To test the applicability of this method of analysis, determinations were made with mixtures of known compositions with the following results: samples as prepared, weights and compositions (in terms of wt. % furfuryl alcohol)—25.0 g. of 20%, 30.0 g. of 30.0% and 17.3 g. of 65.3%; after distillation—22.5 g. of 19.5%, 28.3 g. of 31.7% and 16.2 g. of 64.8%, respectively. The recovery of product was satisfactory and the composition was in good agreement with the mixture as prepared in each case.

Acid Mixtures.—(a) The total acidity of a sample, approximately 1 g., of the mixture of acids obtained from the crossed Cannizzaro reaction was determined by titration with standard alkali (phenolphthalein as indicator).

(b) A sample, usually about 1 g., of the mixture of acids was dissolved in 10 ml. of 10% sodium hydroxide solution, an equal volume of water was added, and the solution was heated almost to boiling where it was maintained during the next step. Saturated potassium permanganate solution was added until a pink color persisted for one minute. The excess permanganate was destroyed by the addition of sodium bisulfite solution. After the mixture had cooled, 15 ml. of sulfuric acid (1:1) and then sufficient sodium bisulfite to dissolve the manganese dioxide, which had precipitated, were added. The mixture was boiled gently under a reflux condenser for one hour. The solution was cooled, the condenser was washed down with a small quantity of ether, and the mixture was transferred to the extractor. After extraction had proceeded for from six to eight hours, the ethereal solution was washed with two 10-ml. portions of water and transferred to a titration flask. All but the last 5 or 10 ml. of ether was distilled off through a 60-cm. Vigreux column; the last portion of solvent was allowed to evaporate at room temperature. The amount of residual benzoic acid was determined by titration.

(c) From the difference between total acidity and benzoic acid, the amount of furoic acid was calculated.

That the method described above gives results of sufficient accuracy for the problem at hand is shown by the following data obtained with mixtures of furoic and benzoic acids of known compositions: mixtures as prepared—34.16, 31.74, 29.78 and 43.24% benzoic acid; found by analysis—34.36, 31.77, 29.96 and 43.14% benzoic acid, respectively.

Results

For mixtures of furfural and benzaldehyde treated as outlined for the crossed Cannizzaro reaction and the reaction mixtures analyzed by the methods described above, the condensed and summarized results shown in Table I were obtained.

TABLE I

DISPROPORTIONATION IN THE CROSSED CANNIZZARO REACTION WITH FURFURAL AND BENZALDEHYDE

Reaction no.	Alcohols recov., wt. in g.	Molar ratio, furfuryl alcohol: benzyl alcohol	Acids recov., wt. in g.	Molar ratio, benzoic acid: furoic acid
1	42.6	0.42:1	49.2	0.44:1
2	51.6	0.47:1	55.4	0.65:1
3	32.1	0.60:1	57.2	0.65:1
4	34.0	0.64:1	55.9	0.72:1
5	38.2	0.67:1	47.7	0.47:1
6			55.5	0.68:1
7			56.6	0.69:1
8			54.6	0.71:1
Ave.	39.7	0.56:1	54.0	0.63:1

The furfural, furfuryl alcohol and furoic acid used in this work were generously furnished by the Quaker Oats Company, Chicago.

Summary

Mixtures of furfural and benzaldehyde were subjected to the conditions under which the Cannizzaro reaction usually occurs.

Satisfactory methods were found for the analysis of mixtures of reaction products.

In the reaction studied, the furfural was oxidized to a greater extent than the benzaldehyde; the ratio was approximately 5:3.

PULLMAN, WASHINGTON RECEIVED APRIL 25, 1944

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, SWIFT & COMPANY]

The Photochemical Inactivation of Trypsin and Papain Solutions in the Ultraviolet Region

BY F. L. KAUFFMAN AND W. M. URBAIN

It has been demonstrated that ultraviolet light will cause the inactivation of many enzymes,[1] but relatively few experiments have been conducted under conditions which allowed quantitative calculations to be made. Quantum yields (molecules inactivated per quantum absorbed) have been calculated for the photochemical inactivation of urease,[2] and can be calculated from the data of Gates[3] for pepsin. The quantum yields for the inactivation of trypsin[4] have been calculated also at certain wave lengths of ultraviolet light.

Bersin[5] has reported that he found ultraviolet rays to have an initial activating effect upon papain solutions. However, further exposure to ultraviolet light caused the papain solutions to become inactive.

The purpose of this investigation was to determine the general qualitative characteristics and the quantitative relationships of the ultraviolet light required for the inactivation of trypsin and papain solutions by using commercially available mercury arc ultraviolet light sources.

Materials and Methods

Solutions containing 0.10% papain were prepared from crude, powdered Ceylon papain obtained from Parke, Davis and Company. The solutions were prepared fresh for each test, adjusted to a *p*H of 6.4, and chemically

(1) C. Ellis, A. A. Wells and F. F. Heyroth, "The Chemical Action of Ultraviolet Rays," Reinhold Publ. Corp., New York, N. Y., 1941, p. 680.

(2) E. W. Landen, THIS JOURNAL, **62**, 2465 (1940).

(3) F. L. Gates, *J. Gen. Physiol.*, **18**, 265 (1934).

(4) F. M. Uber and A. D. McLaren, *J. Biol. Chem.*, **141**, 231 (1941).

(5) T. Bersin, *Z. physiol. Chem.*, **222**, 177 (1933).

activated with sodium hydrosulfite. The trypsin solutions were prepared by dissolving 0.25 g. of trypsin (1:110, from Pfanstiehl Chemical Company) in 1.0 ml. of 0.1 *N* sodium hydroxide and diluting to 1000 ml.

The ultraviolet light sources used were a 15.24 cm. (6 inch), 360-watt General Electric Uviarc (high pressure mercury arc in quartz) emitting radiations as low as 1850 Å.; and the same lamp fitted with a Corning H. R. Clear Corex D #970 filter, eliminating practically all radiations below 2800 Å., while transmitting only a small amount of energy from the mercury lamp between 2800 and 2900 Å. A large portion of the energy above 2900 Å. is transmitted. The third source used was a 76.2 cm. (30-inch), 20-watt Westinghouse Sterilamp having the large majority of its ultraviolet radiations at 2537 Å. Only a 15.24 cm. portion of this latter lamp was used. The lamps were used in such a way that the maximum benefit was obtained from the reflectors supplied by the manufacturers. The lamps were allowed a suitable warm-up period before use.

The enzyme solutions were placed in a shallow glass tray at selected distances of 12.7 to 38.1 cm. directly beneath the tube of the ultraviolet lamp. The solution depth was kept at about 1 mm. Small variations in the depth of solution did not have any appreciable effect on the loss of enzyme activity during irradiation. The light was blocked off while the solution was stirred, and a sample was then taken with a pipet. The light was then allowed to strike the solution again for another period, and the above procedure repeated. The solution was not stirred during irradiation.

Enzyme activity was determined by means of a controlled casein digestion modified from Northrop.[6] The casein was dissolved in a buffer solution containing disodium phosphate, sodium citrate, and boric acid. A given portion of this solution (25-ml. equivalent to 1 g. of casein) was diluted with water to about 180 ml. in a 200-ml. volumetric flask, heated to 40°, and then 10 ml. of the enzyme solution added. The solution was diluted to the mark and digestion for one hour at 40° allowed. After this time, a 100-ml. aliquot was withdrawn and the undigested casein was precipitated by lowering the *p*H to 4.7 with an acetic acid–sodium hydroxide buffer solution. The precipitate was filtered and washed with a 0.00025 *N* hydrochloric acid solution.

The undigested casein was proportional to the nitrogen content of the precipitate. The amount of nitrogen in the precipitate was determined by a Kjeldahl determination. A control determination, carried out in the same manner without the addition of any enzyme, gave the nitrogen content of the undigested casein. The amount of digestion was indicated by the difference between the nitrogen content of the precipitate obtained before and after enzyme treatment.

Energy Calculations

The energies given off in the ultraviolet region (between 4000 and 1850 Å.) by the Uviarc ultraviolet lamp and the Sterilamp are 521 and 28.2 microwatts per sq. cm. at 1 m., respectively, as stated by the manufacturers' bulletins.[7,8] From the percentage transmission figures of the H. R. Clear Corex D #970 filter,[9] the energy given off in the ultraviolet region by the filtered Uviarc lamp was calculated to be 264 microwatts per sq. cm. at 1 m.

The energy produced at any given distance can be calculated from the equation

$$E_1/E_2 = D_2^2/D_1^2$$

in which E is energy and D is the distance between the light source and the object.

(6) J. H. Northrop, *J. Gen. Physiol.*, **5**, 263 (1922).

(7) General Electric Vapor Lamp Company, Hoboken, N. J., Bulletin No. 112, 1938, 4 pp.

(8) Sterilamp Conditioning, Westinghouse Elec. & Mfg. Co., 1941, 12 pp.

(9) Glass Color Filters, Corning Glass Works, Corning, N. Y., 1936, 12 pp.

Results

Results are presented in Tables I to V and Figs. 1 to 3. In Table I and Fig. 1 is shown the inactivating effect of ultraviolet light from the Uviarc on a 0.025% trypsin solution as measured by a casein digestion.

TABLE I

INACTIVATION OF TRYPSIN BY A UVIARC ULTRAVIOLET LAMP AT 38.1 CM.

Time of exposure, min.	Microwatts/sq. cm.	% N in ppt. No. 1	% N in ppt. No. 2	Diff. from control of % N in ppt.
Control	.....	12.34	12.40	
0.00	.....	6.87	6.93	5.47
.25	53,840		7.35	5.05
.50	107,680		7.86	4.54
1.0	215,360		8.47	3.93
3.0	646,080	11.00		1.34
10.0	2,153,600	11.70		0.64
20.0	4,301,200		12.40	.00

It is seen from Table I and Fig. 1 that the Uviarc ultraviolet lamp at 38.1 cm. caused a 0.025% trypsin solution to lose 90% of its activity in about twelve minutes with an expenditure of 2,600,000 microwatts per sq. cm. in the ultraviolet region (4000 to 1850 Å.).

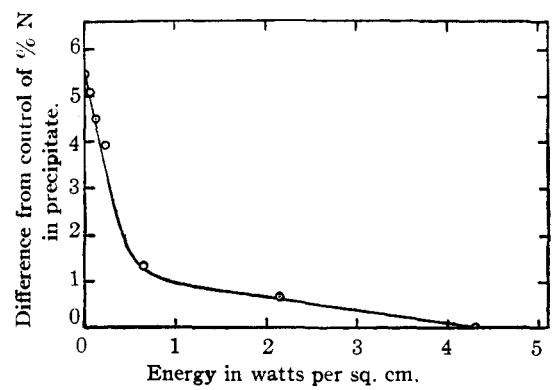

Fig. 1.—Inactivation of trypsin by a 360-watt Uviarc ultraviolet lamp at 38.1 cm.

In Tables II, III, IV and V are shown the effects of ultraviolet light from various sources on a 0.10% papain solution as measured by casein digestions. These results are also shown graphically in Figs. 2 and 3.

It is seen from Tables II and III and from Fig. 2 that the Uviarc ultraviolet lamp at 38.1 cm. caused a 90% inactivation of 0.10% papain solution in fifty-three seconds using about 190,000 microwatts of energy per sq. cm. in the ultraviolet region. Using the same lamp at 19.05 cm., 90% inactivation occurred in twenty-six seconds, requiring about 370,000 microwatts per sq. cm. in the ultraviolet region.

TABLE II

INACTIVATION OF PAPAIN BY A UVIARC ULTRAVIOLET LAMP AT 38.1 CM.

Time of exposure, min.	Microwatts/ sq. cm.	% N in ppt.	Diff. from control of % N in ppt.
Control	.....	12.75	..
0.00	.....	3.57	9.18
.25	53,840	9.38	3.37
.50	107,680	9.74	3.01
.75	161,520	11.77	0.98
1.0	215,360	12.11	.64
1.5	323,040	12.25	.50
2.0	430,720	12.67	.08
3.0	646,080	12.75	.00

TABLE III

INACTIVATION OF PAPAIN BY A UVIARC ULTRAVIOLET LAMP AT 19.05 CM.

Time of exposure, sec.	Microwatts/ sq. cm.	% N in ppt.	Diff. from control of % N in ppt.
Control	.....	12.81	..
0	.....	3.95	8.86
8	114,880	10.64	2.17
16	229,760	11.49	1.32
32	459,520	12.11	0.70
72	1,033,920	12.47	.34
120	1,723,200	12.47	.34

Tables IV and V and Fig. 3 show the effect of the light from the filtered Uviarc ultraviolet lamp and the Sterilamp on a 0.10% papain solution. The filtered Uviarc caused 90% inactivation in seventeen minutes. About 1,850,000 microwatts per sq. cm. of ultraviolet radiation were required. A seventy-seven minute exposure to the Sterilamp was required for 90% inactivation of the 0.10% papain solution. This is equivalent to about 1,600,000 microwatts per sq. cm. of ultraviolet light.

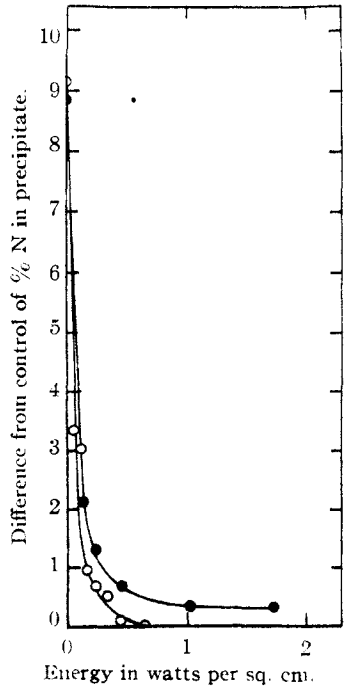

Fig. 2.—Inactivation of papain by a 360-watt Uviarc ultraviolet lamp at: O, 38.1 cm.; ●, 19.05 cm.

TABLE IV

INACTIVATION OF PAPAIN BY A FILTERED UVIARC ULTRAVIOLET LAMP AT 38.1 CM.

Time of exposure, min.	Microwatts/ sq. cm.	% N in ppt.	Diff. from control of % N in ppt.
Control	.....	12.67	..
0	.....	5.95	6.72
1	109,100	9.60	3.07
6	654,600	11.03	1.64
10	1,091,000	11.35	1.32
20	2,182,000	12.11	0.56
40	4,364,000	12.19	.48

TABLE V

INACTIVATION OF PAPAIN BY A STERILAMP[a] ULTRAVIOLET LAMP AT 12.7 CM.

Time of exposure, min.	Microwatts[a] sq. cm.	% N in ppt.	Diff. from control of % N in ppt.
Control	.....	12.61	..
0	.....	2.87	9.72
4	83,830	3.57	9.04
8	167,660	4.90	7.71
15	314,640	5.95	6.66
30	629,280	8.82	3.79
60	1,258,560	11.07	1.54
90	1,887,840	11.84	0.77

[a] A 15.24 cm. (6-inch) portion of the 76.2 (30-inch) Sterilamp was used.

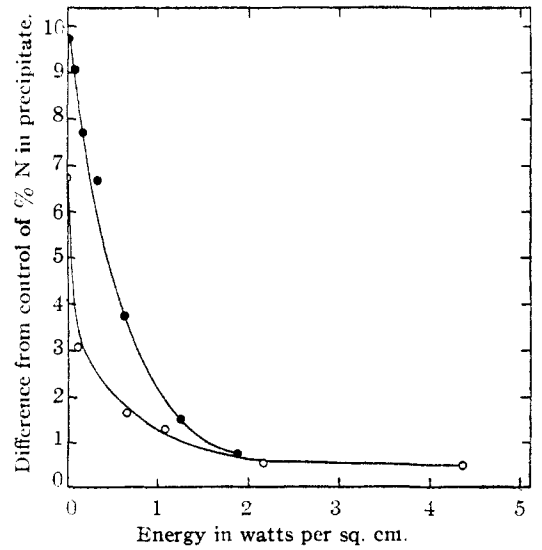

Fig. 3.—Inactivation of papain by A: O, filtered 360-watt Uviarc ultraviolet lamp at 38.1 cm.; ●, 20-watt Sterilamp at 12.7 cm.

Of the ultraviolet sources investigated the one having radiations within the range 1850 to 4000 Å. was the most efficient for destroying the activity of papain solutions. The efficiency of this source (unfiltered Uviarc) was also considerably greater at 38.1 cm. than at 19.05 cm., although the time

of inactivation was less at the latter distance. This greater efficiency is possibly due to the fact that the light is more nearly a point source at 38.1 cm. than at 19.05 cm., causing the radiation from all parts of the radiator to be more effectively utilized. The further the source from the object, the less is the angle of incidence. As the angle of incidence becomes more acute the radiations become more effective. Therefore, the energy multiplied by the time required to destroy enzyme activity does not necessarily give a constant when intensity is varied by changing the distance between the source and the object as was done in this investigation.

The efficiency of a particular source in destroying enzyme activity is related to the type of radiations emitted. Published data[10] on the ultraviolet absorption spectrum of papain show a peak of absorption occurs at about 2750 Å. A relatively small amount of absorption occurs in the vicinity of 2500 Å. and practically no absorption above 2900 Å. Below 2500 Å. there is an increasing amount of absorption to 2400 Å. as far into the ultraviolet as has been investigated.

Since only light that is absorbed is capable of photochemical action, it follows that light having radiations at 2750 Å. should be the most effective in the destruction of the activity of papain.

Light which consisted chiefly of radiations above 2900 Å. (the filtered Uviarc) proved to be much less effective in the destruction of papain activity than the radiations distributed over the whole ultraviolet range. This is also true for radiations largely at 2537 Å. (the Sterilamp). These observations are in agreement with the conclusions reached from the absorption spectrum.

(10) H. H. Darby, *J. Biol. Chem.*, **139**, 721 (1941).

Summary

1. Quantitative data have been obtained for the photochemical inactivation of trypsin and papain solutions using various ultraviolet light sources.

2. The energy required to inactivate a papain solution is dependent on the wave length and the intensity of the ultraviolet light and the angle at which it strikes the solution.

CHICAGO, ILLINOIS RECEIVED APRIL 13, 1944

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, NORTHWESTERN UNIVERSITY MEDICAL SCHOOL]

Elasticity of Keratin Fibers

BY HENRY B. BULL AND MARTIN GUTMANN

Some protein fibers are relatively non-elastic. Silk, for example, is such a fiber and X-ray diffraction studies indicate this fiber to be extensively crystallized with the extended peptide chains lying parallel to each other. On the other hand, there are several protein fibers which under the proper condition show long range elasticity. Notable among these elastic fibers are hair and wool. The elasticity of such fibers can only be understood by considering them as co-polymers of a great many polymerizing units. The presence of these diverse amino acid residues or polymerizing units in the fiber prevents the close fitting of the straightened peptide chains and leads to a great deal of kinking in the fiber; the stretching of such a fiber involves the unkinking of the peptide chains.

We wish to describe in this paper some experiments on the stretching of human hair and to report some conclusions which we have drawn from these experiments regarding the elastic deformation of hair.

Experimental.—Human hair was used in all of our experiments. A homogeneous lot of long natural hairs was obtained from a commercial dealer. The hair was exhaustively extracted with alcohol, with ether and finally washed with water and electrodialyzed. It was dried and stored in a desiccator over calcium chloride.

The tension apparatus used to stretch the hair was designed to apply a load at a uniform rate of increase or of decrease of load. The rate of application and of release of the load was set at 0.470 g. per minute. The adjustment of the length of the stretch of the hair to the load was entirely automatic and very nearly instantaneous. The hair was bathed in water while in the apparatus and the tube containing the hair under examination was surrounded by a water jacket through which water at any desired temperature could be circulated. The temperature selected for our experiments was 25°. It is not our purpose to describe our tension apparatus in detail at this time.

Results.—Figure 1 shows a typical stress–strain curve of a human hair both for extension and for contraction.

It was first pointed out by Speakman[1] that the stress-strain curves for successive extensions of a single fiber were very nearly identical under the same conditions of temperature, moisture, *p*H and rate of loading provided the extension did not exceed about 20% of the original fiber length. From our experience we can add that the agreement between successive 20% extensions improves with each successive extension. We are also able to conclude that not only is the stress–strain curve for 20% extension very nearly reproducible but likewise the stress–strain curve for

(1) Speakman, *Proc. Roy. Soc.* (London), **B103**, 377 (1928).