ON PIGMENT FORMATION IN THE D-BLACK RABBIT.

By Dr P. KOLLER.

(*From the Biochemical Laboratory and the Animal Breeding Station, Cambridge.*)

The mechanism of the origin and formation of pigment has been elucidated by the work of Bertrand (1908), Chodat (1912) and others. They all agree that colour in animals and plants is produced by the action of a ferment or oxidase substance on a colourless chromogen. Von Fürth (1904), Gortner (1911), Przibram, Dembowsky and Breecher (1915), Schultz (1925) and other researchers have demonstrated that the presence of an oxidase (tyrosinase) in the coloured tissues of animals and plants is a *conditio sine qua non* of pigment formation. L. Kaufmann (1923), for example, was able by injection of tyrosinase artificially to supply this oxidase and obtained the formation of melanin pigment.

Our present knowledge of the chemical basis of pigment formation allows us to use the chemical test for genetical analysis. In this connection the work of Onslow (1915) is of special interest. He demonstrated that chemical differences could be found between the two different types of white-coat colour in rabbits and mice, which correspond to differences in their genetic behaviour. In the dominant white coat the pigment-forming mechanism is present, but its action is inhibited by specific "inhibitor" substance. In the recessive white or albino, there is no inhibitor but the pigment-forming mechanism is incomplete.

Onslow used in his experiments the skin of recessive and dominant whites and of recessive black animals, as well as the chocolate form of the latter. He did not extend his work to dominant black, called by Punnett (1913, 1915) D-black, which is dominant to agouti, towards which the normal black is recessive. Our problem was to find out whether, an underlying chemical difference could be found corresponding to the different genetic behaviour of dominant black.

Methods.

In the light of the more recent literature on oxidases and pigment formation (Schultz, 1925) Onslow's method with tyrosinase and peroxide seemed to be inadequate, and it appeared preferable to follow Bloch

(1917, 1924) in replacing these reagents with "Dopa" (1–3, 4, dioxy-phenylalanine).

As experimental animals 2–3 day old rabbits were used. The skin was carefully separated and freed from adherent connective tissue and blood vessels, washed with distilled water and rinsed and dried with a cloth. It was then cut into pieces and ground up in a mortar with pure sand which had been previously wetted with a few drops of water. The pulp was placed in a cloth and pressed out in a press. The juice was separated from solid particles by centrifuging. As material for dominant white or inhibitor, we used the belly of agouti and the white parts of English rabbits.

The extracts of skin were divided into equal parts, which were placed in welled china plates. In three of the extracts a small quantity of solid finely powdered Dopa was suspended. To one of these portions with Dopa a few drops of the extract of white skin from English rabbit or agouti belly was added. To another we added an equal amount of water to test the effect of dilution by water as compared with the effect of dilution by inhibitor extract.

The china plate was placed in a big Petri dish into which also wet cotton-wool was placed. The dish was covered and placed in a thermostat at 37° C. for 4 hours. Then the dish was kept for 8 hours at room temperature and the results noted. The extract of coloured skin alone (used as control) does not change its colour in this time, while the same with Dopa changes to a very deep black-brown colour.

Description of experiments.

The following breeds were used as experimental material: (1) agouti; (2) recessive black; (3) dominant or D-black; (4) dominant white (homozyg. English rabbit); (5) recessive white or albino; (6) chocolate or dilute black; (7) agouti-Dutch.

1. *Extract of agouti skin.*

Extracts were prepared from the coloured coat of agouti and as inhibitor the extracts from the belly of the same agouti was used.

Extract	Reaction
E	–
E + Dopa	+ +
E + Dopa + H_2O ...	± ±
E + Dopa + inhibitor	–

E = extract from coloured skin.
\+ = positive reaction; formation of a precipitate of black colour.
– = negative reaction; no change in colour.

The reaction with Dopa and water was not so strongly positive as with Dopa alone, the water causing a dilution of precipitate colour. The result, however, shows that the agouti coat has all the factors for pigment formation, whereas the belly has in addition an inhibitor enzyme.

2. *Extract of recessive black skin.*

Extract	Reaction
E	–
E + Dopa	+ +
E + Dopa + H_2O ...	± ±
E + Dopa + inhibitor	–

Two kinds of inhibitor were used: one was the agouti belly, in which case a negative reaction was observed. In crosses of agouti and recessive black the F_1 offspring have the dominant white belly. Similarly if we used the extract of dominant white as inhibitor, the reaction was again negative. In crosses of recessive black and dominant white the F_1 offspring are all white.

3. *Extract of dominant or D-black skin.*

Extract	Reaction
E	–
E + Dopa	+ + +
E + Dopa + H_2O ...	+ +
E + Dopa + inhibitor	+ +

The above result shows that the inhibitor (from agouti belly and dominant white) could not prevent pigment formation. Punnett (1915) suggests that in the D-black rabbit there is present an inhibitor enzyme which itself inhibits the action of the inhibitor from agouti belly and dominant white. Therefore when he crossed agouti with D-black in F_1 generation the white belly did not appear and the offspring were all black. In this case the chemical observation is in full agreement with the genetic behaviour.

4. *Extract of dominant white skin.*

Extract	Reaction
E	–
E + Dopa	–
E + Dopa + H_2O ...	–
E + Dopa + inhibitor	–

In order to test more exactly where lies the cause of pigment inhibition, extract from dominant white was used with Dopa. The result in every case was negative, except when the extract was boiled for a few minutes, when a melanin precipitation was observed. It is suggested that

in that case the effect of inhibitor is destroyed by heat and the other components of the extract can, when combined with Dopa, produce pigment.

5. *Extract of recessive white skin.*

Extract	Reaction
E	−
E + Dopa	−
E + Dopa + H_2O ...	−
E + Dopa + inhibitor	−

Dopa cannot produce pigment in recessive white extract and even if we boil the extract the reaction remains negative. But if the extract from recessive black is added a positive reaction appears, because the lacking member of the pigment-forming series is supplied. If we cross, for example, albino (recessive white) with recessive black, the F_1 generation is black, because from the black parent the entire pigmentary system is introduced.

6. *Extract of chocolate skin.*

Extract	Reaction
E	−
E + Dopa	+ +
E + Dopa + H_2O ...	+
E + Dopa + inhibitor	−

The chocolate is a dilute black; in crossing its fate is the same. If we cross it with agouti, the white belly of the latter, which contains the inhibitor, will appear in F_1.

7. *Extract of agouti-Dutch rabbit's skin.*

Three kinds of extract were made: E_1, from the agouti coat; E_2, from the white part of coat; E_3, from the belly.

E_1 behaved in the same way as the agouti; *i.e.* with Dopa it gave a positive reaction. E_2 with Dopa did not give a melanin precipitation and this extract could not prevent pigment formation. E_3 had the same effect as the inhibitor from agouti. In crosses the behaviour of the white part is the same as that of recessive white, which shows that it lacks one member of the pigment-forming system.

Results.

1. Extracts from the coloured coat of agouti, recessive black, chocolate and agouti-Dutch give strong melanin formation with Dopa.

2. Extracts from dominant white skin (belly of agouti, white parts of English rabbit) do not form melanin on addition of Dopa. If added to the extracts of the coat of coloured rabbits mentioned under (1), such an extract completely inhibits pigment-formation.

3. Extracts from the skin of recessive white rabbit (albino) do not form pigment with Dopa and are unable to inhibit pigment-formation of coloured skin extracts.

All these experiments are thus in full agreement with the results of Onslow.

4. Extracts of the skin of dominant black rabbits show a pigment formation similar to that observed in recessive black. If, however, an inhibitor, *i.e.* extract of dominant white skin is added; this is unable to inhibit the pigment formation.

While Onslow's experiments thus adequately explain why albino is always recessive towards recessive black, and dominant white dominant to the same black, our observations give the chemical explanation of the dominant nature of D-black. In this case also the chemical experiments are in full agreement with the genetic behaviour.

It may be mentioned that in a few cases the inhibitor did not completely inhibit pigment formation in recessive black. In this case the animals were found to be heterozygous in regard to their inhibitor factor.

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