combination of these methods. Because of the time required for the completion of such tests, a diagnosis is made usually in retrospect. This same problem is encountered in the laboratory diagnosis of other viral diseases.

Liu (*1*) used the fluorescent antibody technique of Coons and Kaplan (*2*) in the rapid diagnosis of influenza. He observed specific fluorescence when nasal washings were treated with fluorescein-tagged influenza antiserum. Since fluorescence microscopy has been used to demonstrate herpes simplex virus in infected cells and tissues (*3*), its possible use in the rapid diagnosis of herpes simplex virus infections was the object of the preliminary investigation discussed in this report (*4*).

Microscopic slides were prepared from portions of specimens of scrapings from the base of vesicular lesions, from a spinal fluid, or from a nasal swab. These preparations were then fixed in acetone for 10 minutes, washed in physiological saline, and stained with fluorescein-labeled antiserum, which was produced in rabbits in response to subcutaneous injections of herpes-simplex-virus infected extraembryonic fluids of the developing chick embryo. This serum was absorbed with dried mouse-liver powder to remove nonspecific fluorescing material. The conjugation of the antiserum with fluorescein isocyanate and the staining of prepared slides were accomplished according to the method of Coons and Kaplan (*2*). The stained smears were examined with a Bausch and Lomb microscope fitted with a cardioid dark-field condenser and illuminated with a Reichert Fluorex HBO 200 mercury-arc vapor burner and filters.

Portions of the same specimens which were used for making smears were prepared in buffered gelatin saline containing antibiotics and were inoculated onto the chorioallantoic membrane of the developing chick embryo. Six embryos were used per specimen. After incubation of the embryos for 72 hours at 36°C, the chorioallantoic membranes were removed and examined for herpes simplex virus lesions.

Specific yellow or yellow-green fluorescence was observed with eight of the 15 specimens when the fluorescein-stained smears were observed by fluorescence microscopy. In Table 1 it can be seen that typical herpetic lesions were observed on chorioallantoic membrane inoculated with the same eight specimens in which specific fluorescence was observed. No specific fluorescence or herpetic lesions were observed with the other seven specimens. These results demonstrate good correlation with the tentative clinical diagnosis.

The results reported here give evidence that this technique may prove valuable in the diagnosis of vesicular lesions caused by herpes simplex virus. Whether this technique can be applied to spinal-fluid specimens in the diagnosis of herpetic aseptic meningitis or encephalitis is still not known. Establishment of the validity of this method could reduce the time required at present for viral diagnosis and may prove to be invaluable to the dermatologists in differentiation of lesions of herpes simplex virus from those caused by other agents.

Table 1. Rapid diagnosis of herpes simplex virus infections by means of fluorescent antibody studies.

Patient	Tentative clinical diagnosis	Viral isolation		Fluorescence microscopy	
		Specimen	Results	Specimen	Results
B.	Generalized herpes simplex or vaccinia infection	Vesicular fluid	+	Vesicle lesion scraping	+
B. B.	Generalized herpes simplex or vaccinia infection	Vesicular fluid	+	Vesicle lesion scraping	+
Br.	Pemphigus	Vesicular fluid	−	Vesicle lesion scraping	−
E.	Aseptic meningitis	Spinal fluid	−	Spinal fluid	−
G.	Acute herpetic gingivostomatitis and rhinitis	Nasal washing	+	Nasal smear	+
N.	Shingles	Vesicular fluid	−	Vesicle lesion scraping	−
S.	Recurrent herpes of lip	Vesicular fluid	+	Vesicle lesion scraping	+
Pa.	Shingles complicating terminal leukemia	Vesicular fluid	−	Vesicle lesion scraping	−
Ph.	Herpes vulvovaginitis	Vesicular fluid	+	Vesicle lesion scraping	+
L.	Recurrent dermatitis	Vesicular fluid	−	Vesicle lesion scraping	−
Q.	Recurrent herpes of lip	Vesicular fluid	+	Vesicle lesion scraping	+
Sm.	Generalized dermatopaphism	Vesicular fluid	−	Vesicle lesion scraping	−
Sc.	Acute herpetic gingivostomatitis	Vesicular fluid	+	Vesicle lesion scraping	+
U.	Lesions on lips and pharynx	Vesicular fluid	−	Vesicle lesion scraping	−
M. S.	Recurrent skin eruptions	Vesicular fluid	+	Vesicle lesion scraping	+

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4. We wish to express our appreciation to the department of dermatology of the University of Oklahoma School of Medicine for its cooperation in this project. This work was supported in part by a grant (B964C2) from the National Institutes of Neurological Diseases and Blindness.

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Fluorescence of Tryptophan Derivatives in Trifluoroacetic Acid

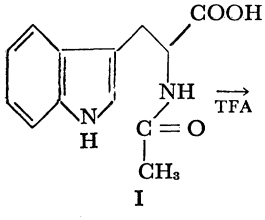
Abstract. Solutions of tryptophan-containing proteins in trifluoroacetic acid (TFA) develop green fluorescence. Studies have been made of the effect of TFA on tryptophan and tryptophan derivatives, and a dihydro-β-carboline has been identified among the fluorescent products.

Proteins and polypeptides are readily soluble in trifluoroacetic acid (*1*). Such solutions are useful for the investigation of the physical and chemical properties of proteins, and it is thus important to understand the extent of chemical degradation brought about by the strongly acidic solvent. This report deals with the effect of TFA on tryptophan-containing materials.

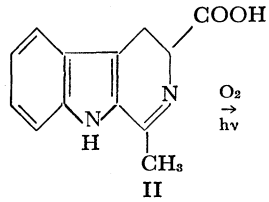
Solutions of tryptophan-containing proteins in TFA, even in the absence of oxygen, gradually develop a green fluorescence. This fluorescence is associated with the protein and is carried with it

on high-speed centrifugation of the solutions. Ovalbumin and lysozyme, for example, develop fluorescence of intensity roughly proportional to their tryptophan content [1 percent (*2*) and 5 to 6 percent (*3*), respectively]. This fluorescence is characterized by a single band having a peak at 530 ± 5 mμ (corrected for detector sensitivity), about 90 mμ wide at half-maximum intensity. Maximum fluorescent lifetimes were determined by flash irradiation and shown to be less than 2×10^{-7} second. The simple derivatives, glycyl tryptophan and acetyl tryptophan, develop fluorescence in the same fashion as the proteins.

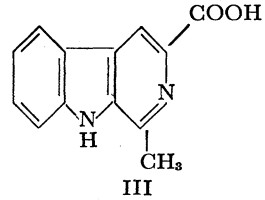
The action of TFA on acetyl-DL-tryptophan was studied in detail. Storage of this substance in oxygen-free TFA results in the growth of a 365-mμ absorption band. Absorption of energy near this wavelength excites fluorescence centered at 535 mμ. The substance responsible for this phenomenon can be isolated in about 10 percent yield from solutions heated to reflux under nitrogen for several hours, and has been identified as 1-methyl-3-carboxy-3,4-dihydro-β-carboline (mp 199° dec.; ultraviolet absorption in ethanol given in Fig 1; *Anal.*, calcd, for $C_{13}H_{12}N_2O_2$: C, 68.41; H, 5.30; N, 12.27; Found: C, 68.13; H, 5.44; N, 12.19.) Although the isolated yield of carboline is poor, it is interesting to note that trifluoroacetic acid can bring about a cyclization reaction of the Bischler-Napieralski type. The low yield is in part accounted for by a transamidation (*4*).



I



II



III

As might be expected, treatment of acetyl-DL-phenylalanine with TFA did not yield detectable quantities of the corresponding dihydroisoquinoline.

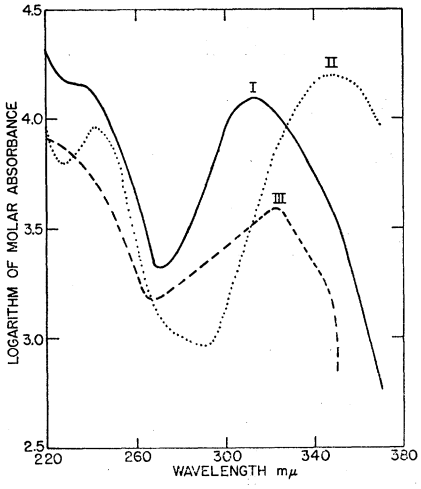


Fig. 1. Ultraviolet absorption spectra in ethanol: I, 1-methyl-3-carboxy-3,4-dihydro-β-carboline, alkaline solution; II, same as I, neutral or acid solution; III, fluorescent product isolated from TFA-treated glycyl tryptophan.

In studies of the fluorescence of the dihydro-β-carboline (II) in TFA solution, it was observed that the green fluorescence was gradually replaced by a blue (470 mμ) emission, with the development of an absorption maximum at about 280 mμ. This same change occurs in 6*N* hydrochloric acid solution and is found to be the result of an oxygen-requiring photodehydrogenation. The product of this oxidation is a mixture of 1-methyl-3-carboxy-β-carboline (III), dec. above 300° (ultraviolet absorption in acidic ethanol: $\lambda_{max.}$ 244, 277, 300, 365 mμ, log ε 4.48, 4.77, 4.21, 4.07. *Anal.*, calcd, for $C_{13}H_{10}N_2O_2 \cdot H_2O$, C, 63.92; H, 4.95; Found: C, 64.29; H, 4.97) and 1-methyl-β-carboline, mp 236°–238° dec. (*5*), identified by spectrum and melting point. Although, judged spectroscopically, the dehydrogenation reaction proceeds without side reactions, only 25 percent of III and 15 percent of the decarboxylated product were recovered in crystalline form.

The 530-mμ fluorescence which develops in oxygen-free TFA solutions of glycyl tryptophan, lysozyme, and ovalbumin is produced by a molecular species which has an absorption maximum at 420 mμ when in TFA solution. (A like fluorescence develops in solutions of glycyl tryptophan in other perfluoro acids, in concentrated sulfuric or phosphoric acids, and in anhydrous hydrogen fluoride.) The fluorescent product from TFA-treated glycyl tryptophan has been purified by extraction into butanol, followed by paper chromatography, but it has not been obtained in crystalline form; it is ninhydrin-active and is readily oxidized in air to dark colored products. Trifluoroacetic acid solutions of this isolated material exhibit the 420-mμ absorption and green fluorescence of the original TFA solutions; solutions in ethanol have the absorption spectrum shown in Fig. 1, but the long-wavelength maximum at 322 mμ is only slightly shifted (to 317 mμ) on acidification, in contrast to the shift to 350 mμ which occurs with known dihydro-β-carbolines. It would be unwise to assign a dihydro-β-carboline structure to this product, the more so since the 420-mμ absorption in TFA is not readily explained in terms of such a structure. Work is continuing on its identification although it is obtained only in small amounts, the bulk of the glycyl tryptophan remaining unchanged on 3 days' storage in TFA.

Tryptophan itself is unstable in TFA when oxygen is present; even traces of oxygen cause such solutions rapidly to become pink, rendering the detection of green fluorescence impossible. In the absence of oxygen, a weak green fluorescence (525 mμ) is observed immediately on mixing; this emission does not increase with time and is perhaps a property of impurities initially present in the solute. Tauber has reported a green fluorescence of tryptophan-containing materials dissolved in perchloric acid (*6*). The present work indicates that this observation may also be made (fluorescence at 535 mμ) on solutions in 70 percent sulfuric acid. In the sulfuric acid solvent, when oxygen has been excluded, an initial green fluorescence grows in quickly but does not further increase with time. It is probably a fair assumption that the green fluorescence of tryptophan in these solvents is the result of oxidation, and, in spite of the coincidence in emission maxima, has not the same source as the fluorescence of glycyl tryptophan, ovalbumin, and lysozyme in TFA.

Reports have been made of the formation of visibly fluorescent photo-oxidation products on ultraviolet irradiation of aqueous tryptophan solutions. These are said to be derivatives of kynurenine (*7*). In TFA, however, kynurenine (*8*) shows no detectable visible fluorescence, and kynurenic acid (*8*) emits at 445 mμ (about 500 mμ in 70 percent sulfuric acid), so that neither is responsible for the green emission observed in our studies. Tryptophan itself has been shown to fluoresce in aqueous solution, but in the ultraviolet region (360 mμ) (*9*).

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Source and Possible Nature of the Odor Trail of Fire Ants

Abstract. Experimental evidence shows that the odor trail of the fire ant *Solenopsis saevissima* (Fr. Smith) is produced as a secretion of the accessory gland of the poison apparatus and released through the extruded sting. Preliminary studies suggest that this substance may be chemically allied to or even identical with the toxic principle of the venom.

Chemical trails laid down by worker ants are essential mechanisms in the organization of foraging and colony migration in many ant species. Yet only recently has much careful attention been paid to the topographic form of these trails, to their anatomical source, and to their chemical nature (*1, 2*). Chemical analyses conducted by Carthy show that in the formicine species *Lasius fuliginosus* (Latreille) the trail substance is a water-soluble anal emission containing uric acid, polysaccharides, and proteins (*2*). These data suggest that the bulk of the material is normal excretory and fecal matter rather than a special glandular secretion. But they do not exclude the possibility that special secretory products, serving as releasers of trail-following behavior, may be present in small amounts.

Now it is possible to show that in the myrmicine species *Solenopsis saevissima* (Fr. Smith) the essential trail substance is produced as a glandular secretion and is released through the sting. Workers of this species lay trails by dragging the tips of their abdomens over the ground with the stings fully extruded. To determine whether venom passed from the sting can induce trail following, a series of experiments was performed in which artificial trails of freshly extracted venom were drawn in the vicinity of foraging workers from a captive colony. In separate trials, venom was either collected directly on sharpened cork tips or (to reduce the possibility of contamination) collected by "milking" the ants with fine capillary tubes and then transferring the fluid to cork tips.

In preliminary tests, foraging workers could almost always be diverted to the artificial venom trails, although their response was generally weaker than it was to true trails laid in the same area by living minor workers. Under the experimental conditions described below, three such trails produced from venom collected directly on cork tips brought forth 6, 8, and 18 workers, respectively, the duration of the effect extending between 1 and 2.5 minutes. Numerous artificial trails made under the same conditions from the wall and contents of the principal gut divisions, as well as from crushed tissue and hemolymph of head, alitrunk, and abdomen (gaster), evoked various intensities of alarm, circling, antennal palpation, and even feeding (in the case of the crop contents), but no distinct trail following.

An attempt was next made to localize the source of the critical trail substance. Three organs are known to empty material through the sting or in its immediate vicinity: the hind-gut, the "true" poison glands (which are paired and empty their contents into the poison vesicle), and the accessory gland of the sting. In a series of experiments these organs were dissected out of freshly killed major and media workers, separated, doubly washed in insect Ringer's solution, and then crushed on separate cork tips to make artificial trails. All three organs were taken from each ant killed, and these were presented in varying sequences to eliminate possible bias in the results due to special sequential effects. As much uniformity as possible was obtained with respect to the number of foraging workers and their trophic "mood" by first allowing masses of workers to accumulate around a freshly killed meal worm (larva of *Tenebrio molitor*) pinned at the edge of the glass plate on which the trails were drawn. Under the particular conditions prevailing at the time of the experiments, a relatively stable concentration of 150 to 200 workers was reached within 10 minutes after the meal worm had been found by the first foraging worker. At this time a fringe of more or less idle workers milled in a tight group around the edge of the meal worm, and it was to these that the artificial trails were drawn.

The results, presented in Table 1, show clearly that the trail substance is concentrated in the accessory gland of the sting. The fact that both the hind-gut and the true poison glands (with vesicle) frequently gave quite negative results suggests that these structures do not normally contain any of the releaser substance at all, only picking it up by contamination during dissection. The relatively high variation in response to the accessory gland preparation may be explained, at least in part, by two irregularities difficult to control: variation in leakage of gland contents during dissection and variation in responsiveness of worker groups. It was noted that when several trials were made on the same day, responsiveness tended to decline progressively.

Table 1. Response of fire ant workers to artificial trails made from various abdominal organs of ten freshly killed workers. The positive responses recorded are those in which workers ran at least half the length of the artificial trails, or approximately 8 cm. The duration is the time interval from the first positive response observed to the last and is given to the nearest half minute.

No. of workers responding		Duration of group effect (min)	
Range	Mean ± standard error	Range	Mean
Hind-gut			
0–18	2.3 ± 1.7	0–1.5	0.4
Poison glands plus poison vesicle			
0–26	8.2 ± 2.8	0–4	1.4
Accessory gland			
31–164	107.5 ± 14.4	3.5–7	5.9

The foregoing results lead to the question: Does the accessory gland substance serve as both releaser and orientator, or is it only a releaser, with venom from the true poison glands functioning as the orienting agent in the trails? To solve this problem, the following experiment was devised. After the experimental conditions described previously had been arranged, an *accessory* gland preparation was drawn in a short sidewise stroke next to the peripheral group of ants, while simultaneously an artificial trail made from the *poison* glands (plus vesicle) was drawn outward from them. In each of five such trials, the ants showed intense excitement, spreading outward in random looping movements, and many new workers were attracted to the scene. But in only one case was the poison-gland trail followed, and then by the relatively small force of 20 workers, representing less than 30 percent of the outward-moving swarm of foragers.

It thus appears that the accessory gland secretion functions as both a releaser and an orientator of trail following. On the other hand, it was noted that occasionally when workers were following accessory-gland trails in large numbers they would also follow nearby old