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 x 14 inches. Cages are mounted on legs about 4 inches high. Galvanized iron pans 2 inches deep and containing wood

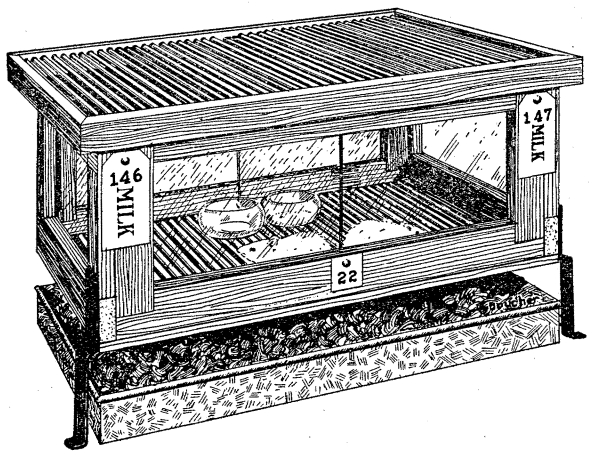

FIG. 5. Two-compartment glass cage designed for the study of nutritional anemia.

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.

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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 acid-formalin 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.

In every case, leaf material prepared in this way is less brittle and cuts much more easily than after the usual treatment (perhaps because it is in alcohol a shorter time), and even when embedded by more or less careless students shows little or no plasmolysis. This method also requires less attention.

The following modification of the method for "dehydrating woody tissue for paraffin embedding" as given in SCIENCE for January 24, 1930, has been used successfully.

After the killing and fixing solution has been washed out, if the material is soft enough to cut easily, it is placed in 10 pts. glycerine + 90 pts. water and left until the water has evaporated. It is then put into equal parts glycerine and butyl alcohol for about 36 hours, followed by pure butyl alcohol for another 36 hours. It is then embedded as described in the article in SCIENCE. Three to six days in the paraffin bath may be necessary to secure thorough embedding.

If tissues are part hard and part soft, they may be softened in C.P. hydrofluoric acid + 95 alcohol, as this method, according to Dr. E. C. Jeffrey, injures delicate tissues less than hydrofluoric acid diluted with water. If tissues are very hard, they may be put into C.P. hydrofluoric acid for three or four weeks, then washed and treated as described.

This method has the advantage of practically avoiding the use of ethyl alcohol, with the result that the material is less brittle and no harder than before dehydration. The longer time in the paraffin bath does not appear to make woody tissues hard and brittle. This method likewise requires less attention.

In dry weather or in dry climates it has been found that the addition of eight or ten parts of glycerine to preservatives such as alcohol-acetic acid-formalin is efficacious in preventing rapid evaporation caused by imperfect cork stoppers.

In cutting wood sections that are likely to curl, if the microtome knife is kept wet with equal parts of glycerine and 95 alcohol, and each section is permitted to remain on the blade a few seconds after cutting and is then transferred to a dish of the same solution, this difficulty is entirely avoided.

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SPECIAL ARTICLES

DATA ON A PROTEIN-ANTIBODY SYSTEM

IT was recently shown by the writers that the entire course of the precipitin reaction between Type III pneumococcus specific polysaccharide and purified homologous antibody could be expressed quantitatively by simple equations that follow the mass law.[1] Since it was of interest to determine whether a similar method of treatment might be applied to a true antigen-antibody system, recourse was had to the compound azo proteins, so fruitfully employed in the solution of other immunochemical problems by Landsteiner and by Avery and Goebel. It was felt that if a protein dye could be synthesized of more intense color and more rigorously freed from its component substances than those used by these workers, a possible means would be at hand for distinguishing between antigen and antibody nitrogen both in the precipitates and supernatants, since the antigen could be determined colorimetrically. A purplish-red disazo protein dye, *R-salt-azo-benzidine-azo-egg albumin,* was finally isolated in a form which satisfied these requirements, the more so as the antibody solutions obtained by sodium sulfate fractionation of rabbit antisera to the dye were substantially colorless.

By methods analogous to those used in the first paper[1] it was found that at the lowest concentration of dye protein used, 1: 67000, the mean ratio between antigen and antibody precipitated was 1: 15 (8 determinations); at the equilibrium point,[1] at which the proportions of the reactants were such that both antigen and antibody were present in solution, 1: 7.5 (6 determinations), and in the inhibition zone, after the maximum precipitate was reached, 1: 3 (14 determinations).

These preliminary data indicate that the composition of the solid phases formed by the precipitin reaction between the dye and its antibody may be expressed by the three limiting equations:

$$\mathrm{An} + \mathrm{Ab} \rightleftarrows \mathrm{AnAb},$$
$$\mathrm{AnAb} + \mathrm{An} \rightleftarrows \mathrm{An_2Ab}, \text{ and}$$
$$\mathrm{An_2Ab} + 3\mathrm{An} \rightleftarrows \mathrm{An_5Ab},$$

in which An = antigen and Ab = antibody. The data so far obtained are insufficient for a decision as to whether the soluble compound formed in the inhibition zone is $\mathrm{An_6Ab}$ or $\mathrm{An_7Ab}$. Further work is in progress and a detailed report will be made when this has been carried out.

Whether or not modification of the above ratios be necessary, it would appear that the precipitin reaction between a true antigen and its antibody is essentially the same as the precipitin reaction in a hapten-antibody system or, for that matter, essentially the same as a typical inorganic precipitation reaction, and may

[1] *Jour. Exp. Med.*, 50: 809, 1929.