areas of infiltration and edema varying in size from 1.0 x 2.0 cm. to 3.0 x 3.5 cm., with central purplish zone of about 1.0 to 1.5 cm. in diameter. These reactions persisted for several days and subsided with residual pigmentation. One of the guinea pigs manifested a typical anaphylactic reaction with dyspnea weakness, involuntary micturition and defacation, and distress lasting 30 minutes, during which time it lay on its side. These final injections were made 36 to 44 days after initial inoculation with tuberculosis of human and bovine origin. The series of five guinea pigs with bovine infection gave results somewhat different. In general, the reactions were not so intense and the size of the lesion was rarely over 1.0 cm. in extent. In one animal local necrosis developed with some erythematous changes in the remainder local infiltration and edema were observed.

These derivatives are being studied in a large series of tuberculous patients, adults and children. There is evidence at hand to show that sensitization can be demonstrated even in terminal cases. Furthermore, typical skin reactions can be elicited in children who have failed to react to human tuberculin while reacting to bovine, and vice versa.

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Observations on the mechanism of the tyrosine-tyrosinase reaction.

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Raper and Wormall[1] in a recent paper conclude as follows:

"Tyrosinase first produces from tyrosine a red substance. This process requires the presence of the enzyme and only takes place in the presence of oxygen. This red substance becomes colorless spontaneously and is then oxidized to form melanin. These

[1] Raper, H. S., and Wormall, A., The tyrosine-tyrosinase reaction, *Biochem. J.*, 1923, xvii, 454.

last two processes take place in the absence of tyrosinase, but may be accelerated by it or by other oxidases found in potato juice."

While this statement may be correct in so far as potato tyrosinase is concerned, it is apparently incorrect as a generalization for all tyrosinase preparations. I have shown[2] that the meal worm, *Tenebrio molitor,* contains a very active tyrosinase which can be prepared in an insoluble form. When this insoluble tyrosinase is used, it is possible to remove the enzyme completely from a system by the simple process of filtration. Consequently it is possible to initiate a reaction, carry it to a definite stage, and then stop the action of the enzyme by filtering the reaction mixture.

Tyrosine and the insoluble tyrosinase were allowed to react until a deep rose color had developed. One-half of the mixture was now removed and filtered twice through "barium" filter papers to remove the enzyme. The other half of the mixture served as a check. Both tubes were set aside in the dark, but open to the oxygen of the air. In a few hours, the contents of the tube containing the unsoluble tyrosinase and tyrosine solution became changed, first becoming violet color and later depositing the black pigment in the usual manner. The filtered portion, on the other hand, remained an unchanged rose color for eighteen days and was then discarded.

Certain evidence has already been presented[3] which indicates that the initial red or rose color is dependent upon the presence of phenolic group in tyrosine while the amino group is necessary for the production of the black pigment. Tyrosine [β (p-hydroxy phenyl) α amino propionic acid] was changed to tyrosol [β (p-hydroxy phenyl) ethyl alcohol] by yeast fermentation according to the method of Ehrlich.[4] The tyrosol treated with soluble tyrosinase from potatoes or mushrooms *(Agaricus campestris)* or with the "insoluble" tyrosinase from meal worms was oxidized with the development of a rose red color, *which remained in solution* and which was not altered on long standing. No insoluble pigment precipitated. The onset of the rose color occurred in solutions of tyrosine and tyrosol after approximately the same

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[2] Gortner, R. A., A contribution to the study of oxidases, *Trans. Chem. Soc.,* (London) 1910, xcvii, 110.

[3] Gortner, R. A., Studies on melanin III. The inhibitory action of certain phenolic substances upon tyrosinase. A suggestion as to the cause of dominant and recessive whites. *J. Biol. Chem.,* 1911, x, 113.

[4] Ehrlich, F., *Ber.,* 1911, xliv, 139.

time interval following the addition of the enzyme so that apparently the same reaction occurs in both compounds. The lack of black pigment formation in the case of the tyrosol experiments is apparently due to the absence of the amino group which would, at least, suggest that the rose-red oxidation colors of a tyrosine-tyrosinase system are dependent upon one type of a chemical reaction and the red-black oxidation colors are caused by a second reaction. It is my experience that the enzyme is necessary to initiate each reaction, and that the second phase of the oxidation will not take place spontaneously, even in the presence of atmospheric oxygen.

273 (2505)

The distribution of water added to blood between the corpuscles and the serum.

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There seems to be uniformity among those who have tried that distilled water injected intravenously or subcutaneously does not act as a diuretic. While the water content of the whole blood or the hemoglobin percentage has been followed the actual water content of the plasma does not seem to have been determined. Owing to the large percentage of solid in red corpuscles it seemed possible that these cells might take up considerable water if the conditions of equilibria were upset. In this paper reference will be made only to experiments done *in vitro*. Two methods were followed. In the first, various amounts of water were added to samples of the same defibrinated blood, and after standing, the various samples were centrifuged and the water estimated in the different sera by weighing. In the second method the defibrinated blood was centrifuged and the serum and corpuscles separated. Portions of the serum were diluted with definite amounts of distilled water and then equal volumes of the centrifuged corpuscles and various sera were mixed and allowed to stand for a couple of hours. The results show that if the amount