TABLE II. Long-Term Effect of ACTH and Cortisone on Whole Blood Coagulation Time.

Patient	Sex	Diagnosis	Daily dosage (mg)	Drug route	Control	Day of therapy 1	2-4	5-7	8-10	11-15	16-21	Day after therapy 1	2	3	4-9	10-15
F.	M	Spinal cord tumor	20	A* I.V.	35	36		36	31	33		34	38		36	
M.	M	Rheumatoid arthritis	20	A I.V.	35	35				34	31	33		38	43	39
J.	F	Acute rheumatic fever	20	A I.V.	36					34		34		35	36	
G.	M	"	20	A I.V.	38	40			31		33					
J.	F	"	400	C* P.O.	33	34	31		33							
B.	F	Rheumatoid arthritis	500	C I.M.	32				33	30	30					

* A = ACTH. C = cortisone.

TABLE III. Whole Blood Coagulation Time in Abnormal Adrenal States.

Patient	Diagnosis	Clotting time (min)
C.	Addison's disease	34
H.	100 mg cortisone/day; bilat. adrenalectomy, 52 days post-operative	34
K.	25 mg cortisone/day; bilat. adrenalectomy, 75 days post-operative	35
A.	Cushing's syndrome	30
M.	After 10 mo continuous ACTH; dermatomyositis; Cushingoid syndrome	37

and pitfalls of the whole blood coagulation time determination have been discussed in some detail elsewhere(5-7). The need for elimination of tissue juice contamination should be especially emphasized. In 3 instances during this study blood did not flow freely into the second syringe and there was some question as to whether the open end of the needle was completely within the vein, having either incompletely entered or pierced it. Clotting times in each instance were less than 22 minutes and immediate repetition of the test using a good draw from another vein gave clotting times well within the normal range. Bubbles and dirty glassware may also accelerate the coagulation process. Oily test tubes may prolong clotting.

Summary. A reproducible sensitive test, which minimizes contamination with thromboplastically active tissue juices is outlined for determination of the whole blood coagulation time. No significant alteration of the coagulation time was found with the administration of ACTH to human subjects or in patients with hypo- or hyper-adrenocorticism when this method was used.

Received June 7, 1951. P.S.E.B.M., 1951, v77.

5. Lee, R. I., and White, P. D., *Am. J. Med. Sci.*, 1913, v145, 495.

6. Quick, A. J., Honorato, R., and Stefanini, M., *Blood*, 1948, v3, 1120.

7. Barker, N. W., and Margulies, H., *Transactions of the Second Josiah Macy, Jr., Conference on Blood Clotting and Allied Problems*, New York, 1949, p. 106.

Studies on Estrogen Tri-*p*-anisylchloroethylene. (18826)

CHARLES R. THOMPSON AND HAROLD W. WERNER.
From the Research Laboratories, The Wm. S. Merrell Co., Cincinnati, O.

Laboratory studies have demonstrated that tri-*p*-anisylchloroethylene (Tace*), like other estrogens, causes estrogenic changes in the mammary gland, uterus, and vagina, sensitizes the uterus to progesterone, produces abortions

* Trademark of The Wm. S. Merrell Co. Tace was prepared by M. G. Van Campen in the Organic Research Laboratories of this Company.

TABLE I. Duration of Estrogenic Activity in Castrate Female Rats. Periods shown are the times at which one-half of the animals no longer exhibited an estrogenic vaginal smear. 18 animals in each series.

Route	Compound	Dose mg	Dose R.U.	Median duration, days
Oral	Tace	1	47	2
		5	238	17
	Hexestrol	1	83	3
		5	415	2
	Diethylstilbestrol	1	333	2
		5	1666	3
Subcutaneous	Tace	1	18	53
	Hexestrol	1	2857	2
	Diethylstilbestrol	1	2500	3

in rats, does not alter thyroid abnormalities from thiourea administration and inhibits the gonadotropic hormone production by the pituitary of castrate female rats in parabiosis.

Preliminary experiments(1) in these laboratories indicated there are important differences between the pharmacology of Tace and the better known estrogens.

Experimental. Duration of action in rats. Data in Table I demonstrate that Tace is long acting following either large oral doses or comparatively small subcutaneous doses and short acting in small oral doses. Hexestrol and diethylstilbestrol are short acting in large or small oral and subcutaneous doses.

Duration of action in monkeys. Results of typical experiments in monkeys (Fig. 1) demonstrate that oral doses of 7.0 mg of Tace and 35 mg of hexestrol produce similar changes in vaginal smears, and uterine bleeding occurs at approximately the same time. A single large oral dose of 70 mg of Tace is long acting. The vaginal changes during the first 15 days are similar to those produced by the 7.0 mg dose of Tace. On the fifteenth day the vaginal cytology is typical of the castrate animal. However, without additional dosing, another period of estrogenic activity commences and the vaginal cytology remains indicative of estrogenic activity until approximately the thirty-fifth day.

Elimination. Results of preliminary work in monkeys and the data for a complete experiment in one monkey and in rats (Table II) demonstrate animals administered hexestrol orally excrete only small amounts of estrogenic material. Elimination is primarily in the urine and is completed within three days. Large amounts of estrogenic material are recovered from the excreta of the monkey administered Tace orally. The greater part is eliminated in the feces during the first 3 days, but considerable amounts are recovered up to 30 days after a single oral dose. Similarly in rats, fecal elimination of estrogenic material is high during the first day, but considerable amounts of estrogenic material are recovered as long as 10 days after oral administration. The recovery of estrogenic material equivalent in potency to considerably more than the activity of the administered dose of Tace means that the excreted estrogen must be a metabolic product of greater potency than the parent compound. This is indicated also by the fact that the estrogen in the feces is short acting. This was demonstrated by administering the fecal estrogen orally to 20 castrate female rats in doses equivalent to 5.0 mg of Tace. The duration of action was 4 days as compared to 21 days for an equivalent dose of Tace. It is conceivable that the estrogen eliminated in the feces during the first few days following oral administration of Tace might have been unabsorbed material. How-

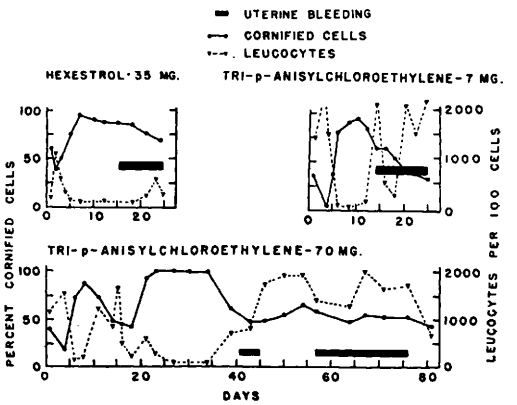

FIG. 1. Responses of castrate female monkeys to single oral doses of Tace (tri-*p*-anisylchloroethylene) and hexestrol.

1. Thompson, C. R., and Werner, H. W., *Fed. Proc.*, 1946, v5, 208.

2. Evans, J. S., Hines, L., Varney, R., and Koch, F. C., *Endocrinology*, 1940, v26, 1005.

TABLE II. Estrogenic Content of Ether Extracts of Excreta of Castrate Female Monkeys and Rats Determined by the Method of Evans, *et al.*(2).

Days after estrogen adminis.		Estrogen recovery expressed as % of dose of estrogen administered					
		Tace		Hexestrol		Control	
		Urine	Feces	Urine	Feces	Urine	Feces
Monkeys*	1	8.4	231	.5	.2	0	0
	2	8.7	200	.2	0	0	0
	3	4.3	30	.1	0		
	4	1.2		0			
	5-7	.9	18	0	0		
	8-10	.6	6				
	11-14	.4	2				
	15-29		6				
	30-36		1.4				
	Total	24.5	494.4	.8	.2		
Rat†	1	.16	110	.2	0	0	0
	5	0	2	0	0	0	0
	10	0	2.7	0	0	0	0

* One monkey received single oral dose of 70 mg of Tace and one monkey single oral dose of 35 mg of hexestrol.

† Avg values for dose groups of 4 rats administered three 5 mg oral doses of Tace and hexestrol in one day.

TABLE III. Avg Estrogenic Concentration in Body Fat of Groups of 4 Castrate Female Rats Administered Three 5 mg Oral Doses of Tace and Hexestrol in One Day. Values are biologic equivalent of estrogen administered determined by the method of Evans, *et al.*(2).

Day following last dose	Mg of estrogen per 100 g fat		
	Tace	Hexestrol	Control
1	11.4	0	0
5	5.9	0	0
10	2	0	0

ever, considerable amounts of estrogenic material must have been secreted into the gastrointestinal tract because (1) estrogenic material is eliminated in the feces for thirty days or more following a single oral dose, (2) the eliminated material is short acting orally unlike Tace itself, (3) estrogenic material equivalent to 20.8% of the administered dose of Tace is recovered from feces of 4 rats during a 24-hour period following a single intraperitoneal administration.

Fat storage. The prolonged effect of Tace following oral administration suggests tissue storage. Since Robson and Ansari(3) reported that α,α-bis(*p*-ethoxyphenyl)-β-phenylbromoethylene was stored primarily in body fat, fat storage was studied following Tace administration. Considerable amounts of estrogenic material are stored in the body fat of the rats which receive Tace (Table III), and the fat concentration gradually decreases over a period of more than 10 days. Hexestrol administration is not followed by storage of estrogenic material in body fat.

Repeated administration. Doses of 5 mg of Tace and 0.1 mg of hexestrol were administered subcutaneously 3 times a week for 13 weeks to groups of 40 normal female rats. A complete report on chronic effects will be made elsewhere. Both estrogens similarly depress the rate of increase in body weight. Uterine and vaginal changes are similar for the two compounds and resemble those reported in the literature for other estrogens. Both substances cause lobule-alveolar growth and evidence of secretory activity of the mammary glands. In general, the mammary gland hyperplasia is more pronounced in animals administered hexestrol than in those administered Tace.

Average weights of pituitaries, adrenals, and ovaries are presented in Table IV. Tace decreases and hexestrol increases the weight of the pituitary. Tace causes a slight increase and hexestrol a pronounced increase in weight of the adrenals. Tace administration results in less ovarian atrophy than hexestrol.

Liver glycogen values (Table V) for rats fed *ad libitum* until sacrificing are not significantly different for the control, Tace, and hexestrol groups. The glycogen content of

3. Robson, J. M., and Ansari, M. Y., *J. Pharm. and Exp. Therap.*, 1943, v79, 340.

TABLE IV. Avg Organ Wt in mg and Standard Errors for Female Rats Administered Repeated Doses of Tace and Hexestrol.

Group	Pituitary	Adrenal		Ovary	
		Left	Right	Left	Right
Tace	8.4 ± .69	25.2 ± 1.26	24.8 ± 1.12	15.2 ± .74	15.1 ± .82
Hexestrol	26.6 ± 1.32	38.4 ± 1.45	37.3 ± 1.33	8.7 ± .46	8.8 ± .60
Control	11.4 ± 1.16	22.1 ± .60	20.2 ± .61	26.1 ± 1.26	26.1 ± 1.41

TABLE V. Glycogen Content of Livers of Female Rats Administered Repeated Doses of Tace and Hexestrol.

Compound	g glucose per 100 g wet tissue and standard errors	
	Fed *ad libitum*	Food withheld 24 hr
Tace	3.5 ± .20	.2 ± .04
Hexestrol	3.1 ± .16	1 ± .24
Control	3 ± .21	0 ± .01

livers of rats receiving no food 24 hours prior to sacrificing are considerably greater for the hexestrol than for the Tace and control groups. Liver glycogen in the fasted group administered Tace is low but significantly greater than the value for the control group. This difference in glycogenetic activity is related possibly to a greater effect of hexestrol on the adrenals.

Because of temporal differences in the action of Tace and hexestrol, strictly comparable physiologic doses cannot be defined. The following evidence suggests, however, that the differences reported for repeated administrations probably represent differences in activity rather than dosage effects: (1) the doses of Tace employed in the chronic experiment were very large, and it is well established that even small doses of the natural and better known synthetic estrogens cause pituitary enlargement; (2) the two estrogens had similar effects on the weight curves and direct estrogen target organs, but Tace had a lesser effect on organs in which the action is mediated through the pituitary.

Gardner(4) also found that Tace differed from α-estradiol benzoate with repeated administration to CC_1 hybrid mice. Animals receiving Tace developed interstitial cell tumors of the testes while animals receiving α-estradiol benzoate did not. He indicated this difference may be related to either a longer survival time of Tace-treated animals or to a more marked effect, directly or indirectly, on the testes.

Summary. The pharmacology of Tace (tri-*p*-anisylchloroethylene), a new compound with estrogenic activity, differs in important respects from that of hexestrol which is typical of the natural and better known synthetic estrogens: (1) Tace does not cause and hexestrol does cause pituitary enlargement in rats; (2) Tace has a lesser but similar effect to hexestrol on organs in which the action of the two compounds is mediated through the pituitary; (3) fat storage resulting in long activity occurs following oral administration of large doses of Tace, while there is no fat storage and action is short following hexestrol administration; (4) estrogenic material equivalent in biologic potency to more than the administered dose of Tace is eliminated with the feces, while only small amounts of estrogenic material are eliminated following hexestrol administration.

4. Gardner, W. U., and Boddaert, J., *Arch. Path.*, 1950, v50, 750.

Received June 15, 1951. P.S.E.B.M., 1951. v77