Fluorescein as an Indicator of Antihistaminic Activity: Inhibition of Histamine-induced Fluorescence in the Skin of Human Subjects

SAMUEL C. BUKANTZ and GUSTAVE J. DAMMIN

Departments of Medicine and Pathology and *Oscar Johnson Institute for Medical Research, Washington University School of Medicine, St. Louis*

As part of a study of the biologic reactions in hypersensitive states, we have been attempting to produce vascular lesions in rabbits by the repeated intravenous injection of histamine as described by Heinlein (*3*) and Merkel (*6*). Because the experimental animals appeared to develop an increasing tolerance for histamine, we sought quantitative methods to determine their histamine-inactivating capacity. Inconclusive results were obtained when the method of Traub, *et al.* (*8*) was employed, and it was incidentally noted that the intradermal injection of histamine did not elicit a skin response in either normal or histamine-injected rabbits. Similar observations on normal rabbits had been reported by Dorsie, *et al.* (*2*).

The possibility of the occurrence of changes in capillary permeability which were not grossly evident suggested the use of tracer substances. The usefulness of fluorescein as such a substance has been established by a number of recent studies (*1, 4, 7, 9*). Accordingly, skin sites prepared in normal rabbits by injection of 0.1-ml solutions of histamine acid phosphate, ranging in concentration from 1: 100 to 1: 10,000,000 (as base), were examined for fluorescence in ultraviolet light (G-E purple X bulb) after intravenous injection of as much as 25 mg of fluorescein/kg.[1] No significant fluorescence was observed, indicating that the normal rabbit skin was not a suitable test organ for the determination of antihistaminic activity.[2]

In continuing our search for a simple quantitative method of measuring antihistaminic activity, it seemed advisable to employ antihistaminic drugs in the skin of fluorescein-injected experimental subjects capable of responding with fluorescence to small quantities of histamine. The first experiment (in the dog, as indicated in Table 1) was performed to determine the range of activity of histamine and the relative activity of two antihistaminic drugs.[3] The results of this experiment reveal that the two antihistaminic drugs studied were of approximately equal activity in preventing fluorescence at histaminic sites, and that a 1: 10,000 solution of the drugs prevented fluorescence by 1: 100,000 and 1: 1,000,000 dilutions of histamine. Further experiments were

TABLE 1

FLUORESCENCE AT DOG SKIN SITES*

Diluent	Dilutions of histamine 1:1,000	1:10,000	1:100,000	1:1,000,000	Saline control
Saline	4	4	3	3	0
NH 188 1:10,000	4	3	0	0	
Benadryl 1:10,000	4	3	0	0	

* One-tenth ml of the designated mixtures of histamine and antihistaminic drug was injected intracutaneously 7 min before intravenous fluorescein (1.1 cc of 5% fluorescein, or 55 mg). Observations were made from 1 to 20 min following intravenous injection. 0 = No fluorescence. Digits represent relative intensity of fluorescence, 4 being maximal.

conducted in 5 human subjects, fixed concentrations of histamine (1: 50,000 in the first, 1: 100,000 in the second, and 1: 125,000 in the last 3) in varying concentrations of each antihistaminic drug being employed. Table 2 reveals that the greater concentrations of antihistaminic drug inhibited fluorescence. The 4 other human subjects

TABLE 2

FLUORESCENCE AT HUMAN SKIN SITES (SUBJECT AS)*

	Time	Saline alone	Histamine in saline 1:100,000	1:1,000,000	1:10,000,000	Dilution antihistaminic drug in histamine 1:100,000 1:5,000	1:10,000	1:20,000	1:40,000	1:80,000	1:160,000	Histamine 1:1,000,000 in saline
Benadryl												
	3:50	0	4	4	3	0	0	±	2	3	3	4
	3:58	±	4	4	3	0	0	1	2	3	3	4
	4:03	0	3	3	2	0	0	±	1	3	3	4
NH 188												
	3:50					±	±	2	3	3	3	
	3:58					±	1	2	3	3	3	
	4:03					±	±	1	2	3	3	

* Skin sites were injected with 0.1 ml of the designated solutions between 3:35 and 3:42 P.M. Fluorescein (3.0 cc of 5% solution) was injected intravenously at 3:45 P.M. Numbers 0–4 indicate intensity of fluorescence.

also manifested an almost identical inverse relationship between the concentration of antihistaminic drug and intensity of fluorescence. It was further noted that, at dimly fluorescent sites, initial fluorescence took longer to develop than at highly fluorescent sites and that the

[1] Fluorescite (C. F. Kirk and Company, New York) was used throughout these studies.

[2] We have subsequently observed intense fluorescence at skin sites prepared with histamine and horse serum in some rabbits sensitized to horse serum. This phenomenon is under investigation, as well as the possible usefulness of the sensitized rabbit for bioassay of histamine and antihistaminic drugs.

[3] NH 188, as well as a grant for this research, was kindly supplied by the Nepera Chemical Company, Inc., Yonkers, New York, and Benadryl, by Parke, Davis and Company, Detroit, Michigan.

fluorescence also disappeared more rapidly from the former sites.

Table 3 summarizes an additional experiment performed to determine the effect of histamine upon the rate

TABLE 3

EFFECT OF HISTAMINE AND HISTAMINE + BENADRYL UPON THE DURATION OF FLUORESCENCE AT HUMAN SKIN SITES FOLLOWING INTRADERMAL INJECTION OF FLUORESCEIN MIXTURES

Mixture Injected	Subjects			
	SCB	GJD	MCJ	VKP
Fl.*	35	45	4†	30
Fl.* + Histamine (1 : 10,000)	4	8	4	10
Fl.* + Histamine (1 : 10,000) and Benadryl (1 : 2,000)	35	25	25	30‡

* Fluorescein 1 : 50,000 dilution in saline.

† Allergic subject (see text).

‡ Intensity of fluorescence at this site much less than at corresponding fluorescein site.

One-tenth ml of each mixture was injected intracutaneously in the forearm. Readings, made in ultraviolet light provided by a G-E purple X bulb were made every 4–5 min throughout the period of observation. Numbers indicate duration of fluorescence in minutes.

of absorption of intradermally deposited fluorescein and the influence of an antihistaminic drug upon this action of histamine. Three sites on the forearm of each of 4 subjects (3 normal, SCB, GJD, VKP, and 1 allergic, MCJ) were injected intradermally with 0.1-ml amounts of the following: (1) fluorescein diluted 1: 50,000 in saline, (2) fluorescein + histamine (1:10,000), (3) fluorescein + histamine (1:10,000) + Benadryl (1: 2,000). In the normal subjects, (1) the fluorescein sites remained visible under ultraviolet light for periods of 30–40 min, (2) the fluorescein + histamine sites no longer fluoresced after 4–10 min, and (3) the fluorescein + histamine + Benadryl remained visible as long as the fluorescein sites. In normal subjects, therefore, fluorescence disappeared rapidly under the influence of histamine, and the antihistaminic drug consistently neutralized this effect. In the allergic subject, the fluorescein site was visible for only 4 min, as was the fluorescein + histamine site, presumably because of local release of histamine or histamine-like substances in the fluorescein site; the fluorescein + histamine + Benadryl site fluoresced for 25 min, demonstrating the neutralizing effect of the antihistaminic drug.

These observations demonstrate that the time of appearance, intensity, and duration of fluorescence at sites injected with histamine may be quantitatively modified by the local presence of antihistaminic drugs. It is anticipated that the application of the dermofluorometer (*5*) to these studies (now in progress) will yield time-intensity curves permitting accurate bioassay of histamine and antihistaminic drugs, whether the latter are injected simultaneously or prior to testing. Such quantitative photometric determinations of fluorescence may also provide a useful tool for the investigation of the degree and duration of humoral and tissue antihistaminic properties following different routes of administration of various antihistaminic drugs.

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Effect of Hormone on Root Formation in *Artocarpus communis*

THOMAS J. MUZIK

Department of Botany, University of Michigan

The tropical breadfruit, *Artocarpus communis*, is usually propagated by means of sprouts which arise from the roots. Shoots arising from the roots of this tree, which extend for a considerable distance from the trunk but remain within a few inches of the soil surface, are separated from the tree when about 12–14″ tall. Sections of the root are planted with the young stem. Branch cuttings are not planted in usual practice.

Experiments in Liberia, West Africa, where the writer was research botanist in the Research Department, Firestone Plantations Company, Harbel, have shown that branch cuttings may be successfully used when treated with hormone. The cuttings dipped in a 1% indole-butyric acid solution gave best results, although others with their bases immersed in a .0002% indole-butyric acid solution for 24 hrs also gave good results Cuttings

TABLE 1

No. of cuttings	Treatment	No. rooted	% rooted
60	Control	0	0
60	24 hrs .0002% indole-butyric	36	60
60	dip in 1% indole-butyric	48	80

were about 12–15″ long, with 3–4 nodes/cutting, about ⅜″ in diameter, and were planted immediately in sand. Banana leaves were laid over the beds for three days after planting to prevent excessive drying. The beds were under a thatched roof which provided light shade. Cuttings were watered daily. Untreated cuttings failed to root, but treated cuttings gave up to 80% success and yielded healthy plants when set out.

The results are given in Table 1.

This method of propagation can be very useful where, as in Liberia, planting stock of breadfruit is not always available.