deflections have been observed in this area and, judging by the observed effects associated with equally intense anomalies elsewhere, local interference with radio reception might be expected.

Financial assistance for the field work was received through a grant-in-aid from the American Association for the Advancement of Science.

GERALD R. MACCARTHY
H. W. STRALEY, III

DEPARTMENT OF GEOLOGY,
UNIVERSITY OF NORTH CAROLINA

## EFFECT OF CERTAIN ENZYMES AND AMINO-ACIDS ON CROWN GALL TISSUES

THE relation of the crown gall of plants (caused by *Phytomonas tumefaciens*) to malignant tumors of animals is deservedly occupying the minds of pathologists. The status of this subject is excellently presented in a recent paper by Riker and Berge.[1] It is apparent that while the main trend of experimental work is toward determining the stimulatory factors in both crown gall and cancers, comparatively little has been done on the therapy of crown gall with the idea of the ultimate application of the results to cancers of animals. Crown gall and different types of sarcomas have been successfully treated by different forms of radiant energy.[2, 3, 4, 5] There seems to be a certain degree of similarity in response of plant and animal cancerous tissues to different types of physical treatment.

The author working with the crown gall on geranium (*Pelargonium zonale*) observed destruction of galls following injection of a mixture of *Erwinia carotovora* (the cause of a soft rot in carrots and other fleshy roots) strains into galls one month old. Gall tissue usually was completely broken down in from four days to a week, depending on the size of gall and environmental conditions. Galls on young tomato (*Lycopersicon esculentum*) and sunflower (*Helianthus annus*) plants were treated similarly and responded in very much the same way. After the destruction of gall tissue on geranium plants there was no new gall observed to appear after one year. Plants were always maintained in a good growing condition. Geranium plants inoculated with *E. carotovora* were never affected by the organism.

[1] A. J. Riker and T. O. Berge, *Amer. Jour. Cancer*, 25: 310–357, 1935.

[2] C. Arnaudi and G. Venturelli, *Rivista di Biologia*, 16: 61–80, 1934.

[3] Georges Lakhovsky, "L'origine de la vie," 175 pp. Gauthier-Villard et Cie. Paris, 1925.

[4] I. Levin and M. Levine, *Jour. Cancer Research*, 7: 163–170, 1922.

[5] J. W. Schereschewsky and H. B. Andervont. Publ. Health Report 43: 927–945, 1928.

This interesting phenomenon led to the supposition that enzymes or other specific compounds might be involved in the elimination of over-growth. With this thought in mind, the author tested diastase, papain, pepsin, cysteine hydrochloride, leucine, iso-leucine, tyrosine and tryptophane.[6] Cysteine hydrochloride was applied in view of the fact that this material was successfully employed in curing Jensen's sarcoma of white rats.[7] All preparations tested were used in the form of 0.1 per cent. water solution or as crystals. Galls employed for treatment were from one to two months old and ranged in size from 3 to 5 cms in diameter and were induced on geranium and sunflower by a rose strain of *P. tumefaciens*. Injection of materials was made by hypodermic syringe in the case of the water solutions. Dry powder (a few crystals in each case) was introduced into a very small incision made in the center of the gall. Sometimes the galls treated with crystals were afterward atomized with sterile distilled water to aid the diffusion of the material. Controls were represented either by injection of sterile distilled water into the galls or by incisions with a sterile scalpel. In all treated cases, except with tryptophane and tyrosine, the galls gradually collapsed, dried and remained on the plant as hard vestiges easily detachable. Pepsin and papain acted very promptly, while diastase and other compounds used mummified the galls of 3 to 4 cms in diameter in from ten days to two weeks. In all these tests there were used from 10 to 20 galls for each treatment, making a total of 180 galls with corresponding controls.

P. A. ARK

UNIVERSITY OF CALIFORNIA, BERKELEY

## SEX DIFFERENCES IN ANEMIC RATS

IN the issue of SCIENCE for January 29, 1937, appeared a note by Margaret C. Smith and Louise Otis describing certain differences observed between male and female anemic rats in their response to various remedial measures. With those supplements incapable of promoting a maximal rate of recovery, the female rats responded better than the male rats. This was interpreted as a true sex difference, and the authors expressed the belief "that ignorance of this fact may explain some of the discrepancies of the same magnitude in the findings in various laboratories relative to the availability of iron in foodstuffs." Also, in 1932 Miss Helen Mitchell[1] observed an analogous phenome-

[6] Chemicals used were of the following brands: Papain-Merck. Diastase, pepsin, leucine and tyrosine-Pfanstiehl. One lot of pepsin from Parke, Davis and Co. Cysteine hydrochloride, isoleucine and tryptophane—Eastman Kodak Co.

[7] C. L. Connor, J. L. Carr and L. Ginzton, *Proc. Soc. Exp. Biol. and Med.*, 34: 374–376, 1936.

[1] *Amer. Jour. Physiol.*, 101: 503, 1932.

non in the rates with which young male and female rats develop anemia on a whole milk diet, from which she concluded "either that the female is endowed with a better prenatal storage of iron or that she uses the iron which is available from endogenous or exogenous sources more efficiently than does the male."

While it is true that male and female rats seem to be used indiscriminately in investigations concerned with nutritional anemia, it is also true that in practically all such work the intake of the basal diet, consisting generally of milk or milk solids, is not under control. It seems to be the general opinion that only the intake of the remedial supplements needs to be controlled, the implication being that a variable intake of the nutrients of milk, which favor the production of anemia, will not modify either the rate with which the anemic condition develops or the rate with which it is corrected. However, in 1930 Nevens and Shaw[2] published a note in this journal in which evidence was submitted that "animals consuming large amounts of milk became anemic more quickly than those limited to small amounts." They used the paired feeding method, involving the feeding of equalized amounts of milk to each of a series of carefully selected *pairs* of rats.

We have fully confirmed the results of Nevens and Shaw and have shown further by the paired feeding method that the response of anemic rats to metallic supplements, in certain concentrations at least, is modified to some extent by the rate of consumption of the basal anemogenic diet, such that the more of this diet is consumed the slower does hemoglobin regeneration proceed.

Since female rats in general grow at a slower rate than male rats and presumably consume less food per day, the question naturally arises whether the sex difference noted by H. S. Mitchell and later by Smith and Otis is a primary difference in iron or copper metabolism, or whether it is merely a sequel of a primary difference in growth impulse.

The experimental results summarized in Table 1 bear directly upon this question. In experiments 1 to 4 inclusive, the basal diet was dried whole milk (Klim). In experiment 5 the basal diet was fresh whole milk. In experiments 1, 2, 3 and 4, one rat in each pair was fed the milk solids *ad libitum*, while its pair mate received two thirds as much as was voluntarily consumed by the first rat. In experiments 1 and 3 the iron and copper supplements were fed in equal daily doses, as indicated, to all rats. In experiments 2 and 4 the supplements were incorporated in the milk solids in the proportions given. The rats in the first four experiments were made anemic by the Elvehjem and Kemmerer method in a prefeeding period. In experiment 5 the rats were taken directly from the stock colony and pair mates were fed equal amounts of whole milk with no supplements. In all experiments rats were paired with reference to initial weight and initial hemoglobin concentration of the blood. In experiments 1 to 4, pair mates were of the same sex, while in experiment 5 each pair consisted of a male and a female, taken, with one exception, from the same litter.

TABLE 1

THE RESULTS OF SOME ANEMIA EXPERIMENTS INVOLVING THE COMPLETE CONTROL OF FOOD AND SUPPLEMENT INTAKE BY PAIRED RATS

| Experiment number | Length of experiments weeks | Metallic supplements to basal milk diet | Number of pairs | Average daily intake of dried milk grams | Body weights | | Hemoglobin in blood | | Average change in hemoglobin level gms 100 cc | Probability of a chance outcome |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Average initial grams | Average final grams | Average initial gms 100 cc | Average final gms 100 cc | | |
| | | Constant daily dose of iron and copper | | | | | | | | |
| 1 | 2 | .2 mg Fe + .02 mg Cu ..... | 6 | 6.65 | 96 | 140 | 5.90 | 10.25 | +4.35 | .0033 |
| | 2 | " " " " " " ..... | 6 | 4.43 | 97 | 105 | 5.95 | 12.20 | +6.25 | |
| 2 | 4 | .5 mg Fe + .05 mg Cu ..... | 7 | 7.45 | 72 | 155 | 4.50 | 15.20 | +10.70 | .16 |
| | 4 | " " " " " " ..... | 7 | 4.97 | 73 | 112 | 4.46 | 16.00 | +11.54 | |
| | | Constant daily percentage of iron and copper | | | | | | | | |
| 3 | 2 | .004 pct. Fe + .00016 pct. Cu . | 7 | 6.92 | 94 | 127 | 5.53 | 11.83 | +6.30 | .015 |
| | 2 | " " " " " " . | 7 | 4.61 | 94 | 100 | 5.57 | 12.86 | +7.29 | |
| 4 | 2 | .008 pct. Fe + 00032 pct. Cu . | 8 | 6.13 | 68 | 108 | 4.23 | 13.92 | +9.70 | .0019 |
| | 2 | " " " " " " . | 8 | 4.09 | 68 | 81 | 4.30 | 15.14 | +10.84 | |
| | | Sex comparison on equal food (fresh milk) intake | | | | | | | | |
| 5 | 4 to 6 | No supplements—males ..... | 6 | ... | 63 | 115 | 11.75 | 6.53 | −5.22 | .085 |
| | 4 to 6 | " " —females ... | 6 | ... | 63 | 125 | 12.20 | 8.17 | −4.03 | |

[2] SCIENCE, 72: 249, 1930.

The results of experiment 1 show that a daily dosage of .2 mgm of iron and .02 mgm of copper promoted a more rapid regeneration of hemoglobin in those rats receiving the smaller intake of milk solids. In only 2 weeks of feeding a difference in recovery concentration of almost 2 grams of hemoglobin per 100 cc of blood developed. In all six pairs the rat on restricted intake recovered the more rapidly, and the probability[3] that this is a fortuitous result is so small (.0033) that it may be neglected. However, in experiment 2, with a daily supplement of .5 mgm of iron and .05 mgm of copper, the result is indecisive, even after 4 weeks of feeding. In 5 of the 7 pairs the rat on restricted food recovered from its anemic condition more rapidly than its pair mate, but in 2 pairs the reverse was true; the statistical analysis (P = .16) indicates that the outcome may have been a fortuitous one. These two experiments on the effect of a variable intake of milk solids are quite analogous to the experiments of Smith and Otis on the comparison of male and female rats in the sense that significant differences were noted only when the supplements were such as to promote a submaximal rate of recovery. They are also in agreement with the theory previously proposed[4] by one of us concerning unbalanced rations, that "the more of them is consumed the poorer nourished will be the animal with reference to the functions with respect to which the rations are unbalanced." Confirmation of this theory has already[5] been reported with rachitogenic diets: the greater the rate of consumption of such diets, the more rapidly does rickets develop. Vitamin $B_1$-deficient diets are also the more toxic the more of them is consumed,[6] as are also diets deficient in vitamin C.[7]

Experiments 3 and 4 show in both cases that for the particular iron and copper concentrations incorporated in the milk solids, the rats on restricted intake recovered significantly more rapidly than the rats on one half again as much food. It may be said that the concentration of iron used in this experiment (.008 per cent.) was lower than that consumed by the restricted rats in experiment 2 (.010 per cent.), but higher than that consumed by the unrestricted rats (.0067 per cent.).

Unfortunately we have not performed a curative experiment with pairs of male and female rats receiving equal intakes of milk solids. However, the rate of development of anemia in paired male and female rats receiving equal intakes of fresh milk was studied in experiment 5. In feeding periods lasting from 4 to 6 weeks, no significant differences were obtained, although in 4 of the 6 pairs the female rat was the slower in developing an anemic condition. The probability of a chance outcome, .085, is however too large to disregard. It will be noted also that on equal intakes of food the female rats gained less in body weight (P = .046), and from available information it may be assumed that their gains contained less of protein, more of fat and less of blood.

It may be concluded that the sex difference in the development of nutritional anemia noted by H. S. Mitchell, as well as that in the recovery from nutritional anemia noted by Smith and Otis, may be partially or entirely the result of a greater intake of the anemogenic basal diet by male rats. To that extent it is merely a sequel of the well-established difference in growth impulse between the male and the female sex. In the same manner, the frequently observed difference between male and female rats in the rate of calcium retention and of the calcification of the bones has been traced in this laboratory[8] to the greater demand for, and consumption of, food by the male.

The control of food intake by comparative animals in nutrition experiments according to some scheme adapted to the problem at hand will generally simplify their interpretation and will make possible a demonstration of a fact or a principle where lack of control can at best establish only a variable degree of probability in favor of it.

H. H. MITCHELL
T. S. HAMILTON

UNIVERSITY OF ILLINOIS

[3] "Student," *Biometrika*, 6: 1, 1908.
[4] H. H. Mitchell, SCIENCE, 80: 558, 1934.
[5] W. E. Watkins and H. H. Mitchell, *Poultry Sci.*, 15: 32, 1936.
[6] G. Amantea and associates, *Atti Accad. Lincei*, 18, 317, 399, 1933; *ibid.*, 20: 134, 1934; *ibid.*, 22: 173, 1936; taken from *Chem. Abst.*, 28, 3764, 1934; 29: 1138, 1935; 30: 6422, 1936. H. G. K. Westenbrink, *Arch. Neerland. physiol.*, 19: 94, 1934; *Ber. ges. Biol.* Abt. B: *Ber. ges. Physiol. u. Pharmakol.*, 79: 585, 1934.
[7] V. Famiani, *Atti accad. Lincei*, 20: 129, 1934; *Chem. Abst.*, 29: 1138, 1935.
[8] B. W. Fairbanks and H. H. Mitchell, *Jour. Nutr.*, 11: 551, 1936.

## CRYSTALLINE CATALASE[1]

WE have prepared crystalline catalase from beef liver. Our method consists essentially in extracting chopped liver wth dilute dioxane, adding more dioxane to the extract to precipitate impurities and then precipitating the enzyme through the addition of still more dioxane. The precipitated enzyme is dissolved in water and crystallizes upon adding ammonium sulfate and cooling. Crystalline catalase has been obtained also from the extracted liver residue by fractionating extracts with ammonium sulfate solution.

Our catalase crystals are slender plates of microscopic size. Presence of the crystals can be observed by rotating the liquid in which they are suspended and

[1] From the Department of Physiology and Biochemistry, Medical College, Cornell University, Ithaca, New York.