cases at concentrations which may be below those producing hyperplasia of the thyroid gland. Thioacetamide appears to be slightly tumorigenic in the rat liver and, in addition, is a very potent producer of nodular cirrhosis.

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Desoxypentosenuclease in Yeast and Specific Nature of Its Cellular Regulation[1]

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Evidence has been obtained in this laboratory of the occurrence in yeast (*Saccharomyces cerevisiae*) of two agents concerned with desoxypentose nucleic acids (DNA), viz., a depolymerase (DNase) and an inhibitor of DNase having interesting specific properties.

When yeast is ground mechanically and the layer of cell debris treated with M NaCl solution, the extract, as was shown recently (*1*), includes a highly polymerized DNA. The same extract has now been found also to contain a DNase in a largely inhibited state, from which it is slowly released on storage at 4°. The increase in activity is about 50-fold within 3 months. Extracts of the ground cells with distilled water contain free inhibitor but no enzyme.

Washed yeast was crushed, distilled water being used as suspending fluid, and the debris extracted with M NaCl, as described previously (*1*). The viscous mixture (500 cc) was kept in the refrigerator for 1–4 months, clarified by centrifugation at 4,000 rpm, dialyzed, and dried from the frozen state in a vacuum. The solution of the residue in 30 cc of water was centrifuged at 20,000 rpm and the supernatant brought to 0.6 saturation with solid ammonium sulfate. The solution of the precipitate, collected at 20,000 rpm, was subjected to a rocking dialysis against ice-cold distilled water for 7 hrs and again centrifuged at high speed. The sediment was washed with water and then extracted with 30 cc and again with 12 cc of M NaCl. The combined extracts, clarified by centrifugation at 20,000 rpm, were dialyzed and evaporated in a vacuum in the frozen state. The DNase preparation weighed 27 mg. Even high dilutions of this agent produced a rapid drop in viscosity of solutions of thymus DNA and of yeast DNA (*1*). It had an activity of about 1,200 units/mg of protein, as defined by McCarty (*4*). In a concentration of 0.6 mg/cc it was free of proteolytic (*5*), nucleotidase, and ribonuclease activities.

Yeast DNase resembled the desoxyribonuclease of pancreas (*3*, *4*) in requiring Mg ion for activation and in being labile to heat; the activity was destroyed completely by heating to 55° for 15 min. It differed, however, from the pancreatic desoxyribonuclease in several important respects. It was insoluble in water but soluble in salt solutions. Its activity optimum lay below pH 6.2; at pH 8.1 only 20% of its activity was retained. The most significant difference consisted in its being specifically inhibited by a yeast fraction which, however, had no inhibiting effect on purified pancreas desoxyribonuclease[2] and on crude DNase preparations from *Neurospora crassa*, germinating barley, and calf thymus, which will be discussed on a later occasion.

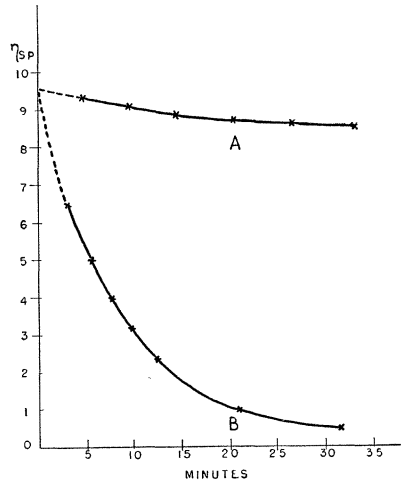

FIG. 1. The specific viscosities of mixtures of thymus DNA (sodium salt) and yeast DNase with (A) and without (B) DNase inhibitor are plotted as the ordinate. The abscissa indicates the duration of incubation of the mixtures before testing, at 30°. Mixture A contained 1.7 mg of DNA, 3 micromoles of Mg, 2.5 units of yeast DNase, and 2.2 mg of inhibitor/cc of veronal buffer of pH 6.6. In mixture B the inhibitor was omitted.

The DNase inhibitor, present in aqueous and salt extracts of ground yeast cells, caused up to 94% inhibition of yeast DNase. A typical experiment is reproduced in Fig. 1. The inhibitor appears to be a water-soluble labile protein, its activity being destroyed in less than 5 min at 55°. It is inactivated by crystalline trypsin, by ficin, and by a proteolytic enzyme preparation from crushed yeast. To the presence of the latter in the inhibitor preparations their inactivation on storage for 8–20 hrs at 30° or for 2–4 weeks at 4° probably is attributable. The inhibitor can be partially purified by precipitation at 0.8 saturation with ammonium sulfate.

[1] This work has been supported by a research grant from the U. S. Public Health Service.

[2] We are very grateful to Dr. M. McCarty for a specimen of this preparation.

Preliminary experiments on the mechanism of this inhibition reaction, which is instantaneous, suggest that it is reversible and noncompetitive. Indications have been obtained that *Saccharomyces carlsbergensis* likewise contains a DNase inhibitor which, however, appears to behave somewhat differently.

A full account of this and related work on other cellular systems, including those of higher organisms, will appear at a later date. But attention may be drawn to the following sequence of autolytic reactions, possibly delicately balanced in the living cell: (1) activation of yeast protease (*2*); (2) digestion of DNase inhibitor; (3) liberation of active DNase; (4) depolymerization of DNA. If, as appears likely, the cleavage of the DNA macromolecules is of importance in the life of the cell, the evidence of the existence within the cell of a specific regulation of this process may be of more general biological interest.

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Effect of Flavonoids (Vitamin P) on Mortality From Total Body Roentgen Irradiation[1]

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In previous papers Clark and Geissman (*1, 2*) reported a test for flavonoids and related compounds (vitamin P-like substances) based on the potentiation of the epinephrine response of isolated mammalian smooth muscle. In a study of the relation of molecular structure to activity, some 70 pure compounds were examined, and the minimum structure essential for high activity was predicted, synthesized, tested, and found to confirm the prediction.

In attempts to extend these and other observations to the intact animal, among other things a study was included of the effects of these compounds on the hemorrhagic syndrome associated with total-body roentgen irradiation. This study was initiated in September 1947, and the purpose of the present communication is to demonstrate the potential usefulness of this approach in further experimental assessment of the antihemorrhagic effects of the flavonoid pigments.

Griffith, *et al.* were the first to demonstrate a beneficial effect of flavonoids in experimental roentgen-irradiation injury. They found a beneficial effect of rutin[2] therapy in accelerating the restorative processes following severe X-ray burns of rats' extremities (*8*) and in preventing the capillary fragility increase following intraperitoneal administration of radon ointment in rats (*7*). They also indicated that rutin probably had no beneficial effect in total-body roentgen-irradiated rats but gave no particulars (*7*).

Rekers and Field (*10*) later reported a decrease in mortality of dogs by rutin therapy before and after 350 r total-body roentgen irradiation.

The beneficial effect of rutin in the case of the X-ray burns of rats' extremities was limited to an acceleration of the restorative processes, since there was no effect on the time of onset or the severity of the lesions. This probably is related to the similar beneficial effect of rutin in the prevention of tissue loss in frostbite gangrene in rabbits' extremities, reported by Fuhrman and Crismon (*4, 5*).

We wish to report the effect of one of several flavonoid substances being studied in several species (rats, mice, guinea pigs) of small laboratory animals subjected to approximately median lethal doses of total-body roentgen irradiation.

Large, healthy guinea pigs of approximately 500-gm body weight were fed on Rockland guinea pig ration[3] and given supplementary ascorbic acid in 0.2% concentration fresh daily in the drinking fluid. One group served as controls and the other as flavonoid treated, for which the drinking water also contained 0.2% "calcium flavonate,"[4] prepared fresh daily. Previous experiments had indicated that single, daily, oral, large doses of flavonoids are not as effective as constant ingestion in the food or water.

After a week of such treatment the animals were given 220–225 r total-body irradiation[5] in a single dose, not including backscatter. They were placed in a multicompartment wooden box, controls and experimentals being arranged alternately in a checkerboard fashion, 4

[1] Supported by a grant from the U. S. Public Health Service. Grateful acknowledgment is made of the suggestions and encouragement of Eaton MacKay, in whose laboratories this work was done.

[2] Quercetin-3-rhamnoglucoside, a common flavonoid active in decreasing capillary fragility, as first shown by Sevin (*11*) and later by Griffith, Couch, and Lindauer (*9*).

[3] Arcady Farms Milling Company, Chicago, Illinois.

[4] A water-soluble preparation from lemon peel, essentially free of water, sugars, and hesperidin. The alcohol extract of the fresh peels is precipitated in alkaline medium with calcium, the precipitate suspended in water and adjusted to an acid pH, reprecipitated by the addition of alcohol, filtered, and the material obtained from the filtrate by evaporation. It gives a cyanidin test about half as intense as rutin on a weight basis. Prepared and supplied by the California Fruit Growers Exchange, Sunkist Building, Los Angeles, California.

[5] GE Model KX-3, 220-kv deep therapy unit. The factors were: 200 kv, 20 MA, ½ mm Cu + 1 mm Al added filtration (HVL, 1.05 mm Cu), 100-cm target distance, 8.5 r/min. The unit is calibrated semiannually by a registered X-ray physicist. The variation in output over the past year has been less than 3%.