A portion of the active destructive agent in raw smelt can be extracted with 5% alcohol. No attempts were made at a quantitative extraction. The residues after alcohol extraction showed high activity. Spitzer *et al.*,[6] on the basis of the chick assay, report that aqueous extracts of carp are inactive while ether extracts showed partial activity. The carp residue after water and ether extraction showed partial activity. Woolley[5] reports that aqueous extracts of carp had 25% of the activity of whole carp suspensions and that 10% NaCl extracts of carp were highly active.

When finely pulverized smelt are thoroughly dried at room temperature, the B_1 destructive factor is lost and subsequent replacement of the original moisture content will not restore this factor. Heating smelt at 100°C for 5 min also causes a complete destruction of the active factor.

The use of certain species of raw fish in the preparation of vitamin B_1 free diets to be used for experimental purposes appears to be worthy of further investigation. Present methods of preparing B_1 free rations by autoclaving for considerable periods of time undoubtedly cause the destruction or alteration of other dietary factors. The use of a fish or a fish extract would circumvent these undesirable effects.

Summary. A viable yeast can protect its thiamine from destruction by a factor present in raw smelt. The antithiamine factor behaves similarly to the antibiotin factor in its inability to penetrate the living cell. The inactivation of thiamine does not appear to be due to the formation of thiamine complexes labile to hydrolysis or to the splitting of the B_1 molecule but rather to an enzymatic degradation of the thiamine molecule. The destructive factor in raw smelt is labile to air drying at room temperatures, heating at 100°C and is extractable in 5% alcohol. Certain raw fish or extracts of these fish may prove valuable in the preparation of vitamin B_1 free diets without the necessity of resorting to prolonged autoclaving.

13856

Estimation of Reduced Degradation Products in Experimental Trinitrotoluene Poisoning.

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Dale[1] presented tentative evidence that administered TNT (trinitrotoluene) in rabbits is partly reduced to dinitro-azoxytoluene and partly to amino compounds. The work of Voegtlin, Hooper and Johnson[2] indicated that in man the portion of the urinary material giving the Webster[3-5] test was the hydroxylamino derivative. It would seem all of these should, under proper conditions, be diazotizable and if coupled with a suitable color reagent, lend themselves to colorimetric estimation. Elvove[6] made a step in this direction, but his method involved reduction of residual nitro groups to nitrite, and application of the Griess reagent. The widely used Webster test suffers from several severe handicaps: (a) the test has

1 Dale, H. H., Medical Research Council of Great Britain, Special Rept. Series No. 58, 1921, 52.

2 Voegtlin, C., Hooper, C. W., and Johnson, J. M., Hygienic Laboratory Bull. No. 126, 1920.

3 Webster, T. A., *Lancet*, 1916, **191**, 1029.

4 Tutin, F., *Lancet*, 1918, **195**, 554.

5 Ingham, J., *Lancet*, 1941, **241**, 554.

6 Elvove, E., *J. Ind. Eng. Chem.*, 1919, **11**, 860.

low sensitivity, hence, is of little value for small quantities, (b) it is negative with the urine of some carnivora such as the cat, (c) it apparently becomes negative in the urine of the human subject with the onset of grave symptoms, (d) it does not by color alone distinguish unchanged TNT from the conjugated degradation products, (e) it is not quantitative. The procedure presented in this paper indicates about the same percentage excretion of reduced products by various animals including those carnivora which give a negative Webster test, it furnishes a method for detecting the residual chromogen in urine after the Webster chromogenic material is extracted, and is sensitive enough to permit tissue and blood analyses.

The test consists essentially in the diazotization of the reduced products formed by the body and subsequent coupling with the color reagent of Bratton and Marshall.[7] Obviously other compounds such as the sulfa drugs or nitro compounds yielding diazotizable groups might interfere. TNT as such does not.

A suitable aliquot of the 24-hr urine (0.05 to 0.3 cc for animals getting 50 mg/kg daily subcutaneously or 0.3% in the diet) is measured into a graduated cylinder, water added to 7.5 cc and 2.5 cc 4 N HCl added. The cylinders are immersed in ice water for 10 min, 1 cc of sodium nitrite (0.1%) added, mixed, allowed to stand 2 min, 1 cc ammonium sulfamate (0.5%) added, mixed, allowed to stand 2 min and 1 cc N-(1-naphthyl) ethylene diamine dihydrochloride (0.1%) added. At the same time standards containing 10, 20, 30, and 40 μg 6-nitro 2, 4-diamino-toluene are prepared and diazotized in the same way. For samples where the concentration is low, color comparison is better if the standard contains an equivalent amount of urine from a control animal. Comparison is made after 5 min. Tissues may be extracted with trichloracetic acid of suitable concentration and the filtrates diazotized. For certain purposes it is best to prepare acetone filtrates (1 part tissue, 3 parts absolute acetone) of the tissues since TNT and some derivatives are very sparingly soluble in acid solution. After evaporation of the acetone at room temperature, the unchanged TNT may be extracted from an aqueous 1% bicarbonate suspension with ether and estimated by the method of Pinto and Fahy[8] or that of Kay.[9] Rabbits apparently excrete a portion of the administered TNT as a conjugated dinitro-hydroxyl-amino-toluene; hence, color comparison is not as satisfactory as for cat's urine. A 20-80 mixture of the amino and hydroxyl-amino compounds might be used as standard. For the ether extract prepared by the Webster method 2,6-dinitro-4-hydroxyl-amino-toluene has been found to more nearly approximate the color. For the dog, 2,4,6-triaminotoluene appears to be the most suitable standard.

With this procedure a rabbit gave diazotizable material in the urine for 3 weeks, and in the liver at necropsy, following a single tolerated dose of 0.4 g per kg. Representative of findings with the method are those for rabbit number 7 dying 48 hr after a single toxic dose, as shown in Table I.

TABLE I.

Distribution of the Reduced Material in the Rabbit 48 Hours Following a Single Toxic Dose of TNT of 500 mg/kg Subcutaneously

Organ or fluid	Mg/100 g or cc as the Diamino Cpd
Blood	6.5
Liver	2.0
Kidney*	5.0
Muscle	1.0
Bile	2.0
Fat	1.0
Lung	3.0
Spleen	3.0
Bone Marrow	2.5
Testicles	0.5
Aqueous humor	0.5
Urine*	330.0

*Webster test positive on kidney and urine.

Fat was the only tissue which on extraction with acetone, evaporation of the acetone, suspension of the residue in 1% $NaHCO_3$ and extraction with ether gave a positive test for unchanged TNT with Webster's reagent.

[7] Bratton, A. C., and Marshall, E. K., Jr., *J. Biol. Chem.*, 1939, **128**, 537.

[8] Pinto, S. S., and Fahy, J. P., *J. Ind. Hyg. and Toxicol.*, 1942, **24**, 24.

[9] Kay, K., *Canadian J. Research*, 1941, **19**, 86 (Section B).

Summary. A method has been described for tracing reduced degradation products of trinitrotoluene in experimental animals. The role of these products in trinitrotoluene poisoning remains to be determined.

13857

Transmission of the Murine Strain of Poliomyelitis to the Syrian Hamster.

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The Lansing strain of poliomyelitis has been successfully passed to the cotton rat and mouse by Armstrong.[1,2] Since certain studies, such as serological investigations, can be performed on these animals with difficulty, it was considered of interest to try to pass the mouse strain to a larger animal. After the mouse-adapted strain had been successfully passaged through 16 Syrian hamster passages, we learned of Armstrong's work where he reports passing the virus through a few hamsters.[3] Since no description of the disease is given it is considered of interest to report our results here.

The 211th Armstrong mouse brain passage was employed to initiate these studies. 0.25 cc of a 1 to 10 mouse brain suspension was inoculated intracerebrally into young hamsters. After 4 days one hamster became paralyzed in both hind legs. The brain and cord were then passed serially to other hamsters and mice. Table I indicates the results obtained.

It is seen that the virus has gone through 16 successive hamster brain passages. Of 75 inoculated hamsters, 46 or 61.3% developed paralysis and died after an incubation period varying from 2 to 15 days with an average period of incubation of 5⅕ days. Only 7 of the paralyzed animals survived. The 1st, 2nd, 4th, 5th, 9th, and 12th hamster brain passages were inoculated into mice and in all instances mice from each series developed typical mouse poliomyelitis after an average incubation period of 7⅓ days.

The disease in the hamster runs the following course. After 24 hr the animal may appear lethargic and irritable while recovering from the effects of the inoculation, but usually they are bright eyed and inquisitively active, quite like a normal animal. The onset of the disease is variable. The first symptoms are usually irritability and malaise followed by a swelling of the eyelids, so that they are nearly closed. Paralysis usually occurs 1 or 2 days after the appearance of the first symptoms. The extent of paralysis is quite variable, from slight irregularities in gait, paralysis of a few toes on one foot to complete paralysis of the legs. Fourteen died of respiratory paralysis while the others died after paralysis of the fore or hind legs. During advanced stages of paralysis, the animal appears moribund, breathes heavily and irregularly and moves only slightly when disturbed. Death, through respiratory paralysis, may occur within 1 or 2 days following paralysis of the legs. In those animals that recovered, there was a permanent paralysis, followed by atrophy and contractures.

Grossly the brain and cord show no abnormalities. Microscopically, there is a diffuse perivascular cuffing of the intrinsic cerebral vessels, occasional foci of diffuse leucocytic infiltration are seen with greatest frequency and greatest intensity in the cerebral peduncles. There is in addition, a focal,

1 Armstrong, C., *Public Health Report*, 1939, **54**, 1719.

2 Armstrong, C., *Public Health Report*, 1939, **54**, 2302.

3 Armstrong, C., and Packchanian, unpublished data, referred to in Vanderbilt Lectures, April, 1941. Armstrong informed us that 2 or 3 passages were made.