TABLE I. Response of Stock Diet Rats to Various Doses of Acetyl-β-Methylcholine.

mg ABM–Cl/ 100 g body wt	Bolus travel (% of total length)	Effect on lacrimation and salivation
0	65 ± 7*(3)†	0
.02	68 ± 13 (3)	0
.05	67 ± 13 (3)	0
.1	69 ± 19 (3)	L‡
.2	56 ± 16 (3)	L and S
.4	68 ± 14 (3)	Copious L and S

* Stand. dev.
† No. of determinations.
‡ L = lacrimation; S = salivation.

TABLE II. Effect of Acetyl-β-Methylcholine on Intestinal Motility of Potassium Deficient and Control Rats.

	Bolus travel (% of total length)	
Dietary K	Non-treated	.2 mg ABM–Cl /100 g body wt
.01%	26 ± 12*(8)†	66 ± 19 (7)
.5 %	70 ± 18 (9)	72 ± 16 (10)

* and † as in Table I.

intestine to respond. This may indicate neuropathology, a decrease in muscle tone under vagal control, impaired neuromuscular transmission, or a decreased sensitivity of the intestinal musculature to parasympathetic stimulation. *In vitro* studies have shown that potassium stimulates glycolysis which is catalyzed by an extract of rat brain(8), that the addition of KCl to brain slices increases aerobic glycolysis(9), and that increasing the potassium content of Locke's solution results in increased synthesis of acetylcholine by brain slices(10). It has also been observed that the exclusion of KCl from Kreb's solution increases the threshold stimulus for mammalian A nerve fibers(11). These data indicate that potassium has an important role in the physiology of nervous tissue. There is also evidence which indicates that potassium is important in the transfer of nerve impulses across the neuromuscular junction(12) and that potassium sensitizes muscles to acetylcholine(13).

Summary. Acetyl-β-methylcholine was observed to stimulate intestinal motility in potassium deficient rats but did not affect the intestinal motility of rats receiving complete diets.

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Estrogenic Action of Some DDT Analogues.* (19903)

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Burlington and Lindeman(1) found chronic 2,2'-bis-(p-chlorophenyl)-1,1,1-trichloroethane (DDT) administration to effect inhibition of

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† Predoctoral Fellow, National Science Foundation.

TABLE I. Dosage and Estrus Response of DDT and Analogs.

Drug	DDT			DMDT			DHDT				
Dosage in mg	15	30	45	15	30	45	10	15	20	25	45
No. of animals tested	6	6	7	6	6	6	6	6	6	6	6
% animals showing estrus	0	0	0	0	0	0	50	75	100	100	100

testis growth and development of secondary sex characteristics in White Leghorn cockerels. These authors also speculated that the inhibition might be due to an estrogenic action upon the part of DDT since they felt the latter to be structurally similar to diethyl stilbestrol. The present investigation had its origin in the fact that DDT does not fulfill the requirements for estrogenic action as hypothesized previously(2,3) and, therefore, if actually a true estrogen, would represent an especially interesting case for both practical and theoretical reasons. In the light of the working hypothesis relating chemical constitution to estrogenic activity(2,3), DDT does possess a relatively large, rigid, and lipoid soluble molecular constitution, but it does not present active hydrogen atoms approximating the hypothesized optimum distance of 14.5 Å.‡ The present investigation reports, therefore, estrogenic tests carried out upon DDT and some related analogues in the ovariectomized rat. Portions of this report have been presented previously in abstract form(4). Burlington and Lindeman(5), in an abstract presented at the same time, reported new evidence concerning the effects of DDT on the blood of the chicken. Their results confirm the present studies indicating DDT is not estrogenic.

Materials and methods. The substances utilized in these studies, with the exception of DDT (obtained through commerce), were prepared by the conventional chloral condensation with appropriately substituted benzene derivatives(6). The estrogenic tests were carried out as previously described(3) by a method essentially similar to the classical procedure of Allen and Doisey(7).

Results and discussion. The results of the estrogenic tests are detailed in Table I. These data indicate that DDT does not possess an estrogenic action. 2,2′-bis-(p-Hydroxyphenyl)-1,1,1-trichloroethane (DHDT) appears to be an active estrogen while 2,2′-bis-(p-methoxyphenyl)-1,1,1-trichloroethane (DMDT) is inactive at doses twice as large as the minimal 100% estrus dose for DHDT. These results, therefore, indicate that DMDT is not demethylated *in vivo* to any appreciable extent to the active estrogen DHDT.

In the light of the original working hypothesis(2,3) it must be pointed out that the presence of electronegative chlorine atoms in the p,p′ orientations should lead to an inactivation of DDT since these atoms prohibit the existence of active hydrogen at these presumably crucial positions in the molecule. Therefore, under the working hypothesis, unless these chlorine atoms could be metabolized to groups possessing active hydrogen, DDT should be inactive as an estrogen. That DHDT is an estrogenic agent possessed of such active hydrogens, in conformity with the working hypothesis in this series, supports the conjecture that the lack of estrogenic activity on the part of DDT is not due to the impossibility of obtaining estrogenic activity in this general structure class.

While DHDT is probably not the only active estrogen derivable from DDT it is of special theoretical interest in the general problem concerning the relation between chemical constitution and estrogenic activity. In particular, one notes that while DHDT is not especially active in comparison with diethyl stilbestrol or estradiol, the comparison of DHDT to its non-halogenated congener 2,2′-bis-(p-hydroxyphenyl) ethane(8) shows that the presence of three chlorine atoms confers a seven-fold increase in potency on a molar basis. This increase in activity of DHDT over its non-halogenated congener may be attributed to at least 3 possible factors consistent with the original working hypothesis. The first effect is noted upon comparison of

‡ All model measurements herein reported refer to values obtained through the use of Fisher-Hirschfelder-Taylor atomic models.

the Fisher-Hirschfelder-Taylor atomic models of the analogues. The greater effective volume of trichloromethyl over methyl tends to maintain the benzene rings in a more nearly rigid configuration by virtue of steric blocking of rotation of the benzene rings. Secondly, by virtue of an inductive effect through the resonating ring system, the chlorine atoms would tend to increase the activity of the p,p'-hydroxyl groups. This latter effect may not markedly influence activity, however, since the trichloromethyl group is not attached directly to the ring but is 'insulated' by one aliphatic carbon atom. Much more effective in increasing estrogenic potency would be the influence of the trichloromethyl group on the p,p'-hydroxyl hydrogens by inductive action through space via the field effect.

In conclusion it must be pointed out that the distance between the active hydrogens of DHDT can never achieve the optimum (14.5 A) and one would not anticipate activity approaching that of estradiol or diethyl stilbestrol.

Summary. 1. DDT and 2,2'-bis-(p-methoxyphenyl)-1,1,1-trichloroethane possess no estrogenic activity in ovariectomized rats in total doses up to 45 mg. 2. 2,2'-bis-(p-Hydroxyphenyl)-1,1,1-trichloroethane possesses seven times the estrogenic activity of its non-halogenated congener 2,2'-bis-(p-hydroxyphenyl)-ethane and this increase in potency may be related to field and steric effects exerted by the trichloromethyl group.

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Effect of Factors other than Choline on Liver Fat Deposition.* (19904)

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The effects of certain amino acids on the metabolic relationship between tryptophan and niacin have been studied extensively (1-6). Recently we were surprised to observe fat deposition in the livers of animals receiving similar diets to those used in the above studies. Since the basal ration contained 0.1% choline and since methionine failed to prevent the occurrence of fatty livers, it appeared that other factors in the ration were involved.

In this paper, we wish to present experiments which demonstrate certain effects of proteins, amino acids and carbohydrate on liver fat deposition.

Experimental. Three-week-old male Sprague-Dawley rats kept in individual cages

TABLE I. Basal Ration for Fat Deposition Studies.

Component	Level in diet (per 100 g ration)
Casein	9 g
L-cystine	.2
Sucrose or dextrin	81.9
Corn oil	5
Salts IV(7)	4
Thiamin • HCl	.2 mg
Riboflavin	.3
Pyridoxine • HCl	.25
Ca pantothenate	2
Choline chloride	100
Inositol	10
Biotin	.01
Folic acid	.02

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