PAPER CHROMATOGRAPHIC SEPARATION OF FLUORESCENT MATERIALS IN NORMAL RAT HAIR*

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During investigations with ringworm in laboratory animals it was observed that the pelage of normal albino rats fluoresces brilliantly under ultraviolet light, (Fig. 1). Besides being fluorescent the hair of albino rats is definitely yellowish when compared to the white hair of other animals. The distinctive body odor of rats also is present at least in part in the clipped hair. The skin of the rat is not fluorescent in the sense being described.

METHODS

Both the fluorescence and the yellow color of the rat hair may be extracted with boiling water, and concentrated by boiling to give a yellow solution with a colloidal turbidity. Extracting the hair in the autoclave at 120°C or autoclaving the boiled extract results in a nonturbid, brownish yellow solution. Neither the fluorescence nor the yellow material was extracted by water or ethanol at 50°C, or by acetone, ether, or chloroform at 30°C. Dilutions of the extract in water, propylene glycol, and glycerol were yellowish green under 3660 Å ultraviolet light, with a slight fluorescent aspect. Dilutions in non-polar solvents such as acetone, ether, chloroform, and benzene were brightly fluorescent, with a color matching that of the rat hair itself. Dilutions with ethanol were intermediate in aspect between that in the polar aqueous solvents and organic solvents. Concentrated solutions dried on filter paper left a bright greenish-yellow fluorescent spot. Dilute solutions left a pale bluish-white spot. The fluorescent factor appeared to possess only limited solubility in organic solvents. The fluorescence of the extract was not affected by extremes of pH at room temperature for short periods. Both the fluorescence and the yellow color of the extract were found to be completely adsorbed by activated charcoal. Precipitation technics have not been successful. Recent work on the chromatographic separation of fluorescent metabolic derivatives of trytophan suggested that similar methods might be applied to the fluorescent material in rat hair. Accordingly, we employed the ascending paper chromatographic technic and developer described by Mason and Berg (1) with satisfactory results.

To find the relationship between the various fluorescent spots obtained, individual spots were eluted with pH 7.6 phosphate solution, reconcentrated by boiling and again spotted on the paper.

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This investigation was supported by a research grant (PHS E502-C) from the National Microbiological Institute, National Institutes of Health, Public Health Service. Laboratory space and animal quarters were made available by the Oklahoma Medical Research Foundation.

Received for publication June 22, 1956.

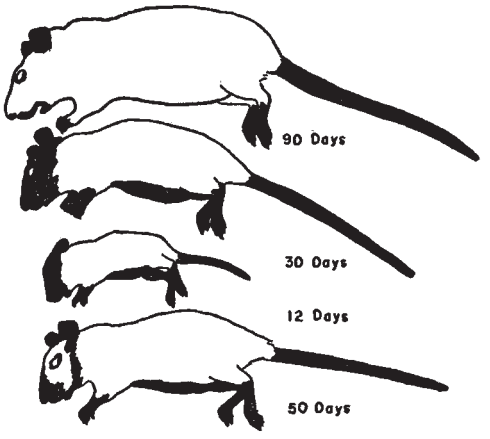

FIG. 1. Fluorescence of normal albino rats. The blackened areas and the vibrissae are non-fluorescent. Observe the characteristic mask and lateral-ventral patches of developing fluorescence in the maturing (50 day) rat, corresponding to the wave of anagen, described by Haddow and coworkers (3, 4).

RESULTS

1. We found that with n-butanol 2, methanol 4, benzene 2, water 2 developer, the principal fluorescence of the boiled extract separated to give a bright bluish green spot (F1) and two paler yellow green spots (F2 and F3) above spot F1. The yellow color of the rat hair separated as a spot partly superimposed on spot F2, (Fig. 2).

2. When we subjected the extract to autoclaving the fluorescent spot F1 and the yellow spot failed to appear and the yellow green spots F2 and F3 were much intensified, and appeared yellow under visible light.

3. When the paper was sprayed with Ehrlich's reagent (p-dimethylamino-benzaldehyde), the bright yellow spot of urea appeared immediately above spot F1, spot F1 developed a salmon color, the yellow spot developed a transient pink color, and the fluorescence of all spots disappeared.

4. The addition of one part of acetic acid to the developer resulted in a marked increase in the Rf values of F2, F6 and the yellow spot, and the appearance of a new spot (F5) between F6 and F2.

5. Spot F5, which like spot F1 develops a salmon color with Ehrlich's reagent, was obtained from the eluent of F1 after boiling and *vice versa* suggesting a possible simple isomeric relationship between them.

6. Spots F2 and F3 were obtained by autoclaving the eluent of spot F1 indicating that they are in fact derivatives of it.

7. Spot F3 was obtained from spot F2, indicating that these two spots are also probably simple isomers. Their identity to the corresponding spots F2 and F3 from acid development was demonstrated by two dimensional development. In the case of spots F1 and F2, equilibrium appeared to be strongly in the direction

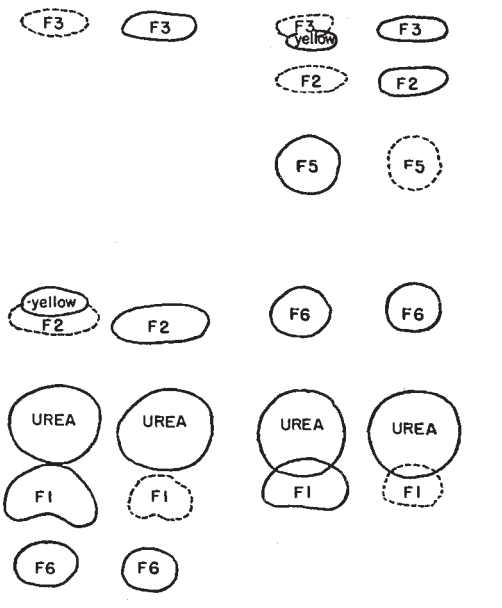

FIG. 2. Paper chromatograms of boiled and autoclaved extracts of rat hair with neutral and acid development. The upper and lower lines indicate solvent limits after 12 hours development.

of F1, but in the case of spots F2 and F3 the relative intensity of the two varied with the extraction and storage of the extract.

8. We found that spot F6 (a weak bluish fluorescence) was not specific for rat hair extract. We obtained it from the boiled extract of guinea pig hair, cat hair, and rabbit hair as well.

9. Comparison with pure samples of kyneurenic acid and anthranilic acid and observation of the spots appearing with Ehrlich's reagent (2), did not support the hypothesis that the fluorescent substances in the rat hair were known tryptophan derivatives.

10. Chromatographic comparison with the extract of fluorescent hair from kittens infected with *Microsporum canis* did not support the hypothesis that rat hair fluorescence and ringworm fluorescence are due to closely related compounds. Similar sharp localization of the fluorescence on chromatograms was not obtained.

11. An extract of nonfluorescent hair from the bellies of 30 day rats (Fig. 1) yielded neither the fluorescent spots nor the yellow spot characteristic of the chromatograms of extracts of fluorescent rat hair.

SUMMARY

The factors responsible for the fluorescence and yellowish color, respectively, of normal albino rat hair were found extractable by boiling water but not by tepid water or organic solvents. Satisfactory separation and evidence of the occurrence of the fluorescent material as an isomeric pair were obtained by paper chromatography.

REFERENCES

1. Mason, M. and Berg, C. P.: A chromatographic method for the detection of tryptophane metabolites. J. Biol. Chem., **188:** 783–788, 1951.
2. Dalgliesh, C. E.: The relationship between pyridoxin and tryptophan metabolism studied in the rat. Biochem. J., **52:** 3–14, 1952.
3. Haddow, A., Elson, L. A., Roe, E. M., Rudall, K. M. and Timmis, G. M.: Artificial production of coat colour in the albino rat—its relation to pattern and growth of hair. Nature, **155:** 379–381, 1945.
4. Durward, A. and Rudall, K. M.: Studies on hair growth in the rat. J. Anat., **83:** 325–335, 1949.