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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5 August 1963

## 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-

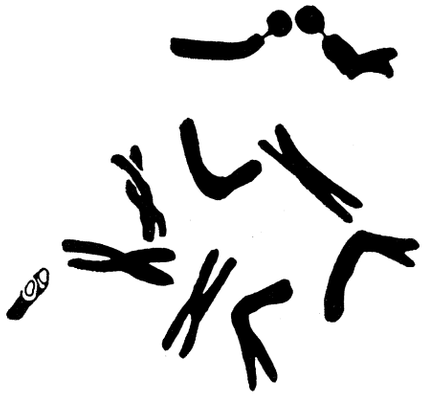

Fig. 1. Chromosomes in the root tip of *T. hirtum*. One pair of chromosomes has satellites. Drawings made with aid of camera lucida. (About × 2200)

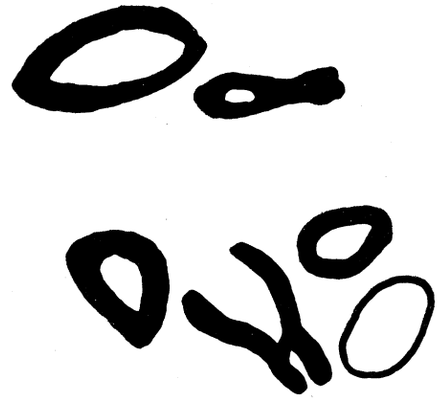

Fig. 2. Chromosomes in meiosis in *Trifolium hirtum*, at the stage of diakinesis. The nucleolus is shown in outline. (About × 1600)

portant clovers described by Hermann (*3*), *T. hirtum* is the only species for which the chromosome number has not been published previously. Cytological studies of *T. hirtum* have now revealed that its somatic chromosome number is 10 (Fig. 1) and that one pair of the chromosomes has satellites (*4*). Preparations of chromosomes were made from root tips which had been treated with 0.002*M* hydroxyquinoline for 2 hours at 4°C prior to fixing. Five different accessions of seed were examined.

A chromosome number of 10 is unusual for the genus: out of approximately 70 species of *Trifolium* for which the chromosome numbers are known, only one other, *T. scabrum* L., has this number. All *Trifolium* species for which the chromosome numbers were reliably counted prior to 1952 were reported to have basic numbers of 7 or 8. In that year Yates and Brittan (*5*) found a chromosome number of $2n = 12$ for some races of *T. subterraneum* L. The 12-chromosome clovers were subsequently separated from the 16-chromosome *T. subterraneum* on the basis of morphological differences, and were designated *T. israeliticum* D. Zoh. and K. (*6*). No other 12-chromosome clover has yet been reported.

Larsen (*7*) reported another new chromosome number in 1960 when he found *T. scabrum* L. to have $2n = 10$ chromosomes. This species was previously reported to have 16 chromosomes (*8*), but the 10-chromosome number has been confirmed independently (*9*). The only other clover found with a possible basic number of 5 is a form of *T. lupinaster* L. with $2n = 40$ chromosomes (*10*). Other forms of *T. lupinaster* were reported by Karpechenko (*8*) and Il'in and Trukhaleva (*10*) to have 48 and 32 chromosomes. Considering these facts, as well as taxonomic position, a form of *T. lupinaster* with a base number of 5 chromosomes does not appear to be probable. Taxonomically, both *T. hirtum* and *T. scabrum* are in the subgenus Trifolium of Hossain (*11*). *Trifolium lupinaster* is in the subgenus Amoria.

*Trifolium hirtum* is, then, the second species of *Trifolium* found to have 10 chromosomes (*12*).

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13 June 1963

## Protein Biosynthesis: Some Alternative Considerations on the Current Hypothesis

Several recent articles give the impression that the problem of protein biosynthesis is largely solved and that the remaining unsettled questions concern specific details (*1*, *2*). The purpose of this discussion is to emphasize that the current formulation still represents a working hypothesis. New findings which do not readily fit in the current visualization should be used to help modify the hypothesis. It is very important at this stage of our understanding to be careful not to select only those findings and interpretations which are consistent.

The essential features of the most generally accepted mode of protein synthesis are as follows.

1) All of the information for making specific proteins is contained in a linear sequence of nucleotides.

2) The information is written in the form of a code which uses 3 (or a multiple of 3) nucleotides to specify a single amino acid. The information may be carried predominantly in two of three nucleotides (*2*, *3*).

3) An exact replica of the DNA sequence is made in the form of RNA with uracil substituting for thymine.

4) This replica called "messenger RNA" attaches itself to ribosomes already existing in the cytoplasm and from this location directs the formation of protein.

5) Amino acid is activated for protein synthesis, and in chemical association with a specific molecule of soluble ribonucleic acid it is brought to a particular location on the messenger. This location recognizes the soluble ribonucleic acid and not the amino acid.

6) The messenger has a short life (2 to 3 minutes) and, after making several proteins, it is degraded. More recently, in order to retain the concept of messenger in those cases where evidence for a rapidly renewed message was not obtained, the possibility of a more stable message is considered.

In regard to point 1, the evidence which demonstrates that nucleic acid is the carrier of genetic information is firmly supported by experiments on bacterial transformation, infection of hosts by virus nucleic acid, and induced and natural mutations which affect the nucleic acids and result in specific substitutions of amino acids. That nucleic