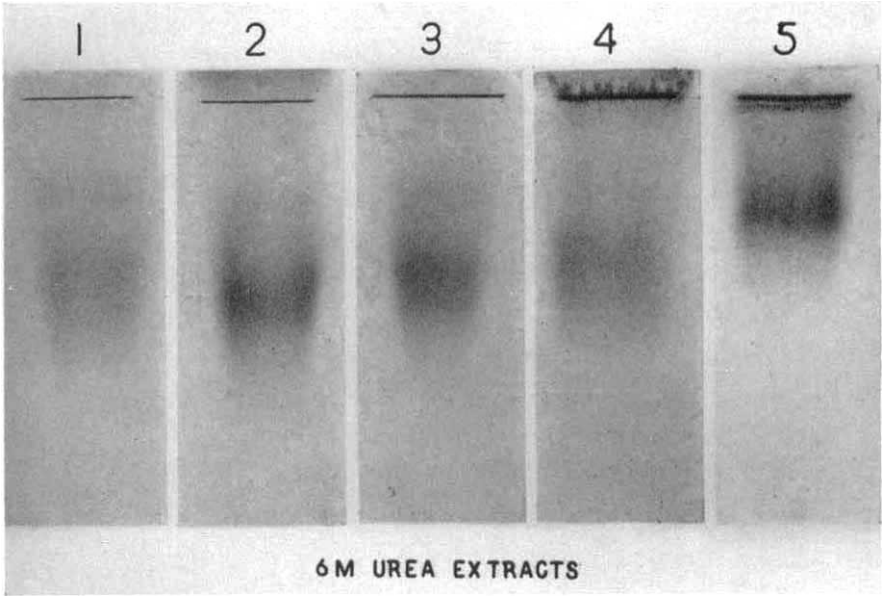

FIG. 2. The abdominal epidermis of five different donors was extracted with 6 M urea. The electrophoretic pattern of each extract shows a similar single widely spread band.

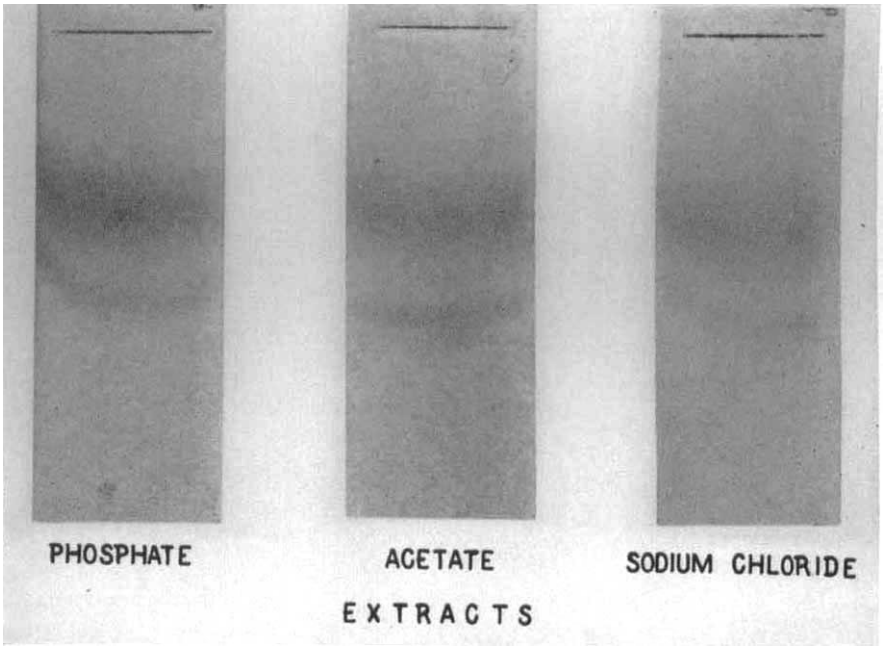

FIG. 3. Extracts of the human abdominal epidermis were obtained with phosphate buffer, acetate buffer, and buffered sodium chloride. The electrophoretic pattern of each extract shows two distinct bands.

human abdominal epidermis by the use of common solvents of proteins, only one component is extracted by 6 M urea. The question of whether or not the single component of 6 M urea extract represents a "specific urea-extractable component" arose. This problem was investigated in the subsequent experiments.

VI. THE AMOUNT OF 6 M UREA-EXTRACTABLE MATERIAL

In order to get more specific data about the material which appears in the 6 M urea extract it appeared of interest to determine the weight of total extractable material and compare it with the known values obtained by extraction with common solvents of proteins. For this purpose the isolated and homogenized abdominal epidermis was repeatedly extracted for 24-hour periods with 6 M urea until no more material passed into solution. The extracts were combined, dialyzed against running distilled water for 48 hours, then evaporated, and the remaining material dried at 110°C. to constant weight. The residue of the epidermis was washed several times in large quantities of distilled water by repeated suspension and centrifugation and finally dried in the same way as the extracted material. The percentage of 6 M urea-extractable material was calculated from the sum of the dry weight of the extracted material and of the residue. The result is shown in Table I together with other data previously obtained (3) with common solvents of proteins. The data show that the 28 per cent value obtained with 6 M urea falls within the same range as those obtained with common solvents of proteins. A close relationship in extractive power of each of these extractants can thus be seen.

VII. THE CHEMICAL NATURE OF THE EXTRACTED MATERIALS

To obtain some information about the chemical nature of the extracted materials their absorption spectra and phosphorus content were investigated. Each of the extracts was prepared and processed in the same way as for paper-electrophoresis studies. The extracts were appropriately diluted with the corresponding buffers so that readings could be made in the Beckman spectrophotometer. The ultraviolet absorption spectra of phosphate buffer, acetate buffer, buffered sodium chloride, and 6 M urea extracts are shown in Fig. 4. It can be seen that each extract exhibited a similar type of absorption curve showing a broad maximum between 260 mμ and 270 mμ wavelengths. This shows that the chemical nature of the absorbing substances in each of these extracts is identical. Since

TABLE I

Percentage of materials extractable from the isolated human abdominal epidermis with various solvents of proteins

Extractant	Percentage of Extracted Material
6 M urea	28
1/15 M phosphate buffer of pH 7.2	20
0.1 M acetate buffer of pH 8.3	25
1.0 M sodium chloride in 1/15 M phosphate buffer of pH 7.2	28

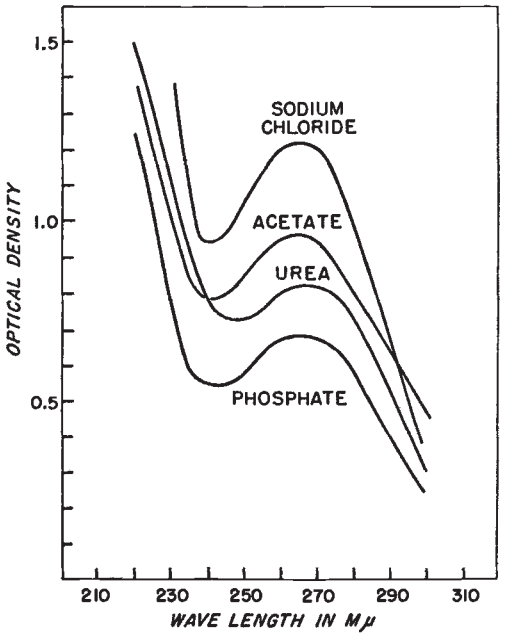

FIG. 4. Absorption spectra of buffered sodium chloride, acetate buffer, 6 M urea, and phosphate buffer extracts of the human abdominal epidermis. Each extract shows maximum absorption between 260 mμ and 270 mμ wavelengths.

it is well known that nucleic acids or nucleoproteins (purine and pyrimidine bases) show a specific absorption at about 260 mμ wavelength while simple proteins (due to their tyrosine and tryptophane content) exhibit absorption maxima at about 270 mμ wavelength, it is very likely that each of these extracts contains mixtures of these substances.

The phosphorus content of the extracted materials was determined according to the method of Fiske and Subbarow (5). The results are shown in Table II. It can be seen that the phosphorus content of the extracts is about the same.

These absorption spectroscopic studies and phosphorus determinations show that substances of similar chemical composition are extracted by both 6 M urea and common solvents of proteins, thus indicating that 6 M urea is a nonspecific extractant of human epidermal proteins.

TABLE II

Phosphorus content of substances which were extracted from the isolated human abdominal epidermis with various solvents

Extractant	Phosphorus Content in Percentage
6 M urea	0.58
1/15 M phosphate buffer of pH 7.2	0.59
0.1 M acetate buffer of pH 8.3	0.67
1.0 M sodium chloride in 1/15 M phosphate buffer of pH 7.2	0.52

VIII. SUCCESSIVE EXTRACTION STUDIES

Although the foregoing studies strongly suggest that 6 M urea did not extract a specific urea-extractable component from the human abdominal epidermis, to still further clarify the question successive extractions using 6 M urea and phosphate buffer were also undertaken and the extracts analyzed.

In one series of experiments the isolated and homogenized epidermis was first extracted for 48 hours with phosphate buffer and subsequently the residue was extracted with 6 M urea for 48 hours. Paper electrophoresis of these extracts showed that while the phosphate buffer extract contained two distinct components the electrophoretic run with the urea extract was blank. This showed that after extraction of the two soluble proteins of the human abdominal epidermis with a common solvent of proteins a further specific urea-extractable component cannot be extracted.

In another series of experiments the homogenized epidermis was first extracted with 6 M urea and subsequently the remaining material was extracted with phosphate buffer. The 6 M urea extract showed the same previously observed widely spread component (see Fig. 2) while the phosphate buffer extract showed no components. This indicated that after extraction of the epidermis with 6 M urea no more material would pass into solution from the residue on subsequent extraction with a common solvent of proteins.

Thus these successive extraction studies further demonstrate that 6 M urea is not a specific extractant of human epidermal proteins and that a specific urea-extractable substance does not occur in a detectable quantity in the human abdominal epidermis.

IX. DISCUSSION

The present study showed that approximately the same amount of material could be extracted from the human abdominal epidermis by the use of common solvents of proteins as could be extracted by 6 M urea. It was also observed that the extracted materials had a very similar chemical composition. These observations strongly suggest that 6 M urea is not a specific extractant of human epidermal proteins; i.e., identical substances are extractable from the human abdominal epidermis by the strong urea solution and by the common solvents of proteins. A significant difference between the extracted substances appeared to be only in the electrophoretic behavior. While the material in the urea extract exhibited only a single electrophoretic component, the material in the extract obtained with common solvents revealed two distinct components.

It is well known that strong solutions of urea can cause many basic changes in native proteins. It has been observed that protein molecules may become depolymerized, that hydrogen bonds between adjacent polypeptide chains can be split, and that the chains may be unfolded. Increase in intrinsic viscosity has also been noted. Most proteins are known to become denatured and their isoelectric point shifted (6).

Because of these marked effects of urea it is possible that during the extraction

procedure the native human epidermal proteins could have formed a protein complex which migrates as a single component in the electric field. In connection with this idea it is interesting to recall that if the two epidermal proteins extracted with phosphate buffer were first dialyzed against 6 M urea for a 48-hour period and then dialyzed against phosphate buffer it was observed that subsequent electrophoretic study revealed only a single component. Further studies would be desirable to determine in just what way and to what degree the epidermal proteins are altered when they are exposed to the action of 6 M urea.

SUMMARY

1. The site and mode of action of 6 M urea on the human abdominal epidermis was investigated by histological studies. It was observed that chiefly the malpighian cells were affected and solubilized by urea.

2. The material extractable from isolated human abdominal epidermis with 6 M urea was found to be very similar in quantity as well as in chemical composition to the material which is extractable with common solvents of proteins.

3. In successive extraction studies with 6 M urea and common solvents of proteins a specific urea-extractable substance could not be identified in the human abdominal epidermis.

4. It was concluded that 6 M urea is a nonspecific extractant of proteins of the human abdominal epidermis.

Acknowledgment. The authors wish to thank Mrs. Margit Matoltsy and Miss Constance A. Balsamo for their valuable assistance during the course of this study.

REFERENCES

1. Rudall, K. M.: The proteins of the mammalian epidermis. Advances in Protein Chem., **7**: 253, 1952.
2. Carruthers, C., Woernley, D. L., Baumler, A. and Kress, B.: Proteins of mammalian epidermis. J. Invest. Dermat., **25**: 89, 1955.
3. Matoltsy, A. G. and Herbst, F. S. M.: A study of human epidermal proteins. J. Invest. Dermat., **26**: 339, 1956.
4. Grassmann, W. and Hannig, K.: Ein quantitatives Verfahren zur Analyse der Serumproteine durch Papierelektrophorese. Ztschr. f. physiol. Chem., **290**: 1, 1952.
5. Fiske, C. H. and Subbarow, Y.: The colorimetric determination of phosphorus. J. Biol. Chem., **66**: 375, 1925.
6. Gortner, R. A.: Outlines of Biochemistry, pp. 444–445. (R. A. Gortner, Jr. and W. A. Gortner, editors). New York, John Wiley & Sons, Inc., 3rd edition, 1949.