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.

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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.

TABLE I.
Hamster Passages of Polio Virus.

Hamster passages						Mouse passages (from corresponding hamster passage)		
		Animals paralyzed (or dead)					Animals paralyzed (or dead)	
Passage No.	No. of animals inoculated	No.	%	Days after inoculation when paralysis occurred	No. of animals recovering from paralysis	No. of animals inoculated	No.	%
1	3	1	33.3	4		6	3	50
2	3	1	33.3	7		4	4	100
3	7	4	57.1	2-4-4-5				
4	6	1	16.6	5		5	4	80
5	5	3	60	3-4-5		5	5	100
6	5	4	80	2-2-4-15	1			
7	3	2	66.6	2-2				
8	4	3	75	3-4-14	1			
9	4	1	25	3		6	5	83.3
10	2	1	50	3				
11	8	7	87.5	3-3-3-4-4-5-5	2			
12	6	6	100	2-2-2-6-6-10	1	7	7	100
13	5	4	80	3-3-3-3	2			
14	6	5	83.3	9-11-11-11-12				
15	3	1	33.3	4				
16	5	2	40	3-15				
Totals	75	46	61.3%	5.2 days	7			

predominantly round cell meningitis. In the sections of spinal cord several small contracted ganglion cells in the anterior horns, suggestive of early necrosis are seen. In addition there is associated polymorphonuclear leucocytic infiltration in the anterior horn segments of several levels. Several vessels within the white matter show marked perivascular cuffing.

Neutralization tests were performed employing hamster brain virus and normal human serum as well as human convalescent poliomyelitis serum. The test animal was the mouse. The positive human convalescent serum neutralized the hamster virus while the normal human serum did not. Likewise, hamster convalescent serum neutralized the hamster virus as well as the mouse virus.

The 5th hamster brain passage was inoculated intracerebrally into 2 monkeys, each receiving 0.5 cc of a 1:10 suspension of brain material. The monkeys showed no signs of illness. After 1 month these monkeys and 1 control monkey were tested with a potent monkey poliomyelitis cord virus, all receiving 0.5 cc of a 1:10 cord suspension, by intracerebral inoculation. The monkeys, previously inoculated with the hamster virus, proved to be immune, while the control monkey developed paralysis and died on the 9th day. The 16th hamster brain passage was inoculated into 2 monkeys, each receiving 0.5 cc of a 1:10 brain suspension, inoculated intracerebrally. In both instances the temperature rose on the 6th day but there was no paralysis. One monkey was sacrificed on the 9th day and a 10% suspension of cord was inoculated intracerebrally into another monkey. This monkey did not develop disease nor was it immune to a subsequent inoculation of potent monkey poliomyelitis virus. The surviving monkey, from the 16th hamster brain inoculation, was tested for immunity after 28 days. This monkey, as well as a control monkey, received 0.5 cc of a 10% suspension of potent monkey cord virus, intracerebrally. The control monkey died of poliomyelitis after 7 days, while the other monkey proved immune.

Conclusions. The Armstrong mouse-adapted poliomyelitis strain was successfully passed serially through 16 Syrian hamster brain passages. The disease in hamsters is usually characterized by paralysis of one or

two limbs and followed by death. A small number of paralyzed hamsters survived, but developed atrophy and contractures of the affected muscles. The pathology in the infected hamsters is typical of poliomyelitis. The hamster strain is infectious for mice. Human convalescent poliomyelitis serum neutralized the hamster strain. Hamster convalescent serum neutralized the hamster strain as well as the mouse strain. The hamster strain inoculated intracerebrally into 4 monkeys produced no paralysis. Three monkeys, subsequently tested with virulent monkey cord virus, proved to be immune.

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Antimicrobial Action of Pyocyanine, Hemipyocyanine, Pyocyanase, and Tyrothricin.

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The isolation of substances from various bacteria, fungi and actinomycetes which in extremely small amounts inhibit or destroy many types of pathogenic bacteria, has aroused considerable interest in recent years. These include such substances as pyocyanase,[1] pyocyanine,[1] hemipyocyanine,[1] tyrothricin[2] (and its components, gramicidin and tyrocidine), actinomycin,[3] streptothricin,[4] gliotoxin,[5] and penicillin.[6]

In view of the present marked interest in antibiotic substances of microbial origin and because of the availability of pyocyanase, pyocyanine and a number of synthetic pyocyanine salts as well as hemipyocyanine, it appeared to be of some interest to compare the antimicrobial activities of these substances with each other and to some extent with tyrothricin.

Hemipyocyanine* (α-hydroxyphenazine), pyocyanine hydrochloride and pyocyanine methosulfate (monomethyl sulfuric acid salt of pyocyanine) were prepared by synthetic methods.[7] Pyocyanine was also isolated as the phenazonium compound and as the hydrochloride and as the perchlorate from cultures of *Ps. aeruginosa*. Pyocyanase which is a crude ether-soluble fatty material composed of a mixture of substances, probably including some pyocyanine, was isolated from the same cultures. Tyrothricin was prepared according to Dubos and Cattaneo.[8]

Bacteriostatic activity was determined by thoroughly mixing varying amounts of each substance with 10 ml of melted brain-heart infusion agar in Petri dishes. After the agar had solidified, the surface was streaked with a loopful of an 18-24 hr broth culture of each test organism. The presence or absence of growth was noted after the plates had been incubated at 37°C for 24 hr. The inhibitory values given in Table I are probably somewhat low since, in most cases, the next dilution used at which growth occurred was twice the inhibitory dilution. It is evi-

1 Kramer, H., *Z. Immunitäts.*, 1935, **84**, 505.

2 Dubos, R. J., and Hotchkiss, R. D., *J. Exp. Med.*, 1941, **73**, 629.

3 Waksman, S. A., and Woodruff, H. B., *J. Bact.*, 1941, **42**, 231.

4 Waksman, S. A., and Woodruff, H. B., *J. Bact.*, 1942, **43**, 9.

5 Dutcher, J. D., *J. Bact.*, 1941, **42**, 815.

6 Chain, E., *et al., Lancet*, 1940, **239**, 226.

* We are indebted to Dr. M. Tishler of Merck & Co., Inc., for supplies of hemipyocyanine and pyocyanine perchlorate.

7 Wrede, F., and Strack, E., *Ber.*, 1929, **62**, 2053; *Z. physiol. Chem.*, 1928, **177**; 1929, **181**, 58; also Hilleman, H., *Ber.*, 1938, **71**, 34. See also Einhorn, A., *et al., Ber.*, 1904, **37**, 106.

8 Dubos, R. J., and Cattaneo, C. J., *J. Exp. Med.*, 1939, **70**, 249.