establish a role for a bacterial virulence-enhancing factor in the pathophysiology of strangulation obstruction, but suggests that synergism between bacteria of one species and products of another can occur in this polymicrobic surgical disease. Further work is needed to determine the nature of the virulence-enhancing factor in strangulation fluids, and whether the factor(s) acts in strangulation obstruction by directly enhancing the virulence of endogenous intestinal bacteria, or by depressing host resistance to infection.

Summary. Sterile, non-lethal ultrafiltrates of strangulation fluids from humans, dogs, and rats promoted a rapid, lethal *E. coli* infection in healthy mice by fewer bacteria than was possible with saline suspensions of the same organisms. *Cl. welchii* and *Cl. sordelli,* similarly suspended, killed only a small proportion of test mice. Sublethal dosages of several substances that might contribute to the virulence-enhancing activity of strangulation fluids were also studied: *E. coli* endotoxin enhanced the virulence of *Cl. sordelli* and *E. coli; Cl. welchii* exotoxin enhanced only *E. coli;* bile and mucin enhanced all 3 test microorganisms. Synergism between bacteria of one species and products of another may contribute to the severity of mixed bacterial infections. The identity of the factor in strangulation fluids is unknown. There is as yet no evidence of a role for a bacterial virulence-enhancing factor in the pathophysiology of strangulation intestinal obstruction.

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Thyro-Adrenal Relationships in Maternal Fetal and Neonatal Guinea Pigs: Effects of Goitrogens* (30829)

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Knowledge regarding placental permeability to maternal hormones and the maturation and functional interrelationships of the mammalian fetal endocrine systems is incomplete. Jost(1) and Mitskevich(2) have summarized pertinent experimental data on the pituitary-thyroid and adrenal systems in the rodent. Critical analysis indicates that there are mutual compensations in hormone function of mother and fetus and that these reflect differences in placental transmission for different hormones, as well as for type of placenta and stage of pregnancy. Information on the thyro-adrenocortical relationship in maternal

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and fetal guinea pig is scant. There is evidence that the pituitary-thyroid axis of the fetus can operate as a feedback mechanism, at least in the later stages of development. Peterson and Young(3) described hypertrophy of the pituitary, as well as thyroid hyperplasia, in fetuses of guinea pigs treated with anti-thyroid agents during pregnancy. These effects were partially counteracted by simultaneous administration of thyroxine to the mother. Postel(4) found that chronic treatment with perchlorate also induced massive thyroid hyperplasia without producing maternal goiter: triiodothyronine administration did not prevent the fetal thyroid hyperplasia, however. Comparable studies on maternal and fetal pituitary-adrenal interrelations in the hypothyroid guinea pig are virtually nonexistent. This study was undertaken to supply such information on the possible effects of goitrogenesis on adrenocortical function in mother, fetus and neonate.

Materials and methods. Guinea pigs, 5-6 weeks pregnant, obtained from commercial sources, were maintained in an animal room (70-76°F) on unrestricted amounts of tap water and Purina Omolene, periodically supplemented with greens. Because of the well-established relationship between ascorbic acid deficiency and adrenocortical secretion in the guinea pig(5,6), precautions were taken to maintain adequate levels by oral supplementation of ascorbic acid (10 mg on alternate days). Propylthiouracil (PTU) was administered in the ration (0.1%) as in previous studies(7). Experimental and control animals were grouped as follows: Groups A and B: untreated mothers and newborn, respectively; C and D: pregnant guinea pigs treated with PTU for 17-30 days and their offspring, all autopsied within 1-24 hours after parturition. Groups E and F consisted of untreated, lactating mothers and 8-10-day-old sucklings. Groups G and H were comprised of nonpregnant adults which were untreated or received goitrogen for 50-70 days.

Guinea pigs were killed by exsanguination. Blood was drawn into heparinized syringes by cardiac puncture in animals under ether anesthesia. After centrifugation, the plasma was frozen until chemical determinations were made. Thyroids, adrenals, gonads and pituitary were quickly removed at autopsy, trimmed cleanly, weighed on torsion balances and then prepared for histological or chemical study. Ascorbic acid content of the left adrenal and left ovary (both gonads in newborn) was determined by the Roe-Kuether method(8). Free 17-hydroxycorticosteroids were measured in 1 ml samples of plasma (duplicate samples in adults) and in the right adrenal, or portions thereof, by modification of Silber's fluorometric procedure(9). After successive extractions with 2, 2, 4-trimethylpentane and chloroform, followed by an alkaline wash, the extracted steroids were mixed with the fluorescing reagent (75% ethanolic H_2SO_4, v/v), centrifuged, and the acid extract transferred to cuvettes. Fuorescence intensity was determined precisely 10 minutes after induction using a Farrand Fluorometer with an exciting wave length of 467 mμ and emitted wave length of 530-540 mμ. Standards containing 0.25, 0.5, 1.0 and 1.5 μg of cortisol were routinely run with the highest standard arbitrarily set at 80. Corrections were applied for reagent blank but not for non-specific fluorescence. Values for 17-hydroxycorticosteroid content of the samples were calculated directly from the standard curve which showed a reproducible linear relationship between intensity of fluorescence and concentration of hydrocortisone over the range tested. It has been established with a variety of methods(10,11,12) that the guinea pig adrenal elaborates several steroids with the 17-hydroxycorticosteroid-configuration but secretes mainly 17-hydroxycorticosterone (cortisol). All results were evaluated statistically using Student's "t." Weights of the endocrine organs are recorded in terms of mg/100 g final body weight. No sex differences were apparent for the various measurements made, consequently values for the newborn were pooled.

Results. Administration of PTU to guinea pigs throughout the last 17-30 days of their pregnancy resulted in significant change in the endocrine system of the offspring (Table I). Body weights of newborn from goitrogen-treated mothers averaged about 10% less than for normal young. Variability was high

TABLE I. Propylthiouracil Treatment and Endocrine Gland Weight in the Guinea Pig.

Group (No. of animals)	Mean body wt, g (range)	Mean organ wt (mg/100 g body) ± SEM				
		Pituitary*	Thyroid	Adrenal	Ovary†	Testis
A. Normal mothers (8)	604	1.9 ± .13	13.1 ± .62	90 ± 9.5§	9.9 ± .9	—
B. Normal young (14♀ : 7♂)	78 (55–110)	5.0 ± .13	28.5 ± 2.8	37.3 ± 3.8	10.0 ± .9	105 ± 7.6
C. Mothers on PTU (8)	681	2.1 ± .12	13.4 ± 1.7	98 ± 14.7§	10.0 ± .7	—
D. PTU—young (18♀ : 7♂)	68 (39–99)	5.5 ± .14‖	566 ± 105‖	28.3 ± 1.4‖	8.0 ± .9	88 ± 11.8
E. Normal mothers, lactating (4)	726	2.2 ± .17	11.0 ± 1.2	68 ± 4.1	8.7 ± 2.0	—
F. Normal sucklings‡ (3♀ : 4♂)	125 (85–137)	4.4 ± .32	22.0 ± 2.0	43 ± 2.9	7.8 ± 1.1	115 ± 17.6
G. Normal, non-pregnant (12)	632	1.6 ± .07	13.8 ± 1.2	57 ± 4.1	8.9 ± .8	—
H. PTU, non-pregnant (12)	619	1.9 ± .03	20.9 ± 2.5	62 ± 4.2	8.5 ± .7	—

* Values for the adenohypophysis in adult guinea pigs and for whole pituitaries in offspring.
† Weight based on left ovary in adults and both gonads in offspring.
‡ 8-10 days old.
§ Mean values are significantly different ($P = <.025$) from corresponding ones in non-pregnant groups (G, H).
‖ Significantly different ($P = <.025$) from mean values in normal offspring (B).

in both groups, however, and there was no indication of cretinism in any of the offspring. Marked hyperplasia of the fetal thyroid occurred. The goitrogenesis was associated with significant hypertrophy of the pituitary and moderate atrophy of the adrenal. Ovarian and testicular weights of goitrous young were also reduced but not significantly so. Despite a 20-fold enlargement of the thyroid in the fetus, goitrogen-treated mothers showed little or no thyroid hypertrophy. Prolonged ingestion of PTU eventually induced goiter formation (Group H). It has already been established that there is an appreciable delay in onset of morphologic change in the thyroids of adult guinea pigs given anti-thyroid agents(7). Adrenal gland weight increased about 50% during pregnancy (Groups A, C). Goitrogen administration did not influence the adrenal hypertrophy of pregnancy nor affect adrenal size in non-pregnant animals. The enlarged adrenal involuted rapidly after parturition (Group E).

Consistent and substantial differences were observed between mother and neonate in the various indices of adrenocortical function (Table II). Maternal plasma concentrations of 17-hydroxycorticosteroids at delivery were at least double those in the newborn and 4-5 times greater than normal (compare Groups A, C, G). Corticoid concentrations in the adrenals of offspring (Groups B, D) averaged one-half to one-third those of mothers. Levels of 17-hydroxycorticosteroids in plasma or adrenal of goitrous guinea pigs (newborn or adult) were not significantly different from those in euthyroid controls. Involution of the adrenal in lactating animals was paralleled by reduction of adrenocorticosteroids toward normal but values in 8-10-day-old suckling guinea pigs were maintained. Despite continued oral supplementation of ascorbic acid to pregnant and lactating animals, ascorbic acid concentrations of their adrenals were consistently lower than in the newborn. Induction of the hypothyroid state in adult animals resulted in significant adrenal ascorbic acid depletion (Group H). No such effect was observed in the young, however, despite the presence of large goiters.

Discussion. The marked fetal hyperplasia of the thyroid and the pituitary hypertrophy induced by PTU treatment in the last few weeks of pregnancy are in accord with the

TABLE II. Effects of Hypothyroidism on Adrenocortical Function in Maternal and Neonatal Guinea Pigs.

Group	Adrenal responses (mean ± SEM)		
		17-hydroxycorticosteroids	
	Adrenal ascorbic acid (mg/100 g)	Plasma (μg/100 ml)	Adrenal (μg/g)
A. Normal mothers	61 ± 12.5	206 ± 28.1*	32.5 ± 6.7
B. " young	103 ± 15.9†	97 ± 8.4†	10.1 ± 1.4†
C. Mothers-PTU	40 ± 6.7	311 ± 19.4*	22.9 ± 3.2
D. Young-PTU	112 ± 8.6†	92 ± 11.6†	10.6 ± 1.3†
E. Normal mothers, lactating	52 ± 21.2	81 ± 14.3	19.4 ± .9
F. " sucklings	115 ± 18.5†	82 ± 12.4	11.2 ± 3.0†
G. " non-pregnant	84 ± 10.9	56 ± 4.3	24.3 ± 3.2
H. PTU, non-pregnant	47 ± 8.0	48 ± 4.4	24.0 ± 1.2

* Means which differ with high significance ($P = .001$) from those in non-pregnant, adult guinea pigs (G, H).

† Values obtained in newborn which differ significantly ($P = .05$ or less) from corresponding ones in mothers (A, C, E).

observations of Peterson and Young(3). The more pronounced morphologic changes in the pituitary-thyroid system as described by them can be accounted for by duration and/or route of administration of the anti-thyroid agent. There is little doubt from this and the earlier studies(2,3,4) that placental passage of goitrogens to the fetus, inhibition therein of thyroid hormone formation, and activation of fetal TSH mechanisms in the pituitary all can occur. It is also significant that fetal goitrogenesis was not accompanied by maternal thyroid hyperplasia. Although serum TSH titers in PTU-treated adult guinea pigs do rise prior to onset of thyroid hypertrophy (7), placental transmission of maternal TSH sufficient to sustain the fulminating thyroid enlargement of the fetus is hardly likely; moreover, we have evidence (to be published elsewhere) that TSH content of the pituitary in goitrous offspring is significantly increased.

The complex relationship between ascorbic acid deficiency and adrenocortical secretion in the guinea pig has received much scrutiny. Severe inanition produces adrenal hypertrophy but not if animals are previously hypophysectomized(13). In growing guinea pigs on a vitamin C-deficient diet, plasma 17-hydroxycorticosteroids are slightly increased; markedly so, if weight loss is appreciable(6). Plasma corticoids reach exceedingly high levels in severely scorbutic animals although their hypertrophied adrenals are virtually devoid of ascorbic acid(5). This relationship must differ in several basic respects for maternal and neonatal guinea pigs. The adrenal and ovary of the fetus apparently can concentrate ascorbic acid to greater extent than do the maternal glands. Whether the fetal endocrines can synthesize ascorbic acid only to lose this capacity at birth cannot be stated.

The fact that circulating levels of adrenocorticosteroids in the neonate are relatively low is consistent with the view that placental transfer of maternal steroids is rather limited. Maternal levels were not determined throughout the gestation period, however, and the high values at parturition could have been temporarily accentuated by stresses engendered by delivery. Plasma corticosteroids were greater in the newborn than in normal, non-pregnant adults, confirming the report of Thornton and associates(14). The fluorometric methodology used by these investigators also revealed changing proportions of "cortisol" and "corticosterone" in plasma of aging guinea pigs. Relatively high corticosterone values found in the neonate decreased sharply in the adult. The decline in plasma cortisol with maturity was much less marked. Our study indicates that the elevation in plasma 17-hydroxycorticosteroids of the newborn coexists with low adrenal concentration. It remains to be determined whether the fetal guinea pig adrenal actually secretes corticosterone in high proportion, if at all, and also what action the high circulating levels of adrenocorticoids may have on ACTH secretion

by the fetal pituitary. The data of Milkovic and Milkovic in the rat(15) suggest that the fetal adrenal rather than the maternal gland is the source of elevated corticosterone levels in the fetal circulation.

The failure to affect adrenal size in the guinea pig with anti-thyroid agents contrasts with numerous positive results for the rat (16). Thiouracils in the latter induce unequivocal atrophy of the adrenal. The effect is attributable to decreased secretion of ACTH(17) rather than to adrenal insensitivity(18). Chronic treatment of rats also reduces plasma and adrenal corticosterone values but these are restored to normal with unilateral adrenalectomy even one year after continuous PTU treatment (unpublished experiments). Kowalewski(19) reported low levels of 17-hydroxycorticosteroids in plasma of guinea pigs (sex?) after long term treatment with thiouracil. There was no evidence of adrenal hypofunction in the goitrous guinea pigs (adults or newborn) of this study, however.

Summary and conclusions. Propylthiouracil administration to guinea pigs during the last few weeks of pregnancy induced pituitary hypertrophy, adrenal atrophy and marked thyroid hyperplasia in their offspring. Adrenal enlargement, without goiter formation, was found in the mothers. A reciprocal relationship was observed between mother and newborn (1-24 hours) regarding ascorbic acid and 17-hydroxycorticosteroid levels. Adrenal and ovarian ascorbic acid concentrations were significantly higher in neonatal guinea pigs, whereas corticosteroid values in maternal plasma and adrenal were 2-3 times greater than in the young. Fetal goitrogenesis did not basically alter the inverse relationship of these parameters of adrenocortical function.

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