

THE INFLUENCE OF EXPERIMENTAL CONDITIONS ON THE RESULTS OF IN VITRO TESTS FOR ANTIFUNGAL ACTION (WITH SPECIAL REFERENCE TO THE EFFECT OF MAINTAINING SOLUBILITY OF THE AGENTS)*

PRELIMINARY REPORT

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The preceding preliminary report (1) pointed out that certain fatty acids and trimethyl cetyl ammonium pentachlorophenate (TCAP) were more fungicidal at pH 5 or 6 than at pH 7 or 8. In further experiments still other factors could be shown to exert powerful influences upon the antifungal action. Thus, the use of different types of vehicles or media brought about great differences in results. The following experiment is an example.

One per cent stock preparations of undecylenic acid were made: (a) in water,** (b) in 2% aqueous triethanolamine, (c) in propylene glycol and (d) in 95% ethanol. Serial dilutions were made of these stock preparations: of (a) in water; of (b) in water with (10 vol. % of) Mc Ilvaine's buffer at pH 8.5; of (c) in equal parts of water and propylene glycol; and of (d) in equal parts of water and ethanol. 0.1 ml. of each of these liquids as well as of the corresponding liquid without the undecylenic acid (blank control) was added to Sabouraud's maltose agar (Difco) and to broth, (1% neopeptone, 4% glucose). The pH of these media was adjusted to pH 5 and 7 with Mc Ilvaine's buffer. The final volumes were 5 cc. Broth and slanted agar tubes were inoculated with a heavy suspension of spores of *Trichophyton gypseum*. The minimum concentrations inhibiting growth are shown in the example on table 1.

TABLE 1

Growth inhibitory concentration of undecylenic acid (mg. per cc.) mixed in various diluents (see text), in agar or broth adjusted to pH 5 or pH 7

MEDIUM	pH	WATER	TRIEHTANOLAMINE WATER BUFFERED AT pH 8.5	PROPYLENE GLYCOL WATER	ALCOHOL WATER
		mg./cc.	mg./cc.	mg./cc.	mg./cc.
Agar	5	0.11	0.055	0.02	0.02
	7	0.20	0.20	0.1	0.1
Broth	5	0.065	0.016	0.013	0.013
	7	0.20	0.14	0.08	0.08

These results again demonstrate that undecylenic acid inhibits more at pH 5 than at pH 7; moreover, higher inhibitory values were obtained when liquids which increase the solubility were employed, and highest titres were found when broth was used as the test medium.

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Received for publication, July 4, 1946.

** All aqueous dilutions were shaken until homogeneous for use.

In a second experiment, preparations (a), (b), (c) and (d) and their blank controls were compared by a diffusion method, in which inhibitory zones were measured on agar plates seeded with *Trichophyton gypseum*: 0.2% concentrations were made in the diluents listed above; filter paper discs 1 cm. in diameter were dipped in the preparations and placed on the surface of 10 ml. of inoculated Sabouraud agar in petri dishes. Table 2 shows the diameters of inhibitory zones, measured after four days.

TABLE 2

Antifungal activity of 0.20% undecylenic acid mixed in various diluents (see text) as shown by inhibitory zones (cm.) in agar adjusted to different pH levels

AGAR ADJUSTED TO pH	WATER	TRIETHANOLAMINE WATER BUFFERED AT pH 8.5	PROPYLENE GLYCOL WATER	ALCOHOL WATER
	cm.	cm.	cm.	cm.
5	3.2	3.6	4.5	9.0
7	0	1.8	2.2	1.6
8	0	0	0	0

These findings are parallel to those shown in table 1. The effect of pH on the inhibitory action of undecylenic acid is again apparent. Likewise, the effect of solubility of the acid is evident. Obviously two factors, an acid medium and a suitable solvent cooperate in producing maximal efficacy of the agent under test.

Under experimental conditions which tend to maintain solubility of undecylenic acid (use of propylene glycol, alcohol, aqueous triethanolamine at pH 8.5), higher inhibitory values were obtained (in both, acid and neutral media) than when aqueous dilutions of the acid were used. The activity under these conditions is again preponderantly one of the free acid in solution; for the tables consistently show that the undecylenate-ion (media at pH 7 or 8) is inferior to the free acid (media at pH 5) in antifungal activity.

Experiments in which similar relationships were observed with other water insoluble fatty acids (heptylic, caprylic, pelargonic, capric) and TCAP will be described in a future publication.

The findings described here and in the preceding report are also of interest in connection with the statements of Rothman and co-workers (2a, b) that odd-carbon, saturated higher fatty acids present in the sebaceous secretion are strongly active against microsporon Audouini. It appears probable that the activity of these acids is greatly enhanced by the sweat secretion, since normal sweat should bring about both low pH and good dispersion, similar to the conditions producing optimal activity in our experiments.

SUMMARY

Experiments are described which indicate that the growth inhibitory activity of undecylenic acid against pathogenic fungi *in vitro* is dependent upon pH and solubility. Thus the types of vehicles and of media chosen can be of decisive influence on the results of studies of antifungal action.

REFERENCES

1. FOLEY, E. J., HERRMANN F., AND LEE, S. W.: The Effects of the pH on the antifungal Activity of fatty acids and other agents. *J. Invest. Dermat.* **8**: Jan. 1947.
2. (a) ROTHMAN, ST., SMILJANIC, A. M., AND SHAPIRO, A. L.: Fungistatic Action of Hair Fat on *Microsporon Audouini*. *Proc. Soc. Exper. Biol. & Med.* **60**: 394, 1945.
(b) ROTHMAN, ST., SMILJANIC, A. H., SHAPIRO, A. L., WEITKAMP, A. W.: Mechanism of Spontaneous Cure of *Tinea Capitis* in Puberty. Read at the 7th Annual Meeting of the Society for Investigative Dermatology, Inc., June 1946, San Francisco, Cal. In Press *J. Invest. Dermat.*