

spatially removed from the substrate of the reaction.

Finally, we have the recent experiments of Bandurski and Galston (unpublished) with albino corn. A mutant strain of corn was obtained in which carotenoid pigments were absent, or if present at all, were less than 0.01 the normal concentration. The coleoptiles of such corn, devoid of carotenoids but possessing the normal content of riboflavin, showed approximately normal phototropic curvature when exposed to about 600 meter-candle-seconds of blue light. This experiment does not, of course, disprove the pos-

sible participation of carotenoids in normal phototropism, but it does show that other pigments (presumably including flavins) can serve as light receptors for phototropic curvature.

Whether or not a flavin pigment is actually involved in phototropism, it is clear that the reactivity of free riboflavin and of flavoproteins toward certain substrates is markedly affected by light. This fact compels us to examine further the possibility that the ubiquitous flavins may be important photoreceptors in biological systems other than higher plants.

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Technical Papers

Static Electricity Elimination During Sectioning with a Microtome

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Paraffin ribbons of tissue sections become charged with static electricity as they are cut on a microtome, and in dry weather the charge may be great enough to pull the ribbon into contact with the metal parts of the instrument, or other objects, making it difficult or impossible to obtain serial sections without loss. This widespread difficulty has vexed biologists and they have tried in a number of ways to lessen it. For example, they have tried grounding the instrument, and using high frequency generators to ionize the air near the microtome. The first procedure helps, but is inadequate, and the generators are somewhat cumbersome to use and they introduce some hazard of shock.

A simple and efficient solution of this problem is to place a surface on which polonium has been plated about an inch from the edge of the knife. The alpha radiation from the polonium ionizes the air and discharges the static electricity as it forms, leaving an easily handled, limp, uncharged ribbon. A further advantage is that the sections do not tend to stick to the knife facet during cutting, and they are less distorted and compressed when cut without the formation of frictional electricity.

A convenient unit (Fig. 1) consists of a polonium-plated, radiating surface (A) recessed into a rotatable head (B) held on a flexible tube mounted on a base (C). When in use it should be placed with the head about 1 in. from the surface of the specimen block (D) and the emitting surface turned to radiate both the surface of the paraffin block and the ribbon as it forms at the knife edge. This arrangement dissipates the charge formed on the surface of the block on its upstroke and on the ribbon from the friction of cutting. Alpha radiation has little penetrating power to damage the specimen or the sections. The useful life of the emitting surface should be somewhat more than a year from the

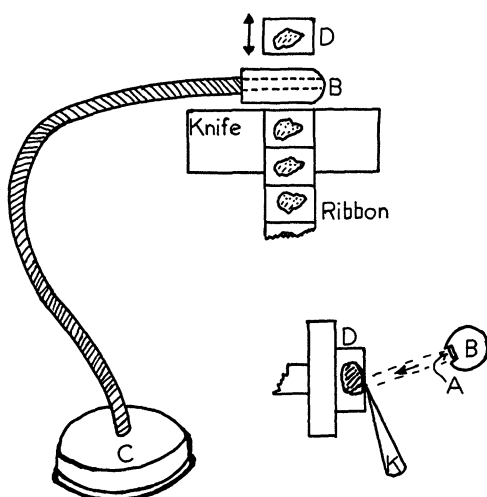


FIG. 1.

time the polonium is plated on the surface. The equipment may be obtained from Mr. Jenkins.

Since alpha rays alone are formed there is no radiation hazard involved from the radiation. Because grease or dirt left on the emitting surface from the fingers will decrease the radiation produced, the polonium strip is protected from contact by being supported within the recess of the head. Should the surface be touched accidentally fingers may become contaminated and should be washed thoroughly with soap and water before eating or smoking, as polonium taken internally is poisonous.

Preparation of Nonprotein Fractions Possessing Adrenocorticotrophic Activity from Sheep ACTH Protein

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The possibility that nonprotein material possessing adrenocorticotrophic activity may be associated with ACTH protein has been investigated. The stability of the protein hormone to boiling, even in 0.1M HCl, its resistance to destruction by strong solutions of NH_4OH , and the retention of activity even after acid and peptic digestion (2), all suggests that the isolated ACTH is indeed an exceptional protein.

Trichloroacetic acid (TCA) precipitation and dialysis have been employed as means of obtaining nonprotein material from an ACTH protein, as prepared by C. H.

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Li (2). All fractions were assayed by the ascorbic acid depletion test of Sayers *et al.* (4).

In the usual TCA experiment 20 mg of the ACTH protein was dissolved in 2 ml of water, and 2 ml of ice-cold 10% TCA was added. The solution was stirred and then centrifuged. The supernatant was decanted and the precipitate was suspended in 2 ml of water; 2 ml of ice-cold 10% TCA was again added. The stirred solution was centrifuged, and the supernatant was added to the previous supernatant. The combined supernatants were considered as the TCA supernatant fraction. The precipitate was resuspended and reprecipitated eight to ten additional times, the supernatants being discarded. The final precipitate was washed three times with 4 ml

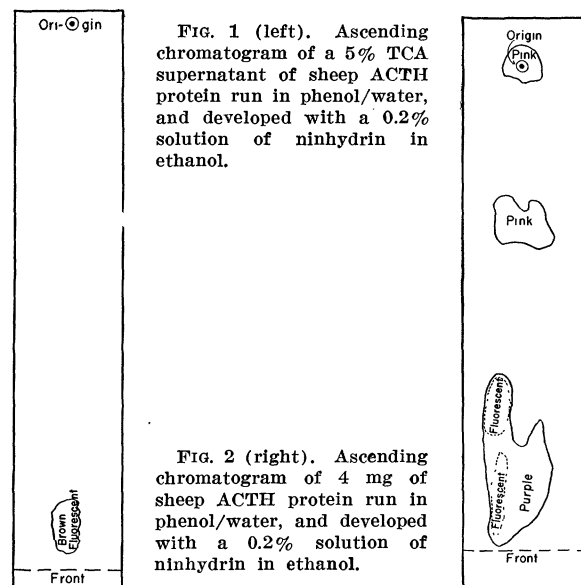


FIG. 1 (left). Ascending chromatogram of a 5% TCA supernatant of sheep ACTH protein run in phenol/water, and developed with a 0.2% solution of ninhydrin in ethanol.

FIG. 2 (right). Ascending chromatogram of 4 mg of sheep ACTH protein run in phenol/water, and developed with a 0.2% solution of ninhydrin in ethanol.

of diethyl ether, and then dissolved in 2 ml of pH 7.5 phosphate buffer. The TCA supernatant fraction was extracted ten times with 4 ml of diethyl ether. The resulting solutions of both the precipitate and supernatant fractions were appropriately diluted, and aliquots were taken for ascorbic acid assay, nitrogen determinations, assay by the repair test of Simpson *et al.* (5), and paper chromatography.

It was found that both the supernatant and the precipitate possessed activity by either assay procedure. By the ascorbic acid depletion method quantitative values were obtained which, when compared to the nitrogen content of each phase, demonstrated that the precipitate contained a smaller amount of activity per unit of nitrogen than the original protein. The supernatant, however, showed a markedly increased amount of activity per unit of nitrogen. In various experiments, utilizing different batches of sheep ACTH protein, this has ranged from six to over ten times that of the original protein. In the various TCA supernatants, from 25% to over 40% of the original activity could be recovered, whereas only 4% of the nitrogen was present in the supernatant fractions. Paper chromatograms of both the TCA precipitate and supernatant fractions (Fig. 1), when run