11662

Convulsive Action of Triphenyl Phosphite.*

ROBERT B. AIRD, WALDO E. COHN AND SIDNEY WEISS. (Introduced by D. M. Greenberg.)

From the Departments of Surgery and Biochemistry, University of California Medical School, San Francisco and Berkeley, California.

Triphenyl phosphite has been used as a convulsive agent in experimental epilepsy.[1] Because it is relatively easy to prepare this compound synthetically[2] and to render it radioactive by synthesizing it from radioactive phosphorus,† it seemed feasible and desirable in our studies on epilepsy to combine the function of tracer substance with that of convulsant. Since it was eventually demonstrated that triphenyl phosphite hydrolyzes and the convulsant effects were due to the phenol fraction which is known to act at the cord level,[3] its use was abandoned. In the course of these studies, however, certain interesting observations were made in connection with the absorption, hydrolysis, toxicity and distribution of triphenyl phosphite. The results seem worthy of brief report, inasmuch as this compound belongs to the same series as triorthocresyl phosphite, which produces a combined system degeneration of the spinal cord[4] and is related to triorthocresyl phosphate, the contaminant responsible for the degenerative changes in cases of Jamaica ginger paralysis.[5]

The toxic doses of triphenyl phosphite as reported by Smith, *et al.*,[4] were confirmed in rats and cats. In dogs 2 doses of as little as 0.5 cc per kilo of body weight, given one week apart, produced the

* Aided by grant from Child Neurology Research (Friedsam Foundation). We wish to acknowledge the helpful advice and criticism of Professor David M. Greenberg.

1 Cobb, S., Cohen, M. E., and Ney, J., *J. Nerv. and Ment. Dis.*, 1937, **85**, 438; *Arch. Neurol. and Psychiat.*, 1938, **40**, 1156.

2 Noack, E., *Ann. der Chemie*, 1883, **218**, 96; Gottlieb, H. B., *J. Am. Chem. Soc.*, 1932, **54**, 748.

† We are indebted to Professor E. O. Lawrence and members of the Radiation Laboratory for the radioactive phosphorus used in this study. It was prepared by bombardment of phosphorus with deuterons accelerated in the cyclotron.

3 Baglioni, S., *Centralbl. für Physiologie*, 1909, **14**, 97; *Z. f. allgem. Physiol.*, 1909, **9**, 1.

4 Smith, M. I., Lillie, R. D., Elvove, E., and Stohlman, E. F., *J. Pharm. and Exp. Therap.*, 1933, **49**, 78.

5 Smith, M. I., Elvove, E., and Frazier, W. H., *U. S. Pub. Health Rep.*, 1930, **45**, 2509.

typical ataxia and weakness in the hind legs within 2 weeks. The early convulsive reaction was found to depend upon the purity of the preparation. A commercial preparation proved most toxic and 2 synthetic preparations, in one of which a high degree of freedom from contamination with phenol was achieved, indicated that the convulsive effects could be closely correlated with the degree of phenol contamination of the preparation.

Smith and his coworkers[4] have adduced evidence purporting to show that the esters of phosphorous acid were absorbed very slowly from the site of injection. Because this might be correlated with the delayed onset of the neurological changes it is a point of considerable importance. In our own studies methylene blue, erythrosin-B, and eosin were found to concentrate at the site of inflammatory reactions, such as are produced by the injection of the esters of phosphorous acid, and are not necessarily related to the specific presence of the phosphites. Only 8% of the radioactive triphenyl phosphite (P^{32}) was regained unabsorbed one hour and 45 minutes after its injection into the peritoneal cavity.

Quantitative determinations of free phenol[6] and radioactive phosphorus (as triphenyl phosphite and hydrolyzed phosphite) were made on the cerebrospinal fluid, blood, motor cortex, diencephalon, and remainder of the cerebral hemispheres of the brains of 8 cats 1½ hours after the intraperitoneal injection of 0.3 cc of radioactive triphenyl phosphite per kilo of body weight. The radioactive preparations varied from 7,500 U‡ to 15,000 U in strength of radioactivity. The results are shown in Table I. One other experiment, in which 0.5 cc of radioactive triphenyl phosphite (30,000 U) per kilo of body weight was injected intraperitoneally, gave results comparable with those shown in Table I and in addition showed 0.011% P^{32} per g of fluid in the cerebrospinal fluid, 0.0032% P^{32} per g of tissue in the muscles and steadily rising values of P^{32} in the blood up to the conclusion of the experiment at one hour and 45 minutes after the injection of the radioactive triphenyl phosphite.

Although the variations of the determinations are wide, it is evident in all instances that only a small amount of radioactive phosphorus (triphenyl phosphite or hydrolyzed phosphite) reached the central nervous system in 1½ hours and at this same time there was a considerable excess of free phenol beyond that normally found in these tissues. If the injected triphenyl phosphite is assumed to be completely hydrolyzed and the phosphorous acid fraction absorbed

[6] Folin, O., and Ciocalten, V., *J. Biochem.*, 1927, **73**, 627.

‡ U—arbitrary unit of radioactivity, approximately equal to 10^{-3} μC.

TABLE I.
Determinations of Radioactive Phosphorus (P^{32})* and Free Phenol (○OH)† 1½ Hours After the Intraperitoneal Injection of 0.3 cc/kg Body Weight of Radioactive Triphenyl Phosphite.

Cat	Dose of P^{32} U	C.S.F. ○OH†	Blood P^{32}*	Blood ○OH†	Motor Cortex P^{32}*	Motor Cortex ○OH†	Diencephalon P^{32}*	Diencephalon ○OH†	Brain Residue P^{32}*	Brain Residue ○OH†
1	15,000	1.84	2.5	7.3	0.3	10.6	0.17	10.5	1.7	4.2
2	15,000	1.13	0.77	4.2	0.3	7.3	0.7	8.6	0.14	0.83
3	10,000	0.95	0.35	2.96	10.0	—	0.4	13.5	0.25	—
4	15,000	1.83	0.89	8.4	2.3	—	2.0	—	0.053	—
5	15,000	2.3‡	0.27	5.9	2.0	5.65	0.23	6.25	2.4	6.04
6	7,500	0.83	0.29	3.2	0.8	12.4	0.1+	5.3	0.12	6.5
7	10,000	0.74	0.70	4.7	7.3	4.96	1.4	5.02	0.60	5.3
8	15,000	0.98	0.19	6.0	0.15	11.0	6.5	5.1	0.30	6.05
Avg		1.32	0.74	5.33	2.89	8.65	1.44	7.75	0.695	4.82
Ratio	○OH/P^{32}		7.2		3.0		5.38		6.93	

*% of dose injected per 100 g of tissue.
†Mg%.
‡Contaminated with blood.

and distributed to a degree comparable to the phenol fraction, the value of the ratio of free phenol, in mg %, to radioactive phosphorus, in % per hundred g, averaged over all 8 cats (average weight 2.75 kg) would be 9. A slightly higher value would be obtained if the free phenol factor is not corrected for the free phenol normally present in the tissues. Inasmuch as the detoxification of phenol (oxidation, conjugation, and elimination) occurs relatively rapidly in the body, the free ○OH/P^{32} ratios of the average observed values should be lower approximations of this theoretical value. This would be true only in case hydrolysis were essentially complete and the absorption and distribution of the hydrolyzed fractions occurred to a comparable degree within the 1½-hour time limit of these experiments. Since the values found in the case of the bloods and brain residues did approximate this theoretical ratio, it is clear that triphenyl phosphite was hydrolyzed readily *in vivo*, that the hydrolyzed fractions were widely distributed, and that this affords an adequate explanation for the fact that the phenol fraction was present in the central nervous system in great excess and in amounts sufficient[4] to account for the early, toxic manifestations which involved the central nervous system. The lower ratios for the diencephalon and motor cortex were due to the relatively higher concentrations of P^{32} found in these tissues, which suggests a selective and preferential absorption of the phosphorous acid fraction by the gray matter of the central nervous system.

The precise distribution in spinal cord, medulla, and cerebellum of the radioactive phosphorus and the concentrations necessary to produce the late, degenerative changes in the central nervous system

were not determined. Since, however, detoxification of the phenol fraction is known to occur rapidly and does not cause degenerative changes in the central nervous system, the late degenerative changes associated with triphenyl phosphite may be presumed to be caused by relatively small concentrations of phosphorus acid acting over long periods. The preferential absorption of the phosphorous acid fraction by the gray matter is of interest in this connection.

11663

Effect of Pantothenic Acid on the Nutritional Achromotrichia.

KLAUS UNNA AND W. L. SAMPSON. (Introduced by H. Molitor.)
From the Merck Institute for Therapeutic Research, Rahway, N. J.

Greying of the fur was first observed by Morgan, Cook and Davison[1] and Lunde and Kringstad[2] in black rats maintained on diets deficient in the vitamin B complex. Addition of preparations containing "filtrate factor",[3] of liver extracts containing pantothenic acid[4] or of brewers' yeast[2] have been reported to prevent the development of greying or to restore the black pigmentation of the hair.

In our laboratory, greying of the hair was obtained in black and piebald rats on a diet consisting of casein, vitamin free, 18%; sucrose, 67%; butter fat, 9%; salt mixture, 4%; cod liver oil, 2%, and supplemented with thiamin, riboflavin, nicotinamide and vitamin B_6. On this diet greying of the fur developed in approximately 80% of the animals within 4 weeks. At this time, the animals reached stationary weights and showed, in addition to the grey symmetrical patterns of the fur, signs characteristic of pantothenic acid deficiency in rats,[5] namely, thinning of the fur, generalized scaly dermatitis, inflammation of the nasal mucosa, blood-caked whiskers, and hemorrhages in various organs, particularly in the adrenal cortex. The addition of graded doses of pantothenic acid (calcium pantothenate, Merck) to the diet demonstrated that a daily supplement of 80 or 100 μg of calcium pantothenate, *e. g.*, doses

1 Morgan, A. F., Cook, B. B., and Davison, H. G., *J. Nutr.*, 1938, **15**, 27.
2 Lunde, G., and Kringstad, H., *Z. f. physiol. Chem.*, 1939, **257**, 201.
3 Morgan, A. F., and Simms, H. D., *J. Nutr.*, 1940, **19**, 233.
4 György, P., Poling, C. E., and Subbarow, Y., *J. Biol. Chem.*, 1940, **132**, 789.
5 Unna, K., *J. Nutr.*, 1940, in press.