AN INVESTIGATION INTO THE EFFECTS OF 2:2-BIS-(*p*-CHLOROPHENYL)-1:1-DICHLOROETHANE (D.D.D.) ON THE MOUSE ADRENAL CORTEX

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2:2-Bis-(*p*-chlorophenyl) -1:1-dichloroethane (D.D.D.), an agricultural insecticide and an analogue of D.D.T., was reported by various workers[1–7] to produce severe cytotoxic atrophy of the zona fasciculata region of the adrenal cortex of the dog when administered over prolonged periods. Verne and Wegmann[8] observed histological damage in the rat adrenal cortex after the administration of the compound during a period of 4 to 6 weeks. Brown[9], working on adult rats maintained on a diet containing 0·1 per cent., reported that the animals rapidly developed signs of some adrenal dysfunction, a decreased response to cold stress being one of the symptoms.

On the other hand several workers were unable to demonstrate adrenocortical atrophy in similar experiments on rats and rabbits[2,3,10,11]. Recently Stoner[12] reported failure to produce degeneration of the zona fasiculata after the injection of the drug in oily solution to rats and mice and, in further experiments based on the study of regeneration of the rat adrenal cortex after medullectomy, he showed that the drug did not exert any striking cytotoxic effect. Stoner suggested that the success of experiments in the dog might be due to the greater susceptibility of the adrenal cortex to toxic agents in a species in which spontaneous adrenocortical atrophy can occur[13]. Sheehan, Summers and Nichols[14] reported on its use in Cushings's syndrome and concluded that the drug did not produce any significant clinical effect on the condition nor did it produce any permanent adrenal atrophy.

In view of the confusion and contradiction of reports in the literature it was decided to investigate further the effects of the drug on the adrenal cortex of the mouse to determine whether cortical atrophy could be produced in that species. In a previous publication it has been demonstrated that adrenalectomised mice are hypersensitive to cold stress[15]. It was therefore decided to determine the effect of prolonged administration, both orally and parenterally, on the survival time of mice exposed to cold stress, using the survival time of the treated animals as a relative index of adrenocortical atrophy.

Methods

Groups of white male weanling mice were maintained on a diet of crushed M.R.C. cubes (diet 41) into which had been incorporated 0·1 per cent. of the recrystallised commercial drug (Rhothane); further groups

fed on the cube diet alone were injected subcutaneously thrice weekly with 20 mg./100 g. of body weight as a 2 per cent. solution in arachis oil. The volume of the injected solution was adjusted weekly to allow for the increase in body weight of the treated groups. Control groups of normal untreated animals and groups of untreated and recently adrenalectomised animals were included in the experiment.

The doses were based on studies performed by Haag, Finnegan, Larson, Dreyfuss, Main and Riese[11], who reported that the symptoms of toxicity were a diminished rate of growth and general emaciation occurring with doses considerably less than the lethal dose. In order to ensure that the dose administered in the current experiment did not produce a general toxic effect, groups of mice were maintained on a diet containing 0·1 per cent for the duration of the experiment and their daily body weight and general condition was compared with untreated control animals. Treatment was continued for 6 weeks, the groups of treated, control and adrenalectomised animals being subjected to cold stress (2·5° C. ± 1·5° C.) in a refrigerator at 2 weeks, 4 weeks and 6 weeks from the commencement of the treatment. The procedure for adrenalectomy and subjection of mice to cold stress has been given elsewhere[15].

Results

The mean survival times of groups of 9 to 12 treated, control and adrenalectomised animals stressed at 2, 4 and 6 weeks from commencement of treatment are given in Table I. The standard error of the mean is given for each value.

TABLE I

THE EFFECT ON THE SURVIVAL TIME OF MICE EXPOSED TO COLD STRESS (2·5° C. ± 1·5° C)

Treatment	Mean survival time of groups of 9 to 12 mice expressed in hours ± standard error of the mean		
	Duration of treatment		
	2 weeks	4 weeks	6 weeks
Adrenalectomised control group.	3·05 ± 0·31	3·11 ± 0·37	3·18 ± 0·23
Normal control group.	5·40 ± 0·38	5·45 ± 1·1	5·08 ± 0·75
Oral 0·1 per cent. of drug in diet.	5·50 ± 0·84	5·25 ± 0·81	5·60 ± 0·92
Parenteral 20 mg./100 g. of body weight thrice weekly subcutaneously.	6·35 ± 0·54	6·60 ± 1·2	6·04 ± 0·89

Statistical treatment of the results given in Table I shows that although after 2, 4 and 6 weeks' treatment the mean survival times of the untreated adrenalectomised groups were significantly different from their respective normal control groups, the treated groups at no time differed significantly from the normal controls. It was therefore considered that at no stage of the experiment did the treatment simulate the condition of adrenalectomy; i.e., it appeared that no significant degree of adrenocortical atrophy had taken place.

General toxic symptoms due to the oral administration of the drug were not evident in the treated groups as indicated by the general condition and the increase in body weight of the animals over the period of the investigation, as compared with that of the untreated controls.

To provide additional information concerning the effect of the drug on the adrenal glands themselves as distinct from the effects of the drug on a function of the glands (the response of the animal to cold stress) the adrenals were removed from each animal, in the above experiment, as soon as possible after death. The adrenals after removal were preserved in formol-saline for a suitable period and when fully hardened they were carefully dissected free from fat and other extraneous tissue and finally weighed. The combined weight of the right and left adrenal in each animal was expressed in terms of mg. of adrenal gland per 100 g. body weight of the animal.

Table II shows the results of this investigation; the mean adrenal weight in mg. per 100 g. of body weight for a group of 9 to 12 animals is given together with the standard error of the mean.

TABLE II

THE EFFECT ON THE ADRENAL WEIGHTS OF MICE

Treatment	Mean adrenal weight of groups of 9 to 12 mice expressed as mg. of adrenal per 100 g. of body weight ± standard error of the mean		
	Duration of treatment		
	2 weeks	4 weeks	6 weeks
Normal control group.	21·55 ± 1·21	22·33 ± 1·26*	18·29 ± 1·36
Oral 0·1 per cent. of drug in diet.	21·90 ± 0·86	19·76 ± 0·94	16·85 ± 0·76
Parenteral 20 mg./100 g. of body weight thrice weekly subcutaneously.	22·18 ± 0·82	21·12 ± 1·51	17·32 ± 1·63

Statistical treatment of the results given in Table II show that treatment for either 2, 4 or 6 weeks failed to produce a significant decrease in the mean adrenal weight per 100 g. of body weight of the treated groups as compared with that of the normal controls. It was therefore assumed that the drug had failed to produce any significant adrenal atrophy.

DISCUSSION

The results of this investigation indicate that, under the experimental conditions observed, the drug administered in the diet in a concentration of 0·1 per cent. or injected subcutaneously thrice weekly in a dosage of 20 mg./100 g. of body weight, for periods of up to 6 weeks, failed to produce an effective atrophy of the mouse adrenal cortex as indicated by the two criteria, the survival time of the treated animal exposed to cold stress (2·5° C. ± 1·5° C.) and the adrenal weights of the treated groups.

The conclusions are in agreement with previous published data based on experiments with rabbits, rats and mice[2,3,10,11,12]. They are, however,

contrary to the results of experiments on dogs [1–7] and also to the decreased response to cold stress in treated rats observed by Brown[9] using similar experimental procedure to that detailed above.

SUMMARY

1. White male mice maintained on a diet containing 0·1 per cent. of 2:2-bis-(*p*-chlorophenyl)-1:1-dichloroethane and mice injected subcutaneously thrice weekly with an oily solution at a dosage of 20 mg./100 g. of body weight, for periods up to 6 weeks failed to exhibit a significantly decreased response to cold stress as compared with the untreated controls.

2. The drug did not significantly decrease the adrenal weights of the treated groups.

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