SPECIAL ARTICLES

THE NATURE OF LYSOZYME ACTION[1]

THE lytic action on certain bacteria of a supposed enzyme, lysozyme, present in tears, egg white and various body tissues and fluids, has been described and studied by Fleming and others.[2] We have tried to determine whether the action of lysozyme is physical or enzymatic. A polypeptide in the form of an amorphous white powder, obtained by the purification of egg white, completely dissolved a suspension of air sarcinae (barium sulfate standard No. 8) in a concentration of 0.12 gamma per cc. An aqueous solution of the purified lysozyme did not change the surface tension of water and had no proteolytic, lipolytic or amylolytic action. It did not activate the action of papain or of the endoproteases of the bacteria. It did not act on lecithin or on the alcohol-ether soluble fraction of the sensitive sarcinae; it gave no evidence of a phosphatase action. It did split off a reducing sugar from ovomucoid and from a polysaccharide obtained by hydrolysis of the test organisms. The corresponding mucoid of the bacteria has not yet been isolated. The defatted bacteria are extremely insoluble, apparently consisting chiefly of a mucoid yielding a large carbohydrate fraction. Cartilage and chitin were not attacked.

Apparently lysozyme is an enzyme or an enzyme mixture which splits a reducing sugar from certain mucoids and from the polysaccharides derived from them. Its occurrence in tears, nasal, bronchial and gastro-intestinal mucus, egg white and semen[3, 4] can thus be understood, the bacteriolytic action being incidental. The same enzyme was obtained from a polypeptide fraction of the sensitive bacteria. The possible relation of this factor to bacteriophage action is being investigated. It is possible that this ferment may furnish an important tool for the study of mucins. It is to be expected that a series of such mucinases[5, 6] will be found in various tissues and organisms. A commercial pepsin preparation was found to split gastric mucin independently of peptic activity. It is possible that the specific bacterial polysaccharides are derived from capsular mucoids and that the enzyme described by Dubos and Avery[7] which decomposes the capsule of Pneumococcus III and hydrolyses its specific polysaccharide belongs to this group of ferments.

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[1] From the Biochemical and Bacteriological Laboratories of the Department of Ophthalmology, College of Physicians and Surgeons, Columbia University, New York City.

[2] A. Fleming, *Proc. Roy. Soc. Med.*, 71: 26, 1932. Review.

[3] Kurzrok and Miller have shown that semen dissolves the mucous plug of the cervical canal.

[4] R. Kurzrok and G. Miller, *Am. Jour. Obstet. and Gynec.*, 56: 15, 1928.

[5] The term "mucinase" has already been applied to a ferment which coagulates mucin. The existence of such a ferment is not, however, well established.

[6] Oppenheimer-Kuhn, "Die Fermente und ihre Wirkungen," Vol. 2, Leipzig, 1925.

EFFECTS OF FEEDING SODIUM BICARBONATE OR LACTIC ACID UPON THE SEX RATIO IN RATS

SEVERAL articles have appeared recently in the newspapers to the effect that the ingestion of sodium bicarbonate during pregnancy influences the sex of the offspring both in the human and in the dog, males being produced exclusively. While there appears to be no physiological basis for such a belief, the necessity of increasing our rat colony gave an opportunity to test the question experimentally.

Animals to be bred were placed upon our stock diet, which consists of a mixture of ground grains, dried milk, mineral salts and cod liver oil, with which was incorporated either sodium bicarbonate or lactic acid. Following breeding, the animals were continued upon the same diet until parturition. The food seemed perfectly palatable, 15 to 20 grams being consumed per day.

The results are given in the following table:

Material and concentration	No. of litters	Males	Females	Total
2½ per cent. sodium bicarbonate	15	61	67	128
5 " " " "	20	85	101	186
2½ " " lactic acid	10	38	42	80
5 " " " "	28	107	128	235
Totals	73	291	388	629

Sex ratio, sodium bicarbonate animals, $\frac{\text{Females}}{\text{Males}} = \frac{168}{146} = 1.15$

Sex ratio, lactic acid animals, $\frac{\text{Females}}{\text{Males}} = \frac{170}{145} = 1.17$

In a group of 14 control litters the sex ratio of females to males was 1.03. Donaldson[1] quotes a table from King which includes data covering some 815 litters, showing a variation in female to male sex ratio of from 1.06 to .66.

[7] R. Dubos and O. T. Avery, *Jour. Exp. Med.*, 54: 51, 73, 1931.

[1] "The Rat," page 25, table 6.

In view of this variation, and of the close agreement between the sex ratios of the two groups in our study, we conclude that, for the rat at least, the sex ratio is not affected by the feeding of base or acid.

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THE EFFECTS OF THALLIUM SULFATE UPON SOILS

QUESTION has lately been raised concerning the ultimate effects, upon the soil, of the thallium compounds used for rodent control. S. C. Brooks[1] has warned land owners of the possibility that the soil might be sterilized by this practise. Obviously, such a problem merits investigation. The writer has developed critical methods during studies on weed control and is preparing a detailed report on tests with thallous sulfate. Meanwhile, he presents the following summary of pertinent results.

Using four California soils, the writer studied the initial toxicity, the decrease of toxicity with time and cropping, the saturation capacity of the soils and the effects of thallium-coated grain upon adjacent plants. Oat seedlings used as indicator plants were harvested 30 days after planting in the greenhouse tests.

In equimolecular concentrations, thallous sulfate proved many times as toxic as arsenic trioxide and sodium chlorate. Whereas toxicity of the latter soil sterilants decreased with successive cropping, that of thallous sulfate remained unchanged through three such treatments.

Thallium toxicity was tested through a range of 25 ppm to 2,000 ppm in the soil solution. It proved greatest in soils of low fertility but could not be correlated with soil type or water-holding capacity. The chemical was strongly fixed in all soils, the saturation capacity of Yolo clay being about 10,000 ppm on a dry weight basis. Leaching with 200 cm of distilled water had practically no effect on the location or toxicity of thallous sulfate in the soil.

Thallous sulfate is an extremely effective soil sterilant, it is strongly fixed and it resists leaching. Although these facts explain Brooks' observations and would seem to justify his warning, their aspect is changed by quantitative studies. Thallium-treated "potted" (hulled) barley had practically no effect upon germination or growth of oats planted in the same soil and spaced within ½ centimeter of the barley grains. When the spacing was decreased to ¼ centimeter, growth was reduced. Except where the dosage was excessive, oat seedlings were unaffected by the application of treated barley to the soil, followed by irrigation.

[1] S. C. Brooks, "Thallium Poisoning and Soil Fertility," SCIENCE, 75: 105–106, 1932.

Thallium-treated grain also affected vegetation in a pasture area very little. The heaviest application, equivalent to 27 pounds of thallous sulfate per acre, reduced growth less than 50 per cent.

In concentrations of 100 ppm or more (on a dry weight basis) thallous sulfate should, apparently, be fully toxic in most soils. At this rate, about 30 pounds would be required to sterilize an acre inch.

Calculations on the area sterilized by the grains comprising a squirrel bait (approximately 20 grams of poisoned grain) show that over 100,000 baits would be required to cover an acre. This is equivalent to 5,000 pounds of poisoned grain bearing 50 pounds of thallous sulfate and might sterilize the top 1½ inches of soil. Under natural conditions, however, the chemical would be fixed in a much shallower layer. The baits, if taken, would be distributed through the top four feet of soil and would have little sterilizing effect.

The disparity between these figures and the amounts used in field practise is striking. As shown by a brief survey of ground-squirrel control in California, the average initial dosage of poisoned grain bearing one per cent. Tl_2SO_4 is about 1/3 pound per acre; later applications are lighter. In one county the dosage has decreased to 1/35 of a pound in five years. The success of this material should permit similar reductions in other regions, so that the amount of chemical becomes totally insignificant as far as soil sterilization is concerned.

The writer observed no loss of fertility in range lands successfully treated for five successive years. The only denuded areas found were the open burrows, fresh mounds and beaten trails of squirrels in untreated fields. Regardless of other objections to thallium compounds in rodent control, the possibility of losing agriculturally valuable land through sterilization seems remote.

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