

STABILITY OF SENSITIZING AND "BLOCKING" ANTIBODIES IN ALLERGIC SERUM FOLLOWING ULTRAVIOLET IRRADIATION^{1,2}

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These studies relate to the stability of sensitizing and of immunizing antibodies in human serum following its exposure to ultraviolet light (UVL) in a manner currently used for the commercial processing of plasma (1). The potency of six urticariogenic samples was determined by the technics of serum-dilution and reagin-neutralization during a 2-year period, comparisons being made between the original and the irradiated portions by means of passive sensitization tests in man. Blocking antibodies were examined in one bleeding.

MATERIALS AND METHODS

Two bleedings were done on each of three healthy adults, after their clinical hypersensitiveness toward one or more inhalant allergens had been confirmed by intracutaneous, conjunctival and serologic tests. No history of jaundice or of transfusion was given by any of them. Whereas all lacked the thermostable, or blocking, antibodies at the outset, one subject was induced to form these by means of a swift booster stimulus consisting of a series of intracutaneous injections with grass pollen antigen on three successive days. All serum specimens were sent to Chicago for irradiation at the Samuel Deutsch Convalescent Serum Center, where Drs. Sidney O. Levinson and F. Oppenheimer (2) applied rays from a low-pressure ultraviolet lamp which emitted about 15 milliwatts per cm² at wave lengths under 2600 Angström units (90% at 2537 Angström units). The exposure time was approximately 1 second. Whereas some of the specimens were exposed to the light within 24 hours after venesection, others had been stored in the cold for as long as 11 months before radiation. A portion of each bleeding was withheld from the rays for control purposes, half being sent to Chicago while the rest was kept under refrigeration in New York City.

Antigens used for titrating the sera were prepared in a standard manner once yearly by exposing ether-defatted pollen, animal dander, or seeds to alkaline saline solution for at least 16 hours. After being rendered bacteria free by passage through fritted glass, the extract was stabilized by admixture with an equal volume of sterile glycerin and labeled according to its content of phosphotungstic acid-precipitable nitrogen (PtaN). No difference in potency was ever found to exist between the old and a fresh preparation, when concurrent titrations were carried out at each transition point.

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Serological titrations of a modified Prausnitz-Küstner (wheal-and-flare) type were done in the skin of normergic volunteers, whose number was restricted so that the factor of individual responsiveness would exert a minimal influence on the outcome of successive experiments. Care was taken to place the intracutaneous injections of control and of irradiated serum in the same positions on opposite sides of the back, and to avoid the use of the same areas for successive experiments—an ideal achieved by the strenuous application of indelible dye. The constancy of the test subject's response was followed by means of overlapping experiments with a standard sensitizing serum and glycerinated allergen.

Details of the 3 varieties of serologic technic used (3) need not be repeated here, but it should be emphasized that all endpoints were based on minimal reactions. The original injection consisted of 0.1 ml, whether diluted serum or serum-antigen mixtures were involved; and each site was tested in 24 hours with .03 ml of antigen containing 1.6 μ g of PtaN per ml. (This is equal to 160 PtaN, or so-called protein, units per ml.) Both serum specimens and allergens were freshly diluted for each experiment, the tubes differing by twofold in either case.

Reagin-neutralization with graded amounts of antigen was carried out with the serum in its original, unheated state and also after being heated to 60 C for 1 or 2 hours. In the former case, titration revealed how much allergen was required to exhaust the sensitizing power of 1 ml of serum, but it was not possible to state what proportion of the antigen was withheld from the reagin by any blocking factor that was present in the sample. If one wishes to gauge these two antibodies separately, it is necessary to employ a second procedure; namely, to destroy one of them by heat and then measure the potency of the residual thermostable, or blocking, antibody. For this purpose, one must "borrow" sensitizing power in the form of serum from an untreated patient. It is then a simple matter to establish the neutralization requirement for the reaginic serum and to note how much extra allergen must be included in the experiment when a measure of the heated, immune serum is involved. Since the reverse experiment has as yet not been achieved, namely to inactivate the blocking factor and measure the residual reagins in an immune serum, one must rely on the serum-dilution technic to give a rough indication of the sensitizing antibody concentration. Since it has been shown (4) that the induced antibodies diffuse rapidly away from normal skin, only the reagins remain to influence the endpoints of diluted serum after 20 hours.

The three serologic procedures listed above (serum-dilution, neutralization in the unheated and in the heated state) were applied to each serum sample. Since only positive findings will be reported, the tests with heated serum will be described for one sample only, no blocking antibody being detected in the others.

FINDINGS

Table I pertains to the 3 donors of blood. It will be noted that various inhalant allergens were involved and a range of clinical susceptibilities, as gauged by the threshold response of the conjunctivae to instilled allergen. The age of the serum when exposed to UVL will be noted to vary from 1 day to 11 months. Thermo-

stable antibodies (TSA) were found only in the second bleeding of patient, F. E. L.

Table II summarizes the results of neutralization tests done on the K.U. bleeding of December 9, 1949, in five normal subjects. In the volunteer, S. W., the control and irradiated portions showed no significant difference in endpoint on the third, fourth, fifth and nineteenth days after venesection. By 13 months, however, there appeared to have occurred a slight deterioration in the treated as compared to the untreated specimen in that the latter required 4.8 μ g of allergen N whereas 1.2 μ g served to desensitize the irradiated material.

Any differences recorded for the subjects, P. O. and P. O. R., were too small to interpret, the serum ages ranging from 11 days to 2 years. Titrations carried out

TABLE I

DONOR			ALLERGEN	MINIMAL RE-ACTIVE DOSE FOR DONOR'S EYE IN Pta-NITROGEN		ANTIBODIES IN SERUM		DATE OF BLEEDING	DATE OF UV IRRADIATION	AGE WHEN IRRADIATED
Name	Sex	Age		μ g/ml	PNU/ml	Reagins	Block-ing			
K. U.	M	40	ragweed pollen	0.040	40	yes	no	12/9/49	12/10	1 day
						yes	no	5/14/51	5/22	8 days
S. E. N.	F	49	cottonseed ragweed	0.005	5	yes	no	3/8/51	5/22	76 days
				0.240	240	yes	no	3/9/51	5/22	75 days
F. E. L.	F	20	grass pollen (timothy)	0.160	160	yes	no	{ 6/7, 6/22, 6/28/50	5/22/51	11 mo.
			horse epidermis	1.280	1280	yes	no			
			dog epidermis	2.560	2560	yes	no			
			grass pollen (timothy)	2.560	2560	yes	yes	7/14/50	5/22/51	10 $\frac{1}{4}$ mo.

in subjects, R. E. I. and A. N. T., 2 years and 10 days after the original bleeding, revealed a possible loss of potency in the irradiated portion during one experiment but no important difference in endpoints for the other individual.

Similar examination of the second bleeding of K. U., prepared on May 14, 1951, brought out no important difference in potency between irradiated and control portion with the exception of the outcome in one recipient, R. E. I., on the 219th day. Whereas 1.6 μ g of N had to be added to the original sample, 0.2 μ g served to desensitize the treated specimen. It will be observed in Table III that another volunteer, P. O. R., who received the same mixtures on this date, revealed no significant difference in endpoints.

When the technic of serum dilution was applied to these two bloods of K. U., alteration of potency in the irradiated sera was suggested by the results of only

TABLE II

Relative stability of ultraviolet-irradiated and nonirradiated reagins

(Based on neutralization endpoints of ragweed-sensitizing serum of K.U. in the normal skin of 5 test subjects)

NORMAL TEST SUBJECT	AGE OF SERUM (DAYS)	NEUTRALIZATION REQUIREMENT IN NORMAL SKIN (μ g Pta-N OF POLLEN ALLERGEN PER ML SERUM)		
		Nonirradiated serum		Irradiated serum
		New York City	Chicago	
S. W.	3	6.4		
	4	9.6		
	4	9.6*		
	4	9.6†		
	5			6.4
	19	4.8	6.4	6.4
	396	4.8	4.8	1.2
P. O.	11	4.8	4.8	6.4
P. O. R.	390		2.4	1.2
	344		1.2	0.8
	740		1.6	1.2
R. E. I.	740		0.4	0.05
A. N. T.	740		3.2	2.4

* Saline solution in serum-antigen mixture replaced with ragweed-reaginic serum, GRI, after it had been heated at 60 C for 1 hour to destroy its pollen-binding power.

† Saline solution replaced with GRI serum after it had been irradiated and heated for 1 hour at 60 C.

TABLE III

Stability of ultraviolet-irradiated and nonirradiated reagins as judged by neutralization endpoints of ragweed-sensitizing serum of K.U. in normal skin

TEST SUBJECT	AGE OF SERUM (DAYS)	NEUTRALIZATION REQUIREMENT OF K.U. 5/14/51 (μ g Pta-N OF ALLERGEN PER ML OF SERUM)	
		Irradiated serum	Nonirradiated serum
P. O. R.	21	0.10	0.20
	219	0.60	1.20
C. O. L.	17	1.20	1.60
	21	0.80	0.60
	35	0.60	0.80
R. E. I.	219	0.20 ?	1.60

2 experiments out of 11 sets done with 5 different recipients. These exceptions will be seen on Table IV among the data listed for S. W. When the early serum

was 396 days old, it could be diluted out to 1:64 and 1:128 in the control samples whereas the endpoint for the irradiated supply was found at 1:24. This relative reduction in potency had also been suggested by the findings on the 4th day of aging, the control sample which had been retained in New York City having a dilutability of 1:128 whereas the treated supply sensitized no farther out than in 1:48 dilution. Differences observed at 390 days in subject P. O. R. were not considered significant.

TABLE IV

Stability of ultraviolet-irradiated and nonirradiated reagins as judged by dilutability of K.U. ragweed-sensitizing serum

TEST SUBJECT	AGE OF SERUM (DAYS)	SERUM-DILUTION ENDPOINTS IN NORMAL SKIN		
		Nonirradiated portion		Irradiated portion
		New York City	Chicago	
Bleeding of Dec. 9, 1949				
S. W.	3	1:128		
	4	1:128		1:48
	19	1:32	1:32	1:32
	396	1:64	1:128	1:24
P. O.	11		1:64	1:64
P. O. R.	129		1:64	1:128
	390	1:64	1:32	1:16
	740		1:8	1:4
R. E. I.	740		1:20 ?	1:20 ?
Bleeding of May 14, 1951				
C. O. L.	17	1:32		1:64
	35	1:128		1:64
P. O. R.	21	1:8 ?		1:4 ?
	219	1:16		1:16
R. E. I.	219	1:10		1:20

The impression that ultraviolet light, in the dosage used, exerted little if any deleterious influence on reagins was confirmed in experiments on 4 other bleedings obtained from 2 donors. For example, the endpoints of 2 sera procured from S. E. N., a cottonseed-allergic woman, were essentially identical when examined in irradiated and control states on 3 occasions in normal subject, C. O. L. The results are summarized in Table V. Table VI relates to another test-subject, E. M. M., who served for ragweed titrations when the S. E. N. specimens were nearly 11 months old. Treated and untreated portions were again similar in potency.

The third donor was F. E. L. who supplied reagins for timothy grass and ragweed pollens as well as for horse and dog epidermis. The 3 bleedings, listed in Table I for June, 1950, were pooled and exposed to UV rays after having been stored for about 11 months. Two weeks and again 6 months after this treatment,

TABLE V

Stability of ultraviolet-irradiated and nonirradiated reagins as judged by neutralization and dilution endpoints of cottonseed-sensitizing serum of S. E. N. in normal skin

TEST SUBJECT	AGE OF SERUM (DAYS)	NEUTRALIZATION REQUIREMENT (μ g Pta-N OF ALLERGEN PER ML OF SERUM)	
		Irradiated serum	Nonirradiated serum
Bleeding of March 8, 1951			
C. O. L.	82	0.40	0.40
	86	0.20	0.20
	100	0.40	0.40
Bleeding of March 9, 1951			
C. O. L.	81	0.10	0.20
	85	0.20	0.20
	99	0.40	0.20
SERUM-DILUTION ENDPOINTS			
Bleeding of March 8			
C. O. L.	86	1:128	1:256
	100	1:512	1:512
Bleeding of March 9			
C. O. L.	85	1:384	1:512

TABLE VI

Stability of ultraviolet-irradiated and nonirradiated reagins as judged by neutralization and dilution endpoints of ragweed-sensitizing serum of patient, S. E. N., in normal skin of E. M. M.

AGE OF SERUM	DATE OF BLEEDING	NEUTRALIZATION REQUIREMENT OF SERUM μ g OF Pta-N (RAGWEED) REQUIRED PER ML OF SERUM		DILUTABILITY OF SERUM	
		Irradiated serum	Nonirradiated serum	Irradiated	Nonirradiated
10 mos., 21 days	3/8/51	4.80	3.20	1:320	1:480
10 mos., 20 days	3/9/51	4.80	4.80	1:320	1:160

both portions of the pooled serum were found, in subject R. E. I., to require 0.6 μ g N of timothy allergen for their neutralization. The dilution endpoints were 1:256 for the control and 1:128 for the exposed portion during the earlier titration, and 1:640 and 1:480, respectively, in the later one.

These findings for the F. E. L. bloods were confirmed in the normal skin of N. A. S., who served for 6 experiments covering 4 different allergens. Table VII will show that the June bloods in nonirradiated state required approximately 3.20 μ g of timothy N for their specific desensitization when they were less than 6 months old, and that a year later this requirement had not dropped for the

TABLE VII

Stability of ultraviolet-irradiated and nonirradiated reagents as judged by neutralization and dilution endpoints of F.E.L. sensitizing serums in normal skin of N.A.S.

AGE OF SERUM IN MONTHS	DATE OF BLEEDING	NEUTRALIZATION REQUIREMENT OF SERUM (μg of Pta-N REQUIRED PER ML OF SERUM)		DILUTABILITY OF SERUM	
		Irradiated serum	Nonirradiated serum	Irradiated	Nonirradiated
Timothy Pollen					
4½	June, 1950		4.80		1:1920
3	July 14, 1950		19.20*		1:2560
5½	June, 1950		1.20		1:2560
5¾	June, 1950		3.20		1:1280
5¾	June, 1950		4.80		1:1920
18½	June, 1950	6.40		1:480	1:640
18¾	June, 1950	3.20			
17¾	July 14, 1950			1:640	1:320
Horse Epidermis					
18½	June, 1950	6.40	1.60	1:320	1:160
17¾	July 14, 1950			1:640	1:640
Dog Epidermis					
18½	June, 1950	9.60		1:640	1:640
17¾	July 14, 1950	25.60	19.20	1:1280	1:1280
Ragweed Pollen					
4½	June, 1950		51.20		1:2560
18½	June, 1950			1:10	1:20
17¾	July 14, 1950			1:32	1:64

* This serum contained blocking antibodies for timothy as well as for the several reagents.

irradiated sample. The July 14, 1950, bleeding followed immunization with grass pollen and possessed blocking antibodies as well as reagents which naturally increased the neutralization requirement. This did not alter the titer which was still in the vicinity of 1:2560, as noted for the sera prepared before therapy. There was a reduction in dilution endpoint in both irradiated and control samples of the June and July sera when they were examined a year later with grass allergen.

In the instance of horse epidermis, there appeared to be a rather higher requirement for the irradiated than for the control portion of the June pooled serum when neutral points were determined at 18½ months of age. The dilution endpoint was essentially the same for both specimens. The July blood gave comparable endpoints for the light-treated and the nonirradiated supplies.

When dog epidermis was chosen as allergen, no difference could be detected in the dilutability of the 2 portions of the June pool. Only the treated sample was examined in neutralization studies. Both types of titration resulted in similar figures for irradiated and nonirradiated material prepared in July, 1950.

The relative potency of the early and later bleedings was again appraised for ragweed allergen. Although desensitization was attempted for one serum only, comparisons were completed by the dilution procedure. These gave no indication of alteration in potency for the ray-treated samples of either the early or the July serum. However, it was noted in passing that a definite loss of potency had taken place during the year that had elapsed since the original serum had been titrated at 4½ months of age. This tendency had also been detected in some of the foregoing experiments, as examination of the tables will reveal.

Thermostable, blocking antibodies were examined for stability toward UVL in the case of one serum. In late June, 1950, the grass-allergic patient, F. E. L., had received an intensive booster stimulus consisting of 5000 PtaN units of timothy pollen administered intracutaneously during a number of hours on 3 successive days. Two weeks later, on July 14, blocking factor was found in her blood as will be shown below. This was suggested when the old method of serum exhaustion was applied to specimens in the unheated state, the immune sample requiring 19.20 µg of pollen N whereas the blood drawn prior to therapy needed only 4.8 µg (see Table VII). It was clearly established by examination of the serum in heat-inactivated state.

Comparisons may be made by noting the endpoints of the control and irradiated portions of the immune serum, listed in Table VIII. For two test subjects, the materials had been exposed to a water bath temperature of 60 C for a period of 2 hours; for the third recipient, the exposure time was 1 hour. Following the admixture of a heated serum with graded strengths of allergen, a measure of the June pool in *unheated* state was added to each tube so as to provide sensitizing capacity. It will be seen that the amount of grass allergen required to satisfy first the heat-stable factors and then neutralize the borrowed reagins was essentially unaltered by the factor of irradiation. When the heated, immune specimen was replaced by the heated "borrowed" sample, less antigen was needed. Indeed, the endpoint of 640 units (6.4 µg N) approximated that recorded earlier for the June pooled supply when it was examined in the unheated state, before irradiation, in the presence of physiological saline solution rather than a measure of the pool in heated form (see line 1 of Table VII). This showed that there was no thermostable factor in the blood procured previous to immunization.

TABLE VIII
Stability of thermostable (blocking) antibodies after ultraviolet irradiation
 (As judged by the neutralization requirements of treated and control portions of a grass-pollen antiserum)

DATE OF EXPERIMENT	TEST SUBJECT	RESPONSE OF SENSITIZED SITES TO TEST WITH 160-UNIT TIMOTHY											
		Composition of serum-allergen mixture used to sensitize site 24 hours before						Timothy pollen in PtaN units					
		Sensitizing serum		Heat-inactivated serum									
		Reaginic	Immune irradiated	20	40	80	160	320	640	1280	2560		
January 23, 1952	N. A. S.	F. E. L., June, 1950 F. E. L., June, 1950 F. E. L., June, 1950	F. E. L., July 14, 1950, yes F. E. L., July 14, 1950, no	3½	3	2½	2 3½ 3	1½ 2½ 3	1- 2 3-1½	1- 1 1½	1- 1 1		
January 30, 1952	N. A. S.	F. E. L., June, 1950	F. E. L., July 14, 1950, yes F. E. L., July 14, 1950, no				1½ 3½ 3	1- 2 1½		1½ 1- 1-0			
January 31, 1952	R. E. I.	F. E. L., June, 1950		3½	1½	±							
			F. E. L., July 14, 1950, yes F. E. L., July 14, 1950, no	3½ 3½ 3½	3½ 3½ 3½	3 3- 3-	3- 3- 3-	1 1- ±	± ± ±				
December 28, 1951	A. N. T.	F. E. L., June, 1950	F. E. L., June, 1950*	3-	3-	1½	1	1-0					
			F. E. L., July, 14, yes* F. E. L., July, 14, no*				3½ 3	3- 2	2½ 1½	1- 1-0	0 0		

* Serum heated for 1 hour at 60 C. All other specimens were subjected to 2 hours at 60 C.

SUMMARY AND DISCUSSION

The effect of ultraviolet rays on the potency of reagins has been judged by the results of serum-neutralization and of serum-dilution in normal human skin. Involved in the study were 6 samples of reagin-bearing serum donated by 3 adults who were allergic to one or more inhalant allergens (ragweed pollen, timothy grass pollen, horse epidermis, dog epidermis and cottonseed). The sera were examined over a long period, ranging from a few days to 2 years or more. Normal recipients of sensitizing mixtures or serum dilutions were restricted to 8 in number, so as to minimize the influence of the individual on the outcome. Thirty sets of experiments were done, each involving some 40 sensitizations.

Comparisons drawn between the light-treated and control portions of the same serum at the conclusion of each experiment revealed that no significant damage had been done by the rays in 17 instances whereas a slight reduction in sensitizing power may have resulted in 4, when the neutralization procedure was employed for the test. With the serum-dilution technic, no difference could be found between untreated and treated portions in 22 trials, a suggestion of lowered potency being noted in 2 experiments. The overall conclusion is that any adverse influence of ultraviolet light on sensitizing antibodies is so slight and infrequently encountered as to be of no practical significance.

In the case of the blocking, heat-stable antibody, limited observations on one timothy-immune serum indicated no loss of antigen-binding power during several experiments on 3 subjects.

The stability evidenced by the reagin, and presumably also the thermostable or blocking antibody, is consistent with the experience of numerous earlier investigators who showed that ultraviolet rays could be employed to inactivate bacteria and viruses (5-8) in the apparatus of either Oppenheimer-Levinson (2) or of Habel-Sockrider (9) without impairment of their immunizing qualities. Blanchard and associates (10) advocated irradiation as a means of protection against homologous serum hepatitis. The procedure has since been adopted by the National Institutes of Health (1) as a minimum requirement for pooled plasma which is destined for use in man. There are no reports in the literature on its application to sera which are to be appraised by tests of the Prausnitz-Küstner variety.

REFERENCES

1. Minimum requirements: normal human plasma, 7th Revision, Fed. Security Agency, Nat. Inst. Health, May 15, 1950, item 3.4.
2. OPPENHEIMER, F. AND LEVINSON, S. O. A new method for the production of potent inactivated vaccines with ultraviolet irradiation. 1. Principles, technique and apparatus. (Publication withheld by the Committee on Medical Research of the Office of Scientific Research and Development, Washington, D. C.)
3. LOVELESS, M. H. *South. M. J.*, **33**: 869, 1940.
4. LOVELESS, M. H. *J. Immunol.*, **38**: 25, 1940.
5. HODES, H. L., WEBSTER, L. T. AND LAVIN, G. I. *J. Exper. Med.*, **72**: 437, 1940.

6. LEVINSON, S. O., MILZER, A., SHAUGHNESSY, H. J., NEAL, J. L. AND OPPENHEIMER, F. J. Immunol., **50**: 317, 1945.
7. HABEL, K. Pub. Health Rep. **62**: part 1, 791, 1947.
8. WOLF, M., MASON, J., FITZPATRICK, W., SCHWARTZ, S. AND LEVINSON, S. J. A. M. A., **135**: 476, 1947.
9. HABEL, K. AND SOCKRIDER, B. T. J. Immunol., **56**: 273, 1947.
10. BLANCHARD, M. C., STOKES, J. JR., HAMPIL, B., WADE, G. R. AND SPIZIZEN, J. J. A. M. A., **138**: 341, 1948.