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20. Supported by PHS grant MH 16674-02. J.-L.D. is a fellow of the Foundations' Fund for Research in Psychiatry, M.O.H. is an International Postdoctoral Fellow of the PHS. We thank Dr. James Macon for developing the separation methods in this laboratory. We thank Drs. Seymour S. Kety, Ross J. Baldessarini, Manfred Karobath, and Charles Popper for their advice and criticism.

* Permanent address: Instituto de Investigaciones Biomédicas, UNAM, and Instituto Nacional de Neurología, SSA; Mexico City, Mexico.

† Present address: Departments of Medical Chemistry and Psychiatry, University of Helsinki, Helsinki, Finland.

1 July 1971

Nonconversion of o,p'-DDT to p,p'-DDT in Rats, Sheep, Chickens, and Quail

Abstract. *The finding of appreciable quantities of* p,p'*-DDT after feeding* o,p'*-DDT to rats led to the proposal of a theory that an isomeric metabolic conversion occurs. The presence of* p,p'*-DDT as an impurity in supposedly pure samples of* o,p'*-DDT is the correct explanation for the appearance of* p,p'*-DDT. Purified* o,p'*-DDT and* ^{14}C*-labeled* o,p'*-DDT yielded no data to support the idea that* o,p'*-DDT is converted to the* p,p'*-DDT isomer.*

Klein *et al.* (*1*, *2*) reported the supposed isomeric conversion of *o,p'*-DDT to *p,p'*-DDT in the rat (*3*). This conversion was based on the finding of appreciable quantities of *p,p'*-DDT after feeding *o,p'*-DDT to rats. Such a conversion would involve either (*1*) splitting off the *o*-chlorophenyl group from the ethane chain with subsequent recombination to form a *p,p'* molecule or (*2*) replacement of the *o*-Cl by H, and chlorination of the para position. Both of these mechanisms appear to be unlikely biological metabolic reactions (*4*). The purpose of our study was to demonstrate that the conversion of *o,p'*-DDT to *p,p'*-DDT does not occur biologically and to provide a more logical explanation for the appearance of *p,p'*-DDT after feeding pure *o,p'*-DDT.

An explanation for the supposed conversion can be given by the presence of *p,p'*-DDT as an impurity in the *o,p'*-DDT which was fed. When a dilute (1 μg/ml) solution of *o,p'*-DDT (over 99 percent pure, Aldrich) was analyzed by gas-liquid chromatography (GLC) (*5*), a single peak with a retention time characteristic of *o,p'*-DDT is observed. When, however, a concentrated (100 μg/ml) solution was analyzed, the presence of *p,p'*-DDT was also noted. Thin-layer chromatography (TLC) of an equivalent sample of this solution and subsequent GLC analysis resulted in essentially quantitative recovery of *p,p'*-DDT from this supposedly pure *o,p'*-DDT solution. Thus, the simple step of analyzing a concentrated solution of *o,p'*-DDT, rather than an extremely dilute one, demonstrated the presence of *p,p'*-DDT.

During the past 2 years, we have conducted experiments in which *o,p'*-DDT was fed to rats (*Rattus norvegicus*), sheep (*Ovis aries* L.), Japanese quail (*Coturnix coturnix japonica*), and chickens (*Gallus domesticus*). Analysis of body lipid revealed that *o,p'*-DDT was the major pesticidal residue but that significant quantities of *p,p'*-DDT were found (Table 1). In all cases, enough *p,p'*-DDT was ingested as an impurity to account for the *p,p'*-DDT found in the animals at the end of the experiment.

Analysis of three batches of commercial *o,p'*-DDT are shown in Table 2. These batches (Aldrich) contained much more *p,p'*-DDT than did earlier samples from the same source, and they contained significantly more *p,p'*-DDT than a sample obtained from the Pesticide Chemicals Branch of the U.S. Department of Agriculture (USDA-ENT 3983). The USDA *o,p'*-DDT has been prepared by isolation from technical DDT 25 years ago in a study of the composition of technical DDT (*6*).

As additional proof that *p,p'*-DDT is not formed from *o,p'*-DDT, pure *o,p'*-DDT was prepared for subsequent feeding trials. Samples (60 mg) of commercial *o,p'*-DDT (Aldrich) were chromatographed on 150 g of aluminum oxide (Merck) in a glass column [27 mm (inside diameter) by 300 mm]. The chromatogram was developed with 300 ml of *n*-hexane, and each 10-ml fraction was analyzed by GLC for the presence of *o,p'*-DDT, *p,p'*-DDT, and *p,p'*-DDE. Those fractions containing only *o,p'*-DDT, usually fractions 13 to 15, were combined to provide pure *o,p'*-DDT for the feeding trials. By this technique, a product was obtained which was 99.974 percent pure *o,p'*-

Table 1. *p,p'*-DDT in rats, sheep, Japanese quail, and chickens after feeding impure *o,p'*-DDT.

Species	*o,p'*-DDT in diet (ppm)	Time (days)	*p,p*-DDT impurity (%)	*p,p'*-DDT intake (mg)	*p,p'*-DDT retained (mg)	Retention (%)
Rat	20	98	0.4	0.118	0.065	55
	40	98	0.4	0.235	0.116	49
	100	22	1.3	0.453	0.322	71
Sheep-ewe	10	120	0.5	8.400	0.625	7
Lamb	10	87	0.5	2.958	1.012	34
Japanese quail	100	45	0.4	0.144	0.104	72
Chicken	150	98	0.6	9.700	5.004	52

DDT (USDA-DCRB-1; see Table 2).

Female rats (215 to 230 g) were fed a control diet or a diet containing *p,p'*-DDT (Aldrich), 100 ppm; *o,p'*-DDT (98.8 percent, Aldrich), 100 ppm; or *o,p'*-DDT (99.974 percent, USDA-DCRB), 100 ppm. After 22 days on the experimental diets, the rats were killed and samples of body lipid and the whole body carcass were analyzed for pesticide residues. *o,p'*-DDT uniformly labeled with ^{14}C (impure mixture, Nuclear-Chicago) was purified by TLC on alumina, and the isolated radioactive *o,p'*-DDT was dissolved in olive oil. Forty-eight hours before the scheduled killing time, the rats receiving the diet containing the Aldrich *o,p'*-DDT received about 5 μc of the ^{14}C-labeled *o,p'*-DDT by stomach tube.

When impure *o,p'*-DDT (98.8 percent, Aldrich) was fed to adult female rats, a sizable amount of *p,p'*-DDT was found in the body lipid (Table 3). The presence of this *p,p'*-DDT could be the basis for a faulty conclusion that this *p,p'*-DDT was formed from the *o,p'*-DDT which was fed. The group fed our purified *o,p'*-DDT (99.974 percent pure) demonstrates conclusively that no conversion occurs (Table 3). The *p,p'*-DDT level in the body fat of these rats was very similar to control levels. If impure *o,p'*-DDT is fed to rats, pesticide residues contain *p,p'*-DDT. If pure *o,p'*-DDT is fed to rats, no *p,p'*-DDT is found.

The lipid extract from the rats fed the impure *o,p'*-DDT and injected with radioactive *o,p'*-DDT was subjected to TLC on alumina, with hexane being used to develop the chromatogram. In this system, *o,p'*-DDT has a greater R_F than *p,p'*-DDT. Approximately 1 percent of the radioactivity recovered from the body lipid was *p,p'*-DDT (Table 4, column 6), a value very similar to the amount originally present in the radioactive *o,p'*-DDT which was fed (column 3). In order to demonstrate further the identity of the radioactive TLC spots as *o,p'*-DDT and *p,p'*-DDT, the extracts and dosing solution were subjected to dehydrohalogenation in KOH to convert the compounds to *o,p'*-DDE and *p,p'*-DDE. The DDE products were then subjected to TLC on alumina in a mixture of 2 percent acetone and hexane. Under these conditions, there is a reversal in the retention times of the *o,p'*- and *p,p'*- isomers, and *p,p'*-DDE has a greater R_F value than *o,p'*-DDE has. Again, the radioactivity recovered as *p,p'*-DDE was similar to that found in the dosing solution and provided no evidence of a conversion of radioactive *o,p'*-DDT to radioactive *p,p'*-DDT. It is also probable that part of the 1 percent of the radioactivity appearing as *p,p'*-DDT or *p,p'*-DDE during the TLC-reversal steps is due to fast-running or tailing of the *o,p'*-DDT. The 1 percent of the radioactivity in the *p,p'*-DDT spot is in marked contrast to the amount of unlabeled *p,p'*-DDT recovered in the body lipid of these same rats which had also been fed the Aldrich *o,p'*-DDT [28.5 percent of the total DDT residue was present as *p,p'*-DDT by GLC (Table 3)]. The administration of radioactive *o,p'*-DDT thus demonstrated, by an additional independent means, that no isomeric conversion of *o,p'*-DDT to *p,p'*-DDT occurred.

Table 2. Analyses of *p,p'*-DDT contamination in samples of *o,p'*-DDT.

Sample	*o,p'*-DDT (%)	*p,p'*-DDE (%)	*p,p'*-DDT (%)
	Aldrich		
092381	98.784	0.026	1.190
101591	98.639	0.023	1.338
110407	98.915	0.026	1.059
	USDA-ENT		
3983	99.477	0.180	0.344
	USDA-DCRB		
1	99.974	0.009	0.017

Since the original reports of Klein *et al.* (*1, 2*), there have been two reports which have directly claimed that *o,p'*-DDT is transformed into *p,p'*-DDT (*7, 8*). Ecobichon and Saschenbrecker in 1968 (*7*) stated that they have shown that *o,p'*-DDT is converted to *p,p'*-DDT but no data were given. In 1969 French and Jefferies (*8*) fed 250 mg of *o,p'*-DDT to homing pigeons (*Columbia livia*) and found *p,p'*-DDT and *p,p'*-DDE in the body fat. A contamination of 0.5 percent *p,p'*-DDT in the *o,p'*-DDT fed would have supplied each pigeon with 1250 μg of *p,p'*-DDT, an amount much greater than the total content of *p,p'*-DDT and *p,p'*-DDE recovered.

Two earlier studies failed to yield evidence of the conversion of *o,p'*-DDT to *p,p'*-DDT (*9, 10*). Mendel *et al.* (*9*) attempted to determine the location of the site of the unusual isomeric conversion of *o,p'*-DDT to *p,p'*-DDT. They incubated *o,p'*-DDT both aerobically and anaerobically with *Aerobacter aerogenes*, but recovered only unchanged *o,p'*-DDT and *o,p'*-DDD. They concluded that this coliform organism was not the mediator of the conversion in the gut of the rat. Recently, Lamont *et al.* (*10*) fed mallards (*Anas platyrhynchus*) *o,p'*-DDT, but since there was no increase in *p,p'*-DDT or its metabolites, they concluded that "the biological isomeric transformation evidently did not take place."

We now have demonstrated conclusively that when pure *o,p'*-DDT is fed to rats, there is no conversion to *p,p'*-DDT. In addition, radioactive *o,p'*-DDT fed to rats did not give rise to radioactive *p,p'*-DDT. Conversely, we have also shown that, if impure *o,p'*-DDT containing small amounts of *p,p'*-DDT is fed to rats, *p,p'*-DDT accumulates. Our data are completely consistent with the view that the supposed isomeric conversion does not occur. The original assumptions of Klein *et al.* (*1, 2*) are based on faulty data concerning the purity of *o,p'*-DDT, and the major conclusion is incorrect.

The correct explanation for the rapid disappearance of *o,p'*-DDT when it is fed and for the appearance of *p,p'*-DDT can now be given. *o,p'*-DDT, the major constituent of commercial

Table 3. Pesticide residues after feeding *p,p'*-DDT, impure *o,p'*-DDT, or pure *o,p'*-DDT.

Group	Body fat concentrations (μg/g)				
	p,p'-DDE	*o,p'*-DDD	*o,p'*-DDT	*p,p'*-DDD	*p,p'*-DDT
Control	0.8		0.1		0.6
p,p'-DDT	36.4			5.7	413.8
o,p'-DDT, Aldrich	2.0	2.6	36.6		14.6
o,p'-DDT, purified	0.9	3.5	72.7		1.0

Table 4. Administration of radioactive *o,p'*-DDT to rats. Values are means of four rats.

Thin-layer chromatography of [^{14}C]DDT	Total dose			Retained in body		
	o,p'-DDT (count/min)	*p,p'*-DDT (count/min)	*p,p'*-DDT (%)	*o,p'*-DDT (count/min)	*p,p'*-DDT (count/min)	*p,p'*-DDT (%)
Untreated	10,086,000	135,000	1.3	793,456	6980	0.9
After dehydrohalogenation	10,117,000	93,500	0.9	831,508	5248	0.6

o,p'-DDT preparations, by virtue of the open positions on the ring bearing the *o*-Cl, is rapidly converted to hydroxy and methoxy metabolites that are rapidly excreted in the feces (*11*). In contrast, *p,p'*-DDT, the minor impurity, is relatively inert metabolically and accumulates in the lipid of the animal body.

Thus, this differential metabolic behavior leads to the simultaneous disappearance of the major component of relatively pure samples of *o,p'*-DDT, with the concomitant appearance of the minor constituent, the *p,p'*-DDT impurity. There is no existing chemical or biological information or data to support the idea that *o,p'*-DDT is converted to the *p,p'*-DDT isomer.

JOEL BITMAN
HELENE C. CECIL, GEORGE F. FRIES
Animal Science Research Division, Agricultural Research Service, Beltsville, Maryland 20705

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11 June 1971; revised 14 July 1971

Methamphetamine-Induced Insulin Release

Abstract. *Administration of methamphetamine or amphetamine to rats and mice produces a rapid increase in the level of immunoassayable plasma insulin not attributable to hyperglycemia. While in the mouse this release of insulin is followed consistently by a profound hypoglycemia, in the rat this response is variable. Studies in vitro demonstrate that insulin is released by a direct effect of methamphetamine on the pancreas.*

Toxicity of *d*-amphetamine in rats and mice is manifested by enhanced motor activity and excitement, followed by depression. The latter state is particularly noted in acutely aggregated mice rather than in animals caged individually (*1*), and it is during this stage of depression that many of the animals die. This terminal lethargy resembles the manifestations of hypoglycemia, and indeed Moore *et al.* noted that such mice showed a marked decrease in blood glucose after intraperitoneal injection of *d*-amphetamine (*1*). These authors attributed the hypoglycemia to stress factors and a syndrome of experimental shock.

Amphetamine and its derivatives, however, are closely related structurally to tranylcypromine, a monoamine oxidase inhibitor that has been demonstrated to cause insulin release and hypoglycemia in the mouse (*2*). This similarity suggested that amphetamine may also produce hypoglycemia by releasing endogenous insulin, and the present studies were designed to evaluate this possibility by examining the effect of methamphetamine and amphetamine on insulin and glucose levels in mice and rats.

Initial studies were carried out on male Sprague-Dawley rats weighing 180 ± 10 g, which were housed in cages 9 by 8 by 6 inches (1 inch = 2.54 cm), two or three per cage; they were allowed free access to food and water. Four hours prior to drug administration, food was removed and animals were aggregated seven to eight per plastic cage (18 by 10 by 6 inches). Methamphetamine hydrochloride (30 mg/kg) in 0.5 ml of normal saline or saline alone was injected intraperitoneally. Swiss-Webster mice weighing 24 to 26 g were used in other studies. Similarly, food was removed 4 hours prior to injection, and the mice were injected intravenously with either methamphetamine (7.5 mg/kg) in 0.2 ml of saline or saline alone. They were then housed four animals per cage (11 by 6 by 5 inches). At appropriate intervals, rats and mice were killed by decapitation, and blood from the carotid arteries was collected in heparinized beakers. Concentrations of blood glucose in barium hydroxide–zinc sulfate filtrates and of plasma glucose were measured by a glucose oxidase method (*3*). Plasma insulin was measured against mouse insulin standards by a modification of a radioimmunoassay method in which the separation of bound and free insulin was performed by precipitating the bound insulin with a goat antiserum to guinea pig gamma globulin (*4*). Methamphetamine had no effect on the measurement of insulin in this system.

Studies of insulin release in vitro were carried out with mouse pancreas tissue by using a slight modification of a previously described incubation system (*5*). In the present studies, after an initial 15-minute preincubation, each piece of mouse pancreas underwent two sequential 30-minute incubations, and portions of each incubation medium were assayed for insulin. In all studies, glucose and insulin values for animals treated with drugs were compared with control animals injected simultaneously with saline, by use of Student's *t*-test.

Figure 1 illustrates the effect of methamphetamine (30 mg/kg, intraperitoneally) on insulin release in the rat. After methamphetamine injection, plasma insulin values rose rapidly, increasing three- to fourfold over control

Table 1. Time relation of insulin and glucose levels in mice. Swiss-Webster mice were injected intravenously with methamphetamine (7.5 mg/kg) in 0.2 ml of saline or an equal volume of saline alone as described in the text. After decapitation, blood from the carotid arteries was assayed for glucose and insulin as described. Each value represents the mean ± standard error of the mean (S.E.M.) of six observations and is compared (Student's *t*-test) with a control group killed at the same time.

Experimental group	Time after injection (minutes)			
	0	2.5	5	10
	Insulin (μunit/ml)			
Control (saline)	25 ± 6	14 ± 3	21 ± 6	27 ± 6
Methamphetamine	25 ± 6	44 ± 7*	85 ± 24†	36 ± 7‡
	Glucose (% zero time control)			
Control (saline)	100	90 ± 10	99 ± 8	95 ± 5
Methamphetamine	100	101 ± 4‡	92 ± 5‡	94 ± 6‡

* $P < .01$, different from control. † $P < .05$, different from control. ‡ Not significantly different from control.