

Table 1. Mean air temperature characterizing four climatic districts in southern California during the summers 1949-55, mean air temperature and total evaporation during the summer season during which four varieties of citrus fruit were grown (1955), and physical characteristics of the fruit peel.

Fruit	Year	Climatic district			
		Coast	Intermediate	Interior	Desert
		<i>Air temperature (°F)</i>			
	1949-55	66.5	71.0	73.2	86.7
	1955	66.6	69.9	74.0	85.9
		<i>Evaporation (in.)</i>			
	1955	7.2	9.1	11.1	16.8
		<i>Peel moisture (%)</i>			
Lemon	1955	448.6	—	388.3	380.9
Grapefruit	1955	—	505.9	388.1	300.3
Valencia orange	1955-56	329.3	—	242.3	273.1
Washington navel orange	1956	—	405.4	309.2	—
		<i>Weight of dry peel per unit volume of fresh peel (g/cm³)</i>			
Lemon	1955	0.1737	—	0.1788	0.1700
Grapefruit	1955	—	0.0973	0.1284	0.1529
Valencia orange	1955-56	0.1726	—	0.2182	0.1982
Washington navel orange	1956	—	0.1500	0.1711	—
		<i>Peel-puncture-pressure (lb/in.²)</i>			
Lemon	1955	1094	1123	—	833
Grapefruit	1955	—	190	254	496
Valencia orange	1955-56	258	—	332	485
Washington navel orange	1956	—	224	423	—
		<i>Storage life</i>			
Citrus		Short	—	Long	—
		<i>Injured by fumigation</i>			
Citrus		Readily	—	Not readily	—
		<i>Injured by oil spray</i>			
Citrus		Readily	—	Not readily	—

selected, in a randomly selected grove of a given variety in a given climatic district. Eleven groves were sampled in a 5000-mi² area; groves of the same variety were separated by the random sampling with the result that two pairs of groves were 30 air-line miles apart, one pair 38, one pair 72, one pair 107, and 2 pairs 133 miles apart.

Variation between individual fruits, heights on tree, trees in a grove, varieties in a climatic district, and between climatic districts (varieties disregarded) was measured statistically. Variables such as tree age, bud sources, pest control, cultivation, cover crop, fertilizer, frost protection, soil type, depth, water capacity, pH, and so forth, and irrigation water quality, irrigation quantity, irrigation interval, nutritional sprays, picking and pruning practice, wind protection, leaf surface, tree height, weed control, and so forth, which in part make up the minimum of 3.67×10^{11} permutations and combinations of variables in citrus culture, were not under control but were segregated as a group and measured as error. Peel moisture and dry weight were determined for each whole fruit peel by

drying the peel to constant weight in an air-draft oven at 65°C. The puncture-pressure of the peel, the measure of tenderness used in this work, was determined by six puncture-pressure tests around the equator of each individual fruit used in the moisture and dry weight determinations.

Table 1 shows that the mean daily temperature for the four months June, July, August, and September 1955 and the six previous summer seasons (that is, for 1949-1955) is progressively higher for each climatic district in the order coastal, intermediate, interior, and desert. Fruit which were growing on the trees during these four months in the summer of 1955 were grown under a similar trend of mean air temperature and air evaporation in the four districts, as is shown in Table 1. In general, the puncture-pressure and dry weight of the peel per unit volume of peel increased from coastal to desert district, and the percentage of moisture in the peel decreased. These factors appear to be correlated with other observations of tenderness to a high degree.

The trends of these three factors with

climatic district, mean air temperature, and air evaporation for the four summer months are shown in Fig. 1. The multiple correlation coefficient between the puncture-pressure of the peel (y), evaporation (x_1), and the reciprocal of peel moisture as percentage of dry peel (x_2) is very high ($R = +0.9898$).

It therefore appears that climatic conditions such as prevailing mean daily air temperatures and evaporation probably induce the same types of morphological responses in fruit as they do in leaves, the leaves probably playing a strong role by withdrawing water from the fruit in periods of water stress as has been long known in citrus.

Differences between mean maximum and mean minimum daily temperatures for the different districts appear not to be the major influence on these responses, because (i) the difference was smaller in the desert district than in any but the coastal district, and (ii) in all districts fruit from the tops of trees where they would be subjected to greater water stress had significantly greater peel puncture-pressures and higher dry weights per unit volume of peel, and lower percentage of moisture, than fruit from the lower regions of the trees.

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Rapid Diagnosis of Herpes Simplex Virus Infections with Fluorescent Antibody

Abstract. A fluorescent microscopy technique is described which may prove useful in differentiating clinically similar lesions into lesions of herpes simplex virus etiology and nonherpetic lesions. Herpes simplex virus was isolated from the specimens which yielded positive fluorescence, and no virus was isolated from the specimens which yielded no fluorescence.

The laboratory diagnosis of a herpes simplex virus infection is dependent upon the isolation and identification of the virus, the observation of type A inclusion bodies, or the demonstration of a rise in specific antibodies, or upon a

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 speci-

mens 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.

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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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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 dermatophagism	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	+

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