THE EFFECT OF ULTRAVIOLET RADIATION ON MONOMOLECULAR FILMS OF EGG ALBUMIN*

by

J. GORDIN KAPLAN AND M. J. FRASER**

Department of Physiology, Dalhousie University, Halifax, N.S., (Canada)

Surely the most intriguing property of most soluble proteins is their ability to form a completely insoluble film one molecule thick upon being adsorbed at the interface between air and water (DEVAUX[1]). The modern approach to the experimental study of these films has largely been worked out in the laboratories of GORTER[2] (Leyden) and RIDEAL[3] (Cambridge), who have drawn many compelling biological analogies from their work[4,5].

Ultraviolet irradiation has become a very important tool in genetic and physiological studies of cell function, due to the fortunately great absorption of this energy by cellular proteins and nucleic acids. Evidence has been adduced to show that enzymes within the cell may exist in an extended condition, analogous to the configuration possessed by proteins while adsorbed at phase boundaries[6]. It thus is desirable to study in detail the effect of this nonionizing radiation on a typical protein adsorbed at the air/water interface, as a first approximation to the interfaces of lower tension existing within the living cell.

EXPERIMENTAL

Materials and apparatus

Two preparations of recrystallized egg albumin (furnished by Worthington Biochemicals, USA) were used throughout these experiments. Pyrex redistilled water and MCILVAINE's phosphate-citric acid buffer were employed. Surface pressure measurements were obtained with a commercially available Langmuir-Adam type apparatus (Central Scientific Co., U.S.A.), which measures the one-dimensional "pressure" or "force" exerted by a monolayer against a mica float. For the sake of greater precision, the platinum ribbons attached to the float were replaced by waxed nylon threads; the possibility of leakage of the film past the threads was checked with talc. Surface pressure is measured in units of dynes per cm, and is therefore dimensionally a tension; it was measured to approximately $\pm$ 0.3 dynes/cm, which was sufficiently precise for the purpose of these experiments. At least 2 minutes were allowed to elapse between change of barrier position and determination of surface pressure.

The trough was filled with a substratum consisting either of distilled water buffered (one part in 10) at pH 4.8 to 4.9, the isoelectric zone of this protein, or of 0.1 *M* NaCl, buffered at the same pH. Egg albumin is known as a comparatively difficult protein to spread[7], and larger films were obtained on the salt substratum. A 0.01 % protein solution was delivered to the surface from an "agla" micrometer syringe (either 0.50 or 0.75 ml); the method of NEURATH[8], in which the tip of the needle is kept from 3–5 mm above the surface, allowing the formed drops to fall to the substratum, was found

* This work was made possible by grants to the senior author from the National Research Council (Canada) and the Defence Research Board (Canada).

** Summer fellow of the National Research Council (Canada).

References p. 596.

to encourage more complete spreading, or more exactly, to yield films of larger area. The crystalline protein was dissolved either in dilute buffer or in distilled water without difference in results. A minimum of 20 minutes was allowed to elapse between spreading the film and beginning an experiment. No attempt was made to regulate the temperature.

Irradiation technique

The ultraviolet source was an ordinary low pressure mercury vapour tube, delivering approximately 85% of its energy at the 254 mμ line; it was mounted, six inches from the trough surface, in a metal housing which completely covered the area to be irradiated. After a film was allowed to spread unconfined for 20 minutes, the movable barrier was advanced until a pressure of 1 dyne/cm was registered; at this point the irradiation was begun, and its effect was followed at frequent intervals by variations in the position of the barrier required to maintain the film at constant pressure. The irradiation was continued for at least 8 hours, following which a "force-area" curve was obtained, by compressing the residual film well beyond its collapse point while noting the pressures corresponding to the observed areas. At first, control (unirradiated) films were done, where possible, on alternate days, but later another trough and surface balance* became available, and control films could be run simultaneously. The results were treated quantitatively, by combining algebraically the control and experimental points to yield a final, corrected curve (*e.g.*, Fig. 1) whose slope served as a measure of the effect of irradiation on the particular film. This treatment of the data is necessary, since the area of a film which is allowed to stand increases, due probably to progressively more complete spreading of the molecules at the surface; to minimize the danger of airborne surface-active contaminants, the control film was housed under a plexiglass cover during a run.

Analysis of film structure

The method of treating the force-area curves has been fully described elsewhere[9], and consists essentially of the following points: 1. the specific area at zero pressure, obtained by extrapolating the linear portion of the curve to the abscissa; 2. the pressure at which the curve becomes linear (linearity point); 3. the pressure at which the curve deviates from linearity in the direction of increased compressibility (collapse point); 4. the slope of the linear portion of the curve (considered, for convenience, as a positive value), which is inversely related to the compressibility of the film molecules. In addition, the presence or absence of a solid thread, or fiber (Devaux effect) was determined by examination of the completely compressed film; the use of these fibers in the study of the biological properties of proteins has been described[10]. These last four criteria are related to certain of the physico-chemical properties of the film molecules[9].

Precautions:

As a check on the absolute accuracy of our technique, force-area curves were run on an extremely pure sample of stearic acid** (melting point 69.4° C), which yielded an extrapolated area of 23.3 sq. A per molecule, which compares reasonably well to the accepted value of 24.4 sq. A per molecule reported by NUTTING AND HARKINS[11]. Other precautions consisted in waxing the trough, float and barrier with paraffin which had been thoroughly extracted with hot sulphuric acid, to remove all spreadable impurities. Whenever the same substratum was used for more than one run, a modified version of the method of cleaning the surface recommended by GUASTALLA[12], involving the use of talc and an air jet, was employed. Only the purest talc may be used, as the cosmetic variety is badly contaminated with surface-active material.

RESULTS

Films spread on distilled water

Fig. 1 represents the area changes with time of a control and an irradiated film, with the resultant addition curve. During the first quarter hour of irradiation practically no change in area of the film is noted, but soon thereafter a linear decrease is seen. After the eight hour reading, data for the force-area curves were obtained, and the presence or absence of a fiber was determined. Fig. 2 shows the force-area curve of a control film, and Fig. 3 that for a typical irradiated film. The data are summarized in Table I, and indicate that the residual films differ significantly from the controls in

* Generously made available by Dr C. C. COFFIN, of the Department of Chemistry, Dalhousie.

** Generously supplied by Dr F. A. VANDENHEUVEL of the Atlantic Fisheries Research Station, Halifax, N.S.

References p. 596.

at least five respects: 1. their extrapolated area is smaller; 2. the linearity point is higher; 3. they are less compressible (greater slope); 4. the collapse point is higher; 5. there is no fiber upon complete compression. While the ability of a protein monolayer to form a fiber does depend on the absolute amount of protein on the surface, the

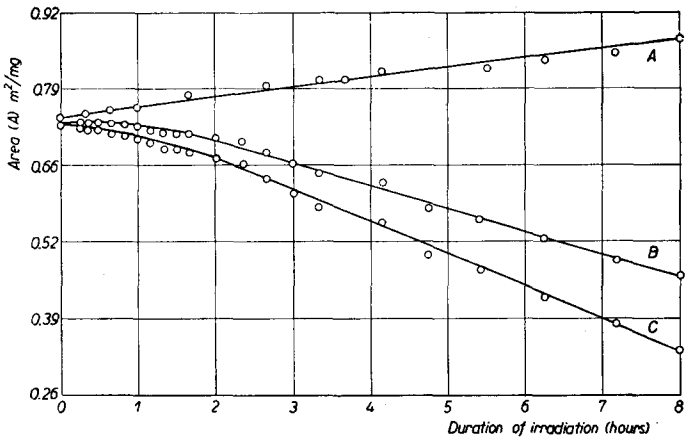

Fig. 1. Area changes with time of irradiated and control films spread on buffered distilled water. A – control film; B – irradiated film; C – corrected curve.

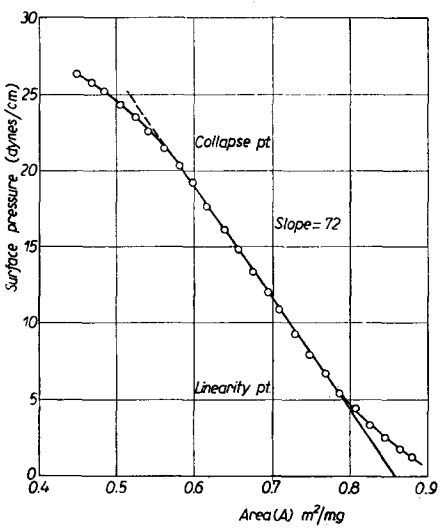

Fig. 2. Force-area curve of control film spread on buffered distilled water.

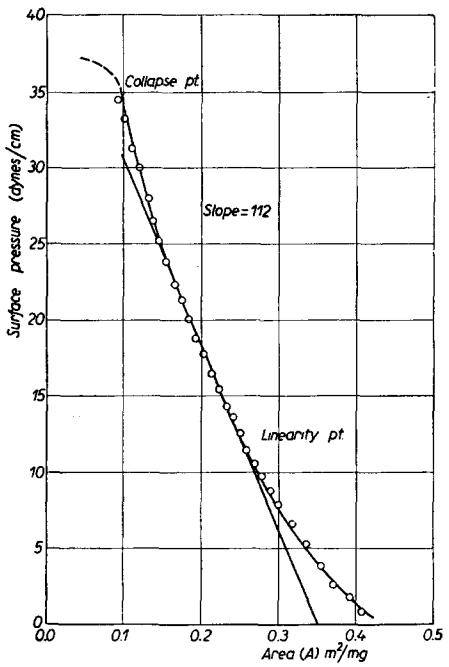

Fig. 3. Force-area curve of irradiated(8 hour) film, spread on buffered distilled water. ⟶

inability of an irradiated film to do so does not follow directly from the loss of adsorbed protein, since a film irradiated for but two hours can no longer form a fiber, whereas unirradiated films of less than half the area can be rolled up into the characteristic, visible, tangible threads previously described[9,10].

References p. 596.

TABLE I

Data for control and irradiated (8 hour) films, spread on buffered distilled water. Columns a–d from analysis of force-area curves, e from qualitative examination of fully compressed films. Standard deviations are shown; significance calculated from t test (FISHER[25]). C – control films; I – irradiated films.

	a Linearity pont (dynes/cm)		*b* Slope		*c* Collapse point (dynes/cm)		*d* Extrapolated area (m^2/mg)		*e* Fibers*	
	C	I	C	I	C	I	C	I	C	I
	6.5	12.0	96	135	25	37	0.67	0.31	++++	—
	6.5	12.0	83	138	20	33	0.68	0.29	++++	—
	3.0	7.0	59	100	20	26	0.85	0.41	++++	—
	5.0	9.5	97	112	21	40	0.63	0.36	++++	—
		10.0		97		40		0.41		—
		10.5		150		32		0.33		—
Average	5.3 ± 1.7	10.2 ± 1.8	84 ± 18	122 ± 22	22 ± 3	35 ± 5	0.71 ± 0.10	0.35 ± 0.06		
Level of significance of mean differences	0.01		0.05		0.01		0.01			

* See footnote, Table IV.

In the case of irradiated films, (see Fig. 3) a curious inflection in the direction of decreased compressibility regularly occurs at 23–26 dynes; this has never been seen in the case of a control film, and suggests that some of the more compressible fragments may be "squeezed" out of the film at higher pressures.

Films on 0.1 M NaCl

Fig. 4 represents the time course of change in area of a control and an irradiated film, and the corrected curve. While the latter is quite similar in appearance to that obtained on distilled water, there are two significant points of difference. 1. the area of the film spread on saline decreases at a much more rapid rate; 2. a definite increase in area is noted during the first fifteen minutes of irradiation, even after the correction necessitated by the expansion of the control film has been applied. This latter difference

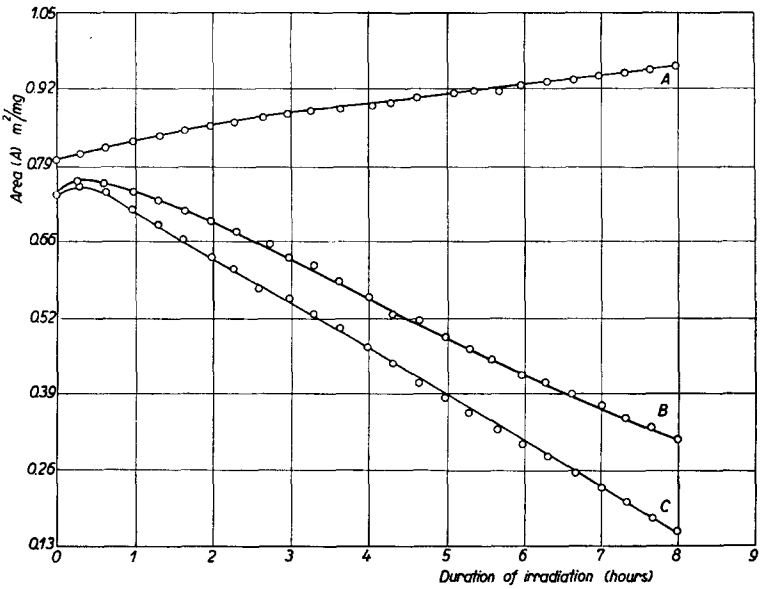

Fig. 4. Area changes with time of irradiated and control films spread on buffered 0.1 *M* NaCl. A – control film; B – irradiated film; C – corrected curve.

is clearly shown in Fig. 5, where the first part of another corrected area-time curve is seen in detail. That these differences are significant may be seen from Table II, where the rates of decrease in area of films on the two substrata are compared, and from Table III, where the corrected percentage change in area after 15 minutes irradiation is shown for both types of film.

The effect of irradiating films for differing durations is presented in Table IV. We have presented most of our force-area data in order to indicate their variability, and while due to smallness of sample size for any given period of irradiation they have not been treated statistically, certain regular changes appear from the outset. Inspection shows that the linearity points of the irradiated films are regularly higher than those of the controls, even after but 5 minutes of irradiation, and after 15 minutes the collapse points seem slightly higher as well, although a larger population would be necessary

References p. 596.

before one could state this unequivocally. On the other hand, the compressibility of the irradiated films seems to remain the same until the 5–8 hour mark. The most clear-cut differences are of a qualitative nature, that is, the aspect of the fibers after compression of the films. The controls produced robust fibers 14 cm long which could easily be lifted entire from the trough by engaging one end with a hooked needle; so sturdy were they that they could be suspended indefinitely from an overhead light, where upon drying, they were often mistaken for cobwebs occurring naturally elsewhere in the laboratory. Fibers from irradiated films, on the other hand, became increasingly fragile and short, and could not be obtained after one to two hours irradiation, even though the areas had decreased only slightly during this period.

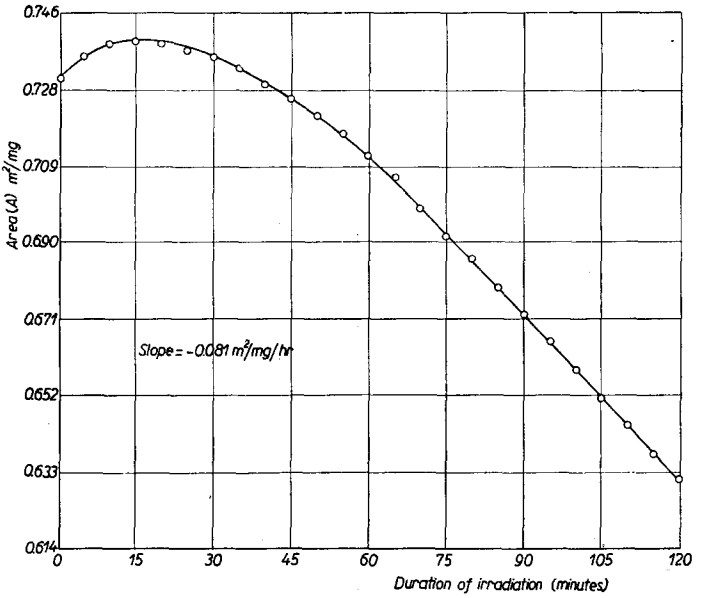

Fig. 5. Area changes with time of irradiated film spread on buffered 0.1 *M* NaCl. Corrected curve shown.

TABLE II

Rates of decrease in area of irradiated films (8 hours), corrected. Calculated as the slope of the corrected curve. 2 of the control curves in each group were combined with an additional irradiated curve, for which no special control films were run.

Spread on buffered 0.1 M NaCl			*Spread on buffered distilled water*		
Sample size		*Rates (m^2/mg/hr)*	*Sample size*		*Rates (m^2/mg/hr)*
Irradiated	10	— 0.087	Irradiated	6	— 0.044
Control	8	— 0.090	Control	4	— 0.043
		— 0.079			— 0.052
		— 0.078			— 0.051
		— 0.081			— 0.039
		— 0.091			— 0.049
		— 0.090		Average	— 0.046 ± 0.005
		— 0.087			
		— 0.079			
		— 0.078			
	Average	— 0.084 ± 0.005			

References p. 596.

TABLE III

Changes in area of films after 15 minutes irradiation at constant surface pressure. Sample sizes are the same as in Table II. Areas are given as position of movable barrier keeping films at constant pressure.

Spread on buffered 0.1 M NaCl			*Spread on buffered distilled water*		
Original area before irrad. (cm)	*Corrected area after 65 min irrad. (cm)*	*Percent change*	*Original area before irrad. (cm)*	*Corrected area after 15 min irrad. (cm)*	*Percent change*
42.4	43.9	+ 4	28.0	27.7	— 1
41.1	42.1	+ 2	28.0	28.0	0
39.2	39.7	+ 1	28.5	27.6	— 3
42.1	42.6	+ 1	39.0	38.5	— 2
39.1	40.2	+ 2	28.5	27.9	— 2
39.1	40.2	+ 3	39.1	39.2	0
42.1	42.9	+ 2		Average	— 1
43.3	44.0	+ 2			
39.8	39.6	— 1			
42.3	43.3	+ 3			
	Average	+ 2			

TABLE IV

Changes in properties with duration of irradiation of albumin films spread on buffered 0.1 *M* NaCl. Each line represents a separate film (or control and experimental pair of films). C – control, I – irradiated.

Duration (minutes)	*Extrapolated area (m^2/mg)*		*Linearity point (dynes/cm)*		*Slope*		*Collapse point (dynes/cm)*		*Fibers**	
	C	*I*	*C*	*I*	*C*	*I*	*C*	*I*	*C*	*I*
5	0.86	0.80	3	7	68	82	18	20	++++	++++
5	0.77	0.63	3	6	65	108	20	23	++++	++++
15	0.81	0.78	1	5	74	71	17	21	++++	+++
15	0.78	0.75	1	4	73	75	17	20	+++	+++
15	0.75	0.71	1	5	82	75	18	20	+++	++
15		0.83		5		72		22		++
30	0.82	0.79	1	6	74	76	18	20	+++	+
30	0.87	0.82	2	7	68	76	18	21	+++	++
30		0.81		6		77		20		++
30		0.88		6		65		23		++
30		0.87		7		65		20		++
60	0.89	0.75	4	6	81	76	22	22	++++	+
60	0.86	0.71	1	7	56	80	19	19	++++	+
60	0.79	0.73	5	7	76	82	17	24	++++	+
60		0.68		3		78		20		+
120	0.72	0.71	1	5	85	73	25	25	+++	—
120	0.82	0.70	3	5	72	77	21	24	++++	—
120		0.76		5		70		25		—
300	0.89	0.45	2	4	65	80	16	18	++++	—
300	0.87	0.58	3	9	74	77	22	34	++++	—
480	0.86	0.28	5	10	72	158	20	32	++++	—
480	0.93	0.32	4	5	64	115	21	20	++++	—
480	0.92	0.34	4	6	63	119	19	26	++++	—

* ++++ Tough thread running full width of trough, picked up in one piece.
+++ Non-continuous thread; possible to pick up parts from trough.
++ Very fragile, slender, non-continuous thread, impossible to pick up.
+ Only tiny "hairs" (3–5 mm) visible.
— No trace of visible or tangible fiber.

References p. 596.

DISCUSSION

Surface activity changes with irradiation

RIDEAL AND MITCHELL[13,14,15] have, in their various papers, stressed their observation that one of the characteristic changes produced upon ultraviolet irradiation of protein monolayers is "at constant total surface area, a rapid rise of surface pressure.."[15]. This corresponds to an increase in area at constant surface pressure and represents an actual *increase* in the surface activity of the protein adsorbed at the interface.

On the other hand, the papers of GORTER[4] and NEURATH[8] have demonstrated a *decrease* in film area, (determined by extrapolation of force-area curves) with duration of irradiation. GORTER attributes the decrease in area to "the gradual disappearance of the spreading protein from the surface", and NEURATH to the collapse of the films. DOGNON AND GOUGEROT[16] noted that the surface *tension* at an interface covered with albumin increased (decrease in surface activity) with duration of irradiation and eventually reached the surface tension of the substratum alone; these latter authors call attention to the disagreement between their results and those of MITCHELL AND RIDEAL cited above.

Our own data (see, for example, Fig. 5) suggest that this disagreement may be illusory, since, under certain conditions, the initial effect of ultraviolet irradiation is to increase the surface activity of the adsorbed protein, but that this is invariably followed by a very marked decrease in surface activity. Exact comparison of our data with those of MITCHELL AND RIDEAL[15] is not possible, since their ovalbumin films were spread on a substratum consisting of phosphate buffer at pH 7; yet it should be noted that these authors employed very brief periods of illumination (several five-minute periods) suggesting that their data are compatible with our own for the initial effect of irradiation on films spread on saline. Even in the case of our films on distilled water, the constancy in area during the first 15 minutes of continuous irradiation suggests the existence of two competing processes, one of which must be an increase in the surface activity of the adsorbed protein, which our methods were not sufficiently precise to detect.

Hypothesis of MITCHELL AND RIDEAL

Another reason for which we hesitate to compare our work with that of these authors is the comparative crudity and inelegance both of our instrumentation and of our formulation of the problem. There are nevertheless certain fundamental differences in interpretation which cannot be explained away on this basis.

In their first paper[17], they have studied the effect of monochromatic UV radiation (2150–3175 A) on films of stearic anilide ($C_6H_5NHCOC_{17}H_{35}$) and demonstrated preferential rupture of the ketoimino linkage, yielding stearic acid and aniline. In this compound, the benzene ring is the chromophore and the absorbed energy is transferred to the CO–NH linkage, which possesses the lowest bond strength in the molecule. This paper also demonstrates the importance of the HARDY-LANGMUIR principle of molecular orientation at interfaces in "controlling the rate of the photochemical reaction".

Their second paper[15] deals with UV irradiation of protein films maintained at constant area. They were able to demonstrate, in the case of insulin, the "photochemical liberation into the substrate of molecular fragments containing aromatic groups"; by intensive irradiation of a film over a substratum containing diazotized *p*-aminobenzoic acid, a faint but definite colour reaction was observed. They suggest "that in these

References p. 596.

photoreactions, both "peptide linkages" adjacent to each side-chain derived from tyrosine, phenylalanine, and possibly also tryptophan, may undergo a process of oxidative photolysis, the end result of which is equivalent to hydrolysis, and the transformed chromophoric residues liberated pass into solution".

This hypothesis is not compatible with our data. It will be noted from Table IV that the areas of films irradiated for 8 hours have decreased to approximately one-third those of the controls; with somewhat longer irradiations, we have reduced the area of films to one-sixth their original value. BERGMANN AND NIEMANN[18] indicate that egg albumin contains some 288 residues; this molecule contains (see[15]) 8 tyrosine residues, 2 of tryptophan and possibly 11 of phenylalanine. If we neglect the very small contributions to absorption of arginine, cystine and methionine etc., (justifiable for a first approximation, see MITCHELL AND RIDEAL[15]), the chromophoric residues account for 7% of the total. It is not possible to attribute the extensive decreases in area observed to the elimination of but 7% of the constituent amino-acid residues. On the contrary, our evidence suggests a much more complicated picture, in which at least 2 distinct photochemical reactions must occur, possibly consecutively, culminating in an extensive decomposition of the molecule into small, soluble fragments.

Interpretation of the data

Our working hypothesis is presented below and summarized schematically in Fig. 6 and in Table V; it is admittedly speculative but has considerable heuristic value, and has already led to successful confirmatory experiments involving UV irradiation of dilute protein solutions[19].

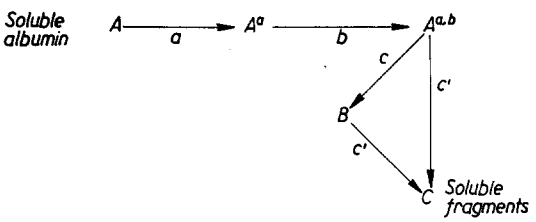

Fig. 6. Schematic representation of working hypothesis. For explanation of symbols, see Table VI, and text.

a. The soluble albumin molecule, A, is unfolded by interfacial action into a molecule, A^a, of drastically altered physico-chemical properties (NEURATH AND BULL[20]); NEURATH *et al.*[21] consider that these film molecules are examples of "a completely denatured protein", in the sense that the component polypeptide chains are either fully extended or are folded

TABLE V

Representation of hypothesis, corresponding to Fig. 6 and text.

Step	*Nature*	*Agent*	*Molecule produced*	*Inferred from*
a	Non-proteolytic	Interfacial forces	A^a – Intact protein, partially unfolded	Altered properties of film molecules
b	Non-proteolytic	Photochemical	$A^{a,b}$ – Intact protein, further unfolded by UV	Initial increase of area of irradiated film
c	Proteolytic	Photochemical	B – smaller molecules, surface-active	Absence of fiber; changed force-area curves
c′	Proteolytic	Photochemical	C – small fragments, not surface-active	Disappearance of film

References p. 596.

in a manner "determined in a haphazard fashion by the interaction of the side-chains". The usual explanation of this non-proteolytic alteration in structure is that the energy existing at the air/water interface can destroy certain of the weaker secondary valences (*e.g.*, hydrogen bonds) which hold the soluble molecule in its tightly rolled-up configuration, resulting in a structure which is less orderly and specific (MIRSKY AND PAULING[22]).

b. The radiant energy, initially absorbed by the protein chromophores, is transferred to adjacent labile bonds (perhaps an H bond, involving the phenolic group of tyrosine residues) not previously broken by interfacial forces; this gives rise to an even more extended and asymmetric molecule, $A^{a,b}$. The increased asymmetry, due to further unfolding of the polypeptide chains, causes the initial expansion of area observed in the case of films spread on saline, and is probably the same sort of process as that which causes the slow expansion of unirradiated films. It is necessary to invoke this step to be consistent with the fact that soluble albumin, previously deformed without proteolysis ("denatured") by heat[7] or by UV irradiation[19] yields films of larger area than does the untreated protein.

Further evidence on this point is provided by the many papers (see review by ARNOW[23]) demonstrating the acceleration of UV "denaturation" of egg albumin in the presence of high concentrations of various salts, including NaCl; this would then be consistent with the observed expansion of irradiated films spread on saline. MITCHELL AND RIDEAL have mentioned that "in photochemical reactions in monolayers, the reaction velocity may be profoundly altered by changes in molecular orientation"[17]. If the initial effect of radiation absorbed by films spread on salt substratum has been to destroy a linkage binding a chromophore, it is tempting to ascribe the subsequent increased rate of destruction of these films to the changed orientation of these chromophore groups with respect to interface and incident radiation, which would be expected to follow from rupture of such a bond.

c. Thereafter, radiant energy absorbed by chromophore side-chains is transferred to the polypeptide main-chain, resulting in the rupture of peptide linkages (proteolysis). That energy absorbed by aromatic chromophores can be transferred through at least 1 or 2 CH_2 groups to break a CO–NH linkage has been demonstrated by CARPENTER[24] by repeating the experiments of RIDEAL AND MITCHELL, but using films of benzylstearylamine and phenylethylstearylamine.

This random photolytic process results in the formation of the fairly large B fragments when the break occurs near the center of the mainchain, and of the small C fragments, when it occurs near the extremities of the chain. It should be emphasized that, in using the designations A, B, C etc., no claim of homogeneity for these categories is intended; indeed, the random nature of the absorption of radiant energy suggests that each theoretical category includes molecules of widely differing sizes and properties. Further, it is evident that the films themselves may be mixed with respect to these categories of molecules.

The presence of the B variety is inferred from the altered force-area curves and the absence of fibers in irradiated films. Other properties attributable to these molecules, besides their surface-activity, are: 1. the attractive forces between the B fragments are less than those of the unirradiated film molecules (high linearity point, high collapse point); 2. they are more rigid, and less easily deformable by compression (low compressibility); c. they are of lower molecular weight (loss of ability to form a fiber). The fact that the linearity and collapse points, the slope (and the presence or absence of

References p. 596.

fibers) are reproducibly and characteristically different for irradiated and control films shows that they must have some underlying physico-chemical significance, even if not that which has been here suggested.

The small C fragments are soluble in the substratum and not surface-active; their appearance is consequently responsible for the gradual decrease in area of the irradiated films.

In a subsequent publication[19], we hope to demonstrate the validity of the scheme presented above, by showing that the effect of UV irradiation on dilute ovalbumin solutions was first, a non-proteolytic alteration in structure ("denaturation") and thereafter a proteolysis with rupture of peptide linkages. In this work, standard procedures of analytical chemistry have been used to verify every step in the proposed reaction sequence.

ACKNOWLEDGEMENTS

The senior author expresses his indebtedness to Dr J. A. McCARTER and Dr J. G. ALDOUS for the gracious loan of much of their equipment and for their advice and interest, and to his colleague, Dr C. B. WELD, for his cooperation in many of the requirements of this research.

SUMMARY

The effect of ultraviolet light on monomolecular films of crystalline ovalbumin at the air/water interface has been studied. Films were maintained at constant pressure while changes in area were followed, correcting for spontaneous expansion of unirradiated control films. During the first quarter-hour of irradiation, the area of films spread on salt substratum increased, while that of those spread on distilled water underwent no change despite modification of certain other properties of the film molecules. Thereafter, the area of the irradiated monolayers decreased at a remarkably regular rate, that for the films on saline being almost twice that for films on distilled water. The decrease in area noted was not simply due to elimination of destroyed protein from the film, leaving only intact protein still adsorbed at the interface, since the properties of the residual film molecules, as determined by analysis of force-area curves, were strikingly different from those of the unirradiated films. The end result of the irradiation is a photochemical proteolysis yielding small, soluble, non-surface-active fragments, but initially a non-proteolytic structural change resulting in further unfolding of the already extended film molecules is believed to occur. To account for these phenomena, a hypothesis is advanced which is consistent with current conceptions of protein structure. The apparent contradiction between the work of the RIDEAL group and that of most other workers may be due to the initial increase in surface activity of irradiated protein films, observed by the former, which is followed by the decrease in surface activity, reported by the latter.

RÉSUMÉ

On a étudié l'effet de la radiation ultraviolette sur les couches monomoléculaires d'ovalbumine cristallisée à l'interface air/eau. Ces couches ont été maintenues à pression superficielle constante pendant l'enregistrement des variations d'aire; des corrections ont été faites pour l'expansion spontanée des filmes témoins, non-irradiés. Pendant le premier quart d'heure d'irradiation, l'aire des couches étendues sur une solution de chlorure de sodium augmentait; par contre, celle des couches étendues sur eau distillée ne changait pas malgré la modification d'autres caractères des molécules du film. Ensuite, l'aire des films irradiés décroissait à un taux d'une régularité remarquable, celui des films sur chlorure de sodium étant à peu près deux fois plus élevé que celui des films sur eau distillée. Ce processus ne correspond pas tout simplement a l'élimination de protéine fragmentée, laissant intacts, toujours adsorbées à l'interface, des molécules non-modifiées, puisque l'analyse de la structure des molécules résiduelles (après irradiation) montre, de façon nette, que ces dernières possèdent une structure tout-à-fait différente de celle des molécules non-irradiées. Le résultat *final* de l'irradiation est donc la protéolyse photochimique de la protéine donnant naissance à de petits

References p. 596.

fragments solubles, dépourvus d'activité superficielle. L'effet *initial* de cette radiation sur la protéine est une modification non-protéolytique de la structure de la molécule (dénaturation), qui a pour résultat le dépliment des molécules déjà étendues sous l'action de l'énergie interfaciale.

ZUSAMMENFASSUNG

Der Einfluss von ultraviolettem Licht auf monomolekulare Filme von kristallisiertem Ovalbumin an der Trennungsfläche Luft/Wasser wurde untersucht. Die Filme wurden unter konstantem Druck gehalten, während die Oberflächenänderungen verfolgt wurden; für spontane Ausdehnung nicht bestrahlter Kontrollfilme wurde eine Korrektur angebracht. Während der ersten Viertelstunde der Bestrahlung nahm die Oberfläche der auf Salzsubstrat ausgebreiteten Filme zu, während diejenige von Filmen auf distilliertem Wasser sich nicht änderte, obwohl sich gewisse andere Eigenschaften der Film-Moleküle änderten. Hiernach nahm die Oberfläche der bestrahlten monomolekularen Schichten mit auffallender Regelmässigkeit ab, wobei die Abnahmegeschwindigkeit für Filme auf Salzlösung fast zweimal so gross war als für Filme auf distilliertem Wasser. Die Oberflächenverminderung war nicht nur auf die Ausscheidung von zerstörtem Protein aus dem Film, wobei nur intaktes Protein an der Grenzfläche adsorbiert bleibt, zurückzuführen; die Eigenschaften der übrig gebliebenen Film-Moleküle sind nämlich auffallend verschieden von denjenigen nicht bestrahlter Filme, was aus der Analyse der Kraft-Oberfläche-Kurven hervorgeht. Das *Endergebnis* der Bestrahlung ist eine photochemische Proteolyse, welche kleine, lösliche, nicht oberflächenaktive Fragmente ergibt; zu *Beginn* scheint eine nicht proteolytische strukturelle Veränderung stattzufinden, welche eine weitere Ausbreitung der bereits gestreckten Film-Moleküle zur Folge hat. Zur Erklärung dieser Erscheinungen wird eine Hypothese vorgeschlagen, welche mit den allgemein herrschenden Auffassungen über die Proteinstruktur im Einklang steht. Der scheinbare Widerspruch zwischen den Arbeiten der RIDEAL-Gruppe und denjenigen der meisten anderen Forscher, könnte darauf zurückzuführen sein, dass die erstere die anfängliche Zunahme der Oberflächen-Aktivität von bestrahltem Protein beobachteten, während die letzteren über die darauffolgende Abnahme der Oberflächenaktivität berichtet haben.

REFERENCES

1 H. DEVAUX, *J. Phys.*, 3 (1904) 450.
2 E. GORTER AND F. GRENDEL, *Trans. Faraday Soc.*, 62 (1926) 2771.
3 A. HUGHES, J. H. SCHULMAN, AND E. K. RIDEAL, *Nature*, 129 (1932) 21.
4 E. GORTER, *Am. J. Diseases Children*, 47 (1934) 945.
5 E. K. RIDEAL, *Science*, 90 (1939) 217.
6 J. G. KAPLAN, *Physiol. Zool.*, 25 (1952) 123.
7 S. STALLBERG, *Trans. Faraday Soc.*, 35 (1939) 1416.
8 H. NEURATH, *J. Phys. Chem.*, 40 (1936) 361.
9 J. G. KAPLAN AND M. J. FRASER, *Proc. Nova Scotia Inst. Sci.*, 23 (1952) part 1, in press.
10 J. G. KAPLAN, *Federation Proc.*, 9 (1950) 69.
11 G. C. NUTTING AND W. D. HARKINS, *J. Am. Chem. Soc.*, 61 (1939) 1180.
12 J. GUASTALLA, Thèses, Université de Montpellier, Imprimerie Nationale, Paris, 1948.
13 E. K. RIDEAL, *Kolloid-Z.*, 61 (1932) 218.
14 J. S. MITCHELL, Communication to the 2nd International Cancer Congress, Brussels, (1937) 420.
15 J. S. MITCHELL AND E. K. RIDEAL, *Proc. Roy. Soc. A.*, 167 (1938) 342.
16 A. DOGNON AND L. GOUGEROT, *Bull. soc. chim. biol.*, 25 (1943) 183.
17 E. K. RIDEAL AND J. S. MITCHELL, *Proc. Roy. Soc. A.*, 159 (1937) 206.
18 M. BERGMANN AND C. NIEMANN, *J. Biol. Chem.*, 118 (1937) 301.
19 J. G. KAPLAN, D. H. ANDREWS, AND M. J. FRASER, manuscript in preparation.
20 H. NEURATH AND H. B. BULL, *Chem. Rev.*, 23 (1938) 391.
21 H. NEURATH, J. P. GREENSTEIN, J. O. ERICKSON, AND F. W. PUTNAM, *Chem. Rev.*, 34 (1944) 157.
22 A. E. MIRSKY AND L. PAULING, *Proc. Natl. Acad. Sci.*, 22 (1936) 439.
23 L. E. ARNOW, *Physiol. Rev.*, 16 (1936) 671.
24 D. C. CARPENTER, *J. Am. Chem. Soc.*, 62 (1940) 289.
25 R. A. FISHER AND F. YATES, *Statistical Tables for Biological, Agricultural and Medical Research*, Oliver and Boyd, London and Edinburgh, 1943.

Received March 31st, 1952