

The animals show no immediate symptoms; but about two hours later there is beginning incoördination, rigidity and respiratory distress, increasing to collapse about the fourth hour, and death about the sixth. An occasional dog survives till the twenty-fourth hour. Autopsy usually shows extensive local hemorrhagic, inflammatory and necrotic changes.

Horse leucocytes similarly injected produce symptoms of the same general nature, though less severe. About two thirds of the dogs injected with horse leucocytes recover. A reinjection of these dogs, however, or the injection of dogs whose meninges have been previously injured by tubercle bacilli, is almost invariably fatal.

The injection of 0.5 c.c. of rabbit leucocytes into the spinal meninges of monkeys produces slight symptoms from which most of the monkeys recover. Larger amounts are usually fatal.

Horse leucocytes are less toxic for monkeys, producing few if any symptoms, even when injected in 1.0 c.c. doses. The toxicity of both leucocytes however increases on repeated injection, the third injection often being fatal. Autopsy in such cases often shows edema of the lungs as the apparent immediate cause of death.

These tests have a bearing on the possible therapeutic uses of leucocytes in meningeal infections.

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**The occurrence, and the significance, of tyrosinase in the reproductive organs of certain amphibians.**

By **ROSS AIKEN GORTNER.**

[From the Laboratory of Biological Chemistry of the Station for Experimental Evolution, The Carnegie Institution of Washington.]

Tyrosinase—the enzyme which oxidizes tyrosin to produce a black, insoluble, pigment-like compound—has been shown by Phisalix (*C. R. Soc. Biol.*, 50, p. 793, 1898) to occur in the skin of the European frog, *Rana esculenta*. Gessard, later (*ibid.*, 56, p. 285, 1904), shows that the same enzyme occurs in the skin of the toad, *Bufo vulgaris*, and in the frog, *Rana temporaria*. In discussing the

importance of the tyrosinase Phisalix says: "It is probable that the oxidase presides over the oxidations within the organism . . . and plays the rôle of fixing oxygen in cutaneous respiration." If such is the case, and the production of the pigment in the skin is only a secondary reaction, or the elimination of a byproduct, the study of this enzyme becomes of much greater importance than merely as a pigment-producer.

In every instance where the mechanism of melanin formation has been elucidated, it has been found that pigmentation is due to the interaction of tyrosinase and a chromogen. It therefore seemed probable that the enzyme might be present in the ovaries of those amphibians which deposit pigmented eggs, and might be responsible for the pigment formation. I have found that tyrosinase is present in the ovaries of the green frog, *Rana clamata*, and the wood frog, *Rana sylvatica*. Owing to the greater ease in securing material of various ages most of the tests were carried out using material from the green frog. *In every instance the intensity, and the rapidity of the development of the tyrosinase test was in inverse proportion to the amount of pigment present in the eggs.* Ovaries which were immature, and contained no pigmented eggs, gave a positive tyrosinase test in a very few hours, partially pigmented ovaries required in some instances as much as 96 hours to produce a positive test, while the ovaries which contained ripe eggs failed to give any indication of the presence of the enzyme in 168 hours. All solutions were kept sterile by the addition of a few drops of chloroform. When partially pigmented ovaries were used the "blank" (no tyrosin added) usually showed considerable darkening, which, however, did not appear in a boiled check, showing that a chromogen was also present. It would, therefore, appear that the pigmentation of the eggs of the frog is due to an oxidation induced by tyrosinase, and that as the pigmentation progresses the amount of tyrosinase decreases, until there is no perceptible amount of the enzyme present in the unfertilized eggs which are fully ripe.

The tadpoles at the moment of liberation from the egg contain tyrosinase, as do also the larvæ of the salamander, *Ambystoma punctatum*. Inasmuch as the ripe unfertilized ova do not seem to contain tyrosinase, it seemed probable that the enzyme might be

added through the sperm, and on testing the testes of adult green frogs during the breeding season, I found tyrosinase to be present, although the coloration was slow to develop (72 hrs.). It is possible that all of the tyrosinase in the ovary was used up in the production of the egg pigment, and that the oxidase for the tadpole is introduced by the male. It is well known that oxidative processes proceed much more rapidly after fertilization and perhaps we may find that in other instances this is due to the entrance of an oxidase with the sperm. Evidences of oxidase action have been found in all of the fertilized amphibian eggs that I have examined, including eggs which contain no pigment.<sup>1</sup> It is also possible that the "poisonous complex," to which Loeb (*Arch. Entwickl. Organ.*, 31, p. 658) ascribes the death of the unfertilized egg, is destroyed by the entrance of an oxidase (perhaps a *specific* oxidase) with the sperm.

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### On two different types of melanin.

By **ROSS AIKEN GORTNER.**

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In investigating the nature of the melanin molecule, I have found that the pigment which is present in black wool is readily soluble in dilute sodium hydroxide, and that it is apparently a protein. To pigments of this nature I have given the name of melano-protein to distinguish them from both the unpigmented proteins and those other melanins, the nature of whose molecule is as yet unknown. The melano-protein which I have obtained from black wool contains no ash, showing that ash is not a part of this pigment, and also proving that this melanin does not contain iron. In some of the preparations of pigment from black wool where less precautions were taken to insure the absence of all contaminating mineral matter, a low percentage of ash was ob-

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<sup>1</sup> In collaboration with Dr. Banta, of this station, I have recently had occasion to test fertilized eggs of *Rana sylvatica*, *Rana pipiens*, *Ambystoma punctatum*, and *Spelerpes bilineatus*.