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Two Characteristic and Sensitive Color Reactions Between Sulfhydryl Compounds and Thymonucleic Acid.*

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Thymonucleic acid reacts with cysteine in H_2SO_4 at 40° in two different ways depending upon which of the two reactants is in excess. The reaction products are colored and can be used for the detection of cysteine in a dilution of $1:10^6$ and of thymonucleic acid in $1:10^4$. Glutathione also gives a positive reaction in a dilution of 1:300,000. The procedure for the two reactions is as follows:

I. To 1 cc of a solution of Na thymonucleate (0.01 to 0.1%) are added, with cooling, 0.04 cc of a 5% solution of cysteine hydrochloride and 5 cc of a mixture of 190 cc of H_2O and 450 cc of concentrated H_2SO_4. The mixture is put into a water bath at 40° for 5 minutes. A deep pink color appears, which is stable for hours. It shows a sharp absorption maximum at 500 mμ. Cystine, homocystine, methionine, and dithiodiglycolic acid do not react even in high concentrations. Glutathione reacts like cysteine in equivalent concentrations. Thioglycolic acid reacts also, though a little less strongly than cysteine. Propyl mercaptan also gives the reactions, but pyruvic acid and acetone do not. When the desoxyribose is destroyed by heating the thymonucleic acid in 2% H_2SO_4 for 2 hours at 100°, the reaction with cysteine disappears completely. All these facts indicate that the colored substance is the product of a reaction between the desoxyribose of the thymonucleic acid and the sulfhydryl group of the cysteine (glutathione, thioglycolic acid).

The reaction can be used for the detection and probably for the colorimetric determination of small amounts of thymonucleic acid. In the range of 0.02-0.08% of thymonucleic acid the color intensity in white light is proportional to the concentration. The specificity and accuracy of the reaction are about the same as that of the diphenylamine H_2SO_4 reaction. The chief advantage which the new reaction seems to possess is the possibility of use even in the presence of large amounts of proteins. This is not feasible with the diphenylamine reaction because proteins and products of their hydrolysis are precipitated by the weak acid used in that reaction. On the other hand, they dissolve easily in the much more concentrated acid used in the reaction with cysteine. However, it must be noted that in the presence of large amounts of proteins the color which develops is not pink but red-brown.

II. The character of the reaction between cysteine and thymonucleic acid changes completely when performed in the presence of a large excess of the latter acid. When 0.8 cc of a 0.002-0.01% solution of cysteine hydrochloride is mixed with 0.2 cc of a 1% solution of Na thymonucleinate, 5 cc of the H_2SO_4 water mixture as above are added, and the mixture held for 3 minutes at 40°, a red-brown color appears with a sharp absorption maximum at 410 mμ. With thioglycolic acid the sensitivity of the reaction is about the same as with cysteine. Glutathione shows the same red-brown color only in higher concentration (0.05%); in lower concentrations, down to 0.005%, the color developed is brown. But even in these lower concentrations the characteristic absorption maximum is at 410 mμ. The brown color cannot be regarded as characteristic, because other substances, like cystine at 0.2%, pyruvic acid at 1%, and even thymonucleic acid alone at higher concentration, show a yellow color, but it seems possible that the absorption maximum at 410 mμ will prove characteristic, for the yellow reaction products formed with cystine and pyruvic acid do not show this maximum. The

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reaction between cysteine and glutathione (but not thioglycolic acid) and thymonucleic acid; can be made about 10 times more sensitive if it is performed in the following way:

To 1 cc of a solution containing cysteine (glutathione) are added, with cooling, 1.7 cc of concentrated H_2SO_4. The well-cooled mixture is then treated with 0.03 cc of a 1% solution of Na thymonucleinate and held at 40° for 3 minutes. The red-brown color with the same absorption maximum appears and can easily be recognized when the concentration of cysteine is only 0.0002%, or that of glutathione is 0.0005%.

Both color reactions of the thymonucleic acid with cysteine are probably due to the formation of some furfuryl compound from the desoxyribose. In fact, furfuraldehyde reacts with cysteine under the same conditions as thymonucleic acid forming a violet red product in presence of an excess of cysteine, and a brown color when the aldehyde is in excess. The latter reaction is much less sensitive than the reaction with thymonucleic acid.

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Experimental Production of Diabetic Cataract in the Rat.

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Two groups of workers describe the appearance of cataracts in animals with experimental diabetes. Dohan, Fish, and Lukens[1] observed this phenomenon after 5 months in a dog with permanent diabetes produced by injection of anterior pituitary lobe extract. Chaikoff and Lachman[2,3] observed it after a year in 8 dogs without pancreas, which were kept alive with insulin and an adequate diet. The spontaneous appearance of cataracts in pancreatectomized rats, with progressive evolution to complete opacity of the lens, led us to investigate factors that might be involved.[4]

Technic and Methods. Male white rats were almost completely pancreatectomized when they weighed 60-80 g. A portion of pancreas was left which varied between 5% to 20% of the weight of the organ. The diet was the same as that given to all the other rats of the Institute, in which no evidence of cataract was detected.

After 7 hours fasting, blood sugar was determined with blood from the tail, following Hagedorn and Jensen's method. The lens was examined through an electric ophthalmoscope after dilatation of the pupil by atropin. A Gullstrand's lamp was used for more minute studies. We classified the lens lesions in 4 degrees of increasing severity, as follows: Type A, alteration of the anterior capsule of the lens (rugosity); Type B, opacity of the lens sutures; Type C, partial opacity of the lens parenchyma, and Type D, total opacity of the lens parenchyma.

The lens and blood sugar in rats were observed under 3 different experimental groups: (1) with 5% of pancreas; (2) with 20% of pancreas; (3) normal, without operation. A fourth group was specially formed with some animals in order to follow the evolution of blood sugar and lens lesions in them.

Results. (1) Rats with only 5% of pancreas: 41 rats were observed. The lesions of the lens appear 50 days after the operation and, generally speaking, get worse as time goes on (Table I). Lesions of Types C and D are rarer because the most seriously affected animals die soon, thus giving no time for the formation of the cataract. The frequency and severity of the lens lesions increase in direct

1 Dohan, F., Fish, C., and Lukens, F., *Endocr.*, 1941, **28**, 341.

2 Chaikoff, I. L., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 211.

3 Chaikoff, I. L., and Lachman, G. S., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 237.

4 Cramer, F. K., and Foglia, V. G., *Rev. Soc. Argent. Biol.*, 1943, **19**.