C57BL/6 preparations band 1 was the most concentrated and band 3 the next highest in concentration. It was evident that in these 14-day Hb^dHb^d preparations band 3 was the major band, while in the Hb^sHb^s hemoglobin of the same age, band 1 was predominant. In Hb^dHd^d fetuses aged 15 days, band 1 (presumably the adult major band) in some, but not all, preparations seemed to have increased in concentration, taking up more stain than band 2; band 3 was still present in relatively high concentration. On the other hand, 15-day Hb^sHb^s fetuses showed a progressive increase in band 1 and a corresponding decrease in all other bands.

It seems reasonable to assume from these data that the development of an adult hemoglobin pattern is delayed in mice with the gene Hb^d; however, the possibility that different strain backgrounds, the presence of the additional adult hemoglobin in such mice, or both, may contribute to this appearance cannot be ignored. Detailed studies of the developing blood pictures of C57BL/6 and AKR/J fetuses are currently in progress. Crossing of the gene for diffuse hemoglobin into a single hemoglobin strain should give the two types of hemoglobin on a similar background and provide interesting information about the effects of background on fetal hemoglobin pattern (7).

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2. Analyses of hemoglobins from adult mice by column chromatography, chain separation, and peptide analysis have demonstrated that mice with the gene Hb^d have two hemoglobins in unequal concentrations (approximately 80:20). These differ from each other in the nature of their β-chains [J. J. Hutton, J. Bishop, R. Schweet, E. S. Russell, *Proc. Natl. Acad. Sci. U. S.* **48**, 1505 (1962)].
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6. Several additional bands, presumably protein contaminants, were developed by Amido Black staining of the gels. These were found in all of the mice examined. It was also interesting that a very fast band was present, moving ahead of the hemoglobin components. This component totally rejected the stain and thus appeared as a white spot against the blue background of the stained gel; it was observed in all fetal preparations but was not present in adults.
7. This research was supported by a research grant CA-01074 from the National Cancer Institute. We thank Mary Hooper for excellent technical assistance.

9 August 1963

New Natural Growth Promoting Substance in Young Citrus Fruit

Abstract. *A naturally occurring compound that induces curvature in the* Avena *coleoptile test has been found in young orange fruits. Cochromatography with C^{14}-labeled indole-3-acetic acid, plus excitation and fluorescence spectra determinations, indicates that this compound is not one of the known indoles or gibberellins.*

A compound which induces curvature of *Avena* coleoptiles but does not appear to be an indole compound has been extracted from young orange fruits. Since this material, which we have called "citrus auxin," does not seem to fit under any of the classes of known endogenous growth regulators: indoles, gibberellins, or phytokinins, it is possible that it may represent a new class of natural growth regulators.

For many years indole derivatives were believed to be the only naturally occurring growth promoters in higher plants. The first description of the occurrence of a non-indolic, gibberellin-like substance in higher plants was reported by Mitchell *et al.* in 1951 (*1*). Since then a number of reports relating to gibberellins or gibberellin-like substances have been published. Crosby and Vlitos (*2*) and Stowe (*3*) reported the existence of non-indolic growth-promoting substances. The first non-indolic growth-promoting substance to be isolated from a citrus tissue was gibberellin A_1 from the water sprouts of *Citrus unshiu* in 1959 (*4*). However, the gibberellins do not cause curvature of the *Avena* coleoptile. In fact, the only compounds which have been known to give a positive reaction in this classical assay for plant growth stimulators have been indole derivatives (*5*).

Peroxide-free ether, saturated with water, was used to extract the "citrus auxin" from lyophilized tissue of Valencia and Navel orange fruits about 10 mm in diameter. A 2-hour extraction was carried out in the dark at room temperature with constant shaking. Extraneous material such as lipids, pigments, and non-acidic compounds was removed from the extract by repeated acetonitrile-hexane and sodium bicarbonate-ether fractionations. By adjusting the *p*H of the bicarbonate fraction to 2.8 before ether extraction, the plant constituents extracted were the acidic compounds.

The concentrated ether extract finally obtained was then fractionated by two-dimensional descending paper chromatography with *n*-butanol-ammonia-water (4:1:1 vol/vol) and isopropanol-ammonia-water (10:1:1 vol/vol). All the extracting, concentrating, and analyzing by chromatography was conducted in the dark, either under a nitrogen atmosphere or in a vacuum.

Fluorescent and absorbing spots were detected by scanning the chromatogram with ultraviolet light (253 mμ, maximal intensity). These spots were eluted in water and their excitation and fluorescence spectra were determined on an Aminco-Bowman Spectrophotofluorometer. Cochromatography with C^{14}-labeled indole-3-acetic acid (IAA) was used to aid chromatographic identification. The *Avena* coleoptile curvature test (*6*) was used as a biological assay of the extracted substances.

The first biological assays indicated that there was a growth-promoting substance in the extract which chromatographed similarly with indole-3-acetic acid. This was the expected observation and one which has been observed in the extracts of many fruits. Upon subjecting this particular material to fluorometric characterization it was observed that it did not have the same fluorometric characteristics as indole-3-acetic acid. The wavelength at which maximum excitation of indole-3-acetic acid occurs is 290 mμ, and its maximum fluorescence wavelength is 360 mμ; "citrus auxin" has a maximum excitation wavelength of 350 mμ and a maximum fluorescence wavelength of

Table 1. Excitation and fluorescent characteristics of growth-regulating substances and related compounds.

Compound	Excitation wavelength (mμ)	Fluorescence wavelength (mμ)
Indole-3-acetic acid	290	360
Indole-3-pyruvic acid	290	350
Indole-3-propionic acid	300	365
Indole-3-butyric acid	290	365
Indole	295	365
α-Naphthaleneacetic acid	310	340
β-Naphthoxyacetic acid	330	350
Gibberellic and gibberellenic acids*	405	450–480
Kinetin	335	410
Reduced pyridine nucleotides	340	457
α-Naphthol	335	455
β-Naphthol	350	460
Citrus auxin	350	460

* Dissolved in 85-percent sulfuric acid.

460 mμ (Table 1). Further chromatography with the two-solvents system and cochromatography with IAA-2-C^{14} indicated that indole-3-acetic acid actually had a lower R_F than that of the "citrus auxin."

Paper electrophoretic migration at *p*H 5.5 and 200 mv gave complete separation of the two compounds; in 5 hours the indole acetic acid migrated 11 cm, while the "citrus auxin" did not move from the origin. Thus, it seemed evident that a compound other than indole-3-acetic acid was present in these fruits, which was active in the *Avena* coleoptile curvature test.

To insure that the "citrus auxin" was not an indole compound, excitation and fluorescence spectra of other indoles were studied, some examples of which are shown in Table 1. Sprince (*7*) lists many indole derivatives which have fluorescent characteristics similar to indole-3-acetic acid, but none similar to the "citrus auxin." Udenfriend (*8*) states that, as a class of compounds, indoles have a characteristic maximum excitation at 278 mμ and a maximum fluorescence at 348 mμ. Since the gibberellins fluoresce only in the presence of sulfuric acid (*8*), the possibility of this compound being a gibberellin could also be eliminated on the basis of fluorometric data. Excitation and fluorescence data of kinetin indicated that "citrus auxin" and kinetin are two different substances (Table 1).

By using our own data, and data reported by others (*7, 9, 10*) we have been able to compare the wavelengths of maximum excitation and fluorescence of over 500 compounds with those of "citrus auxin." The reduced pyridine nucleotides and some naphthol derivatives were found to absorb and fluoresce at wavelengths similar to "citrus auxin." A fluorometric enzymatic assay for reduced pyridine nucleotides, after the method of Estabrook and Maitra (*11*) in which alcohol dehydrogenase and glutamic acid dehydrogenase are used, indicated that a reduced pyridine nucleotide was not the compound which was giving our characteristic fluorescence. Chromatographic experiments also indicated that the reduced pyridine nucleotides would not be found at an R_F similar to that of the unknown compound. The possibility remains that "citrus auxin" is related to one of the naphthols.

It should be stressed that the use of fluorescence spectra was the crucial factor in the recognition of this compound. It is possible that this "citrus auxin" may occur in other tissues and that, in the past, it has simply been thought to be indole-3-acetic acid, since both compounds chromatograph similarly when the one-dimension technique is employed.

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9 July 1963

Genetic Differences in Antibody Production to Determinant Groups on Insulin

Abstract. *Highly inbred strain 2 guinea pigs can produce antibodies to portions of the insulin molecule to which strain 13 guinea pigs cannot produce antibodies. Such differences were not observable within either strain. Consequently it is probable that genetic factors regulate antibody production with respect to the determinant groups toward which antibodies are directed.*

Individual rabbits and guinea pigs produce antibodies which are bound to portions of the insulin molecule to which antibodies from other rabbits or guinea pigs cannot be bound. The technique for demonstrating these differences measures the percentage of antibodies in a test antiserum (AB_t) from one animal which can be bound to an insoluble insulin complex (IC) saturated with antibodies (AB_s–IC) from the antiserum (AB_s) from a different animal (*1*).

It has been possible to demonstrate with I^{131}-labeled antiserums that the binding of antibodies in test serums (AB_t) to antibody-saturated insulin complex is not a result of the dissociation of antibodies from determinant groups common to both the saturating (AB_s) and test (AB_t) antiserums. In addition, the binding of insulin antibodies in AB_t with AB_s—IC has been shown to result from the binding of antibodies to exposed portions of the insulin molecule with which the antibodies in AB_s could not bind.

The determinant groups to which insulin antibodies are directed, therefore, appear to be characteristic for the individual rabbit or guinea pig immunized. Consequently, it is postulated that genetic factors direct antibody production toward specific determinant groups when insulin is the antigen.

This report is concerned with the differences observed in antibodies produced by two highly inbred strains of guinea pigs and a strain of partially inbred rabbits which were immunized with beef insulin that had been recrystallized ten times. Strain 2 guinea pigs produce antibodies to portions of the insulin molecule toward which strain 13 guinea pigs cannot produce antibodies. Such differences were not demonstrable when antiserums from animals of the same strain were tested against each other.

The two strains of guinea pigs were obtained from the National Institutes of Health—strain 2 and strain 13. These strains have complete intrastrain histocompatibility and between strains (*2*) they are not histocompatible. The strain of rabbits was obtained from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine, and had an inbreeding coefficient of approximately 0.75. The animals were immunized with insulin (*3*); insulin antibodies were measured by the method of Arquilla and Stavitsky (*4*) which can reliably detect relative antibody differences of 10 percent (*1*). The insulin complex was prepared by conjugating (via bis-diazobenzidine) insulin to PAB cellulose (paraaminobenzoic ether of cellulose) (*5*).

Binding of antibodies in test antiserums (AB_t) to antibody-saturated in-