that insulin administration results in a decrease in the production of insulin by the islet tissue.[1,2] However, it is apparent that the prolonged use of protamine zinc insulin accentuates this phenomenon to such a degree that a "disuse atrophy" of the pancreas ensues.

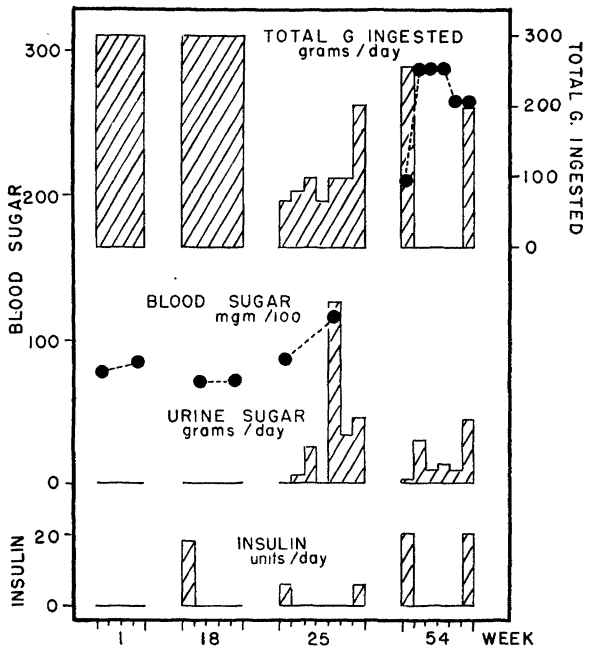

FIG. 2. Illustrating the influence of insulin deprivation at various intervals after the administration of protamine zinc insulin to a partially depancreatized dog.

This compensatory decrease in insulin secretion and its morphological counterpart is made more evident in these experiments, where the amount of pancreas present in the animal is relatively small, though adequate for normal maintenance. It is suggested, therefore, that it might be an extremely dangerous practice to utilize protamine zinc insulin as a prophylactic measure in man, in contrast to its beneficial influence in diabetes mellitus.

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ON CATARACT AND CERTAIN OTHER MANIFESTATIONS OF TRYPTOPHANE DEFICIENCY IN RATS[1]

IT is recognized that tryptophane deficiency leads to lesions in the eyes. Curtis, Hauge and Kraybill[2] in 1932 and Totter and Day[3] in 1941 reported the occurrence of cataract in tryptophane deficient rats, and the latter observers also noted vascularization of the cornea in this condition. We can amply confirm and extend these observations, and we have furthermore noted lesions characteristic of tryptophane deficiency in other organs.

TRYPTOPHANE CATARACTS

In our experiments a tryptophane deficient diet[4] similar to that employed by Totter and Day[3] (No. 5000) was employed. Young rats of about 100 grams in weight, when placed on this diet, developed cataracts with great regularity in from seven to eleven weeks, in contrast to paired feeding control animals on analogous diets containing tryptophane. Two different types of cataract have been observed in the tryptophane deficient animals—an *acute* and a *chronic* type.

The *acute type* starts in the posterior cortex of the lens, spreading within a few days to the perinuclear, nuclear and anterior cortical zones and maturing within two to three weeks. Within a week after the maturation of the cataract the animals die. The acute form of cataract can be arrested in its early stages by supplementing the diet with tryptophane, but some opacities still develop for a time after the supplement is instituted.

The *chronic type* of cataract is confined to the anterior and posterior cortex of the lens and does not mature within the life-time of the animals, which varies from four to nine weeks after the onset of the cataract. In one animal we have observed a combination of the morphological features of both types of cataract. We do not know what factors determine the type of cataract which develops, but our results suggest that the strain of rats studied may be important.

We have observed cataracts *only in growing animals*

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[3] Leo Lehman fellow.

[1] This study was supported in part by grants for the study of amino acids in nutrition made by the Rockefeller Foundation, Merck and Company, Eli Lilly and Company and E. R. Squibb and Sons. This material was presented in part at a demonstration at the meeting of the Federation of American Societies for Experimental Biology in Boston, March, 1942. W. Buschke, A. A. Albanese and R. H. Follis, Jr., *Fed. Proc.*, 1: 175, 1942.

[2] P. B. Curtis, S. M. Hauge and H. R. Kraybill, *Jour. Nutr.*, 5: 502, 1932.

[3] J. R. Totter and P. L. Day, *Jour. Biol. Chem.*, 140: cxxxiv, 1941.

[4] The exact composition of the diet was as follows: Protein (acid hydrolyzed casein concentrate), 147 g; l-cystine, 1.5 g; sucrose, 150 g; starch, 420 g; agar, 20 g; salt mixture (see below), 20 g; Crisco, 190 g; Mead Johnson's cod liver oil substitute, 50 g; water to make to proper consistency. The salt mixture used had the following composition: NaCl, 18.9 g; $CaHPO_4$ anhydrous, 25.0 g; $MgSO_4$ anhydrous, 6.86 g; $KHCO_3$, 44.4 g; KCl, 2.88 g; Fe citrate U.S.P., 2.21 g; $CuSO_4$ anhydrous, 0.24 g; $MnSO_4$ anhydrous, 0.15 g; KI, 0.015 g; NaF, 0.03 g.

on a tryptophane deficient diet. Adult animals have so far failed to develop any evidence of cataract.

OTHER LESIONS IN TRYPTOPHANE DEFICIENT RATS

In addition to cataract formation, we have observed a variety of other lesions in rats on a tryptophane deficient diet.

1. *Vascularization of the cornea,* with characteristic superficial capillary loops, has developed in the majority of our tryptophane deficient rats, both adult and growing rats. This can at least be partially reversed by a tryptophane supplement.

2. *Alopecia* has developed in our animals, both young and old. In many animals, notably the young ones, it has been most conspicuous about the head and back, while in other animals it has affected primarily the back, abdomen and hindlegs. Restoration of hair growth has been observed following the administration of a tryptophane supplement.

3. *The incisor teeth* show defects in the enamel and loss of color; they are brittle and misshapen. This has been observed so far only in growing rats.

4. *Atrophy of the testis and aspermiogenesis* have been observed in male animals.[5]

It is apparent that continuously growing epithelial structures bear the brunt of the lesions in this deficiency. However, the lesions are not confined to such tissues, for there is also found a retardation of growth and a loss of skin turgor which can not be so explained. The animals exhibit a hunched posture such as is encountered in a number of other deficient states.

A CLASSIFICATION OF CATARACTS

On the basis of their morphological characteristics, their mode of development as observed with the slitlamp and their association with particular lesions in other organs we can differentiate the tryptophane deficiency cataracts from:

A. *The diabetic group of cataracts* (cataract in experimental[6] and clinical diabetes,[7] galactose[8] and xylose[9] cataracts). These are characterized in some stages by the prevalence of subcapsular vacuoles (particularly beneath the anterior lens capsule); they are associated with polyuria and polydipsia, with an abnormally high monosaccharide concentration of the blood.[9]

B. *The tetanic group of cataracts* (cataracts in idiopathic[10] and in postoperative parathyroid tetany[11] and rachitic tetany[12]) which are characterized by the zonular *development* of the cataracts in association with a diminished calcium : phosphate ratio in the blood and with the typical neuromuscular manifestations of tetany. These cataracts are occasionally accompanied by the lesions associated with group C and are probably more closely related to the latter group than to group A.

C. The third group of cataracts, in which that of tryptophane deficiency must be included, may be termed the *epitheliodystrophic group,* which includes the cataract attributed to riboflavin deficiency;[13] chronic thallium poisoning;[14] x-ray damage; the cataract of scleroderma and poikiloderma (Rothmund-Werner Syndrome);[15] of atopic eczema (Andogsky-Kugelberg Syndrome)[16] and of myotonia atrophica (Steinert-Batten-Gibb).[17] In addition to the absence of the features of group A, some of these cataracts are characterized by their polymorphism, and all of them are associated with other epithelial lesions. A more complete analysis of the classification of "metabolic" cataracts, including that of tryptophane deficiency, and a bibliography of other syndromes associated with cataract will be reported elsewhere.[18]

Among the members of group C the tryptophane deficiency cataract seems to resemble most closely the cataracts described in riboflavin deficiency and in thallium poisoning. But all the members of this group show striking similarities in their concomitant manifestations. Involvement of the cornea has been observed in connection with each of these conditions of the group, although the corneal lesions have shown some differences. Each of these conditions has been accompanied by alopecia and other skin lesions. Atrophy of the gonads has been described in all these conditions with the exception of atopic eczema. Developmental changes in the teeth have so far been observed only in tryptophane deficiency and in some cases of syndermatotic cataract.[19,20]

COMMENT

Our finding that tryptophane deficiency gives rise to a variety of characteristic lesions lends strong support to the view that this amino acid has specific

[5] Landrum B. Shettles, personal communication.

[6] One of us (Buschke) is at present engaged with Dr. Curt P. Richter in the study of experimental diabetic cataract in rats which has made possible for the first time the comparison of diabetic and galactose cataracts in the same species.

[7] A. Meesmann, Die Mikroskopie des lebenden Auges. Berlin-Wien. Urban-Schwarzenberg, 1927.

[8] H. S. Mitchell and G. M. Cook, *Arch. Ophth.*, 19: 22, 1938.

[9] N. J. Darby and P. L. Day, *Jour. Biol. Chem.*, 123: 503, 1940.

[10] A. Meesmann, Hypocalcaemie und Linse. Stuttgart, F. Enke, 1938.

[11] H. Goldmann, *Graefe's Arch. Ophth.*, 122: 146, 1929.

[12] G. von Bahr, *Acta Ophth.*, 14: suppl. XI, 1936.

[13] C. S. O'Brien, *Arch. Ophth.*, 8: 880, 1932.

[14] A. Buschke, *Arch. f. Derm. Syphil.*, 116: 477, 1913. J. Donski, *Graefe's Arch. Ophth.*, 128: 294, 1932.

[15] A. Siegrist, Der graue Altersstar. Berlin-Wien, Urban-Schwarzenberg, 1928.

[16] W. P. Beetham, *Arch. Ophth.*, 24: 21, 1940.

[17] B. Fleischer, *Graefe's Arch. Ophth.*, 96: 91, 1918.

[18] W. Buschke, *Arch. Ophth.*, in prep.

[19] I. Kugelberg, *Klin. Mbl. Augenheilk.*, 92: 484, 1934.

[20] A. E. Maumence and W. Buschke, unpublished observation.

functions to perform in addition to serving as a building block for body proteins. Although the changes in tryptophane deficiency are not identical with those seen in other conditions causing epitheliodystrophic cataracts, the similarity of the pathological picture is striking and suggests that some common metabolic path is interrupted in these disturbances.

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A VIRUS INACTIVATOR FROM YEAST

THE ability of certain bacteria to inactivate tobacco mosaic virus was reported by Mulvania.[1] Johnson and Hoggan[2] found that a number of fungi as well as bacteria could cause inactivation of tobacco mosaic virus and suggested that this inactivation was most likely due to the utilization of the virus constituents by these organisms. The high rate of inactivation by *Aerobacter aerogenes* and *Aspergillus niger* later led Johnson[3] to suspect that a virus inhibitor was produced by these organisms. Broth cultures of the organisms were found to contain a growth product (or products) capable of inactivating several plant viruses *in vitro* and in dried-leaf tissue. Efforts to separate the active substance from the culture medium yielded discouraging results.[4]

Johnson and Hoggan[2] grew a yeast, *Saccharomyces sp.*, in a medium containing filtered tobacco mosaic virus and broth for 8 days, but could not detect a reduction in the concentration of virus due to the action of the yeast in this mixture. Extraction of an inhibitor from the yeast was not attempted by these workers.

In the work reported below a virus inactivator has been extracted from yeast by autolysis and by autoclaving. A simple method for extracting this substance is as follows: one kilo of frozen baker's or brewer's yeast cake is mixed with 4 liters of distilled water and autoclaved for 30 minutes at 15 pounds pressure. The autoclaved material is filtered through a pad of Celite No. 505. The pale yellow, somewhat opalescent filtrate is treated with two volumes of acetone or alcohol and the resulting bulky, white precipitate is separated from the liquid by centrifugation. The precipitate is dissolved in a volume of distilled water equal to that of the original filtrate. The precipitation and solution in water may be repeated several times, but a small amount of an electrolyte such as NaCl must be added to effect complete precipitation. This partially purified substance was used for much of the preliminary work. Later, for more exact work, the inactivator was further purified by clearing with safranine and neutral lead acetate, followed by heating in 2N HCl. In more detail, 33.3 cc of a 1 per cent. solution of safranin per liter of inactivator were used and the neutral lead acetate (saturated) was added dropwise until no further precipitation took place. Potassium oxalate and $CaCl_2$ were used to free the solution of lead and oxalate, respectively. After precipitating with acetone the supernatant liquid was discarded, the precipitate dissolved in 2N HCl and heated on a boiling water bath for ½ hour. Six to seven volumes of acetone were required to effect the final precipitation. The white precipitate was dried for several days in a desiccator, then ground into a powder before weighing.

TABLE 1

THE INACTIVATION OF PURIFIED TOBACCO MOSAIC VIRUS BY DIFFERENT CONCENTRATIONS OF A PURIFIED INACTIVATOR FROM YEAST

Milligrams of inactivator per 100 cc of a suspension containing 5 mg of virus	Local lesions on 20 half leaves		Per cent. of virus remaining active	Per cent. of virus inactivated
	Treated virus	Untreated virus		
0.303	493	1520	32.3	67.8
0.625	209	1359	14.8	85.2
1.25	164	1614	10.05	89.95
2.50	44	1398	3.14	98.86
5.00	22	1547	1.42	98.58

Each step in the purification process and the effect of different treatments were followed by mixing a solution of the test material with a solution of purified virus. The changes in virus concentration brought about by the inactivator were measured by inoculating half leaves of *Nicotiana glutinosa*. In all cases, one half of each of 20 leaves was inoculated with each treated sample and the other halves with corresponding controls. The ultracentrifugally purified tobacco mosaic virus used in these experiments was kindly supplied by Dr. W. M. Stanley, of the Rockefeller Institute for Medical Research, at Princeton, N. J.

The reaction between the inactivator and the virus is rapid. The results of a typical experiment tabulated above show that with each doubling of inactivator concentration a halving of active virus concentration takes place. This strongly suggests a chemical reaction between one unit of inactivator and one unit of virus rather than an adsorption phenomenon. Furthermore, the inactivator does not combine with heat-denatured virus. The virus-inactivator combination can be broken if the mixture is heated to 99° C. for 10 minutes.

Scions of rose, peach and pear taken from virus-

[1] M. Mulvania, *Phytopath.*, 16: 853–871, 1926.
[2] James Johnson and Isme A. Hoggan, *Phytopath.*, 27: 1014–1027, 1937.
[3] James Johnson, SCIENCE, 88: 552–553, 1938.
[4] James Johnson, *Phytopath.*, 31: 679–701, 1941.