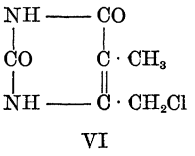
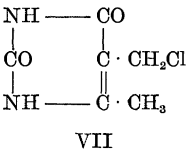


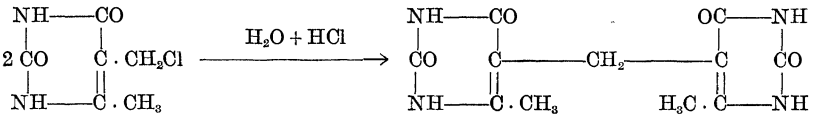
VI



VII

contrast to that observed with the halide represented by formula VII.

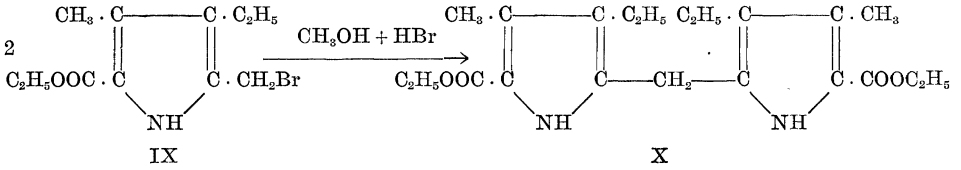
This pyrimidine VII is very unstable when digested with alcohol or water and can be converted practically quantitatively into the dipyrimidine methane derivative



VII VIII

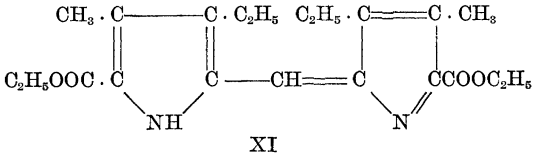
with a specific thiazole (IV) to form the naturally occurring quaternary salt (vitamin B_1) V.[1]

In carrying forward this research program to date our efforts have been confined to the development of new methods of synthesis and to the study of the chemical properties of pyrimidine halides of the uracil type. We now desire to report a recent discovery which reveals a striking difference in chemical behavior between the two isomeric pyrimidine halides expressed by formulas VI and VII. Here we are dealing with primary halide groupings substituted in the 4- and 5-positions, respectively, of the 2,6-dioxy-pyrimidine molecule.

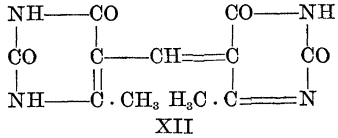


IX X

It was the senior author's experience during his earlier researches on the synthesis of simple nucleoside constructions in 1913–1915 that pyrimidine halides of the type VI are extremely stable and are very resistant to hydrolysis. For example, it was shown that both 4-chloromethyluracil and the pyrimidine halide VI can be heated with concentrated hydrochloric acid at 125–130 without alteration.[2] This behavior is in marked contrast to that observed with the halide represented by formula VII.



XI



XII

This pyrimidine VII is very unstable when digested with alcohol or water and can be converted practically quantitatively into the dipyrimidine methane derivative VIII upon treatment with hydrochloric acid. This unexpected change is quite analogous to the well-known pyrrol reaction applied successfully by Professor Hans Fischer and his coworkers in many cases for the preparation of dipyrrol methanes; and which is illustrated by the following reaction employed for the preparation of the dipyrrol methane derivative X.[3] These dipyrrol methane compounds such as X easily undergo oxidation to the corresponding dipyrrol methenes XI.

Whether our dipyrimidine methane compounds such as VIII may be oxidized to the corresponding unknown dipyrimidine methene derivatives such as XII remains to be determined.

A complete account of the results of this pyrimidine investigation will be presented in future publications from this laboratory.

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THE PREPARATION OF GLUTATHIONE CONTAINING RADIOACTIVE SULFUR

ARTIFICIAL radioactive elements are usually produced as the free isotopes of ordinary elements and may be used in the preparation of compounds suitable for study. The present work was undertaken in order to find a method of obtaining radioactive glutathione to be used later in the study of certain phases of sulfur metabolism in rats. Briefly, this method consisted in growing yeast in a medium containing radioactive sul-

[1] Williams and Cline, *Jour. Am. Chem. Soc.*, 58: 1063, 1504, 1936; 59: 216, 1937; Cline, Williams and Finkelstein, 59: 1052, 1937.

[2] Johnson and Chernoff, *Jour. Am. Chem. Soc.*, 36: 1742, 1914; 35: 585, 1913; *J. Biol. Chem.*, 14: 307, 1913.

[3] Fischer *et. al.*, Ann. 447: 137, 1926; 448: 199, 1926; 459: 85, 1927; 486: 39, 1931; and several other publications.

fur in a form which was easily assimilated by the yeast in its growth. Subsequently glutathione was extracted from the yeast so obtained and found to be radioactive. The technique developed is probably applicable to the preparation of other compounds.

The strain of yeast used was *Saccharomyces cerevisiae,* Frohberg type,[1] which was known to produce considerable quantities of glutathione. The medium used was an adaptation of that employed by Sugata and Koch.[2] They found that sulfur in the form of a sulfate is a true nutrient for yeast and is more easily assimilated than is sulfur in an organic form. In view of this the radioactive sulfur was converted into sulfate and added to the medium in that form. The medium had the following composition:

Refined cane sugar	100.00	grams	per	liter
Asparagine	1.5	"	"	"
Mono ammonium phosphate	3.0	"	"	"
Mono potassium phosphate	2.0	"	"	"
Magnesium chloride	0.25	"	"	"
Magnesium sulfate (radioactive)	0.25	"	"	"

It was found necessary to add bios for good growth; beer wort or a yeast extract made by boiling 50 grams of dried yeast in 500 cc of distilled water for 30 minutes proved to be a convenient source. Unfortuately, analysis of these bios preparations showed appreciable amounts of sulfur to be present. When a 1 per cent. concentration of these extracts, which was found to be a favorable concentration for growth, was used, the amount of sulfur added to the medium in the bios-containing extract was approximately the same as the amount of sulfur, in the form of magnesium sulfate, in the medium. This tended to decrease the activity of the final product. Determinations of the variation of glutathione content with the growth period of the yeast were made by the specific glyoxalase method of Woodward.[3] Maximum glutathione yield was reached after three days, which period of time was therefore chosen as the growth period.

The radioactive sulfur used in this work was prepared by bombardment of roll sulfur by 10 Mev deuterons accelerated by the cyclotron of this laboratory. Under this treatment a portion of the S^{34} is converted into S^{35} according to the reaction

$$_{16}S^{34} + {}_{1}H^{2} \rightarrow {}_{16}S^{35} + {}_{1}H^{1}$$

S^{35} emits beta rays of about 0.107 Mev energy and has a half life of about 88 days.[4] The sulfur was converted into magnesium sulfate by oxidation in a stream of oxygen in the presence of platinum catalysts at 600° C. The SO_3 vapors formed were led into an absorption chamber containing 5 per cent. solution of H_2O_2 and an excess of magnesium oxide, resulting in the formation of magnesium sulfate. The excess of magnesium oxide was filtered out and the solution freed from H_2O_2 by boiling.

Under the bombardment a part of the sulfur was converted into other radioactive elements, principally radioactive phosphorus, which, while not interfering with the growth of the yeast, had to be eliminated when activity measurements were made. When such measurements were to be made, the sulfur was precipitated from the magnesium sulfate solution as barium sulfate, washed thoroughly and dried to constant weight. A portion was weighed in a small nickel boat, a few drops of water added to form a suspension, which was evaporated to dryness to form a thin uniform layer. A drop of egg albumin or gum acacia added to the suspension before evaporation prevented accidental loss of the barium sulfate while measurements were being made.

Since the thin walls of the usual type Geiger counter absorb much of such weak radiation as is emitted by radioactive sulfur, activity measurements were made with a specially designed tube. The nickel boat, containing the sulfur sample, was placed in a side arm which did not permit the rays to reach the counter proper. The tube was evacuated to the desired pressure and the background count taken. The sample was then moved into place with a magnet and the count taken in the usual manner.

After the yeast had grown for three days it was separated from the medium by centrifuging and the glutathione isolated as the highly specific cuprous derivative, by a modification of Pirie's[5] method. Thirty grams of moist yeast containing approximately 30 milligrams of reduced glutathione yielded 20 milligrams of cuprous salt exhibiting the silvery sheen characteristic of this compound in aqueous solution. A portion of the cuprous salt was freed from copper by means of H_2S; the resulting solution was found to be capable of activating the enzyme glyoxalase, thus establishing the presence of free glutathione in the solution. The remainder of the cuprous salt was repeatedly washed by centrifuging until the washings gave no further test for sulfate. A sample of this material, containing 0.3 mg sulfur, gave a count of 150 per minute in the Geiger counter. Another portion, containing 0.25 mg sulfur, was ignited and the sulfate formed precipitated as barium sulfate. This gave a count of 100 per minute. Due to the self-absorption of the beta rays within the different samples, these measurements are only approximately quantitative.

This work would not have been possible without the help and encouragement of Dr. Ellice McDonald, director of the laboratories, and of various members of

[1] *Saccharomyces cerevisiae,* Frohberg type, was kindly furnished by the Fleischmann laboratories.

[2] H. Sugata and F. Koch, *Plant Physiol.,* 1: 337, 1935.

[3] G. E. Woodward, *Jour. Biol. Chem.,* 109: 1, 1933.

[4] W. F. Libby and D. D. Lee, *Phys. Rev.,* 55: 245, February 1, 1939.

[5] N. W. Pirie, *Biochem. Jour.,* 24: 51, 1930.

the staff, and we take pleasure in thanking them. Our thanks are especially due to Drs. A. J. Allen, M. B. Sampson and W. Danforth for the preparation of the radioactive sulfur and to Dr. E. F. Schroeder and Miss G. E. Woodward for the isolation and identification of the glutathione.

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CRYSTALLINE HORSE LIVER CATALASE

ONE of us (O. D. F.) observed that after dialysis for three weeks near the isoelectric point, a solution of horse liver catalase prepared by the method of Agner[1] deposited a small amount of active crystalline material. Not enough of this precipitate was obtained for accurate analysis.

Later, by modifying the method for preparing crystalline beef liver catalase[2] we obtained crystalline horse liver catalase in good yield. The crystals are in the form of very fine needles. After preliminary purification, including fractional precipitation with dioxane, crystallization was induced by slow addition of ammonium sulfate to the properly buffered catalase in a solution containing 3.0 per cent. of dioxane. Catalase crystals have been obtained also from preparations made by Agner's directions up to the point where the material is adsorbed by passing it through a column of calcium phosphate.

A sample of once-recrystallized horse liver catalase has been found to have a *Kat. f.* of 50 to 55 thousand, which is of the order of magnitude of that of the best preparation of Agner. We do not think that the material is yet entirely purified, since the content of iron is 0.2 per cent. instead of 0.09, which would correspond to the hemin iron. It is possible that our material was contaminated with a small amount of a protein high in iron which was observed during the preparation of the catalase crystals, and which is probably identical with the ferritin of Laufberger.[3]

The percentage of hemin, determined by colorimetry and by analysis for hemin iron, is about 0.9, which agrees with the claim of Stern and Wyckoff[4] that horse liver catalase contains about 0.1 per cent. of hematin.

Treatment of the once-recrystallized horse liver catalase with acetone and HCl split off the hemin which dissolved in the acetone. Evaporation of the acetone caused the hemin to precipitate, leaving a small part of the non-hemin iron in the supernatant liquid. Most of the non-hemin iron was in the protein residue.

Our analysis of the sample of once-recrystallized horse liver catalase showed that the copper content was practically negligible, contrary to the finding of Agner that the copper content of horse liver catalase is high enough to be of possible significance.

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3 V. Laufberger, *Bull. Soc. Chim. Biol.*, 19: 1575, 1937.
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INFECTION OF CHICKS AND CHICK EMBRYOS WITH RABIES[1]

IT is the purpose of this communication to present a preliminary report of observations made on chicks and chick embryos inoculated with rabies virus. These investigations were undertaken in order to study the lesions produced in this species and also to determine the changes which might take place in the virus following its adaptation to this unnatural host.

Rabid dog brain passed once through mice was the source of the virus used in this work. Histologically, this dog brain and the mouse-passage brain showed typical Negri bodies. The inoculum consisted of 0.03 cc of 10 per cent. brain emulsion.

Intracerebral inoculation of day-old chicks was followed by signs of rabies after 19 days on the first passage, and 4 passages did not reduce this period appreciably. There was considerable variation in the incubation period and also in the duration of the disease; some animals died within 2 days after the first signs of the disease, while others showed definite paralysis for 2 weeks before death. All these chicks went through a stage of excitement before the onset of flaccid paralysis.

At autopsy no gross lesions were demonstrable. Microscopic examination of brains and cords revealed many Negri bodies, some quite small, others huge. Non-specific changes in the form of acute necrosis of ganglion cells and massive perivascular accumulations of lymphocytes and large mononuclear phagocytes were present throughout the central nervous system.

Portions of brain from the 4th intracerebral passage in chicks were ground and emulsified and 0.03 cc were inoculated intracerebrally into 13-day-old chick embryos. The eggs were opened and the embryos were inoculated according to the technique which had been developed in this laboratory. After 4 days one embryo was sacrificed and its brain was inoculated into the brains of other embryos; subsequent passages have been made every 6 or 7 days, and the virus is now in its twelfth generation.

We have not allowed any chick embryos inoculated by the intracerebral route to hatch. Several embryos inoculated by this route have died between the sixth

1 Aided by grants from the John and Mary R. Markle Foundation.