Failure of Phosphorylated Hesperidin to Influence Fertility in Rodents

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Phosphorylated hesperidin has been reported to have a powerful inhibitory effect upon fertility when administered orally in man (*1*) and orally or intraperitoneally in rats (*2*). In contrast to these findings, we have observed no antifertility action in rats or mice by either route in several series of tests.[1]

The phosphorylation of hesperidin may lead to a great variety of products, depending upon the method and conditions employed. In the above reports no definitive or analytical data were given for the material used in the tests. In June 1952 we prepared several samples of phosphorylated hesperidin using the technique of Beiler and Martin (*3*). One of these products (designated Ortho) was administered to rats in the well-controlled experiment described below to determine whether it had any effect upon the pregnancy rate. Later in the year, through the kindness of B. F. Sieve, we received samples of the material used by him in his extensive tests in the human being (*1*). This compound also was subjected to the same tests.

The test for interference with the pregnancy rate was conducted as follows. Mice or rats were pretreated with the experimental compound for 5–7 days before the mating period began. Females and males were grouped in a ratio of at least 3:1. A mating period of 15–28 days followed, during which time accurate records were kept of the weight, food consumption, and condition of the animals. This period was followed by a postmating period of 20–25 days. Records were kept of the number and size of litters and of the date of parturition. In our colony over a period of years the fertility rate for our strains of animals under no treatment has been 85–90% for rats and 70% for mice; rats and mice fed by stomach tube, about 50–60%, and for mice receiving intraperitoneal injections, about 40–50%. Nevertheless, complete controls were used, in which the control group was given water or saline by stomach tube or injection to parallel those experimental groups undergoing such treatment.

TABLE 1

FERTILITY RATES IN RODENTS

Material	Species	Route	Sex treated	Level mg/kg/day	Nos. of ♀'s littering		% ♀'s littering	
					Control	Exptl	Control	Exptl
Phosphorylated Hesperidin (Ortho)	Mouse	Oral (stomach tube)	Both	100–125	4/7	12/16	57.0	75.0
	Rat	Oral (diet)	Both	100–125	7/8	14/16	87.5	87.5
Phosphorylated Hesperidin (Sieve)	Mouse	Oral (diet)	Both	50–70	10/15	11/15	67.5	73.3
			♀	50–70	10/15	12/15	67.5	80.0
			♂	50–70	10/15	13/15	67.5	86.7
		I.P. injection	Both	40–50	6/15	7/14	40.0	50.0
			♀	40–50	6/15	9/15	40.0	60.0
			♂	40–50	6/15	6/15	40.0	40.0
	Rat	Oral (stomach tube)	Both	100–125	4/8	8/15	50.0	53.3

Table 1 summarizes the nature and results of the trials. The levels of compound fed were considerably higher than those given by other investigators. Even the lowest oral feeding level in mice exceeded by a factor of some 5 the daily mg/kg level given in the human trials. Examination of the results indicates beyond question that there has been no interference with pregnancy through the oral or intraperitoneal administration of these compounds. Indeed, there appears to be a slight increase in fertility of the treated animals, but the χ^2 values show that the difference between experimental and control groups lacks statistical validity. These results are obviously at variance with those reported for the human being. The reasons for this are not immediately evident, although the possibility may be considered that there exists a wide species difference between rodent and man in the antifertility effects of phosphorylated hesperidin.

Sieve (*1*), commenting on the antifertility effects of phosphorylated hesperidin in the mouse, stated that "it can be concluded from actual experiments that there is definite impairment of fertility of the mice under treatment of phosphorylated hesperidin." However, analysis of the data presented in his article reveals that the P value calculated according to Mainland (*4*) is so large as to make this conclusion dubious

[1] Just after this paper was prepared for publication, Chang and Pincus (SCIENCE, 117, 274, 1953) reported similar failure to achieve an antifertility effect in rats with a commercially prepared sample of the compound.

on the basis of the small number of animals used. The data of Martin and Beiler (*2*) cannot be subjected to analysis as they appear in their report.

The relationship of the phosphorylated hesperidins used in fertility trials to structure, antihyaluronidase potency, capillary permeability, phosphorus content, species, and mode of administration remains obscure, since significant correlations between these factors and fertility inhibition have yet to be reported.

References

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The Toxicity of Chlordane Vapors

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The vapor toxicity to warm-blooded animals of the insecticide chlordane (1,2,4,5,6,7,8,8-octachloro-4,7-methano-3a,4,7,7a-tetrahydroindane) has been a subject of controversy for the past several years.

Experimental evidence presented in this paper offers an explanation for the variable results shown by previous authors and shows a significant lack of toxicity to mice resulting from chlordane vapors.

Lehman (*1*) was unable to maintain pigeons placed in a room which had been treated with chlordane, although it was first thoroughly scrubbed and aired. Frings and O'Tousa (*2*) reported that mice could not survive in air which had first passed through chlordane. Injury was also noted in mice that had been confined to a chamber whose sides had been treated with chlordane. On the other hand, Nickerson and Radeleff detected no injury to pigeons (*3*) or leghorn cockerels and pullets (*4*) that had been confined 30–60 days in a box whose inner surfaces had been treated with chlordane. Since the only controlled investigation indicating significant toxicity of chlordane vapors to warm-blooded animals is that of Frings and O'Tousa, the author undertook, with their cooperation, an investigation patterned exactly after theirs. The only uncontrollable variable was the source and time of manufacture of the chlordane.[1] Twenty female Swiss albino mice in a wire mesh cage were placed in a treatment chamber 12 × 20 × 36 in. and subjected to 14 days of continuous exposure to a current of air (18 ml/sec) which had first passed through 105 ml of chlordane in a saturation train. No deaths occurred nor did any mice show signs of anorexia, blindness, or loss of coordination. At autopsy, organs and tissues were normal.

1 All chlordane used in the present investigation was supplied as technical chlordane (1068 chlordane) by the Velsicol Corporation, Chicago, Illinois, and as AAEE Reference Standard chlordane by the Wisconsin Research Foundation, Madison, Wis.

The experiment was repeated 4 times, once using AAEE Reference Standard chlordane for 14 days and 3 times using three different current production batches of chlordane for 25 days each. No symptoms of toxicity were noted, and no deaths occurred. No gross pathological changes were observed, but microscopically the liver showed minimal changes such a some reticulation and oxyphilia of the cytoplasm, and the lungs showed slight congestion with some proliferation of bronchiole lining cells. Kidneys were normal. These results were at such variance with those reported by Frings and O'Tousa that further investigation was certainly indicated.

Early samples of chlordane frequently gave off irritating volatile materials but in production, this characteristic has long since been eliminated by the more complete removal of unreacted ingredients, chief among which was hexachlorocyclopentadiene. Possibly the chlordane (Octaklor, 1947 production) used by Frings and O'Tousa contained a considerable quantity of unreacted volatile material which may have been primarily responsible for the reported symptoms and high rate of mortality among the mice. In order to test this hypothesis, hexachlorocyclopentadiene[2] as utilized in chlordane production was added in varying quantities to 1068 chlordane being sold commercially. Experiments were then conducted in which female mice were subjected to air passing through these mixtures (Table 1).

TABLE 1

MORTALITY AMONG MICE SUBJECTED TO VAPORS OF CHLORDANE (CH) PLUS ADDED HEXACHLOROCYCLOPENTADIENE (HX)

		No. Mice	Mortality ratio	Comments
I	Ch, 90% Hx, 10%	20	20/20	All dead within 24 hr
II	Ch, 92.5% Hx, 7.5%	20	20/20	All dead within 48 hr
III	Ch, 95% Hx, 5%	20	20/20	Symptoms present at 4 days Deaths between 10th and 25th days
IV	Ch, 97.5% Hx, 2.5%	20	6/20	Symptoms present at 4 days Deaths between 20th and 25th days
V	Ch, 100% Control	20	0/20	No symptoms of toxicity.

In every case except that of the chlordane control, external symptoms followed the same pattern described by Frings and O'Tousa, namely, cessation of feeding and drinking, huddling together, lethargy, apparent blindness, and loss of coordination. The mixtures were also irritating to the eyes of workers in the laboratory. Onset and severity of symptoms were directly proportional to the volume of added hexachlorocyclopentadiene.

2 Supplied by the Velsicol Corporation.