FIG. 1.

or 20 ft, line at a distance of 6 m, or 20 ft. If such is the case, it is recorded as 6/6 or 20/20. The statement "20-20" vision refers to this fraction. The chart is best illuminated by 80–100 ft-c.

By adopting the principles used in developing the Snellen Test Types, but using larger objects and greater distances, it is possible to determine the point at which individual features in a landscape cease to be differentiated. It is assumed that the illumination of the object observed is of the same order of magnitude as that used for the Snellen Type Tests for visual acuity. Furthermore, the geomorphologist is postulated to have 20/20 vision. For example, a cliff 100 ft high, at a distance of 13.06 miles, will subtend an angle of 5′, as does the letter of the Snellen "20-20" line viewed at its standard distance of 20 ft. It will be just perceived as a discontinuity of form by a person with "normal" vision. This statement must be understood as semiqualitative, as illustrated by Fig. 1 when viewed by the reader at a distance of 20 ft. In this illustration, the Snellen letter is a sharply defined black figure on a white background (*11*), which offers maximum contrast. The horizon line of the profile, on the contrary, consists of an undulating form, so that only abrupt declivities are equally conspicuous. In actuality, the sky and landscape offer a black-and-white contrast only under most unusual conditions of atmospheric clarity and lighting. When reduced illumination, haze, or subtility of contour obtains, greater changes in relief are necessary for perception.

The preceding discussion leads to a simple rule for field observation: a 100-ft cliff at 13 miles will be just perceptible under optimum conditions. At one-half the distance, a 50-ft cliff will subtend the same angle and offer similar geometric contrast. Under poor illumination, a precipice several times this scale would be necessary for discernment. With the dispersion of light caused by haze, a further allowance should be made, particularly for distant skylines. Therefore, although a horizontal surface separated from another such bench by a 100-ft cliff could be seen by a physiographer with "normal" visual acuity under ideal atmospheric conditions, it might easily be overlooked. The two surfaces, in this case, would be described erroneously as a single-planed surface of low relief. From consideration of the physical and concomitant external factors that may produce optical deception, it is apparent that descriptions of topographic relief based on the eye alone are not reliable.

The physiologic limitation of the human eye is offered as a plausible reason for different physiographic descriptions of identical upland areas. Lack of consideration and evaluation of this factor may explain why some geomorphologists have seen but one "even and continuous surface" in uplands, whereas others have identified a number of beveled surfaces separated by small vertical intervals. The former dismiss as minor (or perhaps do not see) the minute details noted by the latter.

It thus appears that "the optical deception" of hilltop accordance that Davis would not admit may very well be a fact. Like his colleagues in other fields of science, the physiographer finds that his eyes have a finite limit of reliability, and he is therefore driven to search for other methods of checking observations of topographic forms than by eye alone—namely, map analysis. Although he may be deprived of the comfort that what he sees is real, nevertheless, the foregoing simple formula may be of some assistance in determining the approximate height of features, where distances are known, by providing a scale of relief in which 100 vertical ft at 13 miles' distance will be barely perceptible under ideal conditions.

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## Intracellular Localization and Distribution of Carbonic Anhydrase in Plants[1]


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Steemann Neilson and Kristiansen (*1*) have recently reported that in the aquatic plants *Fontinalis dalecarlica* L. and *Elodea canadensis* Mich. carbonic anhydrase is limited to the chloroplast sediment obtained by centrifuging the filtered leaf brei. This observation agrees with that of Day and Franklin (*2*), who found that carbonic anhydrase is confined to the chloroplast sediment obtained from leaves of *Sambucus canadensis* L. As Steemann Neilson and Krist-

[1] This investigation was conducted in the Plant Science Laboratories of the Division of Applied Biology, National Research Council of Canada, Ottawa. Issued as N. R. C. No. 2318.

iansen pointed out (*1*), these results indicate that the enzyme is located in or on the chloroplasts, contrary to those of Bradfield (*3*), who found the enzyme only in the leaf cytoplasm of herbaceous land plants. Neish (*4*), who provided the first evidence of carbonic anhydrase in green leaves, found the enzyme distributed between the chloroplast and cytoplasm fractions.

Using a manometric technique, modified after the boat method (*5*), we have reinvestigated the distribution and intracellular localization of carbonic anhydrase in land and aquatic plants. Our enzyme unit E.u. is the ratio $\frac{R-Ro}{Ro}$ (*5*), determined at 10° C by adding 0.5 ml 0.2*M* $NaHCO_3$ in 0.02*M* NaOH to 1.0 ml 0.2*M* phosphate buffer (pH 6.8), with and without enzyme solution. Leaf extracts were prepared by grinding and expressing the juice through nylon. The chloroplasts were sedimented by centrifuging at low temperature for 15 min at 15,000 *g*. The carbonic anhydrase activities of the uncentrifuged extract, supernatant, and resuspended chloroplast fractions were determined, using an enzyme-limited system. The enzyme was found in the leaves of 21 of the 23 species tested. Cysteine (0.01*M*) had little effect on the observed enzyme activity. The activity observed in the leaves of land plants varied from 5–20 E.u./ml leaf extract in monocotyledons (*Triticum vulgare* Vill., *Panicum miliaceum* L., *Hordeum vulgare* L. and *Tradescantia fluminensis* Vell.) to 120–160 E.u./ml in the most active dicotyledons (*Tetragonia expansa* Thunb., *Spinacea oleracea* L., *Tropaeolum majus* L., and *S. canadensis* L.). The major part of the carbonic anhydrase was found in the supernatant fraction of most of the examined species, including *S. canadensis* L. The aquatic plants and *S. racemosa* L. were exceptional in that the enzyme was apparently limited to their chloroplast sediments. Concentrated chloroplast suspensions prepared from the aquatic plants (*Potamogeton* spp., *Myriophyllum* spp., and *Elodea canadensis* L.), however, showed very low activities in either the presence or absence of 0.01*M* cysteine (*3*). Enzyme activity could be detected when the chloroplasts were suspended in 1/12 of the original volume, as compared to the thirtyfold concentration employed by Steemann Nielson and Kristiansen (*1*). The activity was reduced below the limits of measurement when chloroplasts from the aquatic species were suspended in the original volume of liquid, and usually could not be detected in the uncentrifuged leaf extracts. It seems unwise to draw conclusions as to the intracellular localization of carbonic anhydrase on the basis of observations on aquatic leaves (*1*) which contain less than 1% of the activity found in the leaves of land plants. The fact remains, however, that aquatic species such as *E. canadensis,* which can use $HCO_3^-$ from the surrounding medium for photosynthesis, do contain detectable amounts of carbonic anhydrase, as reported by Steemann Nielson and Kristiansen (*1*).

The apparent restriction of carbonic anhydrase to the chloroplasts of the land plant *S. racemosa* L., which had previously been reported for *S. canadensis* L. by Day and Franklin (*2*), was found to be an artifact. A natural flocculating agent in the cell sap, believed to be tannin, causes the cytoplasmic proteins and chloroplasts to be deposited together when centrifuged. Negligible protein remains in the supernatant liquid after centrifuging. On resuspending the crude chloroplast sediment in water and adjusting the pH upward from 6.1 to 8–10, it was observed that the cytoplasmic proteins, including carbonic anhydrase, were dispersed, and that the major part of the activity remained in the supernatant fraction after centrifugation. Similarly, when *S. racemosa* leaves were crushed in dilute alkali, flocculation of the cytoplasmic proteins was prevented, and the greater part of the carbonic anhydrase remained in solution when the chloroplasts were removed by centrifuging. In other land plants a minor part of the enzyme activity was often recovered in the chloroplast sediment. This residual activity, however, was almost entirely removed by washing the chloroplasts with water. It is therefore concluded that the carbonic anhydrase of land plants is localized in the leaf cytoplasm and that this may be equally true of the aquatics.

Interest in plant carbonic anhydrase stems from the role it may play in photosynthesis. If carbon dioxide is used as $HCO_3^-$ ions in photosynthesis, carbonic anhydrase would be required to catalyze carbon dioxide hydration at the rate of photosynthesis in strong light (*6*). Our observations include several indicating that carbonic anhydrase is connected with photosynthesis and that it is an adaptive enzyme. Bradfield (*3*) has already reported that plants showing high carbonic anhydrase activity in their leaves do not show detectable activity in their roots. We have observed that the white parts of variegated *Tradescantia* leaves contain 50% less carbonic anhydrase per ml extract than the green parts. Albino barley leaves contain 75% less carbonic anhydrase than normal barley leaves of the same size and age. On excluding light from *Tropaeolum majus* and *Petroselinum hortense* plants for 4 days, the carbonic anhydrase activity of the slightly chlorotic leaves was 55% and 30% less than that of the controls. Very young leaves of *Tetragonia expansa* are lower in carbonic anhydrase than mature leaves, expressed as E.u. per ml extract or per g fresh weight. The capacity for photosynthesis and Hill reactions is known to undergo a similar increase in the early stages of leaf development (*7, 8*).

The cyanide-sensitive reaction(s) of photosynthesis are believed to be connected with the cytoplasmic fixation of carbon dioxide rather than with the photochemical production of reducing power by the chloroplasts. (The Hill reactions of isolated chloroplasts are not inhibited [*9*], whereas the dark fixation of $CO_2$ by living cells can be abolished by cyanide [*10*].) We have observed 50% inhibition of plant car-

bonic anhydrase in crude extracts, and 75% inhibition in dialyzed extracts by $10^{-3}$ *M* KCN. We believe that plant carbonic anhydrase contributes to, but is not entirely responsible for, the cyanide-sensitivity of photosynthesis in land plants. A detailed presentation of these and related investigations of carbonic anhydrase in plants appeared in Section C, *Canadian Journal of Research.* (C 28, 673 [1950]).

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## Animal Protein Factor for the Rat Present in Crude Casein and its Relationships with Vitamin $B_{12}$[1,2]


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The existence of unknown factors essential to the nutrition of many organisms and present in small amounts in animal protein, and consequently generally denominated animal protein factors (A.P.F.), has been substantiated by several researches, mainly in the field of poultry science. Cary and Hartman *et al.* in 1946 (*1*) reported that an unidentified factor, soluble in hot alcohol (*X* factor), and found in crude casein and liver extracts, is required by the rat for growth, reproduction, and lactation. Further studies (*2*) presented evidence that factor *X* is present even in certain leafy foods. Shortly thereafter, Zucker and coworkers (*3*) reported that a new factor, present in animal protein, and absent from vegetables, soluble in water, dilute acid, alkali, and alcohol-insoluble, is indispensable for rats. This factor was named "zoopherin." Zucker and Zucker (*3*), feeding rats on a complete, purified diet, devoid of zoopherin, observed a high mortality in the newborn, resulting from hemorrhagic lesions in the upper part of the stomach.

For two years we have investigated the effects on rats of a factor present in crude casein. The observations collected, the lesions noted, the kind of diet used, and relationship of the factor to vitamin $B_{12}$ lead us to publish the results.


[1] We wish to thank Cesare Barbieri, of the American Committee, University of Bologna, New York, for having supplied us with the vitamin $B_{12}$, a product of the American Roland Corporation, used in this investigation.

[2] We acknowledge the assistance of M. A. Dina, of the Pathological Anatomy Institute of the University of Bologna, in the histological examinations of the lesions described.


Rats of our strain were fed the Randoin and Causeret (*4*) diet, which is not a synthetic diet, but rather a natural and extremely varied nourishment. This diet consists of:

| | Percentage |
|---|---|
| Ground cereals (wheat, maize, barley, oats, rye) | 88 |
| Wheat germ | 5 |
| Crude casein | 5 |
| Wheat germ | 1.5 |
| Crude casein | 0.5 |

Twice a week the animals also received carrots and vegetables *ad lib.* (salad, lettuce, cabbage, cress, broccoli) and dry yeast. With such a diet Randoin (*4*) and one of us (*5*) have observed optimal growth, reproduction, and lactation for several generations.

Crude casein was the only source of animal protein in this diet. Experiments were then carried out replacing crude with purified casein, made as follows. Casein was suspended in water containing 0.5% acetic acid and a few drops of chloroform. The ingredients were thoroughly mixed, and after slow decantation the liquid was discarded and replaced with new water. The procedure was repeated three times a day for 2 weeks. Finally, the casein was centrifuged down and desiccated *in vacuo*. The animals were kept in special cages in order to avoid possible coprophagy.

A first group of 25 female rats fed a Randoin diet containing purified casein exhibited normal growth, as well as regular reproduction and lactation. The young born of such females (1st generation) exhibited high mortality: 89 out of 126 young rats died (70.6%). The surviving rats reached an almost normal growth: 7 females were able to reproduce. Absolute mortality (100%) was shown by the young of these females (2nd generation). Death did not occur during the first few days of life, but usually between

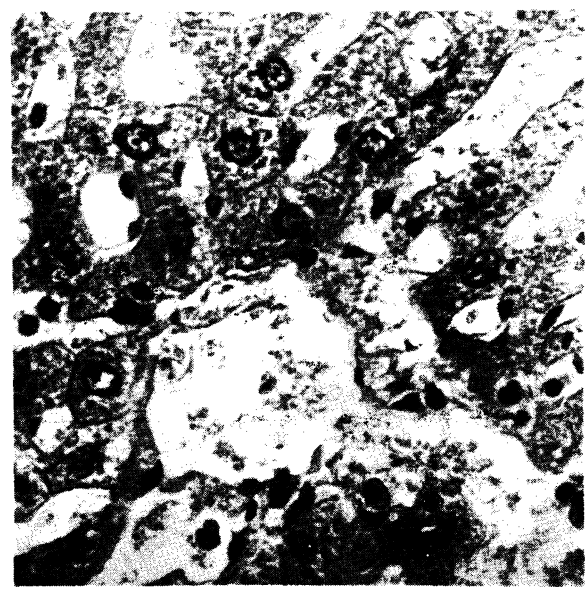

FIG. 1. Normal liver (×720).