TANNING OF FATTY ACID, AMINO ACID AND PROTEIN MONOLAYERS BY METAL IONS

By J. H. Schulman and M. Z. Dogan
Ernest Oppenheimer Laboratory, Dept. of Colloid Science,
University of Cambridge

Received 6th July, 1953

Fatty acid monolayers are made insoluble by metal ions only at the pH at which the basic metal ions are found. This interaction is accompanied by a large increase in the area per molecule of fatty acid and solidification of the monolayer. By a chemical analysis of these " tanned " monolayers it was shown that in the case of chromium ions and straight chain fatty acids one ion is attached to one fatty acid molecule. If the area of fatty acid is made greater by branch chain substitution a further chromium atom can be anchored into the monolayer per fatty acid molecule.

The structure of these two-dimensional solid lattices can be shown to be brought about by the interlinking between neighbouring molecules by hydrogen bonding between the hydroxyl groups attached to the basic metal ion and ketonic groups in the carboxyl group in the fatty acid. Steric conditions in the formation of these two-dimensional lattices vary with each metal ion, but the greatest possibilities are shown with chromium and copper.

Long-chain hydrocarbon amino acid monolayers behave similarly, but no tanning is observed with long-chain amine monolayers. Protein monolayers in which there is a

carboxyl group in the protein molecule such as serum albumin, but not gliadin, react in direct analogy to the fatty acids, with the basic metal ions other than copper. These monolayers expand and solidify with time effects showing that the steric factors involved in the cross-linking between the hydroxyl groups in the basic metal ions to neighbouring protein chains are important in the tanning of the protein monolayers. Copper, in direct contrast, does not react with the carboxyl group in the protein monolayer but with the imidazole group, thus tans gliadin and methaemoglobin monolayers readily but not serum albumin.

It has been shown by Wolstenholme and Schulman [1, 1a] and Thomas and Schulman [2] that metal ions such as Fe, Al, Co, Cu, Zn, Pb, interact with fatty acid and long-chain sulphate monolayers over the pH stability of the basic metal ions. Ions such as $U_2O_5^{2+}$ and Ca^{2+} form insoluble soaps in the ionized and not in the basic ionic form owing to steric hindrance both from the shape and size of the long-chain ionic compounds and from the metal ions themselves.

Thus various steric factors and pH of the solution play an important part in controlling the interaction between metal ions and ionic groups in orientated monolayers. The nature of the reaction is first an adsorption of the basic metal ion on to the carboxyl or sulphate ionic group in the monolayer and then a link between the adlineated hydroxyl groups by hydrogen bonding to build up a two-dimensional solid lattice. On this structure the long-chain hydrocarbon compounds are spaced. In contrast the simple metal soap monolayers are readily soluble. It has been further shown by Smith and Schulman [3] and Cuming and Schulman [4] that adsorption of these long chain ionic compounds from solution on to the solid surfaces of the metals or minerals composed of the metals mentioned above takes place under identical pH and stereochemical conditions as with the monolayer interactions. It is the purpose of this publication to test the analogy between the interactions of basic metal ions and monolayers of fatty acids, amino acids and different proteins with the tanning of collagen by chromium ion solutions. It has already been shown by Schulman and Rideal [5] and Cockbain and Schulman [6] that there is a direct analogy between the interaction of large molecules or polymers containing spaced phenolic or silicic acid groups and long-chain amine and protein monolayers, and vegetable tanning.

EXPERIMENTAL

METHOD.—This consisted of measuring the solidification area and the force/area curves of monolayers of the various fatty acids (straight and branch chain), stearyl tyrosine and protein such as gliadin, bovine serum albumin, insulin and methaemoglobin with the metal ions in the underlying solution at a concentration, chiefly, of M/2000. Addition of salts such as Na_2SO_4 and NaCl were added where necessary to enable a protein such as methaemoglobin to spread to a monolayer and also to discharge the amine ionic groups of the protein monolayer to overcome the positive potential barrier in order to permit the positive basic metal ions to adsorb and interact with the negative carboxyl groups.

The pH was controlled by addition of N/10 NaOH or N/10 HCl to a CO_2 solution containing the metal salt, or by sodium bicarbonate and sodium acetate. The mechanical properties of the interacted monolayers were qualitatively observed by blowing talc powder on the surface of the monolayer.

CHEMICAL ANALYSIS OF INTERACTED MONOLAYERS.—Chemical analysis was carried out on straight and branch chain fatty acid (myristic and 4 : 10-butyl decyl acetic acid) monolayers spread on chrome alum solutions in order to find out the composition of the basic chromium ion fatty acid complexes. A Perspex trough of 100 × 17 × 1 cm was used and 12 monolayers were spread and removed. After spreading a monolayer the area of solidification was recorded and the film then compressed. The monolayer was collected with a glass scoop and was dried with a filter paper before transferring it to a weighing crucible; this eliminated the inclusion of the solution in the film. It was heated in an oven to 100° C and weighed to a constant weight in a microbalance. The crucible

was then heated to redness before chromium determination in order to dispel the fatty acid.

The following procedure was adopted for the chromium analysis. The sample was fused with Na_2O_2 to convert chromium into chromate. The latter in acid solution (H_2SO_4) gave a pink colour with the reagent diphenyl carbazide. The intensity of the colour was determined on a Spekker colorimeter. This method gave reproducible results. In parallel with fatty acid monolayers, hexadecyl alcohol monolayers spread on M/2000 chrome alum and were collected to find out the quantity of chromium ions held to the monolayer by physical adsorption. There is no interaction between metal ions and alcohol monolayers. Approximately 2 mg of monolayer were collected by these methods.

A convenient method of determining the amount of fatty acid collected would be to hydrolyze the chromium soap in acid and then respread the fatty acid on the Langmuir trough and determine its area and hence its weight. This method was not used in the following determination with the chromium interactions, but was used by Thomas in his copper ion fatty acid interactions. A summary of Thomas' work will be given. He obtained a 65-70 % recovery of the amount of film spread, a result very similar to that found for the chromium experiments. The weight of the films collected was about 2 mg.

RESULTS AND DISCUSSION

INTERACTION OF M/2000 CHROME ALUM ON LONG CHAIN FATTY ACID MONOLAYERS.—It has been shown by Wolstenholme and Schulman [1, 1a] that myristic

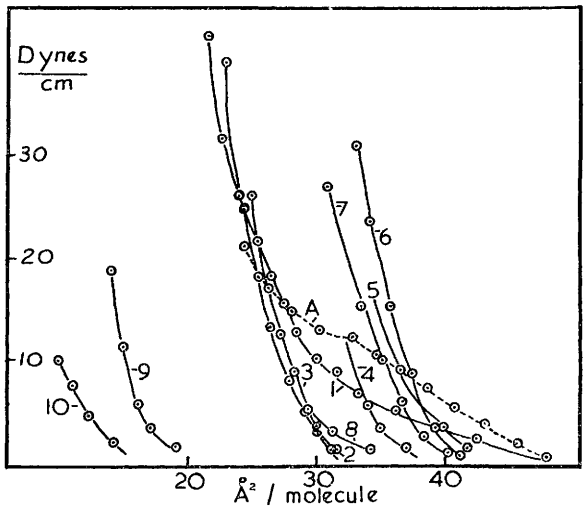

FIG. 1.

A. pH 1·971, 7·5° C
1. pH 3·25, 17·5° C
2. pH 3·72, 14° C
3. pH 4·39, 17·5° C
4. pH 4·80, 18° C
5. pH 5·40, 19° C
6. pH 5·65, 14·5° C
7. pH 5·87, 15·5° C
8. pH 6·20, 17° C
9. pH 6·42, 15·5° C
10. pH 6·73, 17° C

acid or branch chain fatty acid monolayers readily proceed to go into solution at pH 3 or greater. They are made insoluble by the formation of the basic metal soaps, steric hindrance can prevent the hydrogen bonding between the hydroxyl groups in a decreasing order of magnitude, for the following ions, Ca^{2+}, Co^{2+}, Fe^{3+}, Al^{3+}, Cr^{3+}, Cu^{3+} and thus prevent the formation of the insoluble complex.[1a]

In fig. 1, 2 and 3 the interaction of M/2000 chrome alum solutions has been shown with myristic acid, α-methyl myristic acid and butyldecylacetic acid, by a study of their force area curves at different pH. The largest area of solidication of the monolayer occurs around pH 5·3 and is 42 Å², 53 Å² and 80 Å² respectively as the size of the fatty acid molecule increases, further the pH range of solidification diminishes as the cross-sectional

area of the fatty acid is increased. Without the chromium ions in solution the liquid fatty acid films would be very soluble at pH 5·3.

This is summarized in fig. 4, showing also the variation of the area per molecule at solidification with changing pH for the different metal ions. Fig. 5 shows clearly that

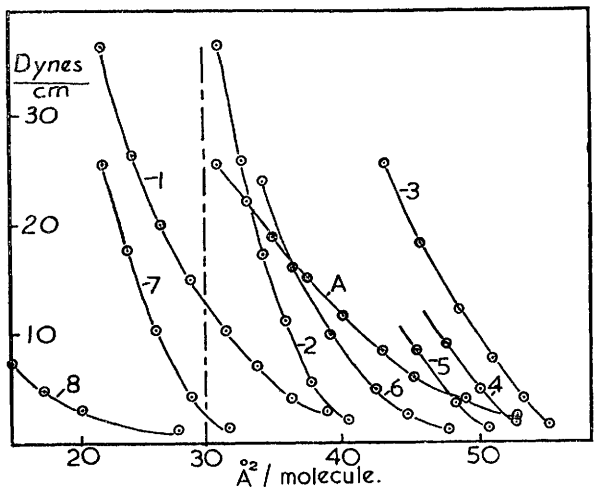

FIG. 2.—α-Methyl myristic acid and M/2000 chrome alum interaction.

1. pH 4·30, 14° C
2. pH 4·80, 14° C
3. pH 5·14, 14° C
4. pH 5·44, 15° C
5. pH 5·71, 15·2° C
6. pH 5·89, 13·8° C
7. pH 6·11, 14·3° C
8. pH 6·38, 16° C
A. pH 2·94, 16° C

at pH 5·3 for chromium ion solutions the maximum number of $Cr(OH)_2^+$ ions (calculated from the data of Pourbaix [7]) is available, this number diminishing rapidly on increasing pH. It thus could be supposed that at pH 5·3 for chromium solutions the fatty acid monolayers are solidified by the formation of a chromium monosoap molecule interlinked with its neighbours either side by the hydrogen bonding of the two hydroxyl groups

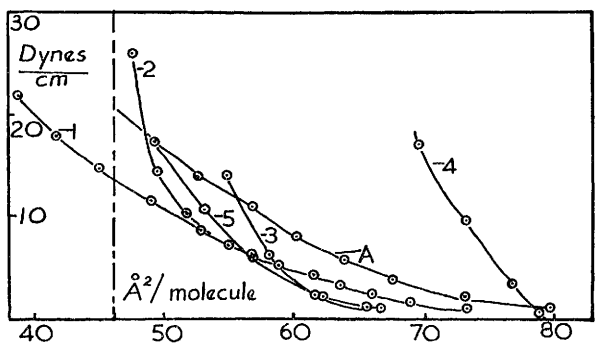

FIG. 3.—4 : 10 acid and M/2000 chrome alum interaction.

1. pH 4·72, 13° C
2. pH 5·05, 14·6° C
3. pH 5·14, 14·5° C
4. pH 5·53, 15° C
5. pH 5·86, 16·5° C
A. pH 2·05, 16° C

(see fig. 6). As the cross-sectional area of the fatty acid molecule is increased by branch-chain substitution another basic chromium ion can bridge the gap in the chain polymer.

A further cross-bridging can be envisaged by the C=O group in the carboxyl group of the fatty acid interlinking with one of the hydroxyl groups of the neighbouring molecule. A close-packed solid network consisting of linear chain polymer bonded by these two

forms of hydrogen bonding and by the van der Waals forces of the hydrocarbon chains can be seen as the structure of the two-dimensional lattices (fig. 6). This picture of the structure of the " tanned " two-dimensional solid chrome fatty acid lattice receives strong support from the chemical analysis of myristic and 4 : 10-acid monolayers spread on M/2000

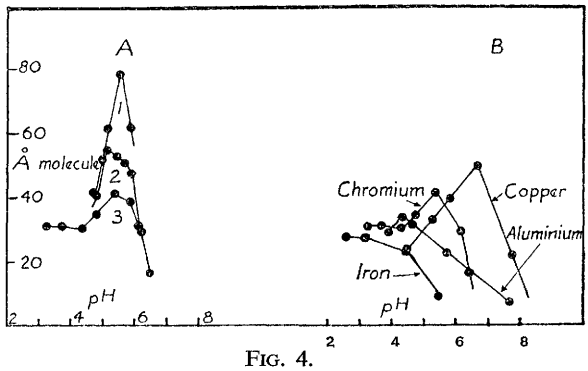

FIG. 4.

A. Effect of M/2000 chrome alum on fatty acids.

B. Effect of pH on area of solidification

1. 4 : 10 fatty acid $C_{10}H_{21}CHCOOH$ (with C_4H_9) — cross-sectional area 46 Å²/mol.
2. 1 : 12 fatty acid $C_{12}H_{25}CHCOOH$ (with CH_5) — cross-sectional area 30 Å²/mol.
3. Myristic acid $C_{12}H_{25}CH_2COOH$ — cross-sectional area 20 Å²/mol.

chrome alum solution. It will be shown in the following section that one chrome atom is analyzed for one myristic acid molecule and two chrome atoms for one butyldecyl-acetic acid molecule in the solid monolayers at pH 5·3.

CHEMICAL ANALYSIS OF THE FATTY ACID MONOLAYERS SPREAD IN M/200 CHROME ALUM. —The results of the experiment are shown in table 1. Octadecyl alcohol monolayers

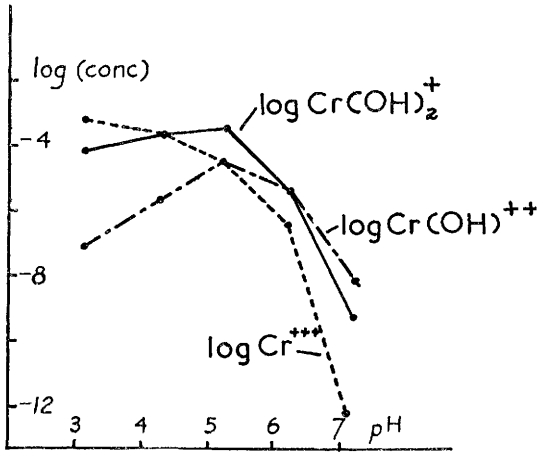

FIG. 5.—Basic ions in M/1000 chromic solution.

do not interact with metal ions. Therefore, Cr determined with these monolayers would be due to physical adsorption only. At pH 4·9 and 4·95 no Cr was found; at pH 5·35. chromium hydroxyl started to precipitate, hence Cr was included in the alcohol monolayers This physically adsorbed Cr was taken into account with the evaluation of Cr content

of the long chain fatty acid monolayers at pH 5·35. 72 % of the long-chain alcohol monolayers could be collected. The percentage recovery for myristic acid or 4 : 10-acid monolayers could not be determined directly owing to presence of Cr. Cr may be adsorbed as $Cr(OH)^{2+}$, $Cr(OH)_2^{2+}$ or $Cr(HCO_3)^{2+}$ and in addition may contain sulphate

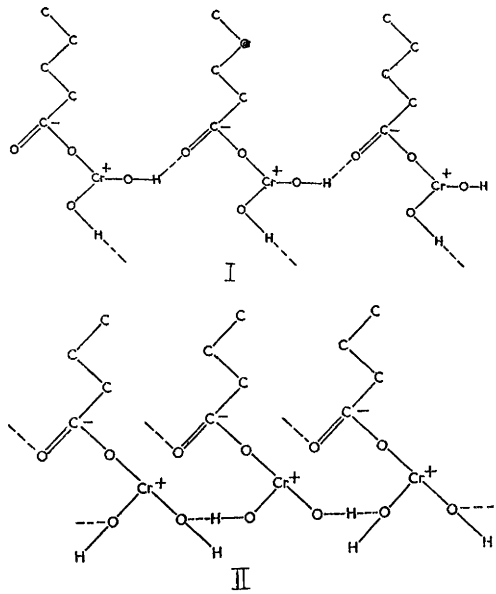

FIG. 6.—Two types of hydrogen bonding in the two dimensional tanned lattices.

TABLE 1

(*a*) myristic acid monolayers on M/2000 chrome alum

pH	amount spread (mg)	amount weighed (mg)	Cr determined in the sample (mg)	Cr/myristic acid (assume 60%R)	Cr/myristic acid (assume 70% recovery)
5·0	1·39	1·6	0·175	0·92	0·83
4·9	1·785	1·8	0·150	0·68	0·55
4·95	1·79	2·1	0·220	0·90	0·81
5·35	1·885	2·4	0·400	1·05	0·90
5·35	1·885	2·0	0·440	1·20	1·03

(*b*) 4 : 10-acid on M/2000 chrome alum

pH	amount spread (mg)	amount weighed (mg)	Cr determined in the sample (mg)	Cr/4:10 acid (assume 60% R)	Cr/4:10 acid (assume 70% R)
5·35	1·50	1·8	0·469	1·9	1·6
5·35	1·54	1·6	0·39	1·45	1·25

(*c*) octadecyl alcohol on M/2000 chrome alum

pH	amount spread (mg)	amount weighed (mg)	Cr determined in the sample (mg)	recovery %
4·9	2·68	2·0	—	72·0
4·95	2·35	1·7	—	72·5
5·35	1·885	1·7	0·16	—
5·35	1·885	1·5	0·11	—

ions from the sub-solution. Some of the fatty acid monolayers could not be recovered owing to the fact that with these systems the film tended to stick to the glass slides so high recovery could not be obtained. In working out Cr/fatty acid ratio, two recovery figures were assumed 60 % and 70 %. Thomas [2] has shown that by hydrolyzing the collected basic copper soap and respreading the fatty acid, that a 70 % recovery was obtained.

From an analysis of known and equivalent quantities of chromium salts by this analytical method, the results of the collected chromium were about 15 % too low. Corrections can be made for this, but it can be seen (table 1) that at the optimum pH for solidification 5·35 about one atom of chromium is collected for one myristic acid (area 42 Å^2) molecule in the monolayer and 2 atoms of chromium for the branch chain fatty acid molecule (area 80 Å^2) where another chromium dihydroxide ion is required to bridge the gap in the solid lattice produced by the substituted branch chain. The fatty acid molecules in the monolayer are only partially dissociated at pH 5·3. By formation of an insoluble basic metal soap with the available fatty acid ions the equilibrium would be changed and more fatty acid ions would become available until the whole monolayer would be in the insoluble basic metal soap form. This gives strong support to the type of structure given for the tanned fatty acid monolayers in the previous section.

INTERACTION OF STEARYL TYROSINE MONOLAYERS WITH M/2000 CHROME ALUM AND M/2000 COPPER SULPHATE IN THE PRESENCE OF M/100 SODIUM SULPHATE.—Fig. 7 shows the stearyl tyrosine monolayer on M/100 Na_2SO_4 solution at different pH's. The presence of Na_2SO_4 is necessary since the positive basic metal ions cannot approach the zwitter

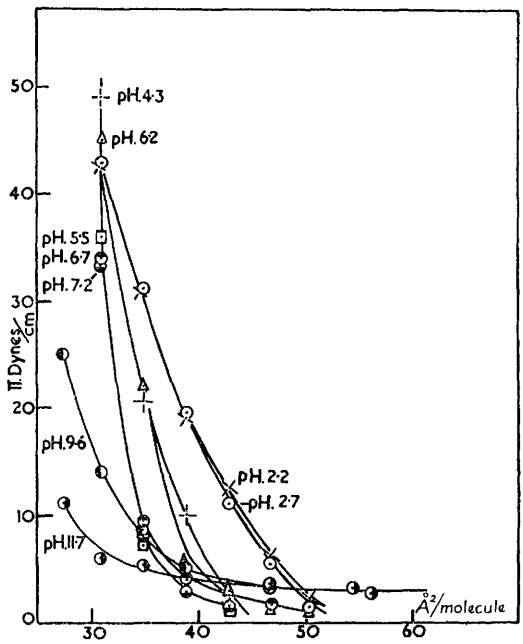

FIG. 7.—Stearyl tyrosine monolayers on M/100 Na_2SO_4 solution.

ion monolayer in the presence of the positive amine group. The sulphate ion removes the positive potential barrier. This becomes very important in the case of the proteins. Fig. 8 and 9 show the effect of chromium ions and copper ions in the underlying solution. Here again the reaction is similar to those already seen for the fatty acid monolayers. The films become tanned and solid at very increased areas per amino acid molecule at the pH of the basic metal ion formation, 5·25 for chromium and 6·26 for copper.

The striking interaction of the copper ions with the amino acid monolayers is interesting in view of the experiments in the following section. These show that the copper ions do not interact with the carboxyl groups in protein monolayers.

The metal ions chromium and copper as acetate solutions show no interaction with long-chain amine monolayers. Complexes can only be formed with the amine group in its associated form, and the metal ions are not in solution but precipitated at the alkaline pH necessary.

THE INTERACTION OF MONOLAYERS OF PROTEINS WITH METAL IONS Cr, Cu, Fe and Al. —*Bovine serum albumin monolayers.*—The serum albumin was dissolved in distilled water, 1 mg/ml, containing 1 % ethanol as a spreader. The protein monolayer spread quickly to consistent values on distilled water or buffers. They were spread on the chromium

ion solutions at different pH's and it immediately became evident that whereas the interaction and solidification were immediate on fatty acid and amine acid monolayers time effects were observed with the protein monolayers. The spreading values became consistent after 1 h. This time effect may be due to steric factors in the linking of the various carboxyl groups in the protein molecule chains orientated at the surface, similar to the tanning of the branch chain fatty acids, by the basic chromium ion bridges.

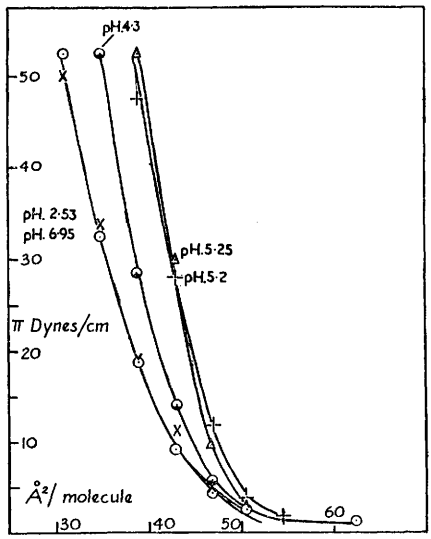

FIG. 8. — Stearyl tyrosine monolayers on M/2000 chrome alum M/100 Na_2SO_4.

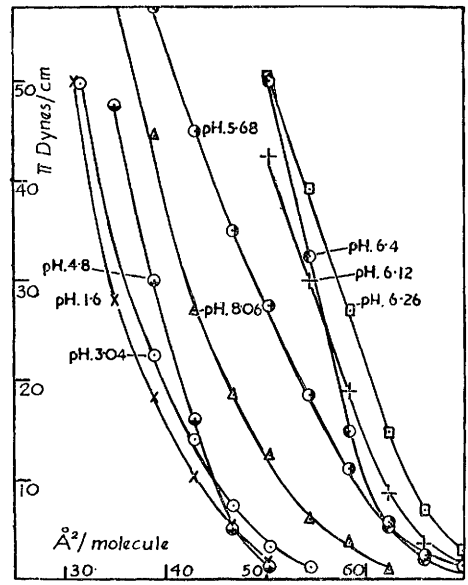

FIG. 9.—Stearyl tyrosine monolayers on M/2000 $CuSO_4$, M/100 Na_2SO_4.

FIG. 10.—Bovine albumin films.

A. pH 3·8, M/100 $Na_2SO_4 10H_2O$ 18° C
1. pH 2·1, M/2000 chrome alum 18° C
2. pH 3·7, „ „ „ 20° C 1 h.
3. pH 4·21, „ „ „ 17·5° C 1 h.
4. pH 4·76, „ „ „ 18·5° C 1 h.
5. pH 5·26, „ „ „ 18·5° C 1 h.
6. pH 5·71, „ „ „ 17·5° C 1 h.
7. pH 6·07 „ „ „ 18·5° C 1 h.

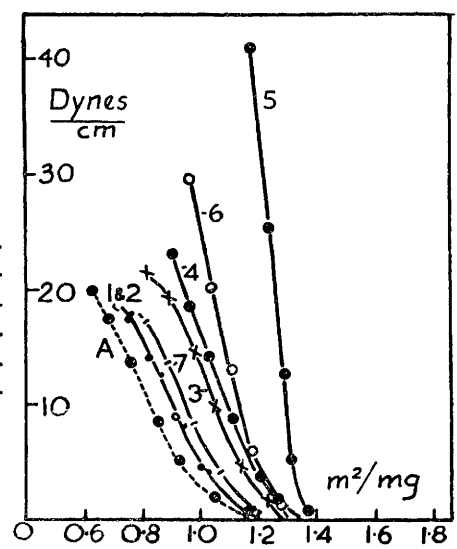

Fig. 10 shows quite clearly that the optimum expansion and solidification of the protein monolayer takes place at pH 5·26, which is identical with the tanning pH of fatty acid monolayers. Curve 5 shows a tanned serum albumin film without visco-elastic properties, rigid and incompressible standing very high pressures before collapsing.

The surface potential against area (m^2/mg) curve shows likewise that the structure is a solid in which no reorientation of the polar groups takes place on compression, the surface potential remains constant at 200 mV (curve 5). At pH 2 the surface potential changes from 150 mV to 350 mV on compression over curve 1 showing no tanning, and changes from 200 mV to 300 mV at pH 4·8 for curve 4, showing partial tanning.

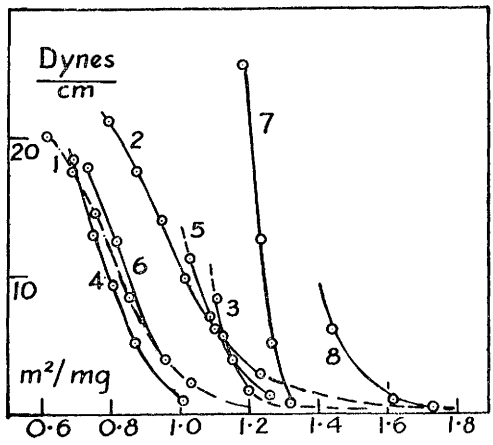

FIG. 11.—Bovine albumin films.

1. pH 3·8: M/100 Na_2SO_4, 21° C.
2. pH 2·38: M/100 ferric chloride, 21° C (2 h).
3. pH 2·86: M/100 ferric chloride, 21° C (1½ h); M/10 Na_2SO_4 . 10 H_2O.
4. pH 4·2: M/100 $AlCl_3$. 6 H_2O, 21° C (1 h).
5. pH 4·4: M/100 $AlCl_3$. 6 H_2O, 20·5° C (1 h); M/10 Na_2SO_4 . 10 H_2O.
6. pH 5·52: M/100 $CuSO_4$. 5 H_2O, 20° C.
7. pH 5·26: M/2000 chrome alum, 18·5° C (1 h).
8. pH 7·15: phosphate buffer; tannic acid 80 mg/l.; rigid unstable film after 1½ h.

Fig. 11 shows that again by analogy with the fatty acid tanning other metal ions such as Fe and Al only tan the serum albumin protein monolayer at the pH at which the basic metal ions are formed, pH 2·8 and pH 4·2 respectively, enabling the hydroxyl cross-bridges to be built in the tanned two-dimensional lattice. It can be observed that tanning only takes place in the Na_2SO_4 solution, which overcomes the positive potential barrier of the amine groups in the protein monolayer.

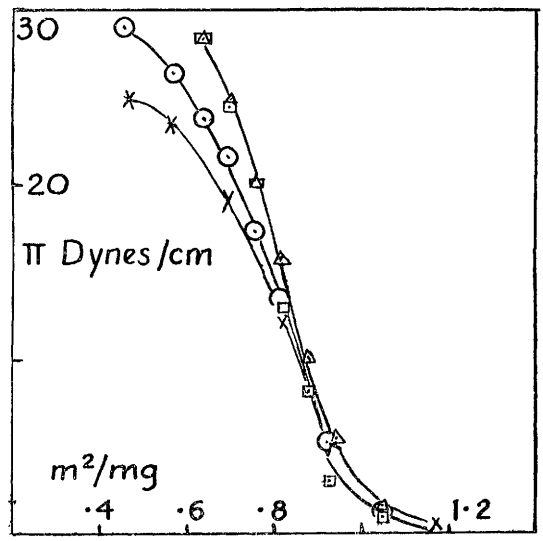

FIG. 12.—Serum albumin monolayers on M/2000 $CuSO_4$. 5 H_2O; M/100 Na_2SO . 10 H_2O_4 after 1 h.

× pH 3·81, sat. CO_2
△ pH 5·42, sat. CO_2
⊡ pH 6·57, sat. CO_2
⊙ pH 7·24, sat. CO_2.

In fig. 11 as comparison, both the tanning by chromium ions and that given by tannic acid are shown (curves 7, 8). The spaced phenolic groups in the tannic acid molecule, as described by Schulman and Cockbain,[6] bridge across the amine groups in the monolayer and this expands and rigidifies the protein monolayer by analogy with the immobilizing of the carboxyl groups by the basic metal ionic lattices.

Fig. 12 also shows the astonishing result that copper ions have no effect on serum albumin monolayers. The reason for this is not clear since copper solutes at the basic metal ion pH of 6·3 tan fatty acid and amino acid monolayers very readily, expanding these films to large areas per molecule. It may be that the basic copper ion can only

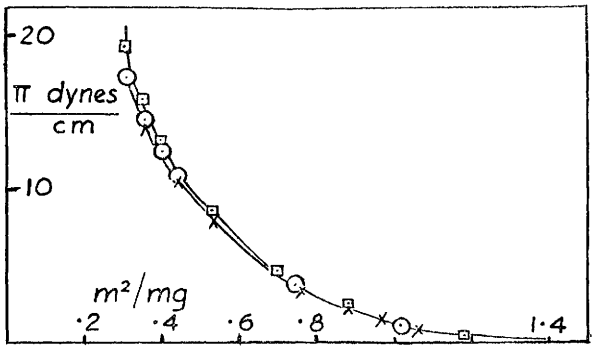

FIG. 13.—Gliadin on chrome alum. M/2000 chrome alum, M/100 Na_2SO_4; (sat. CO_2, pH 5·5).

× after 10 min; ⊙ after 30 min; ⊡ after 60 min.

exist in the monohydroxide form, whereas all the metal ions which tan, such as Fe, Al, Cr, can exist in the dihydroxide form and can thus have other dimensions to form cross-linkages with the carboxyl groups in the serum albumin monolayer. This is much more evident when a protamine such as gliadin is used.

Gliadin monolayers.—These monolayers were spread from 60 % ethanol solutions and it can be seen from fig. 13 that chromium ions have no effect on the gliadin monolayer; it is known that there are very few carboxyl groups available in gliadin. On the other hand, there are a large number of amine groups in gliadin with which chromium ions as

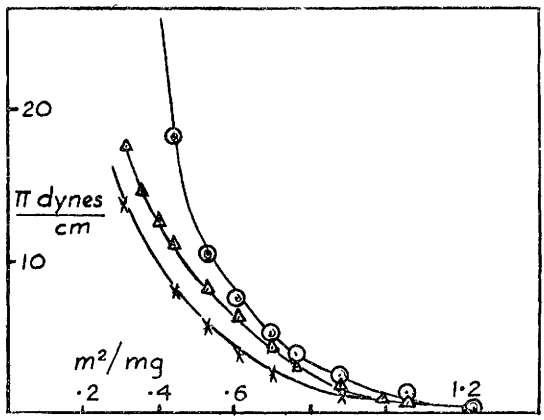

FIG. 14.—Gliadin monolayers.

△ pH 5·79 M/100 Na_2SO_4 10 H_2O (1 h).
⊙ pH 6·6 (after 1 h) subsolution,
M/2000 $CuSO_4$ 5 H_2O,
M/100 Na_2SO_4 10 H_2O.
× on distilled water.

seen from their inactivity on long-chain amine films are unreactive, but copper ions appear to be able to react with the positive groups as seen in fig. 14. This is even more strikingly shown with monolayers of methaemoglobin in which there are a considerable number of imidazole (histidine) 33 groups compared to 16 in serum albumin.

Methaemoglobin monolayers.—Methaemoglobin does not spread on distilled water nor is it adsorbed from solution at the air/water interface as shown by the non-frothing of haemoglobin solutions. But methaemoglobin readily spreads on salt solutions. Copper, as can be seen from fig. 15, is very reactive on methaemoglobin but does not expand the film as do the other basic metal ions. If the protein is spread on the copper sulphate solution at the active pH range 6·15-6·7 buffered with CO_2, prevention of the spreading takes place presumably by reaction and because the isoelectric point of methaemoglobin is near that of the pH of formation of the basic copper ion, where minimum spreading could be expected. These films are strongly rigidified. Some small expansion does take place with this type of tanning if the copper solution is injected under a spread methaemoglobin film. This is due primarily to the incompressibility of the reacted monolayer and not to the expansion and solidification as found with the fatty acids or chromium ion tanning. The question of the buffer salts in relation to the copper tanning is important since $Cu(OH)_2$ precipitates at pH 5·8 and basic copper carbonate at pH 6·4. The basic copper carbonate solutions have been found by Thomas [2]

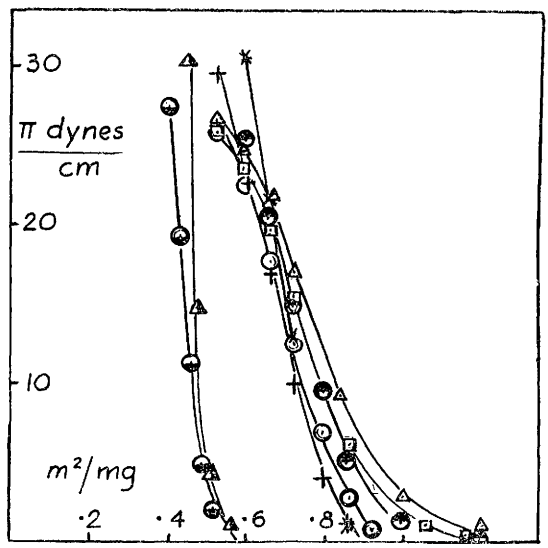

FIG. 15.—Methaemoglobin monolayers on

M/2000 $CuSO_4 . 5 H_2O$; M/100 $Na_2SO_4 . 10 H_2O$ (1 h).

△ pH 3·0 × pH 4·9 [CO_2 sat.] + pH 7·23 [CO_2 sat.]

⊡ pH 4·04 △ pH 6·15 [CO_2 sat.] ◓ pH 9·4 [CO_2 sat.]

⊙ pH 4·9 [CO_2 sat.] ◒ pH 6·71 [CO_2 sat.]

to be much more reactive than solutions buffered with sodium acetate. This does not occur with the chromium salts. Very large expansions have been found with fatty-acid tanned monolayers with copper solution buffered by CO_2 and NaOH whereas only small expansions occur when they are buffered with sodium acetate. Similar structures as shown in fig. 6 can be built up.

The reaction of copper with the histidine groups in the protein monolayer apparently has an optimum again at the basic metal ion pH of 6·1-6·7. This is very surprising since a positive amine group is being attacked instead of the carboxyl. The neutralization of the imidazole group overlaps with this pH. The reaction of copper ions with methaemoglobin is strong (see fig. 15), a reduction of 40 % of the area of the monolayer is observed at the optimum pH whereas the reaction with chromium ions is smaller in comparison (see fig. 16). This could be due to the fact that the isoelectric point, pH 6·8, of methaemoglobin is above the basic metal ion pH for chromium.

Insulin monolayers.—Insulin containing both imidazole and carboxyl groups is reacted upon by both copper ions and chromium ions at the basic metal ion pH (see fig. 17). The tanning is not so marked as with serum albumin by chromium ions.

Table 3 summarizes the basic metal ion interactions with the various protein monolayer according to the availability of carboxyl or imidazole groups in the proteins. This is compared to the bulk reaction with gelatin or collagen.

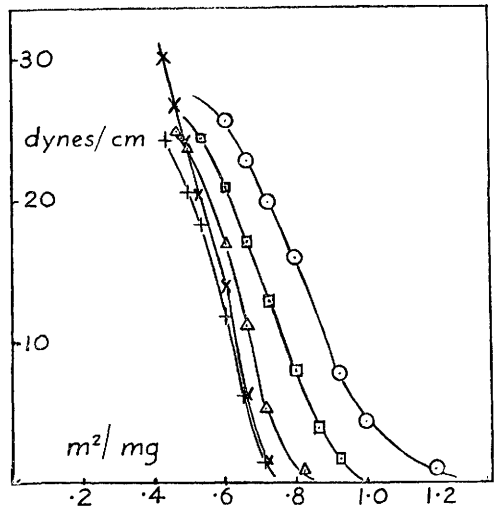

FIG. 16.—Methaemoglobin monolayers on chrome alum.
M/2000 chrome alum; M/100 Na_2SO_4 10 H_2O
⊙ pH 2·92 ⊡ pH 4·48;
× pH 5·47; △ pH 6·12;
+ pH 7·0.

COMPARISON OF MONOLAYER REACTIONS WITH THE BULK TANNING.—Elod and Schackowsky [8] have investigated the solubility of gelatin films with metal ions. The pH range of insolubility of the metal complexes with gelatin coincides closely with the pH range of interaction of the basic metal ions with fatty acid monolayers and serum albumin monolayers. The metal ions investigated are Ca^{2+},

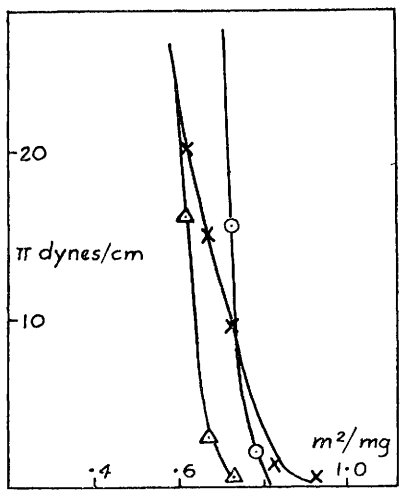

FIG. 17.—Insulin monolayers.
× pH 5·40 substrate M/100 Na_2SO_4 . 10 H_2O (sat. CO_2).
△ pH 6·4 (1 h) substrate M/1000 $CUSO_4$ 5 H_2O M/100 Na_2SO_4 10 H_2O (sat. CO_2)
⊙ pH 5·14 substrate M/2000 chrome alum (sat. CO_2) M/100 Na_2SO_4.

$U_2O_5^{2+}$, Fe^{3+}, Al^{3+}, Cr^{3+}. It has been shown by Thomas and Schulman [3] that $U_2O_5^{2+}$ does not interact with fatty acid monolayers at the basic metal ion pH owing to steric considerations but because of the size of the $U_2O_5^{2+}$ ion insoluble soaps can be formed, in contrast to the other metal soaps. The pH of interaction of gelatin with UO_2^{2+} and fatty acid monolayers is identical (see table 2). This table can be directly compared with fig. 4.

TABLE 2

metal	pH of optimum insolubility of gelatin film
$Ca(NO_3)_2$	7·5-9·0
$UO_2(NO_3)_2$	4·1-5·0
$Th(NO_3)_2$	3·5-4·0
$FeNH_4(SO_4)_2$	2·3-3·0
$Al_2(SO_4)_3$	4·0-6·0
$Cr(NO_3)_2$	4·5-5·0
$CrCl_3$	4·5-5·0
$Cr_2(SO_4)_3$	3·5-4·0

Further, there is direct evidence in bulk reactions that copper ions do not interact with the carboxyl groups but with the imidazole groups.[9] There is much evidence that the carboxyl groups in collagen are responsible for the effect of chromium ions on its solubility, hydrolysis by trypsin and its thermal properties and that this is due to the bridging of the carboxyl groups in the protein by the basic metal chromium ion lattices.

TABLE 3.—METAL ION PROTEIN MONOLAYER INTERACTION

	Fe	Al	Cr	Cu
long chain tyrosine			+++	+++
gliadin			0	++
insulin			++	++
methaemoglobin			+	+++
bovine serum albumin	+++	+++	+++	0
gelatin (bulk)	+	++	+++	+

One of us, M. Z. Dogan, is indebted to the Mining Research Institute of Turkey for a personal grant.

1 Wolstenholme and Schulman, *Trans. Faraday Soc.*, 1950, **46,** 475.
1a Wolstenholme and Schulman, *Trans. Faraday Soc.*, 1951, **47,** 788.
2 Thomas and Schulman (unpublished), J. G. N. Thomas, *Diss.* (Cambridge, 1953).
3 Schulman and Smith, *Kolloid-Z.*, 1952, **126,** 20.
4 Cuming and Schulman, *Symp. Mineral Dressing* (Inst. Min. Met., 1952).
5 Schulman and Rideal, *Proc. Roy. Soc. B,* 1937, **122,** 29.
6 Cockbain and Schulman, *Trans. Faraday Soc.*, 1939, **35,** 1.
7 Pourbaix, *Thermodynamics of Dilute Aqueous Solutions* (Edward Arnold and Co., London, 1949).
8 Elod and Schackowsky, *Kolloid-Z.*, 1935, **72,** 221; *Kolloid-Z., Beih.*, 1939, **31,** 1.
9 Tanford, *J. Amer. Chem. Soc.*, 1952, **74,** 211.
10 Gustavson, *Advances in Protein Chemistry*, 1949, **5,** 354.