TABLE II. Additivity of Enzymatic Activity of Intestinal Juice and Added Enzymes.

Sample	Activity as measured by optical density at 280 mμ Found	Calculated	% recovery
Exp. I			
Chymotrypsin (2 ml)	.214		
Trypsin (2 ml)	.457		
Intestinal juice (2 ml)	.288		
Chymotrypsin (1 ml) & intestinal juice (1 ml)	.233	.251	93
Trypsin (1 ml) & intestinal juice (1 ml)	.344	.373	92
Exp. II			
Chymotrypsin (2 ml)	.265		
Trypsin (2 ml)	.449		
Intestinal juice (2 ml)	.041		
Chymotrypsin (1 ml) & intestinal juice (1 ml)	.156	.153	98
Trypsin (1 ml) & intestinal juice (1 ml)	.278	.245	113
Mean recovery of 2 experiments:			
Trypsin & intestinal juice			103%
Chymotrypsin & intestinal juice			96%

half hour was 63% for chymotrypsin, and 8% for trypsin (Fig. 1).

When both enzymes were incubated in aqueous solution at pH 7.5, 79% of the chymotrypsin and 69% of the trypsin remained active after one-half hour (Fig. 2). Since the relatively rapid inactivation of trypsin cannot be attributed to the pH of the intestinal juice alone, these results suggest that trypsin inhibitors(2,3,5) from the pancreatic juice are still active in the intestine. It is also possible that the enzyme proteins are destroyed by proteolytic activity *per se*.

Summary. The stability of trypsin and chymotrypsin was determined in human intestinal juice. On a weight per weight basis at the end of one-half hour as much as 63% of chymotrypsin was still enzymatically active while only 8% of the trypsin activity could be detected. In fact, 30% of the chymotrypsin still remained at the end of 2 hours.

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Glycyrrhetinic Acid on Female Response to Exogenous and Endogenous Sex Steroids.* (27093)

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The anti-estrogenic activity of beta glycyrrhetinic acid in immature intact as well as adrenalectomized rats has been reported(1). The previous study(1) was concerned with the restriction of the uterotrophic response to estradiol benzoate in immature rats with intact gonads. This investigation was undertaken to determine whether the antagonism was limited to the uterine response to exogenous estrogens and whether its effect was dependent upon the presence of gonads or pituitary.

Methods and materials. Rats of the Wistar strain were maintained under constant temperature (25°C) and lighting (12 hours light) and were fed a Rockland rat diet and water *ad libitum*. Hypophysectomized rats received 24 hours after surgery were

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given 5% glucose in the drinking water. Beta glycyrrhetinic acid† and some of the steroid hormones‡ were prepared separately or together in an ethanol-sesame oil vehicle (1). The estradiol benzoate given to hypophysectomized rats and the estrone and estriol were prepared separately in sesame oil. PMS§ was dissolved in isotonic saline. All injections were made subcutaneously and the control animals received an equal volume of vehicle.

The uterotrophic responses of intact, ovariectomized (23 days old) or hypophysectomized rats (24 days old) to exogenous steroids (Table I) were determined after a 3-day injection period(1). Ovariectomy had been performed 5 days prior to use; hypophysectomy had been performed 2 days before use.

The effect of glycyrrhetinic acid on the vaginal estrous response to estradiol benzoate was determined in adult (100-150 g) ovariectomized rats 2 weeks after the second priming dose with estradiol benzoate. Only rats which had responded to 2 successive courses of priming at 19 and 26 days post-operatively were used. Vaginal smears were taken at 48 and 72 hours and stained with Shorr's differential stain(2).

The uterotrophic response to endogenous sex steroids was determined in immature rats receiving equine gonadotropin (PMS) or hypophyseal gonadotropin from a castrated male donor parabiont. PMS treated rats (23 days old) were given a 3-day injection period with a total dose of 6 mg of glycyrrhetinic acid and 1 or 10 I.U. of PMS or a 7-day injection period with a total dose of 16 mg of glycyrrhetinic acid and 10 I.U. of PMS. Parabiosis was performed on 23-25-day-old rats and treatment of the female recipient with drug or vehicle was started at 24 hours post-operatively. Rats were sacrificed on the 10th post-operative day after a total dose of 6 mg of glycyrrhetinic acid or on the 9th post-operative day after a total dose of 16 mg of glycyrrhetinic acid.

TABLE I. Glycyrrhetinic Acid on Uterotrophic Response of Immature Rats to Exogenous Steroids.*

Hormone	mg G.A.	N	Uterine wt, mg ± S.E.	P
		Intact		
0	0	5	13.8 ± .8	
Estrone .3 μg	0	10	24.9 ± 1.4	
" "	6	11	20.7 ± 1.4	<.05 >.02
" 3.0 μg	0	4	54.4 ± 1.0	
" "	6	5	43.8 ± 3.8	<.02 >.01
Estriol .6 μg	0	5	28.1 ± 1.7	
" "	6	5	21.8 ± 1.0	<.02 >.01
T. P. .6 mg	0	25	38.9 ± 2.0	
" "	6	15	38.6 ± 3.3	
" "	15	9	35.7 ± 1.6	
Prog. 3.0 mg	0	10	40.2 ± 1.6	
" "	6	9	37.7 ± 3.1	
		Ovariectomized		
0	0	7	15.1 ± 1.8	
0	6	6	15.2 ± 2.3	
E.B. .3 μg	0	14	73.7 ± 4.0	
" "	6	17	57.8 ± 2.7	<.001
		Hypophysectomized		
0	0	13	13.5 ± 4.5	
0	6	11	11.8 ± 4.2	
E.B. .3 μg	0	30	43.0 ± 1.8	
" "	6	33	42.9 ± 1.2	

* 23-24-day-old rats treated for 3 days. Ovariectomy performed at 18 days, used at 23 days. Hypophysectomy performed at 22 days, used at 24 days. Doses represent total amount. Dose represented by 0 indicates animals received an equal volume of vehicle.

Results. Uterotrophic and vaginal estrous responses to exogenous steroids. Glycyrrhetinic acid significantly antagonized the uterine growth response to estrone and estriol in immature intact rats, but had no effect on uterine response to testosterone and progesterone (Table I). Glycyrrhetinic acid also produced a significant decrease in the uterotrophic response of ovariectomized rats to estradiol benzoate (Table I).

Vaginal estrous response to estradiol benzoate was significantly decreased by glycyrrhetinic acid when the drug dose was 20,000 times the estrogen dose. Though 1.0 μg of estradiol benzoate produced 100% cornification in control animals, 20 mg of glycyrrhetinic acid reduced this estrous response to

† Beta glycyrrhetinic acid was supplied by S. B. Penick Co., N. Y.

‡ Estradiol benzoate and testosterone propionate were supplied by Organon Inc., Nutley, N. J., estrone and estriol by Parke Davis and Co., Detroit, Mich., and progesterone by the Schering Research Division, Bloomfield, N. J.

§ Pregnant mare serum (Gonadogen) was supplied by Upjohn Co., Kalamazoo, Mich.

31.25% ($P<.001$) and 43.75% (P .001) in 2 separate experiments. There was no evidence of glycyrrhetinic acid toxicity.

Uterotrophic response of immature rats to endogenous steroids.

Glycyrrhetinic acid did not antagonize the uterotrophic response to endogenous sex steroids regardless of the source of gonadotropins or the dose. Six and 16 mg of glycyrrhetinic acid was not anti-estrogenic in immature female parabiont recipients nor PMS stimulated animals. However, 16 mg produced a 16.78% increase ($P<.01$) in uterine weights of the parabiont recipients but not the PMS stimulated animal. There was no apparent increase in ovarian weight.

Discussion. The estrogen antagonism with glycyrrhetinic acid does not depend upon gonads nor adrenals(1), but does appear to depend upon the presence of the hypophysis. The anti-hysterotrophic action is restricted to exogenous estrogens, but the anti-estrogenic effect is not limited to the uterine target organ. The vaginal mucosa is less sensitive than the uterus to the estrogen antagonism and the minimum effective ratio of drug to estradiol benzoate was 5 times the minimum anti-hysterotrophic ratio(1).

The lack of antagonism of endogenous steroids may infer that the mechanism of action of glycyrrhetinic acid lies in interference with absorption or metabolism of exogenous estrogen rather than through altering target organ response. This effect may be mediated through the hypophysis.

Augmentation of the uterotrophic response of female parabionts may have been due to a pituitary-adrenal inhibition during prolonged drug administration such as has been observed with glycyrrhizin(3,4). This lowering of the adrenal cortical hormone level can augment the estrogen response(5). However, the adrenal cortical hormone level of the PMS stimulated rat may not have been changed due to the effect of PMS on the adrenal gland itself(6).

Summary. Beta glycyrrhetinic acid restricted the response of the female accessory reproductive tract to exogenous steroidal estrogens (estradiol benzoate, estrone and estriol). It antagonized the uterotrophic and vaginal estrous response of ovariectomized rats to estradiol benzoate, but had no effect in hypophysectomized rats. It did not interfere with the response to other exogenous uterine stimulating steroids nor to endogenous steroids. High doses of glycyrrhetinic acid, increased the uterine response of immature parabiont recipients though similar doses had no effect in the PMS stimulated animal.

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Chlorpromazine Inhibition of the Positive Intermediary Potential.* (27094)

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Gasser and Graham(1) described a positive potential of long duration recorded from spinal cord. They termed this the positive intermediary potential and associated it with alterations in the central excitatory state. Using different technics, Lloyd and McIntyre (2) reinvestigated the positive intermediary potential and found that it resulted from an increased negativity which originated in secondary neurons of the spinal cord as a resid-

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