be seen from this table that (1) sulfanilamide at 37°C delayed the growth, but did not completely prevent it, even when small inocula (1/6,250,000 dilution of a 48 hours culture) were used; (2) sulfanilamide at 43°C completely and continuously inhibited visible growth of the enterococcus; (3) sulfanilamide at 43°C was bacteriostatic toward much larger numbers (1:50 dilution of a 48 hours culture) of enterococci than at 37°C.

This increased bacteriostatic activity of sulfanilamide in concentration of 1% at 43°C could be demonstrated with 3 strains of hemolytic and 3 strains of non-hemolytic enterococci, even when relatively large numbers of microörganisms (1:50 dilution of a 18 to 48 hours culture) were used for inoculation. With one strain of non-hemolytic enterococcus, growth was not completely inhibited but definitely retarded by sulfanilamide at 43°C.

It is important to mention that the growth of the enterococci in the control broths was not markedly delayed or suppressed at 43°C in comparison to that obtained at 37°C. This observation supports the view of White,[10] namely, that the increase in activity of sulfanilamide toward hemolytic streptococci cannot be explained solely on the basis of a deceleration of the growth rate at higher temperature.

In conclusion, at 43°C sulfanilamide in concentration of 1% is markedly more bacteriostatic toward both hemolytic and non-hemolytic enterococci, than at 37°C.

## 11093

## Tyrosinase in Feather Germs.

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The presumable enzyme system involved in melanin formation by growing feathers does not seem to have been investigated, although it might be supposed to be a tyrosinase or dopa-oxidase, by analogy with melanogenesis in mammalian skin and hair roots, amphibian skin, insect hypoderm, etc. That a tyrosinase *is* present, whatever its rôle, in feather germs of black and red chickens, and absent or somehow masked in the germs of certain white breeds, is shown by the following study.

Feather germs, in which the rhachis tip had not yet (or just re-

cently) broken through the sheath, were plucked from New Hampshire Red, Black Minorca, hybrid black (from Barred Plymouth Rock ♀ x R.I. Red ♂) or White Leghorn chickens. At this stage the germs were about 2-3, 1.5-2 and 1-1.5 cm long in reds, blacks and whites respectively and weighed about 75-100, 40-50 and 35 mg each. The feather sheath was split longitudinally, the papilla (which forms most of the mass of the germ) removed, and the barbs scraped off the inner surface of the sheath. About 150-750 mg of live barb tissue were thus obtained from 25-50 feather germs of one color type. They were immersed in 2 cc of M/120 pH 7.4 phosphate buffer and ground with a small amount of sand. In some cases the resulting mixture was centrifuged for 15 minutes at about 2000 r.p.m. and the supernatant fluid (approximately 1.5 cc) divided equally among 2 to 4 serological tubes. In other tests the mixture was divided and the portions centrifuged separately so that subsequent reactions occurred in the presence of precipitated solid barb material. Reagents were added to each tube in such concentrations and quantities as to bring the reaction mixtures to the compositions shown in Table I. Where urethane was not used a small crystal of thymol was added. The tubes were plugged with cotton and set in an incubator at 37°C.

TABLE I.

Tests for Tyrosinase in Colored Feather Germs and for Tyrosinase Inhibitor in Dominant White Germs.

| | Composition of test mixture* | | | | Melanin formation | | |
|---|---|---|---|---|---|---|---|
| | | | | | Positive trials | Doubtful | Negative |
| 1. | black† | | | | 0 | 0 | 3 |
| 2. | ” | | tyrosine | | 7 | 2 | 2 |
| 3. | ” | | ” | urethane | 8 | 0 | 0 |
| 4. | ” | | ” | KCN | 0 | 0 | 9 |
| 5. | ” | boiled | ” | | 0 | 0 | 4 |
| 6. | ” | | ” | white | 1 | 1 | 4 |
| 7. | ” | | ” | ” boiled | 2 | 0 | 3 |
| 8. | red | | | | 0 | 0 | 2 |
| 9. | ” | | tyrosine | | 4 | 1 | 2 |
| 10. | ” | | ” | urethane | 3 | 0 | 2 |
| 11. | ” | | ” | KCN | 0 | 1 | 3 |
| 12. | ” | boiled | ” | | 0 | 1 | 1 |
| 13. | ” | | ” | white | 2 | 2 | 1 |
| 14. | ” | | ” | ” boiled | 3 | 1 | 1 |
| 15. | white | | tyrosine | | 0 | 0 | 4 |

* Volume of each mixture, about 1 cc. Concentrations in mixtures: tyrosine, 0.1-0.2%, including undissolved crystals; phosphate buffer pH 7.4, M/40; cyanide, M/500; urethane, 5%. Each portion of extract, from about 50-750 mg of live tissue, *i. e.*, juicy barb portions of 10-50 feather germs.

† Lines 1-7 are the combined data from Black Minorca and hybrid black barbs.

The freshly-prepared extracts were ordinarily very clear, slightly grayish if from black, pale orange from red, pinkish from white germs. They remained so, or became whitish and somewhat opaque, where negative reactions are recorded in Table I. In trials recorded as positive, whether from black or red germs, a disc of dense brown-black or black appeared at the top of the liquid in 6-30 hours and spread slowly downward. That this pigment was really melanin was not tested although it seemed very probable from the composition of the mixtures.

The reactions were not very constant among comparable mixtures. Of 30 tubes containing colored germ extract and tyrosine, with or without urethane (Table I, lines 2, 3, 9, 10), 22 showed definite melanin formation, 6 were negative and 3 doubtful. The same lack of reproducibility has been found in mammalian skin extracts.[1,2]

In spite of the defects of the method, one conclusion can scarcely be escaped: birds, or at least chickens, like rabbits,[1] mice,[2] and amphibian larvae[3] do not lack tyrosinase. Five extracts of colored barbs (lines 1 and 8) without added tyrosine did not form melanin; but 22 of 31 extracts with tyrosine did. Tyrosine alone at pH 7.4 is well known to be relatively stable; and 5 of 6 mixtures of tyrosine with boiled extract (lines 5, 12) did not react; the sixth was dubious. Together these results show only that colored extracts contain a heat-labile substance necessary for melanin formation from tyrosine. That the substance is an enzyme of the oxidase class is suggested by the cyanide effect on its action (lines 4 and 11): 12 of 13 tyrosine-extract-cyanide mixtures failed to form melanin and one gave a doubtful reaction. The behavior of the extracts thus fits the classical definition of tyrosinase: an oxidase system in the presence of which tyrosine forms melanin, without added peroxide.

Several other more uncertain conclusions are suggested by the data of Table I. (1) Red feather germs seem to contain a tyrosinase like, if not identical with, that of black germs, producing *black* pigment *in vitro* as do red guinea pig hair follicles in dopa solution.[4] (New Hampshire Reds have some black or red and black feathers but the germs used were from regions of red plumage.) (2) White Leghorn germs contain little or no tyrosinase, or else have also a tyrosinase inhibitor as Hadley[5] and others seem to have supposed, on genetic grounds. (3) Such a substance, if it exists, is present in too small

1 Pugh, C. E. M., *Biochem. J.*, 1933, **27**, 475.

2 Charles, D. R., *Genetics*, 1938, **23**, 523.

3 Figge, F. H. J., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 569.

4 Russell, W. L., *Genetics*, 1939, 645.

5 Hadley, P. H., *Agric. Exp. Station R. I. State Coll. Bull.* 11, 1913, **155**, 151.

amounts to give a clear tyrosinase inhibition when extracts of roughly equal amounts of black and white tissue are mixed (lines 6, 7, 13, 14). (4) Five percent ethyl urethane seems to accelerate and perhaps intensify the tyrosinase action of feather germ extracts: positive reactions in the presence of urethane appeared at 5-18 hours; without urethane, at 20-40 hours. This effect may come about by blocking out certain reducing systems involving dehydrogenases.

The variability of results is perhaps explained by the morphological aspect of pigment formation in feather germs. The melanophores in which the melanin seems to be produced are only a small part of the barb tissue. They are fully active, possibly, only in a narrow circumferential band at the base of the germ. If these cells alone contain an appreciable amount of tyrosinase, extract of 100 mg of barb would represent only a few mg of tyrosinase-containing tissue. It might be expected that much or all of the enzyme would sometimes be destroyed during extraction.

*Summary.* Extracts of black chicken feather germs show a cyanide-sensitive, heat-labile tyrosinase activity, forming melanin from tyrosine without added peroxide, in about 80% of trials when one cc of the reaction mixture contains extract of 50-750 mg of young barb tissue. Extracts of red feather germs show a similar activity in about 60% of cases, forming black rather than red pigment. Extracts of White Leghorn germs do not inhibit the tyrosinase from roughly equivalent amounts of black germ.

11094 P

## Inhibition of Experimental Dental Caries by Fluorine in the Absence of Saliva.*

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Although the inhibition of dental caries by fluorine has been demonstrated in endemic areas[1, 2] and in laboratory rats fed on a

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1 Black, G. V., and McKay, F. S., *D. Cosmos*, 1916, **58**, 129.

2 Dean, H. T., *Pub. Health Rep.*, 1938, **53**, 1443.