11. O. H. Wheeler and J. L. Mateos, *J. Org. Chem.* **21**, 1110 (1956).
12. D. Larsen and F. W. Heyl, *J. Am. Chem. Soc.* **56**, 2663 (1934).
13. H. Ichiba, *Sci. Papers Inst. Phys. Chem. Research (Tokyo)* **28**, 112 (1935).
14. S. Bernstein and E. S. Wallis, *J. Org. Chem.* **2**, 341 (1937).
15. D. Larsen, *J. Am. Chem. Soc.* **60**, 2431 (1938).
16. R. J. Anderson, *ibid.* **46**, 1450 (1924).
17. We are indebted to J. M. Chemerda for a sample of this compound.
18. A. C. Ott and C. D. Ball, *J. Am. Chem. Soc.* **66**, 489 (1944).

11 March 1957

Formation and Auxin Activity of Indole-3-Glycolic Acid

It is usually assumed that the plant growth hormone, indoleacetic acid, arises in plant tissue by some transformation of the amino acid tryptophan (*1*). This belief has become widespread despite the fact that the best yields in numerous enzymatic experiments have never exceeded a few percent (*2*). We therefore felt it necessary to examine other possible biosynthetic pathways for indoleacetic acid.

In view of the facts that tryptophan is known to arise in various biological systems via an enzymatic coupling of indole with serine (*3*) and that indole is known to couple also with D-glyceraldehyde-3-phosphate (*4*), we examined the possibility that indoleacetic acid or some close analog could arise by a direct union of indole with a two-carbon fragment. We found that indole and glyoxylate, in aqueous solution, reacted nonenzymatically to yield a Salkowski-positive compound later characterized as indole-3-glycolic acid (IGA, Fig. 1). This compound is active in several bioassays used for detecting auxins.

The reaction between indole and glyoxylate was carried out as follows. Aqueous solutions of indole and sodium glyoxylate (*5*) were mixed so that they contained 0.2 μmole of indole and 60 μmole of sodium glyoxylate in 1 ml of solution. After the mixture had been incubated for 1 hour at 30°C, residual indole was extracted with toluene and assayed by a method similar to that described by Yanofsky (*6*). Under the conditions described, approximately 25 percent of the indole disappears in 1 hour. If, to a duplicate tube, one adds 4 ml of Salkowski reagent, a color appears which is similar to but not identical with that given by indoleacetic acid and which is different from the color produced by indole.

For chromatographic investigation of the product, 0.4 ml of 0.005*M* indole was mixed with 1.6 ml of 0.2*M* sodium glyoxylate and incubated 2 hours at 30° C. Samples of this reaction mixture were applied to Whatman 3-MM paper, and the solvent (isopropanol, ammonia, and water, 80/5/15) was allowed to ascend 30 cm beyond the applied spots. The paper was then air-dried and sprayed with Ehrlich's aldehyde reagent. The chromatograms showed a brilliant rose-colored spot which had an R_F of 0.37 (the R_F value for indoleacetic acid under these conditions is 0.47; for indole, 0.96).

The product of the indole plus glyoxylate reaction was confirmed as indole-3-glycolic acid by cochromatography with authentic indole-3-glycolic acid that was synthesized by a new independent method, and also by a comparison of the R_F values of these two materials in various solvent systems. Indole-3-glyoxylic acid was first prepared by the method of Giua (*7*), who had erroneously designated the product as indole-2-glyoxylic acid. The correct nature of the product was established by Kharasch *et al.* (*8*), and later confirmed (*9*). The indole-3-glyoxylic acid was reduced by hydrogenation of the sodium salt over palladium, or more slowly over 5-percent palladium on charcoal, and by use of sodium amalgam. Sodium indole-3-glycolate is stable, easily purified, and obtainable in good yields, but the free acid decomposes rapidly to colored products in air (*10*) and has not been isolated in pure form.

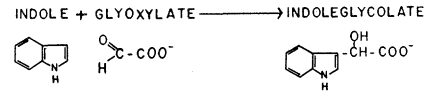


Fig. 1. Reaction of indole and glyoxylate.

The sodium indole-3-glycolate, recrystallized from methanol-ether, is a white solid with a decomposition point of 360°C. Analytic data, obtained from the Weiler and Strauss Microanalytical Laboratory, Oxford, England, are as follows. Formula, $C_{10}H_8O_3N$ Na (213.17). Calculated: C 56.33; H, 3.78; N, 6.57; Na, 10.79. Found: C, 55.68; H, 4.10; N, 6.50; Na, 10.73.

Synthetic sodium indole-3-glycolic acid, as well as the reaction product, has an R_F of 0.16 in benzene-propionic acid-water (100/70/5), 0.76 in 20-percent (wt./vol.) aqueous potassium chloride, 0.82 in *n*-butanol-acetic acid-water (4/1/1), and 0.01 in *n*-butanol-pyridine-dioxane-water (14/4/1/1). A brilliant rose-colored spot was obtained with Ehrlich's aldehyde reagent.

Pure sodium indole-3-glycolic acid, as well as mixtures of indole and glyoxylate, show distinct activity in auxin bioassays, causing extension growth of subapical etiolated pea epicotyl sections, root initiation on excised etiolated pea epicotyl sections, and inhibition of axillary buds (Table 1). It may be noted from Table

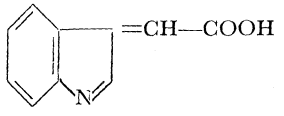
Table 1. Activity of indole-3-glycolic acid in auxin bioassays. When no values are given, no measurements were made.

Substance	Concentration	Extension growth* (mm of growth per section ± standard error)	Root initiation† (average number of roots per section)	Lateral bud development‡ (relative growth by visual estimate)
Control		3.08 ± .19	3.4	++++
Indoleacetic acid	$1 \times 10^{-6}M$	7.32 ± .32		
Indoleacetic acid	$1 \times 10^{-5}M$	5.82 ± .29		
Indoleacetic acid	$1 \times 10^{-4}M$	5.31 ± .22	7.4	++
Glyoxylate	$4 \times 10^{-3}M$	2.96 ± .18		
Indole	$4 \times 10^{-4}M$	3.79 ± .19		
Indole-3-glycolic acid	$1 \times 10^{-6}M$	3.54 ± .21		
Indole-3-glycolic acid	$1 \times 10^{-5}M$	3.65 ± .22		
Indole-3-glycolic acid	$4 \times 10^{-5}M$	4.81 ± .11		
Indole-3-glycolic acid	$1 \times 10^{-4}M$	5.04 ± .36	6.6	+++
Indole-3-glycolic acid	$4 \times 10^{-4}M$	6.73 ± .21		
Indole-3-glycolic acid	$5 \times 10^{-4}M$		14.3	+
Indole-3-glycolic acid and glyoxylate	$1 \times 10^{-4}M$, $4 \times 10^{-3}M$	5.39 ± .19		
Indole-3-glycolic acid and indole	$1 \times 10^{-4}M$, $4 \times 10^{-4}M$	8.00 ± .23		
Reaction mixture§	$4 \times 10^{-5}M$	3.50 ± .20		
	$4 \times 10^{-4}M$	7.65 ± .24		

* Eight-millimeter subapical sections of 7-day etiolated pea epicotyls floated in 5 ml test solution containing 1 percent sucrose and 0.01*M* phosphate at *p*H 6.1, 13 sections per flask, 20 hours' incubation in the dark.
† Ten-centimeter sections of 8-day etiolated pea epicotyls including second node, incubated 24 hours with basal ends in 2 ml of test solution containing 0.005*M* phosphate at *p*H 6.1, and then 7 days in frequently renewed 2-percent sucrose, all in the dark; 11 sections per flask.
‡ Same procedure as that for root initiation, but apical end in solution for the first 24 hours.
§ The reaction mixture was prepared by mixing 1.6 ml of $5 \times 10^{-3}M$ indole with 0.4 ml of 0.2*M* sodium glyoxylate and incubating for 1 hour at 30°C. Under these conditions, not all the indole disappeared. The molarity given represents that of the indole added.

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*).

J. B. Greenberg
A. W. Galston
Josiah Willard Gibbs Research Laboratories, Department of Botany, Yale University, New Haven, Connecticut

K. N. F. Shaw
M. D. Armstrong
Laboratory for the Study of Hereditary and Metabolic Disorders, University of Utah College of Medicine, Salt Lake City

References and Notes

1. S. A. Gordon, *Ann. Rev. Plant Physiol.* 5, 341 (1954).
2. S. G. Wildman, M. G. Ferri, J. Bonner, *Arch. Biochem.* 13, 131 (1947).
3. E. L. Tatum and D. M. Bonner, *Proc. Natl. Acad. Sci. U.S.* 30, 30 (1944); C. Yanofsky, in *A Symposium on Amino Acid Metabolism*, W. D. McElroy and B. Glass, Eds. (Johns Hopkins Press, Baltimore, Md., 1955); J. B Greenberg and A. W. Galston, *Plant Physiol.* 31, xxvi (1956).
4. C. Yanofsky, *J. Biol. Chem.* 223, 171 (1956).
5. The sodium glyoxylate used in these experiments was kindly furnished by I. Zelitch of the Connecticut Agricultural Experiment Station. Glyoxylate may be prepared by the methods of D. E. Metzler, J. Olivard, and E. E. Snell [*J. Am. Chem. Soc.* 76, 644 (1954)] and of N. S. Radin [*Biochem. Preparations* 4, 60 (1955)].
6. C. Yanofsky, in *Methods in Enzymology II*, S. P. Colowick and N. O. Kaplan, Eds. (Academic Press, New York, 1955).
7. M. Giua, *Gazz. chim. ital.* 54, 593 (1924).
8. M. S. Kharasch, S. S. Kane, H. C. Brown, *J. Am. Chem. Soc.* 62, 2243 (1940).
9. M. E. Speeter and W. C. Anthony, *ibid.* 76, 6208 (1954).
10. J. W. Baker, *J. Chem. Soc.* 1940, 458 (1940).
11. G. Klein, Ed., *Handbuch der Pflanzenanalyse III/1 Spezielle Analyse II* (Springer, Vienna, 1932), pp. 665–6.
12. G. H. N. Towers and D. C. Mortimer, *Can. J. Biochem. and Physiol.* 34, 511 (1956).
13. A. Fischer, *Planta* 43, 288 (1954).
14. J. A. Bentley *et al.*, *Biochem J. (London)* 64, 44 (1956).
15. This research was supported in part by research grants from the National Institutes of Health, U.S. Public Health Service and the American Cancer Society.

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-

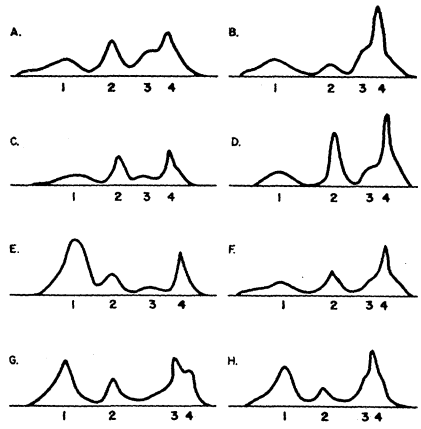


Fig. 1. Plasma electrophoretic pattern of eight normal parakeets, showing wide variations in the quantity of the four protein components.

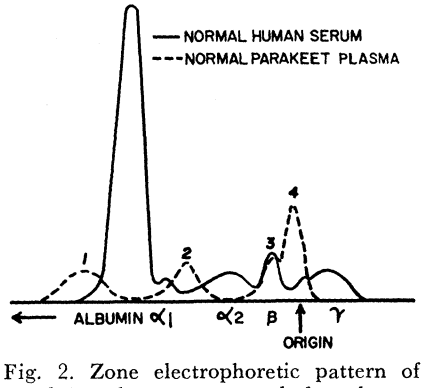


Fig. 2. Zone electrophoretic pattern of parakeet plasma compared for electrophoretic mobility with normal human serum.