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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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-

Table 1. Lack of antibody variation in guinea pigs of the same strain. Values are percentage of AB_t which is bound to AB_s–IC.

AB_t	Strain 2 (AB_s–IC)		AB_t	Strain 13 (AB_s–IC)	
	C-9	C-17		D-15	D-32
C	< 0.5		D	< 0.5	
C-18		< 0.5	D-15		< 0.5
C-19		< .5	D-24		< .5
C-25		< .5			
C-26		< .5			

Table 2. Variations in antibodies produced by strain 2 (C) and strain 13 (D) guinea pigs.

Guinea pigs AB_t	Percent of AB_t bound to AB_s–IC saturated with:	
	AO1	AO2
C/D	95.0/0.5	99.5/0.5
C17/D15	69.7/0.5	57.0/0.5
C18/D24	41.0/4.1	35.0/0.5
C19/D32	30.0/5.5	36.0/0.5
C25	25.6	22.9
C26	54.5	59.0

sulin aggregates (AB_s-IC) was estimated by comparing the antibody levels in nonincubated test antiserums (AB_t) with aliquots of the same antiserums which had been incubated with AB_s–IC.

It has not been possible to demonstrate antibody differences among animals of the same strain. Table 1 summarizes the results obtained from experiments designed to test antibody differences within each of two strains of guinea pigs. Five different AB_t (C, C-18, C-19, C-25, and C-26) from strain 2 guinea pigs were tested against two samples of AB_s–IC each of which had been saturated with one of two (C-9 or C-17) AB_s samples from individual strain 2 guinea pigs. The test antiserum C was a pool from four strain 2 guinea pigs; test antiserums C-18, C-19, C-25, and C-26 were obtained from individual strain 2 guinea pigs. None of the AB_t from strain 2 contained significant amounts of antibodies (10 percent or more) capable of binding to the AB_s–IC which had been prepared with antiserums from two animals of the same strain.

Similarly, AB_t from three (D, D-15, and D-24) strain 13 guinea pigs were each tested against two insulin aggregates saturated with AB_s from two (D-32 and D-15) animals. Test antiserum D was a pool from four strain 13 guinea pigs; test antiserums D-15, D-24, and D-32 were obtained from individual strain 13 guinea pigs. Again, no differences toward the determinant groups to which these antibodies were directed could be demonstrated among animals of the same strain by the method used.

Differences in antibodies produced by guinea pigs of strain 2 and strain 13 were demonstrated when individual AB_t were tested against insulin aggregates saturated with antibodies from strain III rabbits. Table 2 summarizes the results obtained when six strain 2 (series C) AB_t and four strain 13 (series D) AB_t were tested against two AB_s–IC each of which had been saturated with antiserum AO1 and antiserum AO2 respectively from Bar Harbor strain III rabbits.

All of the strain 2 antiserums contained significant amounts of antibodies which were bound to portions of the insulin molecule to which neither of the two strain III rabbits could bind. On the other hand, none of the strain 13 antiserums contained significant (10 percent or more) amounts of antibodies which could bind to the insulin aggregate saturated with antibodies from the two partially inbred rabbits.

Consequently, strain 2 guinea pigs produce antibodies to portions of the insulin molecule whereas the strain 13 guinea pigs cannot produce antibodies to those same portions. These results are consistent with the hypothesis that genetic factors regulate antibody production with respect to the determinant groups toward which insulin antibodies will be directed.

No binding of antibodies in AB_t from strain 2 guinea pigs to AB_s–IC prepared with antiserum from strain 13 guinea pigs was observed. Nor was binding of antibodies in AB_t from strain 13 guinea pigs to AB_s–IC prepared with AB_s from strain 2 noted. The differences in antibody-binding sites on insulin between these two strains of guinea pigs were only noted when AB_s–IC prepared with strain III rabbit was used. In order to explain these results, it is postulated that the insulin binding sites of the antibodies from strain 2 guinea pigs are in close proximity to the binding sites of the antibodies from strain 13 guinea pigs.

Consequently, because of overlap and steric hindrance, AB_t from strain 2 guinea pigs will not bind to AB_s–IC prepared with strain 13 antiserum and vice versa. The binding sites of the antibodies produced by strain III rabbits are much closer to the binding sites of the antibodies produced by strain 13 guinea pigs but quite distant from the antibodies produced by strain 2 guinea pigs. Therefore, the AB_t from strain 13 will not bind and AB_t from strain 2 will bind to AB_s–IC prepared with AB_s from strain III rabbit.

It is not known whether the genetic regulation observed with antibodies produced towards insulin is a generalized immunological phenomenon. In this regard, Levine, Ojeda, and Benacerraf (*6*) were able to demonstrate differences in the antibodies produced by the same strain 2 and strain 13 guinea pigs when immunized with poly-L-lysine conjugates to which various haptenes were attached. Therefore, animals may, possibly, produce antibodies only to certain antigenic determinants depending upon some genetic factors that are not well understood (*7*).

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Chromosome Number of Rose Clover, Trifolium hirtum

Abstract. *The somatic chromosome number of the cultivated* Trifolium hirtum *All.* (*rose clover*) *is 10. Satellites occur on one pair of the chromosomes. Out of approximately 70 species of this genus for which the chromosome numbers are known, only one other has a chromosome number of 10.*

The value of *Trifolium hirtum* All. (rose clover) as a soil-building range legume has been demonstrated by Love and Sumner (*1*) and Williams *et al.* (*2*). Out of the 20 economically im-