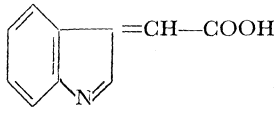
1 that indole and indole-3-glycolic acid show marked synergism in promoting extension growth, and that much more indole-3-glycolic acid than indoleacetic acid is required to give maximal extension growth.

In view of the natural occurrence of indole (*11*) and glyoxylate (*12*) in various organisms, including higher plants, the possibility should be further examined that the coupling reaction and the product here described are of importance in the auxin economy of the plant. Indole-3-glycolic acid could function as an auxin per se, or could serve as a precursor of indoleacetic acid, either via direct reduction of the a-hydroxyl group, or, alternatively, via transannular dehydration to 3-carboxymethyleneindolenine,



followed by reduction.

The natural occurrence of indole-3-glycolic acid in various plant sources has been claimed (*13*), but the R_F values listed do not agree with our data. The occurrence of indole-3-glycolic acid as a breakdown product during paper chromatography of indolepyruvate has also been reported (*14*), the product in this case having an R_F close to that reported here (*15*).

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13 February 1957

Electrophoresis of Plasma Proteins in the Parakeet

Previous studies on electrophoresis of avian plasma or serum proteins have been limited to the goose, hen, turkey, pullet, and cockerel (*1*). The present investigation (*2*) of the plasma electrophoretic pattern of the shell parakeet (*Melopsittacus undulatus*) was undertaken during a study of the effects of spontaneous (*3*) or transplanted (*4*) pituitary tumors on these birds.

The parakeets were exsanguinated by cardiac puncture. After separation of the plasma, 10 mm³ was applied to Whatman 3-mm filter paper on a conventional vertical principle electrophoretic cell of our own design utilizing Veronal buffer at *p*H 8.6, μ 0.05 with 5 percent glycerine. Migration was permitted for 4 hours, using 150 to 170 v delivering 15 to 20 ma for paper measuring 10 by 14 cm. Pooled human serum was used for mobility reference. After heat fixation the papers were stained with bromphenol blue for protein and Sudan black B for lipoprotein. Scanning of the papers was accomplished with an automatic photoelectric cell recording apparatus with simultaneous area integration (Spinco Analytrol).

As can be seen in Fig. 1, there is considerable variation in the quantity of these four fractions in the normal parakeets that have been studied. By the methods used, no essential differences were discernible between the electrophoretic patterns of serum and plasma from the same birds. Although it appears probable that the protein composition of parakeet serum should differ from that of the plasma, the method of filter-paper electrophoresis is not adequate to demonstrate such a difference. Plasma was used in all studies because of the ease of handling the small samples of whole blood available. Each of the four components has an affinity for Sudan black B, although differing in intensity. Component 1 stains darkly with lipid stain, component 2 is barely perceptible, and components 3 and 4 stain as a single component of lesser intensity than component 1. Lipid staining also is seen at the point of application of the plasma, showing a nonmigrating component, probably chylomicrons.

Four protein components were recognized in normal parakeet plasma; these have been named components 1, 2, 3, and 4 in order of decreasing mobility (Fig. 2). Component 1 migrates faster than human albumin and represents 32 percent (range, 17 to 63 percent) of the total protein. Component 2 travels at a rate intermediate between human α_1- and α_2-globulin and occupies 16.5 percent (range, 6 to 28 percent) of the total area scanned. Component 3 is in juxtaposition to component 4 in a migratory area of human β-globulin, the former representing 22.5 percent (range, 8 to 45 percent) and the latter 29 percent (range, 17 to 48 percent) of the total plasma protein.

These normal patterns are strikingly altered in the plasma of parakeets bearing either primary or transplanted pituitary tumors; the quantity of protein observed to migrate as component 2 is greatly increased, representing 59 percent (range, 45 to 65 percent) of the total protein. To date, this has been ob-

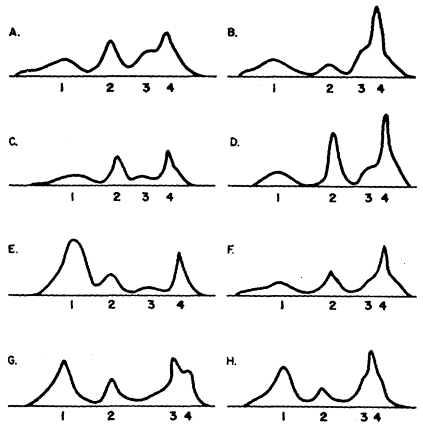

Fig. 1. Plasma electrophoretic pattern of eight normal parakeets, showing wide variations in the quantity of the four protein components.

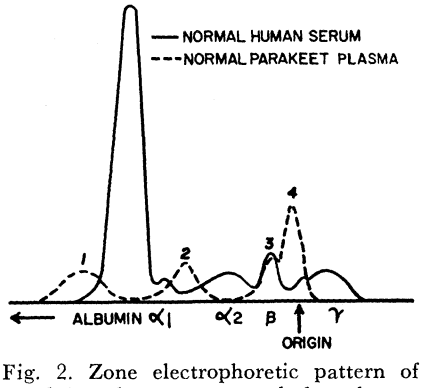

Fig. 2. Zone electrophoretic pattern of parakeet plasma compared for electrophoretic mobility with normal human serum.

served in all parakeets that bear these tumors and has not been found in parakeets with other primary and transplanted tumors such as fibrosarcoma and methylcholanthrene-induced carcinoma nor has the increased protein associated with an elevated serum calcium such as has been described in estrogen-treated roosters been found (*5*).

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18 February 1957

Citrus Fruit Grafting

The grafting of a citrus fruit from one plant to another has not been reported previously, as far as I am aware. This technique should be useful in studying many problems, particularly when fruits are desired on small plants. A technique previously described (*1*) in which cuttings with fruits attached were rooted and used for experimental purposes has many useful applications but does not provide the flexibility offered by grafted plants.

For example, by employing the grafting technique, it is possible to study the influence of leaves on fruit development and composition by producing various combinations of leaves and fruits. Nitsch (*2*) suggested that organic acids are probably produced in the leaves and translocated to the fruits. On the other hand, Sinclair and Eny (*3*) and Sekhara Varma and Ramakrishnan (*4*) suggested that the large amounts of organic acids in lemon pulp are not translocated from the leaves but formed from the sugars in the juice vesicles. By grafting a sweet lemon fruit on a sour lemon plant, it was thought that information about the accumulation of organic acids in lemons might be obtained. The results of preliminary experiments of this type using sweet and sour lemons, both of which belong to the species *Citrus limon* (Linn.) Burmann (*5*), are presented here.

The Faris sweet lemon, used in these experiments, is distinguishable from sour forms in the bland, insipid taste of the juice, which has less than 1 percent acid in tree-ripened fruit in contrast with 4½ to 6 percent acid in the Ross Eureka, a commercial variety. On 25 May 1956, eight immature Faris sweet lemons were successfully grafted on Ross Eureka lemon plants growing in the greenhouse in number 10 cans. The fruits were picked and juiced when mature, between 20 July and 6 December. For comparison, Ross Eureka lemons that had been similarly grafted on 4 May were used. Vegetative branches appearing on the scions about 3 weeks after grafting (Fig. 1) were removed from half of the plants.

Analyses were made of the juice from the fruits for percentage titratable acid, *p*H, total soluble solids (refractometric reading), and sugars. The sweet lemon fruits grafted on sour lemon plants generally remained low in acid content. However, on those plants which had leaves from the sweet lemon scion in addition to leaves on the sour lemon stock, the acid content of the fruit (1.31 percent) was somewhat greater than it was on plants from which scion sprouts had been removed (0.32 percent). Whether this difference in acid content was in response to the combination of foliage, improved graft union, or some other cause remains to be determined.

Concurrently, another experiment was started in which Ross Eureka lemon plants were grafted with either Faris sweet lemon scions or Ross Eureka scions. When the vegetative shoots were about 2 to 4 feet long, sweet or sour lemon fruits were grafted on the tops in four combinations. All scion sprouts were removed as they appeared so that a single source of foliage existed on each plant. The fruits were grafted on 3 August, 1956, and on 10 September when the first fruits became yellow, one fruit of each of the four combinations was picked. At approximately monthly intervals, the remaining fruits matured and were picked, so that on 6 December the last set of four fruits was removed from the plants.

Results of analyses of the fruits showed that all the sweet lemon fruits remained low in acid concentration whether they were nourished by leaves of sour (0.52 percent acid) or sweet (0.44 percent acid) lemon plants. Also, the sour lemons remained high in acid concentration whether they were nourished by leaves of sweet (5.18 percent acid) or sour (5.18 percent acid) lemon plants. Aside from the characteristic of a lower acid concentration, the sweet lemons had a much higher reducing sugar concentration (5.17 percent) than did the sour lemons (1.45 percent).

The total soluble solids in the two types of fruits were about the same. After the acid and sugars had been subtracted from the total soluble solids only one or two percent soluble solids remained undetermined. The difference in undetermined soluble solids between

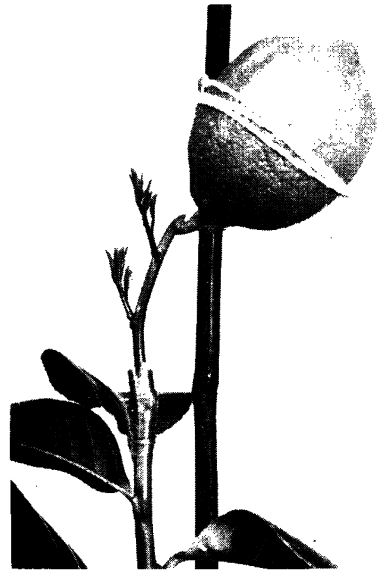

Fig. 1. Lemon fruit grafted 4 May 1956, photographed 29 May. Sprouts on the scion indicate successful graft union. Fruit was 51.4 mm in diameter when grafted and 65.2 mm when mature on 11 December; the calculated increase in volume is 63 percent.

sweet and sour fruits was only a fraction of the difference in acid concentration, thus indicating that acid was lacking in the sweet fruit rather than that it was present in a nontitratable salt form.

The failure of a sweet lemon, such as the Faris, to accumulate high concentrations of organic acids, when grafted on a sour lemon plant, such as the Ross Eureka, indicates that the high concentration of organic acids in a lemon is more complex than that which would result merely from the translocation of acid from leaves to fruit and accumulation in the latter site.

Further studies are required to determine to what extent leaves can modify fruit composition. By using the technique of grafting fruits, it will be possible to extend the study and to employ orange, grapefruit, and other foliage to produce lemons and other citrus fruits.

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25 February 1957