with water that impressions of the worker's fingers are recorded if the latter are damp. Wiping the fingers with alcohol, xylene or other "dryers" is helpful in avoiding this trouble.

By this method the cost of a slide is not more than one third that of an etched slide of similar appearance prepared with transparent "inks."

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SPECIAL ARTICLES

AUTOCATALYTIC ACTIVATION OF TRYPSINOGEN IN THE PRESENCE OF CONCENTRATED AMMONIUM OR MAGNESIUM SULFATE

THE writers have described[1] the isolation from fresh cattle pancreas of a crystalline protein ("chymo-trypsinogen") which is transformed by a minute amount of active trypsin into an active proteolytic enzyme "chymo-trypsin." The course of the activation reaction is monomolecular and its rate is proportional to the trypsin concentration. Chymo-trypsinogen can not be activated by entero-kinase while the mother liquor from the chymo-trypsinogen crystallization is activated by entero-kinase but not by trypsin under ordinary conditions.

Subsequent experiments have shown that a protein fraction which has a very slight activity can be obtained from this inactive mother liquor. This fraction becomes highly active, as measured by the digestion of hemoglobin or casein, if allowed to stand for several hours in the form of a suspension in 0.5 saturated ammonium or magnesium sulfate at about pH 7.0 and 30° C. The activation follows the course of an autocatalytic reaction except for a prolonged lag period. The final specific activity is about 80 per cent. of that of crystalline trypsin. If a fresh suspension is inoculated with some of a perviously activated suspension activation occurs very rapidly. Active trypsin may thus be "propagated" by inoculating a suspension of the inactive protein with active material.

The suspension is prepared for activation as follows. The mother-liquor from the chymo-trypsinogen crystallization previously described is precipitated by bringing to 0.7 saturated ammonium sulfate and filtered. One gram of this filter cake is dissolved in 7.5 ml M/5 phosphate or borate buffer pH 8.0 and then 7.5 ml saturated ammonium sulfate is added. The suspension contains about 1.5 mg of protein nitrogen per ml.

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[1] M. Kunitz and J. H. Northrop, SCIENCE, 78: 558, 1933; *Jour. Gen. Physiol.* (in press).

CRYSTALLINE PROGESTIN

THE preparation from corpus luteum extract of crystalline material possessing progestin activity has been reported by Fels and Slotta,[1] Fevold and Hisaw,[2] and Allen.[3] None of these workers gives details as to the physical and chemical properties of their preparations. In a joint investigation carried on in the Rochester and Columbia laboratories we have succeeded in isolating several crystalline compounds from the product obtained by Allen's procedure. The main constituent of the mixture is a physiologically inactive compound, A, melting at 190° and possessing the composition $C_{21}H_{34}O_2$. This compound is a hydroxy ketone; its phenylurethane, p-nitrobenzoate and semicarbazone have been prepared.

A compound, B, with the formula $C_{21}H_{30}O_2$ crystallizing from ether-petroleum ether in blunt prisms with a melting point of 128° proved to possess the characteristic physiological properties of the hormone. It causes progestational proliferation in the uterus of the castrated rabbit in doses from 0.5 to 1.0 mg. A potency of 1 rabbit unit per mg has been tentatively assigned to this compound. Since the compound yields a crystalline dioxime, both oxygen atoms must be present in the form of carbonyl groups. The ultra-violet absorption spectrum of this compound shows a single band with a maximum at 240 mμ, which according to Menschick, Page and Bossert[4] is characteristic for α,β-unsaturated ketones. Compound A in the same concentration does not absorb light in the photographic region.

Furthermore, a compound, C, melting at 120–121° and crystallizing from ether-petroleum ether or dilute methyl alcohol in needles, has been isolated. This substance is also physiologically active; its potency is the same as that of Compound B within the limits of accuracy of the assay. Its ultra-violet spectrum is identical with that of Compound B. On combustion it gives the same figures for hydrogen as B, but somewhat lower carbon figures. On treatment with semicarbazide both compounds C and B yield apparently the same amorphous semicarbazone, which is

[1] E. Fels and K. H. Slotta, *Klin. Woch.*, 10: 1639, 1931.

[2] H. L. Fevold and F. L. Hisaw, *Proc. Soc. Exp. Biol. Med.*, 29: 620, 1932.

[3] W. M. Allen, *Jour. Biol. Chem.*, 98: 591, 1932.

[4] W. Menschick, J. H. Page and K. Bossert, *Ann. Chem.*, 295: 225, 1932.