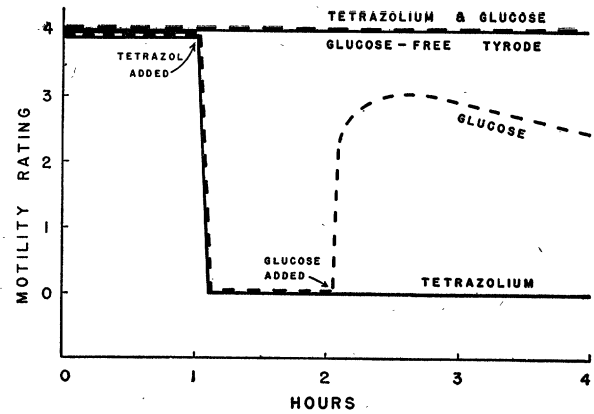

FIG. 1. Inhibition of rabbit sperm motility by 2,3,5-triphenyltetrazolium chloride in absence of glucose (lower curve). Addition of glucose to tetrazolium-treated sperm permits motility.

(Fig. 1). If motility is thus impaired, it may be reacquired by the addition of glucose within an hour following tetrazolium treatment. To this extent the reaction is reversible.

The direct counteraction by glucose of the tetrazolium suggested that the salt might be undergoing a reduction by the sugar, thereby rendering it unable to take part in the hydrogen transfer mechanisms of the cells. Tests with various sugars, however, and with photoreduced (red) tetrazolium indicate that this is not the case. Glucose and mannose are equally effective in preventing the inhibitory action of the tetrazolium; galactose and maltose are less effective; fructose, sucrose, and lactose are least effective; and ribose has no effect whatever. The molar ratios of glucose and triphenyltetrazolium chloride may be varied over a tenfold range without changing the reaction. A small quantity of tetrazolium inhibits motility; likewise a small amount of glucose, even in the presence of a large excess of tetrazolium, can maintain motility. No protective action against the tetrazolium was displayed by solutions ($M/100$–$M/1000$) of succinate, malate, fumarate, or malonate. The study has thus far been conducted primarily with 2,3,5-triphenyltetrazolium chloride and its effects on rabbit sperm, but both neo- and ditetrazolium chlorides produce the same types of response, and in fact give equivalent inhibition at lower concentrations. The reactions on rabbit sperm have been paralleled on human and bovine sperm.

Rabbit spermatozoa can generally maintain motility for periods up to 2 days at room temperature if provided with either glycolyzable energy sources (e.g., glucose, fructose, or mannose), or with an adequate oxygen supply. Under anaerobic conditions (tank nitrogen) sugar is essential (if sugar is absent oxygen is required) to support motility for more than a few minutes. These systems are sensitive to the glycolytic inhibitors, iodoacetate and fluoride, and to the oxidative poisons, cyanide and azide. If tetrazolium is used in combination with these compounds, motility is impaired in suspensions in which glucose is present but in which its utilization is prevented by iodoacetate or fluoride. On the other hand, so long as glucose is available, cyanide and azide sensitivity remains the same in the presence and absence of tetrazolium.

Attempts to elucidate the mechanism of action of tetrazolium salts on mammalian sperm have established these facts: (1) motility is impaired immediately following the addition of the salt to the sperm suspension; (2) the reaction need not proceed to the red formazan stage; (3) the inhibition of motility can occur if colored photoreduced tetrazolium is used; (4) the reaction can be reversed by the addition of suitable carbohydrate; (5) the reducing value of the sugar does not seem to be critical, although its enzymatic utilization by the cells may be; and (6) the inhibition is not counteracted by succinate, malate, fumarate, or malonate. It is justifiable to conclude at present, therefore, that the tetrazolium effect on sperm motility is not merely a reduction accompanying dehydrogenase activity, but is a physiological inhibition of a different order. The physical and physiological aspects of this impairment are being further investigated.

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On a Thiazolone Compound from Thiamine Disulfide

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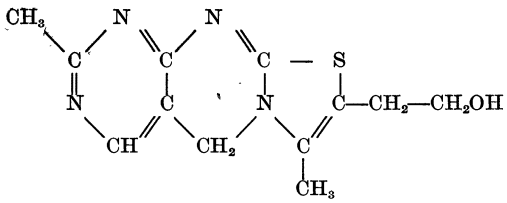
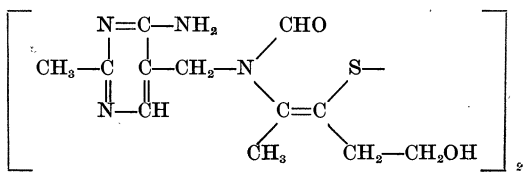
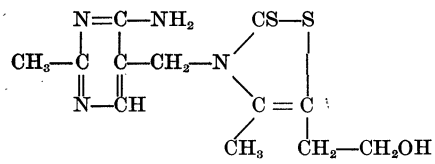
In 1940 Zima *et al.* (*1*) obtained thiochrome (I) and a product melting at 233°–4° by heating thiamine disulfide (II) in ethylene glycol. From the analytical



I



II



IV

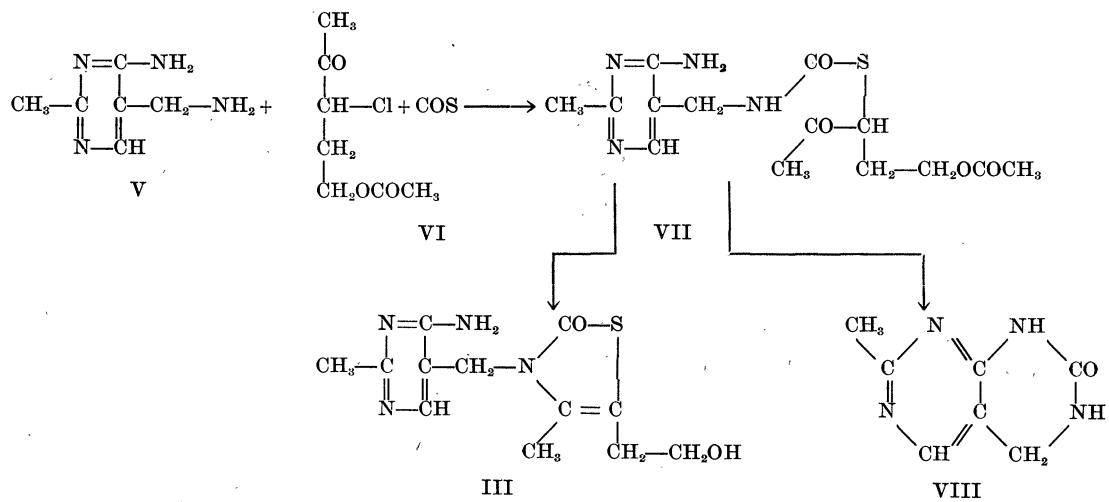
data they assumed the new product to be *N*-(2′-methyl-4′-amino-pyrimidyl-(5′))-methyl-4-methyl-5-β-hydroxyethyl-thiazolone (III). However, it has so far been believed that III might be formed as the first product when thiamine is oxidized with alkaline potassium ferricyanide, but spontaneous elimination of water to give I might make its isolation difficult. Therefore, the present investigation was undertaken to ascertain whether such a compound (III) really exists and, if so, to identify it by synthesis. Having previously synthesized the corresponding thiathiazolone compound (IV) (*2*), we employed the same method for the preparation of Type III.

Carbon oxysulfide was passed into a solution of 2-methyl-4-amino-5-aminomethyl-pyrimidine (V) and γ-aceto-γ-chloro-propyl acetate (VI) in alcohol containing a little ammonia. An exothermic reaction occurred, to yield S-(α-aceto-γ-acetoxy-propyl) *N*-(2-methyl-4-amino-pyrimidyl-(5))-methyl-thiocarbamate (VII), colorless needles from alcohol, mp, *ca* 250° (dec), (softening at *ca* 170°).[1]

Anal calcd for $C_{14}H_{20}O_4N_4S$: C, 49.40; H, 5.92; N, 16.46. Found: C, 49.42; H, 6.25; N, 16.02.

Compound VII was also obtained by the interaction of VI and ammonium *N*-(2-methyl-4-amino-pyrimidyl-(5))-methyl-thiocarbamate, the latter being prepared from carbon oxysulfide, V, and ammonia. When VII was heated at 170° C or treated with an aqueous solution of caustic alkali, it was converted into 2-oxo-7-methyl-1,2,3,4-tetrahydro-pyrimido-(4,5-d)-pyrimidine (VIII) (*3*). The same kind of reaction was also observed with α-aceto-γ-acetoxy-propyl *N*-(2-methyl-4-amino-pyrimidyl-(5))-methyl-dithiocarbamate (*2*), an intermediate of IV.

On the other hand, treatment of VII with HCl caused thiazolone ring closure and hydrolysis of the acetyl group to give the desired thiazolone (III) as follows: A solution of VII in *ca* 5% HCl was heated on a water bath for 10 min, and the resulting solution was made alkaline, whereupon III separated as fine



V VI VII III VIII

crystals, which crystallized from dilute alcohol in colorless needles melting at 233°–234°.

Anal calcd for $C_{12}H_{16}O_2N_4S$: C, 51.41; H, 5.75; N, 19.99. Found: C, 51.31; H, 5.87; N, 20.29.

The thiazolone (III) thus obtained and the one prepared from II in the manner of Zima *et al.* showed no depression of melting point when fused with each other, and proved to be identical in crystal form, solubilities, etc. Both were acetylated with acetic anhydride and pyridine to give the same acetyl derivative melting at 174°.

Anal calcd for $C_{14}H_{18}O_3N_4S$: C, 52.17; H, 5.63; N, 17.38. Found: C, 52.31; H, 5.65; N, 17.30.

It was found difficult to convert III into I by heat-

[1] The melting point depends on rate of heating.

ing III in a high-boiling solvent, heating as such above its melting point, or by other treatments, so that the hitherto believed mechanism of thiochrome formation from thiamine was proved not to be correct.

Recently Sykes and Todd (*4*) attempted the synthesis of the same compound (III). Although they got the same intermediate (VII) from VI and potassium *N*-(2-methyl-4-amino-pyrimidyl-(5))-methyl-thiocarbamate, they failed to convert it into III.

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A New Occurrence and Description of the Fossil *Arthrophycus*

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A new occurrence of the hitherto doubtful or unclassified fossil *Arthrophycus* is reported here. It was found last summer making up most of the west wall of an abandoned quarry in the Tuscarora on the north side of McKee Gap near Roaring Spring, Blair Co., Pa. The fossil occurs in tremendous profusion in a slabby, mottled, red-to-grey sandstone having a nearly vertical dip, near the Clinton contact. Fig. 1 shows a slab (with an 18-in. scale) removed from the wall. Fig. 2 is a closer view of another slab. The fossiliferous sandstone slabs, which are up to 2 ft thick, are separated by fissile, dark-grey shale, which is barren of *Arthrophycus*. The same fossil was found only sparingly in the lower Tuscarora near the Juniata contact at the crest of Tussey Mountain almost directly east of McKee Gap. Both localities are similar to other occurrences described for the Medina group.

The earliest description of this species is found in Amos Eaton's report of 1820 (*1*) where it is accurately described but not named, and is assumed to belong to the Vermes. In 1831 Richard Harlan (*2*) described the same fossil under the name of *Fucoides alleghaniensis*, or *F. brongniartii*, implying its algal origin. R. C. Taylor (*3*) in 1834 reprinted Harlan's report emphasizing the fossil's plant character, and again in 1835 reported in detail on the beds in which fucoids occur (*4*). T. A. Conrad (*5*) in 1839 described the specimen as *F. harlani*. The generic name *Arthrophycus alleghaniensis* was given by Hall in 1852, and H. R. Goeppert (*6*) somewhat later in 1852 named it *Harlania halli*. Finally, in 1893, Joseph F. James (*7*) decided that, according to accepted nomenclatorial procedure, the specimen's name *Arthrophycus alleghaniensis* (*Harlan*) *Hall*, as given by Hall, should be retained.

Admittedly, owing to the complete lack of internal structure, the classification of the specimen is problematical, yet its status as a plant seems to have been strongly indicated over one hundred years ago and time and again during the past century. Nevertheless

FIG. 1.