found in the basal cells of the tissue that had been incubated in tyrosine, an equal amount of melanin was found in the basal cells of the control that had been incubated in buffer alone. A similar distribution of melanin formation was seen in sections from the same specimens when dopa-phosphate buffer was used in place of tyrosine-phosphate buffer.

Heating the tissue to 100° C for 10 min completely abolished the ability of the melanoblasts to convert tyrosine to melanin.

When the irradiated slices of skin were incubated with 0.01 M sodium diethyl-dithiocarbamate for 6 hr prior to incubation with tyrosine, the reaction was completely inhibited. Sodium diethyl-dithiocarbamate has been shown (*16*) to inhibit the oxidation of tyrosine catalyzed by mouse melanoma tyrosinase by combining with copper, which is necessary for enzymatic activity.

The deposition of granules of melanin in the cytoplasm of the cell body and dendritic processes of melanoblasts after incubation of human skin with tyrosine solutions indicates the presence of tyrosinase activity in these cells. The oxidation of dopa to melanin is also catalyzed by the melanoblasts, since incubation of sections of human skin with dopa results in deposition of pigment in these cells. Bloch's statement that the melanoblast contained only a specific "dopa oxidase" is apparently not supported by these findings. The data support the view that tyrosine can act as a precursor of melanin in human skin.

Human epidermal tyrosinase apparently exists in an inactive or partially inhibited state in normal unirradiated skin. Under the described experimental conditions irradiation of the skin with ultraviolet radiant energy, and possibly other types of radiation (such as roentgen rays, which are known to produce clinical pigmentation), is required before human melanoblasts can convert tyrosine to melanin. The mechanism by which ultraviolet radiant energy activates the enzymatic reaction is unknown. At least two factors appear to be involved. First, trace amounts of dopa, which are known (*15*) to accelerate greatly the tyrosine-tyrosinase reaction, may be formed in the melanoblast or surrounding cells. The dopa may be formed by direct photochemical oxidation of tyrosine present in the tissues and then catalyze the enzymatic oxidation of tyrosine to melanin. The conversion of tyrosine to dopa by ultraviolet radiant energy in the absence of the enzyme has been demonstrated (*2, 6, 18*). Second, ultraviolet radiant energy may decrease the concentration of normally occurring sulfhydryl groups in the epidermis. Rothman, Flesch, and others (*8, 19*) have shown that inhibition of the tyrosine-tyrosinase reaction *in vitro* by extracts of human epidermis is attributable to the presence of sulfhydryl groups. This inhibitory action was absent after irradiation of the epidermal extracts with ultraviolet radiant energy. Sulfhydryl groups inhibit tyrosinase by combining with the copper which is required for enzyme activity.

The inhibition of the human tyrosinase reaction by sodium diethyl-dithiocarbamate suggests that this enzyme, like tyrosinase in plants (*12, 13*), insects (*1*), and in mouse melanomas (*16*), requires copper for its activity.


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## Zinc Precipitation of Plasmin


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The work of Vallée (*1*) on the various zinc concentrations in certain tissues has given significance to the study of the effects of low concentrations of zinc on blood proteins. During a study of plasmin activity in ulcer exudates, I noted that the activity was lower in those ulcers treated with zinc oxide. This observation suggested studying the effect of zinc on plasmin *in vitro.*

Plasmin,[1] assaying 1 unit per mg, can be completely precipitated and inactivated by small quantities of zinc. A method of obtaining this inactivation is given here.

One ml of a solution containing 30 mg of plasmin (30 units) was added to a tube containing 198 μg of $ZnSO_4 \cdot 7H_2O$ in 1 ml of distilled water. This represents 45 μg of ionized zinc, assuming it is all present in the ionized form. A precipitate formed that was removed from the solution by centrifugation. The supernatant was then tested for plasmin activity against gelatin in a viscometer. No activity was detected over a period of 4 hr. Varying degrees of precipitation were noted with 22.5 μg, 11.25 μg, 5.6 μg, and 2.8 μg of zinc. At 1.6 μg no precipitation was noted. Testing of the supernatants after sedimentation of the precipitates showed a loss of activity inversely propor-

[1] Supplied by Eugene Loomis, of Parke, Davis & Company Research Laboratory.

tional to the amount of precipitate that had been formed. The supernatant from the plasmin treated with 1.6 μg of zinc showed the same activity as the untreated plasmin.

The experiment was repeated using homogenized egg albumin[2] as a substrate, and again it was found that 45 μg of ionized zinc was sufficient to precipitate 30 units of plasmin, so that no activity remained in the supernatant fluid.

A zinc chloride solution was prepared so that 1 ml again contained 45 μg of zinc, and this was also effective in completely precipitating or inactivating plasmin. The precipitate was redissolved by dialyzing it against tap water for 4 hr. Plasmin activity was demonstrated in this solution, although recovery was not complete. Dialysis of plasmin against tap water results in the loss of some plasmin activity.

The technique promises to be of value in the crystallization of this enzyme.

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[2] Courtesy of Otto Schales.

## A Simple Technique for Observing Carotid and Brachial Artery Pulse

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Various methods have been used in the laboratory for measurement and observation of the carotid pulse. In most cases the methods employed utilized various delicate pieces of apparatus, such as double membrane tambours and kymograph drums. In many cases irregular recordings of the carotid pulse are obtained because of inertia in the equipment used. Students rarely actually visualized the pulse they were studying.

A simple technique for visualizing the carotid pulse and the brachial pulse has been developed in this laboratory. The simple apparatus necessary, as shown in Fig. 1, consists of a test tube *A*, two graduated glass tubes (with a bore of about 1 mm) *B* and *C*, a three-holed rubber stopper, two pieces of rubber hose (about 18 in. long) *D* and *D′*, and two glass funnels *E* and *E′*. (Funnels are 1.5 in. in diameter at the mouth.) The test tube is almost completely filled with water *F*, and a few drops of methylene blue, neutral red, ink, or other coloring material are added to the water, so as to obtain a slightly colored solution. Each of the graduated glass tubes is inserted in one of the openings in the rubber stopper. The rubber stopper with tubes is inserted into the test tube. A piece of rubber hose is attached to each of the glass tubes. A funnel is then inserted into the free end of each piece of rubber hose.

When either funnel is pressed against the neck over the carotid artery, air in the tubular system is bubbled out

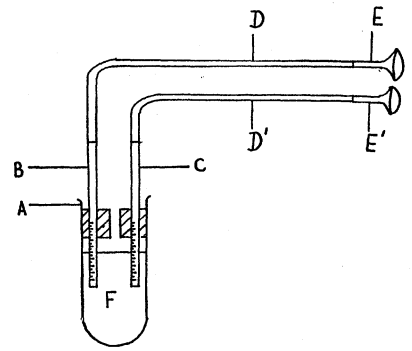


FIG. 1. Schematic drawing of apparatus used for observing carotid and brachial artery pulse.

at the submerged end. If a little more than sufficient pressure to detect carotid pulse is exerted, then slight release of pressure will draw fluid into the system to any desired height. When pressure is properly adjusted, the pulse will drive the air column through an excursion of several mm (as is noted on the graduated portions of the glass tubes).

The other funnel may be placed over the brachial artery near the bend of the elbow, and with somewhat greater pressure, the pulse in the brachial artery may be likewise observed.

If a 1-in. strip of old inner tube rubber approximately 18–24 in. long has a hole punched near its middle, through which the funnel stem is inserted, then the funnel can be held against the carotid by elastic tension, provided the tubing is drawn around the neck and fixed by means of a hemostat or clamp. A protecting roll of cloth at one side of the opposite carotid artery should be used to avoid decreased cranial flow, which may otherwise occur. It is possible that, when one funnel is thus fixed over the carotid artery, the same individual can hold the other funnel against the brachial artery. Thus the menisci of both pulse pressures can be obtained simultaneously and compared as to excursion and sequence.

## Cretaceous Rocks in the Kamishak Bay Area, Cook Inlet, Alaska

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South of Kamishak Bay in the Kamishak Hills between the Kamishak and Douglas rivers, at least 2,000 feet of Cretaceous sediments rest with possible disconformity upon Upper Jurassic Naknek beds. The Cretaceous is predominantly greenish-gray, medium-grained silty current-bedded sandstone, similar in many respects to the underlying Jurassic. Near the base, bluish-gray concretionary limestone is present locally, but at other places the presumed base is marked by a thin pebble conglomerate, including well-rounded fragments of horn-