

Chittenden and Hindhede for nearly ten years without noting any decrease in the tendency to catch colds unless the protein restriction amounted to protein starvation. Then the addition of salt to the diet and occasional increases in the protein intake (due to instinctive compulsion) gave rise to considerable edema. Furthermore, the susceptibility to colds was increased and pleurisy developed under such circumstances in the winter of 1917-1918. During the next year, as a result of a change in diet occasioned by military service, I found that the continued use of a higher protein intake decreased the tendency to develop distinct edema from additions of salt or more protein. However, the army diet did not confer immunity to colds, nor did evidence of slight edema decrease to the extent that it decreased later with the carnivorous diets or with a low carbohydrate diet such as I have adopted more recently to control both hydration and the susceptibility to colds.

The restriction of the carbohydrate intake as a means of keeping the hydration of the organism at a low level is based upon the fairly well-established fact that along with carbohydrate a considerable amount of water is stored. At least, with the carbohydrate reserve kept at a relatively low level, I find that ordinary colds do not develop. A limitation to about five hundred calories from carbohydrates in a diet of about twenty-five hundred calories daily seems satisfactory. The protein intake has been kept adequate (1.2 to 1.6 gm. per kg. of body weight) and the balance has been made up with fats. With this regimen, I have caught no distinct cold but have noticed a little sneezing from time to time, or an excessive secretion of nasal or pharyngeal mucus which generally cleared up within a few hours without anything being done about it.

It may seem idle to speculate at this time about the mechanism of catching a cold. But presumably, with the organism hydrated to a high degree and with the tissues in general already under some degree of tension, a sudden cooling of the skin may throw an overload of fluid on internal structures, including the upper respiratory tract. Undoubtedly the process is not simple. A specific nervous or vasomotor reaction seems to be involved in the chill which often initiates a cold. A factor in the development of this reaction may be an increased sensibility of the cutaneous nerves in nutritional edema.

Although quantitative data on this subject were not secured, I have repeatedly observed the variations in skin sensibility with changes in the degree of hydration. In shaving, the "pulling" due to a razor which is not very sharp is far more painful with edema than under normal conditions. A difference was often

seen here between morning and evening, as the hydration of the face which is greatest in the morning (after reclining) decreases during the day with the maintenance of the upright position. This change may, however, become reversed if the diet used during the day leads to a considerable retention of fluid. Pinching of the skin has shown similar fluctuations in sensibility in other skin areas. This has been so constant that I am inclined to regard the return of normal sensibility as a better index of the complete disappearance of edema than other tests. In using the edema test of McClure and Aldrich, the pain incident to puncture with the needle was also greatest with the most pronounced edema. This discouraged the use of the method. The change in sensibility may be qualitative rather than quantitative, as the pain in edema reminds one of the pain of mild inflammation. The theory that there is a nervous factor in the development of edema is therefore hereby supported.

The above explanations would harmonize the theories that refer colds to dietary excesses with those that refer them to changes in the weather or to exposure. The oft-quoted freedom from colds of Arctic explorers would be regarded as the consequence of a decreased carbohydrate intake rather than of outdoor life or relative absence of bacteria. The effectiveness of remedies, such as food restriction, sweating and bed rest would be explainable as being due to dehydration. However, it is hard to understand why sodium bicarbonate should be beneficial, as Cheney<sup>6</sup> recently reported. Perhaps the other measures he uses are more directly responsible for the results. Cheney's implication of the protein intake as a cause of colds is supported if it is remembered that this is a protein excess in a diet otherwise excessive.

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## SCIENTIFIC APPARATUS AND LABORATORY METHODS

### PARAFFIN SECTIONS OF TISSUE SUPRA- VITALLY STAINED

THE use of Nile-blue sulphate for staining living *Amblystoma* embryos which will furnish grafts for transplantation in the study of many problems in embryonic development has been popular for some time (Detwiler, 17).<sup>1</sup> Its main drawback has been that

<sup>6</sup> Cheney, V. S., *Amer. Jour. Pub. Health*, xviii, 15, 1928.

<sup>1</sup> S. R. Detwiler, "On the Use of Nile-blue Sulphate in Embryonic Tissue Transplantation," *Anat. Rec.*, V, 13, 493-497, 1917.

the color of the grafted tissue remains only while the embryos are alive and that the limits of the transplanted cells can no longer be traced by the stain in embryos which have been fixed and passed through alcohols. During the course of blood work in which supravital technique was employed, Miss Fazil  Shevket noted a method described by McJunkin (25)<sup>2</sup> for the examination in paraffin sections of tissue supravitaly stained. This method with slight modifications was tried out by Miss Kathryn Stein on *Amblystoma* embryos stained in Nile-blue sulphate and gave good results. Because it may be of value to other workers in this field, the method, quoted from McJunkin (pp. 313-314), is given below.

The tissue stained supravitaly in toto is fixed for twelve hours in a Zenker-formol solution, consisting of 15 cc of 40 per cent. formaldehyde and 85 cc of Zenker's fluid without acetic acid. It is then cut into pieces not to exceed 3 mm in thickness and transferred to Zenker's fluid without acetic acid for 12 to 25 hours. Bits of the tissue are then placed in pure absolute acetone for 1 hour (2 changes), in benzol for 20 minutes and in 52  C. paraffin for 20 minutes. Sections of the desired thickness are cut and attached to the albumin-coated slides by allowing them to dry overnight at room temperature. To stain, the paraffin is removed with xylol (10 seconds) and pure acetone (10 seconds). After immersion in water (5 seconds) the slide is stained very lightly with hematoxylin (Harris without acetic acid) for about 5 seconds and immersed in tap water (5 seconds). The section is then dehydrated with pure absolute acetone for 10 seconds, at once immersed in xylol for 10 seconds and mounted in balsam. To limit the action of the acetone and xylol to these times the slides are stained singly and the solutions run over them from a dropping bottle. A 0.05 per cent. aqueous solution of methylene blue may be substituted for the hematoxylin.

This method has been used on *Amblystoma* embryos up to stage 40 and the stained tissue showed clearly. There appears to be no reason for its not being applicable to older embryos provided the color is still visible in the living animal. The staining with Nile-blue sulphate was done in the usual way (Detwiler, 17)<sup>1</sup> and the embryos to be examined were fixed according to McJunkin's scheme and sectioned. It was found that they could remain in acetone longer than one hour without harm and that xylol could be substituted for benzol. An hour for infiltration did not appear to be detrimental. It also seemed to be unnecessary to stain the sections with hematoxylin unless the nuclei were to be examined particularly because the

<sup>2</sup> F. A. McJunkin, "The Origin of the Mononuclear Phagocytes of Peritoneal Exudates," *Amer. Jour. Path.*, V. 1, 305-324, 1925.

outlines of the vitally stained tissue were evident in the sections after mounting.

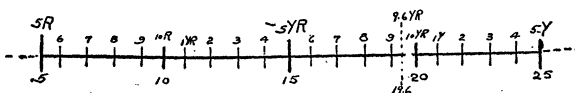
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### COLOR DISCS IN SOIL ANALYSIS

In a note<sup>1</sup> regarding color discs used in soil analysis, G. B. Bodman suggests that a ratio,  $\frac{\text{"Red"}}{\text{"Yellow"}}$ , as determined by color disc analysis is found to bring out the apparent striking color differences of two soils.

The ratio,  $\frac{R}{R+Y}$ , the proportion of R to the total color, will give better results, while the use of the notation of the Munsell color discs in the above ratio will give still better results in that it expresses the hue as the eye sees it. For example, the red disc being used in soil work is R 4/10, and the yellow, Y 8/9. The first figure in the notation represents the brilliance of color on a scale of 0 to 10, black to white, the second figure chroma, or color saturation, 0 being neutral, 10 expressing strong chroma.<sup>2</sup>



By translating the hue letters R and Y to figures, i.e., R=5, Y=25 (the hue circle being completed in 100 steps, YR=15, GR=35, G=45, BG=55, B=65, PB=75, P=85, RP=95, back to 5R), and letting x equal the first hue (clockwise), while z equals the second, use the formula (which is essentially the same as  $\frac{R}{R+Y}$ ):

$$z - \frac{S(A_1 \cdot B_1 \cdot C_1)}{S(A_1 \cdot B_1 \cdot C_1) + S(A_2 \cdot B_2 \cdot C_2)} (z - x),$$

when A = per cent. area, B = brilliance, and C = chroma. The first area, A<sub>1</sub>, applies to the hues taken in a clockwise direction, that is, to red, while A<sub>2</sub> applies to yellow. S is the summation sign.

For example, if the percentages matching a certain soil color are 10 per cent. red, 15 per cent. yellow, 70 per cent. black, and 5 per cent. white, when the Munsell notation for red is R 4/10, and that of yellow, Y 8/9, the relative hue may be found by substituting in the formula above:

$$25 - \frac{(10 \cdot 4 \cdot 10)}{(10 \cdot 4 \cdot 10) + (15 \cdot 8 \cdot 9)} (25 - 5) = 19.60.$$

<sup>1</sup> SCIENCE, April 27, 1928, p. 446.

<sup>2</sup> Cf. Colorimetry Report of the Optical Society of America, 1920-21, *J. O. S. A. and R. S. I.*, Vol. VI, No. 6, August, 1922, pp. 534-5 for definition of hue, brilliance and chroma.