has the same appearance as normal skin. In marked contrast is the white ash left by incineration of the nests of so-called naevus cells. Reddish colored ash, indicative of iron, is not seen. When, however, the mole undergoes malignant degeneration and gives rise to a melanocarcinoma, the ash acquires a bluish color in addition to the large amount of flat white ash.

Carcinomata of the skin whether of basal or squamous cell type, show a greater concentration of blue ash in the nests of malignant epithelial cells. This coloration is the same in nuclei and cytoplasm. In squamous cell carcinomata the epithelial pearls and areas of cellular degeneration show a predominantly white ash. The amount of ash decreases in epithelial pearls and increases in areas of cellular degeneration.

Discussion. In incinerated tissues the white ash is either calcium or magnesium. Policard believes it possible that bluish ash indicates the presence of sodium or potassium. Exact determination of this is yet to be made, but with the evidence at hand one may safely say that there is a fundamental chemical difference in the mineral salt content of benign and malignant neoplasms. This differentiation appears in early proliferative changes, considered by some as precursors of malignant neoplasms.

6550

Isolation of a Crystalline Depressor Substance from the Brain.

C. J. WEBER, J. B. NANNINGA AND RALPH H. MAJOR.
(Introduced by E. A. Doisy.)

From the Department of Internal Medicine, University of Kansas School of Medicine.

It has been shown[1, 2, 3] that a depressor substance is present in certain extracts from brain tissue. Major and Weber[2] presented evidence that the active depressor substance was not histamine choline or acetyl choline. Major, Nanninga and Weber[3] presented further evidence that the depressor activity of these brain extracts

[1] Major, Ralph H., and Weber, C. J., *J. Pharm. and Exp. Therap.*, 1929, **38**, 367.

[2] Major Ralph H., and Weber, C. J., *J. Pharm. and Exp. Therap.*, 1930, **40**, 247.

[3] Major, Ralph H., Nanninga, J. B., and Weber, C. J., *J. Physiol.*, 1932, **76**, 487.

was not due to either histamine, choline, acetyl choline, adenosine or adenylic acid. We have recently obtained from these brain extracts a crystalline compound which possesses a marked depressor action upon the blood pressure of dogs under ether anesthesia. We have employed the following procedure to obtain the active depressor substance in crystalline form.

After removal of the acetone from the crude acetone extract of brain, 60 gm. of potassium carbonate was added to each 100 cc. of the crude extract. This was extracted with an equal volume of 95% alcohol and the extraction repeated 4 times. This, after standing overnight in the ice-box, was filtered; the filtrate evaporated under partial vacuum to a consistency of a thick syrup and then redissolved in 95% alcohol. Concentrated sulphuric acid mixed with 50 parts of 95% alcohol was added to this solution, producing a heavy flocculent precipitate. The sulphuric acid alcohol mixture was added until no further precipitation was obtained, the solution filtered, neutralized with NaOH and again filtered. The filtrate was then evaporated under a partial vacuum to a thick syrupy consistence, dissolved in water and treated with phosphotungstic acid, which produced a copious white precipitate. This precipitate was separated by filtration, the filtrate evaporated under low pressure to the consistency of a thick syrup and then dissolved in absolute alcohol.

This alcoholic solution, after the separation by filtration of a slight insoluble precipitate, was subjected to fractional precipitation with water-free ether. The addition of one volume of ether produced a precipitate which was dissolved in absolute alcohol and then treated with 0.1 volume of ether. This procedure again produced a precipitate which was separated by filtration. The filtrate was treated with 0.3 volume of ether, the precipitate removed and the filtrate then treated with one volume of ether. This again produced a precipitation, the precipitate was dissolved in absolute alcohol and 0.3 volume of ether added. The precipitate forming was dissolved in absolute alcohol and this solution was treated with concentrated sulphuric acid in 50 parts of absolute alcohol until no further precipitation occurred. This precipitate was removed, one volume of ether added to the filtrate, the precipitate again removed and 3 volumes of ether added to the filtrate. This procedure again produced a precipitate which was filtered off and for the sake of convenience we shall designate them as the final precipitate and the final filtrate.

The final precipitate was dissolved in absolute alcohol and treated

with 5 volumes of ether. A precipitation occurred and the precipitate was found under the microscope to consist of small needle-like crystals, which were quite soluble in absolute alcohol and could be readily precipitated by 5 volumes of ether.

The final filtrate containing 3 volumes of ether was treated with an additional 3 volumes of ether, which produced a marked cloudiness. On standing in the ice-box long needle-like crystals appeared. These crystals also were readily soluble in absolute alcohol and the addition of 3 volumes of ether again produced the long needle-like crystals.

The small crystals showed a marked depressor activity after 7 recrystallizations from absolute alcohol and no loss of activity could be detected as the result of these recrystallizations. The long needles likewise showed no loss of depressor activity after 6 recrystallizations.

The larger needles after being dissolved in water, the water removed and then being dissolved in absolute alcohol, came down, upon the addition of ether, in the form of small needle-like crystals apparently identical with those obtained from the final precipitate.

Both large and small crystals have the same depressor activity. 0.1 mg. of each kind of crystal caused a fall of 8 mm. Hg. in blood pressure in a dog weighing 12 kilos, under ether anesthesia, while 0.5 mg. produced a fall of 40 mm. Hg. Both types of crystals obtained by precipitation with ether on exposure to the air are rapidly converted into a gummy mass. Aqueous solutions of the crystals give a negative Pauly reaction, a negative Sakaguchi reaction and are quite active in an atropinized dog.