threw himself wholeheartedly into the intimate life of the college, entering with zest into its activities, educational, scientific and social, and contributing a valuable element to its discussions by his independent, yet sympathetic, point of view and a breezy disregard of conventions which now and again shocked more conservative minds.

It was unfortunate that the refusal of the university to provide an official house in the Parks for the Professor of Astronomy made his relations with the university less cordial. This and the remoteness of astronomical studies from the ordinary curriculum rendered his influence in Oxford less than it ought to have been. But those who knew him well were aware of the keen interest which he took in its studies and general welfare, and also in the lives of his friends and colleagues. No one was more ready to give active help to his friends in any time of trouble. It was characteristic of his generous temper and his sympathy with struggling causes that from the first he gave his vigorous support to the Workers' Educational Association and the organization of tutorial classes. In this work his optimism and persistence were invaluable.

Here, as in everything else he took up, his help was direct and personal. He had the courage and strong will of a true Yorkshireman, and he also shared the North Country love of music; he was an assiduous member of the Oxford Bach Choir, and an active supporter of musical enterprises. A lover of the open air, he had a close acquaintance, by bicycling, boating and walking, with the beautiful country near Oxford, and in other parts of England, and his scientific expeditions gave him a wide acquaintance with the oversea dominions and with foreign countries. In these activities he was fortunate in having a wife who shared in his tastes and in much of his travel. The astronomical world is weaker by his death, and he has certainly left in his college and among his friends in the university a place which can not be filled.

Professor Turner was elected a fellow of the Royal Society in 1896, and was president of the Royal Astronomical Society in 1903 and 1904. From 1913 to 1922 he was one of the general secretaries of the British Association. He was given the honorary degree of D.Sc. by the Universities of Leeds, Sydney, Wales and Strasbourg, that of D.C.L. by Durham, and that of LL.D. by California. He was also a corresponding member of the French Institute, and he received the Bruce gold medal of the Astronomical Society of the Pacific in 1927.—*The London Times*.

## SCIENTIFIC APPARATUS AND LABORATORY METHODS

## AN IMPROVED METHOD FOR THE STUDY OF NUTRITIONAL ANEMIA IN THE WHITE RAT

RAPID progress in solving the mysteries surrounding the underlying causes of pernicious anemia has been made within the past four years.<sup>1</sup> One of the phases of this problem which has attracted the attention of many investigators is the study of nutritional anemia in the white rat.

Young rats at the weaning age become anemic when fed 4 to 6 weeks on whole milk only, for at this stage the store of iron in the body is at a low ebb. It has been noted in some instances, however, that anemia did not appear unless milk was fed over a prolonged period or even to the second generation.<sup>2</sup> The authors were surprised to find that under the conditions in their laboratory a number of rats fed whole milk only or whole milk plus cod-liver oil grew well and for the most part were about normal in hemoglobin content (Fig. 1). Studies were therefore conducted to learn why the conditions under which these animals were kept were not conducive to anemia.



FIG. 1. Contrary to expectations, young rats fed whole milk and cod-liver oil made good growth and in most cases were normal in hemoglobin content. The solid curves show live weight and the broken lines the amounts of hemoglobin in the blood.

The cages employed in the early work were constructed of galvanized iron wire, having a mesh small

<sup>&</sup>lt;sup>1</sup> SCIENCE, 71, (No. 1850): x, June 13, 1930.

<sup>&</sup>lt;sup>2</sup> W. M. Happ, Bul. Johns Hopkins Hosp., 33: 163, 1922.

enough to retain most of the feces. There were places on the cages where the zinc had been worn off. The rats were observed to gnaw these wires vigorously.

Questions were therefore raised regarding the effect upon hemoglobin formation of several factors, including the material of which the cage was composed, the access of the rat to fecal material, the feeding of codliver oil, and others.

Cages of different material were therefore constructed. In these the wires or rods forming the bottoms were spaced one half inch or more apart to prevent retention of feces in the cage. Later all cages were equipped with legs to prevent the animals from reaching shavings or feces below the cages. Paired feeding trials carried out with animals kept in cages of different materials showed that the low hemoglobin values of young rats at the weaning stage rose quickly to normal when the animals were placed in iron wire cages and remained at high levels (Fig. 2). The ani-



FIG. 2. Rats can secure enough minerals from cages in which iron wire is exposed to prevent anemia.

mals in glass, copper and well-galvanized iron wire cages gradually became anemic. Some cures of anemic rats have been effected by placing them in a cage of common iron wire.

With the evidence at hand that the material used in construction of the cages may have a pronounced effect upon the condition of rats kept in them, other factors were studied, using cages in which the animals could come in contact with nothing but glass.

Cod-liver oil was found to have no effect, the hemoglobin values of young animals fed fresh whole milk and cod-liver oil declining rapidly. This result has also been noted by Krauss.<sup>3</sup>

The literature is lacking in detailed reports of the feeding methods used in different laboratories. Apparently animals are fed once daily in some cases, twice daily in others and in one instance once daily six days per week. The effect upon hemoglobin titer of the amounts of milk consumed was studied (Fig. 3). Lot 1 was fed milk once daily six days per week.

<sup>3</sup> W. E. Krauss, Jour. Dairy Sci., 12: 438, 1929.



FIG. 3. Consumption of large amounts of milk induces anemia more quickly than small amounts.

Growth was rapid and hemoglobin values declined less rapidly than in Lot 2, which was fed milk ad libitum twice daily seven days per week. Lots 3 and 4 were fed in the same manner as Lot 2, except that they received only three fourths and one half, respectively, the amount consumed by Lot 2.

Carefully controlled paired feeding trials in which the control animal was fed milk twice daily ad libitum, and its mate three fourths the amount, also showed that the hemoglobin values in the animals fed the smaller amount declined less rapidly.

As already noted, the galvanized wire cages used in the first trials had a mesh of such size that many of the feces were retained in the cages. The effect of the presence of feces in the cages was studied in paired feeding trials in which the animals were kept in specially constructed glass cages. The bottom of the cage for the control animal consisted of glass rods spaced about one half inch apart, while in the cage of the pair mate the glass rods were close enough together to retain the feces. The hemoglobin values of all animals declined rapidly, but those of the animals having access to feces declined somewhat less rapidly (Fig. 4). A more efficient utilization of food by rats



FIG. 4. Access to excreta delays somewhat the progress of anemia.

having access to excreta and shavings was noted by Griffith.<sup>4</sup>

In view of these findings, a special laboratory technique for the study of nutritional anemia has been developed.

The cages are constructed of wood and glass in such a way that the animals can come in contact with noth-

4 W. H. Griffith, Jour. Biol. Chem., 85: 751, 1930.

ing but glass. Tops and bottoms consist of glass rods spaced about one half inch apart. The top is removable. Sides are lined with window glass. The floor space allowed each animal is about  $10 \times 14$  inches. Cages are mounted on legs about 4 inches high. Galvanized iron pans 2 inches deep and containing wood



FIG. 5. Two-compartment glass cage designed for the study of nutritional anemia.

shavings fit loosely below the cages to receive the excreta. Shavings are changed twice weekly. Cages are washed once weekly, a washing powder solution being used, followed by rinsing with tap water, then distilled water, and drying with a clean towel.

Glass sponge dishes (common office type) are used as feed dishes. These are practically non-spillable, easily washed and not readily broken. These are washed twice daily, using a stiff brush with washing powder solution. They are rinsed in tap water, drained on a drying rack made of glass rods, then rinsed in water redistilled from glass, and again drained.

The whole milk used is obtained by milking the cow directly into a glass funnel and glass jug. It is placed in a refrigerator shortly after milking. A fresh supply is obtained daily.

Hemoglobin determinations are made every two weeks, using a colorimeter with Newcomer hemoglobin attachment. This is checked for accuracy by means of the Van Slyke oxygen capacity method.

The plan of feeding animals in pairs is employed in most cases. In this plan two litter mates of the same sex and weight constitute a pair. Milk is fed twice daily, the amounts fed being adjusted so that the amounts eaten by both animals are the same. The specially treated milk or special experimental routine is given one animal, the other constituting a control or check animal. Differences in food intake, which often greatly complicate the interpretation of results, are thus avoided. It is possible in this way to attribute differences in growth and hemoglobin levels to the experimental condition under investigation.

## SUMMARY AND CONCLUSIONS

Experiments in our laboratory have demonstrated that white rats fed only fresh whole milk secured enough minerals from their cages to prevent anemia.

Animals consuming large amounts of milk became anemic more quickly than those limited to small amounts.

Access to feces delayed somewhat the onset of anemia.

These results probably explain some of the conflicting data reported in the literature and also raise a question regarding the correctness of conclusions drawn from experiments in which recognition was not given the factors shown in this study to be important.

> W. B. NEVENS D. D. SHAW

UNIVERSITY OF ILLINOIS

## SOME NEW METHODS AND COMBINATIONS IN PLANT MICROTECHNIQUE

It is a common experience to find beginning students in plant histology failing to secure good sections of ordinary or thin leaves embedded in paraffin, because of serious plasmolysis or imperfect infiltration.

It occurred to the writer that the glycerine process used in the Venetian turpentine method for extremely delicate tissues such as algae might be adapted to the paraffin method to assure dehydration without plasmolysis. This year, the following method has been tried with excellent results even by students that are below average.

In the case of leaves that have been killed in an aqueous solution, the material is first thoroughly washed, then put into 10 pts. glycerine +90 pts. water, in a flat open dish, and left until the solution becomes about as thick as pure glycerine. It is then washed in 95 ethyl alcohol and put through two changes of absolute ethyl alcohol, followed by: absolute alcohol 3 pts. xylol 1 pt.; absolute alcohol 1 pt. xylol 1 pt.; absolute alcohol 1 pt. xylol 2 pts.; pure xylol. It is then embedded in paraffin in the usual way.

Leaves that have been killed in an alcoholic solution, however, after being washed in a similar concentration of alcohol, are put into a solution of glycerine mixed with a water percentage equal to the alcohol percentage of the killing solution. For instance, material killed in a 50 alcohol-acetic acidformalin solution is first put into equal parts of glycerine and water. With this exception, the method is the same as that used following an aqueous killing solution.