

TABLE 2
VARIABILITY OF PROGENY ROWS OF ALFALFA, EACH
GROWN FROM SELF-FERTILIZED SEED OF A SINGLE
PLANT. THREE PLANTS EACH OF THE
HIGHEST, OF INTERMEDIATE, AND OF
THE LOWEST VARIABILITY OF
EACH CHARACTER

Variability group	Plant height	Plant width	Angle of erectness	Diameter of stem	Length of leaflet	Width of leaflet	Color of bloom
Highest	38.2	45.6	50.7	25.7	27.5	23.7	12.19
"	36.7	43.8	46.2	22.0	26.8	29.6	10.64
"	30.8	39.4	44.6	21.1	18.9	28.8	10.15
Intermediate	20.8	22.9	21.1	14.2	12.6	16.7	4.35
"	19.9	20.8	23.1	13.8	11.9	16.7	3.83
"	18.4	21.2	19.5	13.3	11.2	15.5	3.33
Lowest	9.3	11.0	8.6	8.5	7.1	11.2	1.22
"	9.6	11.2	7.8	6.1	6.6	10.3	1.10
"	7.9	7.5	7.0	4.5	4.8	9.0	0.0

Inbreeding which tends to purify the various sub-varieties, or strains which make up a variety, allows for the appearance of any abnormalities which in open pollination (such as occurs in the field) are obscured by being in a crossed condition with normal types. In the first inbred generation, three rather distinct sorts of abnormal plants have appeared.

Each of two progenies, both from the same parent strain, showed several plants on which the leaflets had tiny brown markings not characteristic of any recognized disease when examined by a plant pathologist. The leaves, partly folded and partly crinkled, suggested that small sections of the leaf structure had been lost or had died after development had started. Sufficient study has not yet been made to enable more to be said at the present time.

In three other progenies there occurred some distinctly shorter plants extraordinarily leafy but which would produce only about half as much hay as the parent variety. One strain of nearly normal size showed in a large degree this same condition of extreme leafiness.

In a single progeny almost half the plants had the blossom replaced by a freak vegetative development that resembles somewhat the appearance of the flower buds on a plant of seed onion. This condition occurs naturally in the field at rare intervals. Here a progeny was found in the first inbred generation in which the character was thousands of times more fre-

quent than is found in the commercial varieties or strains.

Some strains are becoming pure for erectness, while others are pure for a nearly flat position; others are showing a strong tendency to coarseness and others to a suggestion of "vininess" in the upper branches. This last character suggests a faint resemblance to twining branches such as occur on vetches and some beans.

There is also considerable evidence that seeding ability in certain strains is governed in part by the genetic constitution of the plants.

While an immense amount of careful scientific work would be required to develop and maintain economic strains, the promise is such as to warrant its being given a thorough trial.

GEORGE STEWART⁴

NON-ACID-FAST TUBERCLE BACILLI

NON-ACID-FAST organisms long have been known to exist as a phase in the life cycle of the acid-fast group. Until recently no pure cultures of the non-acid-fast varieties have been obtained. It has been thought that the occasional non-acid-fast rod and the forms described as Much's granules¹ were involution forms which had lost their acid-fast property. From Kahn's recent work,² it would appear that these granules were the precursors of numerous young organisms. Recently Dreyer and Vollum³ reported the cultivation of a non-acid-fast strain of human tubercle bacilli which showed optimum growth at the bottom of veal peptone bouillon medium. These organisms were fully virulent.

In this communication we describe a non-acid-fast, almost a virulent type of organism which has much in common with the acid-fast strain from which it was isolated.

A two-months-old culture of H-37 strain of human tubercle bacilli developed a group of yellow-orange, chromogenic colonies in the midst of other normal-appearing colonies. These chromogenic colonies appeared to have the same morphological characteristics as the known acid-fast ones surrounding them. They were made up of numerous non-acid-fast rods and granules and were Gram-positive. There were also present a few acid-fast rods and granules, and some of the non-acid-fast rods contained from one to three acid-fast granules.

Three subcultures were made at different times from the original colonies. The cultures grew rap-

⁴ Formerly Agronomist; now Senior Ecologist, Intermountain Forest and Range Experiment Station, U. S. Forest Service.

¹ H. Much, *Beitr. z. Klin. d. Tuberc.*, 8, 85, 1907.

² M. Kahn, *Amer. Rev. Tuberc.*, 20, 150, 1929.

³ G. Dreyer and R. L. Vollum, *Lancet*, 220, 1015, 1931.

idly, attaining a maximum growth in ten days. During the first five days they were first yellow-white, then yellow, and by the tenth day they were a deep orange color. Each of these subcultures had the general morphological characteristics of the acid-fast group. They were composed of numerous rounded colonies, and occasionally one or more of these was piled on one another. The edges of the growth were usually smooth and at times became indented as the growth progressed. The staining characteristics were the same as the original colonies. Dilution cultures were made to obtain single colonies. After dilution growth appeared in two days. This was in the form of white, rounded colonies which did not start to assume the yellow color before the fourth or fifth day. By the tenth day they had attained a maximum growth and a deep orange color. The rounded colonies, by the third or fourth day, showed from two to five lobulations which gave the appearance of dividing starfish eggs. None of these showed the crusting projections found in many of the colonies of the acid-fast group. All appeared on the surface of the medium and did not cause lysis of it. By the end of the sixth week each colony had developed a fine, veil-like growth, orange in color about its base. These were very similar to those seen about the bases of many single colonies of acid-fast organisms. At the tenth day, sixth week and third month the organisms stained the same as those of the original colonies, except that there were fewer acid-fast rods to be found.

The organism grew easily and rapidly on blood agar; it made almost a complete growth in five days, and showed no further growth after ten days. On this medium it had lost its chromogenic power and appeared as dirty white, slightly heaped-up colonies. Often these had a moth-eaten appearance and irregular borders. They had about the same appearance on plain agar, although some piled up in clean white, rounded groups. On these media they were entirely non-acid-fast and were of a coccoid, granular type, slightly larger than ordinary cocci, although a few rods the size of acid-fast organisms still were present.

The organism gave a luxuriant growth on Long's medium. It formed a pellicle on which rounded, granular, light-orange colonies developed. Many of these granular colonies sank to the bottom of the flask, but others developed in their place on the pellicle. These again were almost wholly non-acid-fast rods, although a few acid-fast granules were present. The growth was less luxuriant on plain and dextrose broth, and in these two types of medium it was found at the bottom of the tube as a dirty white precipitate. Growths of this sort were made up of the non-acid-fast, coccoid granular organism.

Five guinea-pigs were inoculated with suspensions of the second generation of the chromogenic organism. Each pig was given 1/50 of 1 mgm subcutaneously in the right groin. Four weeks after this inoculation, all were in excellent condition and were reinoculated with 1 mg of the same culture. This was again given subcutaneously in the right groin.

Of these five animals, two at autopsy had no demonstrable tuberculosis. The other three had caseous nodes at the sites of inoculation and varying numbers of tuberculous lesions in spleens, livers and lungs. Acid-fast rods were demonstrated in smears from the caseous material, as well as in smears of the affected organs. The amount of tuberculosis each animal had was much less than would be expected following a much smaller dose of our parent strain of H-37 human tubercle bacilli.

We believe that this organism is a non-acid-fast mutant of our strain of H-37 human tubercle bacilli. Although chromogenic, it maintains cultural characteristics similar to the acid-fast group when grown on special media. Its growth on simple media is composed almost entirely of non-acid-fast granules. Granular forms may be the primary type of organisms of the acid-fast group, with the chromogenic organism as an intermediate phase, and the acid-fast bacillary forms the typical, well-known varieties.

We also have evidence that sterile Berkefeld filtrates of cultures of the chromogenic organism will cause normal cultures of human and bovine tubercle bacilli and timothy grass bacilli to lose their acid-fastness and grow as forms similar to the coccoid granular type of the chromogenic mutant.

FRANKLIN R. MILLER

THE LABORATORIES OF THE
ROCKEFELLER INSTITUTE FOR
MEDICAL RESEARCH,
NEW YORK

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