Biochemical Pharmacology, Vol. 19, pp. 3045–3047. Pergamon Press, 1970. Printed in Great Britain

Age and the sensitivity of chicks to the delayed neurotoxic effects on some organophosphorus compounds

(*Received* 3 *March* 1970; *accepted* 24 *June* 1970)

IT IS well known that some organophosphorus esters produce delayed neurotoxic effects in man, hen and other species. Ataxia develops in adults 10–14 days after administration of the compound and is very prolonged: it is quite distinct from the comparatively brief period of muscle weakness associated with acute anti-cholinesterase effects.

When the delayed neurotoxic effect of a single dose of di-iso-propylphosphorofluoridate (DFP) was first studied on hens it was noted that young chicks were not sensitive.[1] The insensitivity of chicks to the same lesion produced by tri-orthocresylphosphate (TOCP) was also noted by Bondy *et al.*,[2] who found that a single dose of 1 g/kg TOCP by mouth had no effect on chicks aged 10, 20, 30, 40 or 50 days, but did produce the delayed ataxia when given to chicks aged 72 or 100 days. Since 1953, a number of different experiments have been done in this laboratory in which chickens of different ages have been given a single dose of DFP and these are recorded here for the first time. Recent work on adult hens treated with neurotoxic organophosphorus compounds has shown that a characteristic protein fraction in the brain is phosphorylated within a few hours of dosing.[3] The protein has been shown to be an esterase.[4] This esterase is present in chick brain and is inhibited after a single dose of neurotoxic organophosphorus compound, although no ataxia develops. A possible explanation is that enzyme inhibition does not persist—due either to more rapid reactivation or synthesis of new enzyme. The persistence of inhibition and the effect of repeated doses has therefore been investigated.

Materials and methods

Young chicks of various breeds were purchased commercially and reared in the laboratory on stock diets until tested. Monoorthocresyl diphenyl phosphate (MOCP) prepared by Dr. H. Bondy (Coalite Co. Ltd.) was given undiluted by mouth. DFP (Boots Pure Chemical Co.) and Butafox (*N,N'*-di-*n*-butylphosphorodiamidic fluoride) supplied by C.D.E.E. Porton, were injected subcutaneously as freshly prepared solutions in 10 per cent ethanol. Twenty min before each injection of DFP or Butafox each bird received 10 mg/kg atropine and 0·1 mg/kg eserine in aqueous solution by subcutaneous injection. The eserine protects the cholinesterases from irreversible inhibition and was particularly useful in experiments where repeated doses of direct-acting inhibitors were given.

Control birds for each group were observed and their weights regularly recorded. The neurotoxic disability was graded as follows: O, No effect; A, Slightly abnormal gait; B, Severely abnormal gait; C, Can stand but frequently collapses; D, Unable to stand. "Neurotoxic" esterase was assayed in homogenates of whole brain using the differential assay with phenyl phenylacetate substrate as described by Johnson.[4]

Results

A single dose of 1·0 mg/kg DFP to hens aged 12 months or more will invariably produce disability (Grade C or worse) within 10–14 days. Table 1 shows that chicks (7–49 days old) may be injected with DFP (2–5 mg/kg) without any disability appearing; during the period 60–100 days from hatching chicks show a striking increase in their susceptibility to the neurotoxic effects of DFP. Furthermore, when they are first dosed at 100–130 days of age the effects are both more severe and more long lasting.

For repeated dosing of young birds two neurotoxic compounds were chosen which do not produce such severe cholinergic effects as DFP. These were Butafox a direct enzyme inhibitor which is neurotoxic to adult birds at doses of 0·3 mg/kg or less,[5] and MOCP a triarylphosphate requiring metabolic conversion to form the active neurotoxic compound. The certain single neurotoxic dose of MOCP for adult hens was 0·1 ml/kg by mouth.[6] Table 2 shows that when the dose of either compound is repeated several times at short intervals then young chicks develop ataxia. The discrepant marginal response of chicks given four and five doses of Butafox respectively (Table 2) is curious but the difference was not great. The Table also shows that the condition in the chick tends to regress quite quickly except in the most severe cases: this contrasts with very slow regression in adults. Severely ataxic chicks had

TABLE 1. DEGREE OF DELAYED NEUROTOXIC DAMAGE IN CHICKS GIVEN A SINGLE DOSE OF DFP AT DIFFERENT AGES

Age at dosing (days)	Dose (mg/kg)	No. of chicks	Disability (No. of chicks) at different times after dosing
7	5	5	0 (5) up to 30 days
28	2	6	0 (6) up to 42 days
42–49	2	27	0 (27) up to 20–30 days
55–59	2	20	A (3) up to 60 days 0 (17)
62–63	2	10	A (7) up to 18 days B (3)
66–70	2	15	B (15) at 12 days, A (15) at 30 days
70	5	5	B (4) at 15 days, A (5) at 30 days 0 (1)
76–77	2	19	B (13) at 15 days: (A (13) at 70 days; 0 (6)) A (6)
82	2	8	B (8) at 15 days and at 44 days
100	2	11	D (8) at 14 days C (3)
130	5	5	C (5) at 12 days: D (3) at 21 days; C (2)
200	5	3	D (3) at 17 days

All birds were given eserine/atropine before DFP and disability was assessed as described in the materials and methods section.

TABLE 2. THE EFFECT ON CHICKS OF REPEATED DOSES OF BUTAFOX OR MOCP GIVEN AT 2–3 day INTERVALS

		Dosing schedule							
Day No.		Butafox (mg/kg)				MOCP (ml/kg)			
1	0·3	0·3	0·3	0·3	0·3	0·5	0·5	0·5	0·5
4	0·2	0·2	0·2	0·2	0·2		0·1	0·1	0·1
5		—	—	—	—		—	0·1	0·1
6		0·2	0·2	0·2	0·2		0·1	0·1	0·1
7			—	—	—		0·1	0·1	0·1
8			0·2	0·2	0·2			0·1	0·1
10				—	—				0·1
11				0·2	0·2				
13					0·2				
		Disability assessment							
15	O,O	O,A,B	A,A,B	O,O,A	O,O,O, A,A	O, A	B	C, B, C	B, C
22	O,O	O,O,A	A,A,B	O,O,O	C,B,D*,D,C	O, O	D	D*,C, D	D*,C
28	O,O	O,O,A	O,A,A	O,O,O	C,A,–, D,C	O*,O*	D*	–, D*,D	–, C
40	O,O	O,O,O	O,O,O	O,O,O	C,A,–, D,*D			–, –, A	–, A
63					B,O,–, –, D			–, –, O	–, O*

Chicks were initially 32–35 days old. Eserine/atropine was given before Butafox but not before MOCP and disability was assessed as described in the materials and methods section. Each assessment refers to a single chick dosed as indicated.

* Birds were killed at this time and tissues were examined histologically.

TABLE 3. THE EFFECT OF DFP ON THE "NEUROTOXIC ESTERASE" OF HEN AND CHICK BRAIN

	Hens	Chicks
Total number assayed	12	9
Brain weight g (Mean ± S.D.)	3·30 ± 0·16	2·29 ± 0·28
Brain protein content mg/g brain (Mean ± S.D.)	95 ± 8	93 ± 13
Normal activity of "Neurotoxic esterase" (nmole phenol/min/g brain) Mean (and range)	70(68–73)	83(72–96)
Days after DFP dose	% Normal activity	
1	8,9,18	16,23
2	20,31	23
3	29	—
4	53	57
7	62	70
9	50	59

Dosed birds received a single dose of DFP (2mg/kg s.c.) after atropine/eserine as described in Methods. Birds were killed at various times and the esterase activity in homogenates of whole brain was assayed. Chicks were 49 days old when dosed. No chick survivors (3) became ataxic but all hen survivors (4) did.

characteristic lesions in both peripheral nerve and spinal cord. An experiment done many years earlier indicated the importance of close spacing of the repeated doses: a dose of 1 mg DFP/kg at weekly intervals from 10 days after hatching did not cause neurotoxic signs until chicks had reached an age at which they were becoming sensitive to the effect of a single dose.

Table 3 shows that the activity of "neurotoxic esterase" is not greatly different in hen and chick brain; activity in both is reduced to a very low level by a dose of 2 mg/kg of DFP and the rate of return of activity after a single dose is also very similar. This is contrary to our working hypothesis and the essential difference between chick and adult hen in their response to neurotoxic organophosphorus compounds is still not understood. It is possible that phosphorylation of the esterase disturbs neuronal metabolism in the adult more than in the young or that the chick with growing axons has greater ability than the adult to adapt to such disturbance.

The effect of age on the sensitivity to neurotoxic organophosphorus compounds is not confined to chickens; it has been shown that kittens are insensitive to doses of the metabolite of TOCP which are certainly neurotoxic to the adult cat.[7] It would be interesting to know more about other changes which may take place during the critical period when the response of the chick to neurotoxic organophosphorus compounds changes so strikingly.

M.R.C. Toxicology Unit,
Woodmansterne Road,
Carshalton,
Surrey

M. K. JOHNSON
J. M. BARNES

REFERENCES

1. J. M. BARNES and F. A. DENZ, *J. Path Bact.* **65**, 597 (1953).
2. H. F. BONDY, E. J. FIELD, A. N. WORDEN and J. P. W. HUGHES, *Br. J. Ind. Med.* **17**, 190 (1960).
3. M. K. JOHNSON, *Biochem. J.* **111**, 487 (1969).
4. M. K. JOHNSON, *Biochem. J.* **114**, 711 (1969).
5. D. R. DAVIES, P. HOLLAND and M. J. RUMENS, *Biochem. Pharmac.* **15**, 1783 (1966).
6. W. N. ALDRIDGE and J. M. BARNES, *Biochem. Pharmac.* **15**, 541 (1966).
7. J. D. TAYLOR, *Toxic. appl. Pharmac.* **11** (1967).