Although there are a number of magnetometer and metal-detection systems available, the unit we first used was a Waugh magnetometer type W-2, which was produced during World War II to bench-check steel aircraft components for residual magnetism. It required minor modifications for working into the chopper input of an Offner model D electroencephalograph. The detector responds to the absolute intensity of a steady magnetic field in a particular direction and will register zero when oriented in a plane perpendicular to the direction of the ambient field. The system is calibrated by measuring the effect of rotating the pickup a given amount about a vertical axis from its null point with respect to the earth's field. The field to which the instrument is responding for calibration is presumed to be the product of the horizontal intensity and the sine of the angle of displacement. On the basis of the published value of 0.25 gauss (*3*) for the horizontal intensity of the earth's field in California, 0.2 mgauss/cm of pen travel seems to be ample over-all sensitivity, the periodic field variations with motility present characteristically approaching 1 mgauss peak to peak.

The magnets used are 3/16-inch diameter by ½-inch long General Electric Company sintered alnico-5 rods furnished cut to length. These magnets are not treated in any special way to increase or preserve the degree of magnetization they have when received. They are prepared for ingestion by coating with polystyrene. Subjects report neither awareness of them after ingestion nor any kind of after effect, and the data (*4*) indicate that the ingested magnet probably remains within the stomach of most subjects for periods of 2 hours or longer. The field variations recorded can be shown to be a function not only of periodic amplitude of magnet movement, but of mode of motion, orientation, and position with respect to the detector.

The method appears to have many uses beyond the recording of stomach motility. For example, it is currently being used to detect particular patterns of movements in small laboratory animals; the magnets are fixed under the skin. The Waugh type W-2 magnetometer has been modified and improved by the current manufacturers (*5*) and by William T. Kyle, Electronic Facility, College of Engineering, University of California, Los Angeles. Information concerning the improved circuit we now employ may be obtained from Kyle until it is published.

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References and Notes

1. Individuals too numerous to mention have contributed to discussions of this problem. John Dinning of the UCLA College of Engineering is to be credited with suggesting the successful solution.
2. This project was supported in part by USPHS grant M788.
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Stigmasterol in Cigarette Smoke

The presence of phytosterols in tobacco leaf (*1, 2*), seed (*3*), and smoke (*4*) has been previously reported; in only one case was a structure assigned to the sterol, that of γ-sitosterol isolated from *Nicotiana tabacum* (*2*). We wish to report the isolation of stigmasterol, a compound whose melting point differs from that of the other sterols recorded, from cigarette smoke (*5*).

The smoke generated from regular size, American, blended cigarettes was fractionated as we have described earlier (*6*). Fraction M (200 g) was chromatographed on silica gel; a mixture of 84 percent benzene and 16 percent ether eluted material whose infrared spectrum had absorption bands in the 9 to 11-μ region similar to those of many sterols (*7*). These bands were used to guide further isolation work. Fractional recrystallization from 1/1 benzene and methanol was followed by three chromatographies on acid-washed alumina, fractional recrystallization from 4/1 methanol and ethanol, and recrystallization from 1/4 benzene and petroleum ether (30° to 60°C), whereupon 35 mg of colorless crystals, mp 165.0 to 168.5°C, $[\alpha]_D^{20}$ – 40.2° (*8*) was obtained (stigmasterol, mp 170 to 171°C, $[\alpha]_D^{17}$ – 45.8° (*9*). The compound gave a positive Liebermann-Burchard test, and a precipitate with digitonin; it absorbed 2.0 moles of hydrogen (*10*) (assuming a molecular weight of 411); it exhibited a "false energy" maximum (*11*) at 206 mμ in the ultraviolet, ruling out the possibility of conjugated double bonds; and it had an infrared absorption spectrum consistent with that of stigmasterol. The reduction product had a melting point of 136.8 to 137.3°C $[\alpha]_D^{20}$ 25.3° {stigmastanol, mp 137°C (*12*), $[\alpha]_D^{20}$ 24° (*13*)}; its acetate melted at 131.2 to 132.4°C and had a rotation of $[\alpha]_D^{20}$ 16.2° {stigmastanyl acetate, mp 129.0 to 129.5°C $[\alpha]_D^{20}$ 15.4° (*14*)}; and its benzoate had a melting point of 135.0 to 136.0°C $[\alpha]_D^{20}$ 19.7° {stigmastanyl benzoate, mp 135.0 to 136.°C $[\alpha]_D^{20}$ 19.6° (*15*)}.

The acetate and the benzoate of the original sterol were prepared; the former, after several recrystallizations from 4/1 methanol and benzene, had a melting point of 140 to 141°C and a rotation of $[\alpha]_D^{20}$ – 51.9° {stigmasteryl acetate, mp 140 to 141°C $[\alpha]_D^{20}$ – 50.3° (*16*)}. It did not depress the melting point of an authentic sample of stigmasteryl acetate (*17*), and it had an infrared spectrum identical with that of the authentic material; the benzoate, after repeated recrystallization from 6/1 methanol and benzene, melted at 161.0 to 161.5°C $[\alpha]_D^{20}$ – 24.3° {stigmasteryl benzoate, mp 160.5 to 161.5° $[\alpha]_D^{20}$ – 24.5° (*18*)}.

The crude steroid fraction from fraction M contains other steroids which we have not as yet isolated in pure form. A sterol has also been obtained from fraction K (*6*); the data are: mp 149.5°C $[\alpha]_D^{20}$ – 63°, positive Liebermann-Burchard test; precipitate with digitonin; acetate, mp 137.0 to 138.0°C; benzoate, mp 124.5 to 125.0°C. The quantity isolated was too small for us to effect rigorous purification, and these values may be open to revision.

Khanolkar, Panse, and Divekar (*2*) found the sterol glycoside which they isolated from tobacco leaf to be readily hydrolyzed by dilute hydrochloric acid. The possibility therefore exists that, inasmuch as an acid extraction step was utilized in our fractionation of the cigarette smoke condensate, stigmasterol may be present in smoke in part, or in whole, as a glycoside. The quantity of sterol isolated corresponds to 0.5 μg per cigarette; this figure must be taken only as a lower limit inasmuch as considerable losses were sustained in the isolation process.

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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.

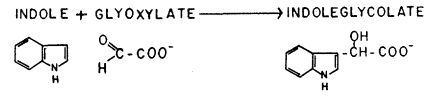

Fig. 1. Reaction of indole and glyoxylate.

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.