Neurotoxicity by the dividing administration of critical dose of phosvel

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Summary. Phosvel, an organophosphorus insecticide, produces delayed neurotixicity in hens. A change in the toxicity, and in the residue of insecticide in the fat, were observed when the critical dose which was demonstrated in the case of a single dose was subdivided.

It is widely known that a few of the organophosphorus compounds produce delayed nerve damage in exposed individuals, but even now the biochemical mechanism of the toxicity is unknown[1]. Phosvel (leptophos) is one of the organophosphates which possess such a characteristic neurotixicity as described by Abou-Donia et al.[2] and by us[3]. From its properties, Davies et al.[4] suggested that phosvel might be deposited in the fat of animals. Actually, we could detect phosvel in the fat of hens more than 3 weeks after the oral administration of a single dose. Since it was thought that organophosphorus compounds are metabolized rapidly in vivo, the administration of subdivided doses has scarcely been used experimentally to determine their toxic effect. Phosvel, however, proved to be deposited in the fat, so that we supposed that the toxic effect might possibly increase with the subdivided administration of the critical single dose. The relationship between the toxic effect and the concentration of phosvel in the fat after this treatment regimen are discussed.

Materials and methods. The commercial phosvel in Japan contains 34% of pure phosvel, [0-(4-bromo-2,5-dichlorophenyl)O-methyl phenylphosphonothioate]. The pure ingredient was precipitated as a white crystalline substance by mixing the commercial product with 4 parts methanol at room temparature[5]. The crystalline precipitate was identified by means of standard phosvel which had been supplied by a manufacturer, using gas chromatography. Comparing the retention time of both chemicals, the purity of the prepared precipitate proved to be 93.7%.

25 white leghorn hens, 21 months of age, with an average weight of 1.74 kg, were used in the 1st experiment. As we reported earlier[3], it seems that the single oral dose of 250 mg/kg b.wt of hens is approximately the critical dose which causes delayed neurotoxicity. Hens were subdivided into 5 groups, each comprizing 5 animals. Group 1 was given a single dose of phosvel dissolved in salad oil, at the rate of 250 mg/kg b.wt ('250 group'). Group 2 was dosed continuously for 2 days at the rate of 125 mg/kg a day ('125×2 group'). The groups 3, 4 and 5 received the compound at the rate of 83 mg/kg, 50 mg/kg and 25 mg/kg b.wt per day during 3, 5 and 10 days ('83×3', '50×5' and '25×10 group'). Consequently, each group was given the same total amount.

In the 2nd experiment, 33 hens, 24 months of age and weighing 1.66 kg on the average, were divided into 6 groups, 5 groups of 6 birds and 1 group of 3 birds. The former groups received the same treatment regimen as in the 1st experiment, whereas group 6 served as controls. The experimental groups, each of 6 hens, were subdivided into 2 subgroups. One subgroup was sacrified on the 1st day and the another subgroup on the 8th day after its final treatment. The phosvel in the adipose tissue of the hens was analyzed by gas chromatography under the conditions we reported before[5,6].

Results and discussion. 1st experiment. On the 1st day after administration, it was found that hens of the '250 group' and the '125×2 group' were lethargic and had a poor appetite, whereas no abnormality was observed in the others. During the following 6 days, however, no abnormalities could be observed in any group. On the 8th day after the final treatment, ataxia was first observed in 2 hens of the '50×5 group', and the other animals of this group also showed an awkward gait on the following day. Locomotor ataxia developed successively in 4 hens of the '83×3 group'

Table 1. 1st experiment. Ataxia, paralysis and death of hens given a total amount of 250 mg/kg of phosvel, using 5 different schemes of administration

Dose (mg/kg/day)	Duration of administration	No. of hens	Ataxia (paralysis) No. of hens	Days after final dose until ataxia	Death No. of hens	Survival hens after 45 days
250	1	5	0 (-)	-	1*	4
125	2	5	1 (1)	19	0	5
83	3	5	4 (4)	10-14	3	2
50	5	5	5 (5)	8- 9	3	2
25	10	5	3 (3)	8-10	1	4**

* Death by accident, not paralysis. ** Survival hens after 21 days.

Table 2. 2nd experiment. Concentration of phosvel in adipose tissue of hens on days 1 and 8 after 5 different schemes of administration of a total amount of 250 mg/kg

Dose (mg/kg/day)	Duration of administration	Concentration of phosvel (ppm*) A. 1 day after dosing Mean (No. of hens)	Range	B. 8 days after dosing Mean (No. of hens)	Range	Ratio B/A
250	1	9.32 (3)	5.09-17.43	2.23 (3)	1.93-2.08	0.24
125	2	18.13 (3)	10.06-23.73	1.76 (3)	1.19-2.73	0.10
83	3	18.71 (3)	10.73-23.02	1.56 (3)	0.65-2.06	0.08
50	5	9.54 (3)	7.90-12.13	2.35 (3)	1.94-2.76	0.25
25	10	4.60 (2)**	4.46-4.73	1.83 (3)	0.76-2.81	0.40
Control	-	Not detected				

* Petrolem ether-soluble fat basis. ** Fat of 1 hen was not available.

between day 10 and 14 after the last dosing. For the '25 × 10 group', 3 out of 5 birds showed ataxia between day 8 and 10. On the other hand, only 1 of the '125 × 2 group' showed signs of ataxia on day 19, and no hen became abnormal in the '250 group'. Finally, 13 hens were attacked by ataxia during the observation period. Sooner or later all of them developed paralysis. 7 hens in a serious condition died by the 11th day after the onset of ataxia, probably as a result of respiratory failure. The results are summarized in table 1.

2nd experiment. Samples of fat were collected from the abdominal cavities of hens (adipose tissue of gastrocolic omentum). The concentrations of phosvel in the fat of hens on the 1st and the 8th day after the last treatment are shown in table 2. On the 1st day, the phosvel concentration was highest in the '125 × 2' and the '83 × 3 group', followed by the '50 × 5 group'. The mean value of the '250 group' was only half that of the '125 × 2' and the '83 × 3 group'. On the other hand, the '50 × 5 group' showed maximum phosvel concentration on day 8, and the level of the '250 group' was slightly less. The trend of the mean values on day 8 was not similar to that on day 1. Phosvel was not detected in the control group.

Concerning the toxicity of phosvel, it is necessary to consider the amount of a single dose, and the frequency of doses, as well as the total dosage, because, by the administration of the critical dose divided and administered at different times the toxicity was intensified in some groups and alleviated in other groups.

Phosvel was most absorbed by the fat with 3 doses of 83 mg/kg just after the final treatment but the residue in the fat was most pronounced with 5 doses of 50 mg/kg after the 'latent period (8 days)'. It may suggest that a large single dose tends not to cause effective absorption and retention.

Although the data of the 2nd experiment did not always explain the variation of neurotoxicity observed in the 1st experiment, it was suggested that the absorption of phosvel by fat and its concentration behaviour might depend on the treatment regimen; toxicity might be related not only to the level of phosvel in the fat but to the course of concentration during the 'latent period'.

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Embryonic development of an insect myocardium[1]

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Summary. Evidence from light and electron microscopic studies indicates that differentiation of myocardial cells in moth embryos begins at 6 days post-oviposition and is completed on eclosion. Fibrillogenesis, and development of cytoplasmic organelles and membranes are described. The heart is fully differentiated and functional at eclosion.

Cardiac cells that comprise the myocardium of the adult moth *Hyalophora cecropia* share many basic physiological properties common to cardiac muscle in phylogenetically higher forms[2], and indeed, fundamental phenomena common to all excitable cells and tissues have been studied on this preparation. Ultrastructural studies have likewise confirmed that the cellular matrix of the moth myocardium has both similarities and dissimilarities to vertebrate hearts. This paper describes investigations undertaken to study the development of this insect myocardium during the embryonic phase and to relate the findings to those reported for higher forms[3,4].

Material and Methods. The cecropia moth progresses through a 4-stage metamorphosis, the 1st stage being completed in the egg. *H. cecropia* eggs hatch into the larval forms in approximately 10 days at 23 °C and for this study embryos were removed at intervals of 3, 4, 6, 7, 8 days post oviposition (dpo) and fixed for examination in the electron microscope. Hearts of newly-emerged larvae were also fixed in situ and excised for the electron microscope.

Results. The adult form of the tubular heart consists of striated muscle arranged in a helical fashion with a total wall thickness of only a single cell. It is derived embryonically from ventrally located mesodermal cardioblast cells that migrate dorsally and assume a crescent shape as they fuse in the dorsal midline of the abdomen[5].

Fusion of the crescent-shaped, undifferentiated, mononuclear cardioblasts occurs at approximately six dpo, at which time they contain sparse amounts of rough endoplasmic reticulum, randomly scattered elongate mitochondria, measuring 6.5 µm × 1.5 µm, with ill-defined cristae, abundant free ribosomes and conspicuous basal lamina (surface coat). Irregularly shaped membranous vesicles, measuring 0.1–1.0 µm in diameter, which originate from the clearly defined Golgi bodies, are dispersed throughout the sarcoplasm. Microtubules lie parallel and just beneath the sarcolemma which faces the hemocoele. Microtubules contact and orient perpendicularly to the sarcolemma where 2 newly-fused myocardial cells contact each other. A significant amount of cellular projections containing numerous microtubules, presumably from undifferentiated pericardial cells, occur around the entire perimeter of the myocardium, and are in contact (fasciae adherentes) with the myocardial basal lamina (surface coat) and the sarcolemma (figure 1). The function of such an intercellular interaction is unknown at the present time; however, they may be responsible for the cardioblast assuming their crescent shape. These projections completely disappear by the time of eclosion. At 7 dpo Z-bands and thick and thin filaments can be identified just below the sarcolemma which faces the hemocoele (figure 1). The organized myofilaments and Z-bands are oriented in the longitudinal axis of the myo-