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Antibacterial Substances in Placentas and Serums of Mothers and Newborn Infants. (25792)

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The protection of the human fetus against infection has been an intriguing subject for many years. Since the placenta interposes a continuous though narrow wall of tissue between the blood of the fetus and that of the mother, and since the chorionic cells of the placenta may be phagocytic(1,2), the simplest hypothesis would assign the role of a barrier to the placenta. The final destruction of invading organisms would be relegated to the antimicrobial substances of maternal and fetal bloods(3,4,5,6), and perhaps to some inadequacy of fetal tissues as a culture medium(7) for certain organisms. However, some reports (8,9,10,11) suggest that the placenta may contain antimicrobial substances distinct from those of the blood. The present report records concentrations of certain antibacterial substances in the blood of mothers and newborn infants and attempts to determine whether there are additional antibacterial factors in the placenta itself.

Materials and methods. Venous blood was obtained from mothers, and blood from newborn infants was collected from the placental portion of the severed umbilical cord immediately after birth. After the blood had clotted at room temperature serum was separated by centrifugation in the cold and stored at –20°C. Placenta were frozen at –20°C, allowed to thaw, comminuted in the cold for 3 minutes using a Waring blendor, and centrifuged at 2000 rpm for 1½ hours at 5°C. The sterile, dark red supernatant was stored at –20°C. Some placental preparations were treated with bentonite to remove lysozyme (12) or mixed with washed, heat killed bacteria to absorb specific antibodies. Bactericidal activity of the serums and placental materials were tested against *Escherichia coli, Shigella flexneri, Salmonella choleraesuis, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus viridans* and a beta hemolytic *Streptococcus pyogenes*. Before testing, each organism was cultured for 24 hours in brain heart infusion broth (Difco). One-tenth ml of a 10^{-7} dilution of organisms suspended in 0.85% sterile NaCl solution was added to duplicate tubes containing either 1.0 ml cold serum, placental material or saline control. Mixtures were incubated for 30 minutes in a water bath at 37°C. Two ml molten nutrient agar (Baltimore Biological Labs.) was added to each tube, the contents mixed and allowed to solidify. *Pseudomonas* and *Salmonella* grew poorly below the surface of the agar. Mixtures containing either of these organisms were poured directly, therefore, onto the surface of nutrient agar plates. Colonies were counted after incubating tubes or plates for 24 hours at 37°C. By comparing colony counts of saline controls with counts of tubes containing placenta or serum, percentage of bacteria killed could be calculated. Hemolytic complement titers were expressed as the final dilution of serum or placental material required to lyse 50% of 1.5 x 10^8 optimally sensitized sheep erythrocytes in a reaction volume of 1.5 ml after 30 minutes of incubation at 37°C. Triethanolamine buf-

TABLE I. Bactericidal, Lysozyme, Properdin, and Complement Activities of Maternal Serums and Their Corresponding Cord Serum and Placenta.

Extract or serum	Bactericidal effect as % of *P. aeruginosa* killed in 30 min.	Lysozyme content (μg/ml)	Properdin (units/ml)	Hemolytic complement (C'H50/ml)
M*	98	7.0	8	233
C	96	8.4	4	146
Pl	78-95	13.4	1	13
M	100	5.8	16	<10
C	100	26.0	8	144
Pl	100	12.4	1	18
M	100	7.0	12	140
C	99	10.4	<2	68
Pl	100	9.4	2	17
M		5.6	8	231
C	100	6.4	4	100
Pl	75	8.4	1	20
M	90	4.4	8	48
C	90	6.4	<2	77
Pl	85	8.4	1	28
M	99	4.6	4	228
C	99	8.8	4	100
Pl	76	12.0	.5	<10
M	98	3.1	4	238
C	84	6.6	2	160
Pl	54	4.2	.5	22
M	98	4.6	8	100
C	100	6.6	2	—
Pl	99	14.4	2	26
M	94	4.2	<2	<10
C	100	8.0	3	114
Pl	50	9.6	2	25
M	100	4.0	4	112
C	75	7.4	<2	67
Pl	30	.5	<2	22
M	100	7.6	2	260
C	97	8.6	2	91
Pl	99	16.0	.5	26
M	100	5.3	8	196
C	100	9.0	<2	81
Pl	97	12.4	.5	17

* M, maternal serum; C, cord serum; Pl, placenta.

fered saline(13) containing Mg^{++} and Ca^{++} was used as the reagent diluent. Hemolysis was measured spectrophotometrically and results were plotted utilizing the probit transformation(14,15) to calculate the 50% hemolytic unit of complement (C'H50) per ml of sample. Lysozyme activity was determined by spectrophotometrically measuring the clearing of a suspension of *Micrococcus lysodeikticus*(16,17). Properdin assays were performed by the zymosan assay method of Pillemer, *et al.*(18). To detect properdin in placental extracts it was first necessary to precipitate properdin as euglobulin and resuspend it to one-fourth original volume in endpiece(13).

Results. Determinations of properdin, lysozyme, and bactericidal action against *Pseudomonas aeruginosa* were done on 20 maternal sera and the corresponding infant serums and placentas. In addition complement was determined in 12 of these cases. Representative results are shown in Table I. The maternal serums usually gave properdin values nearly equal to those of pooled normal serums and varying from 2 to 16 units. Infant serums from the umbilical cord had less properdin, and placental preparations had very small amounts, perhaps because of destruction of properdin by mechanical agitation. Lysozyme was found in all serums and placentas in concentrations resembling those in normal sera and ranging approximately from 5 to 25 μg/ml. In every case the serum of the newborn baby had a greater concentration of lysozyme than that of the mother. Complement titers in maternal sera were found to vary within the range for normal adults; complement titers were lower in cord sera than in maternal sera and greatly reduced in placental preparations, probably because of the agitation of the latter in the Waring blendor. Rarely, there was an unexplained deficiency of complement in maternal serum, but not in the corresponding placenta or neonatal serum, a finding which could not be correlated with the patient's blood group or obstetrical history. All serums and placental preparations demonstrated bactericidal activity. This activity against *Pseudomonas aeruginosa* could not be correlated closely with levels of properdin, complement or of lysozyme (Table I).

When tested against 7 different organisms, each placental preparation showed a distinct spectrum of bactericidal activity (Table II), particularly against *Shigella flexneri, Escherichia coli* and *Pseudomonas aeruginosa,* but no detectable activity against *Salmonella choleraesuis, Streptococcus pyogenes* or *Streptococcus viridans.* Only one placenta and cord serum showed activity against *Staphylococcus*

TABLE II. Antibacterial Spectra of Placentas and Serums.

Serum	*Staph. aureus*	*Strep. viridans*	*Strep. pyogenes*	*Esch. coli*	*Shigella flexneri*	*Salmonella choleraesuis*	*Pseudomonas aeruginosa*
M*	0	0	0	+	+	0	+
C	0	0	0	+	+	0	+
Pl	0	0	0	+	+	0	+
M	—	0	—	—	0	—	—
C	0	0	0	+	+	0	+
Pl	0	0	0	+	+	0	+
M	0	0	0	+	+	0	+
C	0	0	0	+	+	—	+
Pl	0	0	0	+	+	+	+
M	0	0	0	+	+	0	+
C	0	0	0	+	+	0	+
Pl	0	0	0	+	+	0	+
M	0	0	0	+	+	0	+
C	0	0	0	+	+	0	+
Pl	0	0	0	+	+	0	+
M	0	0	0	+	—	0	+
C	+	0	0	+	0	0	+
Pl	+	0	0	+	0	0	+
M	0	0	0	0	0	0	0
C	0	0	—	—	+	0	—
Pl	0	0	0	+	0	0	0
M	0	0	0	+	0	—	—
C	0	0	0	+	0	0	+
Pl	0	0	0	+	0	0	0

* M, maternal serum; C, cord serum; Pl, placenta.

+, more than 50% of organisms killed in 30 min. at 37°C.; 0, few or no organisms killed; —, not tested.

aureus. Several placentas were tested against *Candida albicans* and found completely inactive. The antibacterial spectrum of each placental preparation was almost identical with that of the neonatal and maternal serums from the same patient.

Heating at 56°C for 30 minutes completely inactivated the antibacterial substances of the placenta. *Pseudomonas aeruginosa* and *Escherichia coli* survived as well in heated placental material as in 0.85% NaCl solution.

Absorption with killed bacteria also resulted in loss of bactericidal activity, but usually against the absorbing organism only. A placental preparation absorbed with *Escherichia coli* killed *Pseudomonas* but not *Escherichia coli*. A preparation absorbed with *Shigella* was ineffective against *Shigella* but remained strongly bactericidal for *Escherichia coli* and *Pseudomonas*. When the antibacterial effect of a placental preparation had been lost by specific absorption, it could be restored by addition of heat-inactivated placenta (Table III).

Variations in pH, from 6.01 to 7.88 had little effect on bactericidal activity of placental extracts. Treatment with bentonite reduced concentration of lysozyme to less than 0.5 μg/ml without affecting bactericidal activity. Likewise, wide variations in concentration of properdin and complement had little effect (Table I).

Discussion. The human placenta, familiar as a source of gamma globulin, has been shown to contain lysozyme, properdin, and complement. The presence of these substances was expected, as they are normal constituents of blood, and the placenta is a very vascular organ. Since levels of properdin and complement were higher in maternal than neonatal serum, it may be conjectured that both factors are passively transferred through the placenta to the fetus and not produced or concentrated by the placenta.

On the other hand, lysozyme was present in greater concentration in the blood of the infant than in that of the mother. This unexpected finding suggests that lysozyme is either

TABLE III. Bactericidal Activity of Placental Preparations against *Escherichia coli.*

	Dilution of placenta				
	1:4	1:8	1:16	1:32	1:64
Placenta	+	+	+	+	+
" absorbed with *E. coli*	0	0	0	0	0
" heated 56° 30 min.	0	0	0	0	0
Heat-inactivated placenta + placenta absorbed with *E. coli*	+	+	+	+	0

+, definite bactericidal effect, more than 80% of organisms killed; 0, no definite effect, more than 80% survived.

selectively transmitted across the placenta or produced in the fetal organism or its membranes. It is of interest that in certain instances, antibodies also have been found in greater concentration in cord serum than in maternal serum(19).

No evidence of a distinct antibacterial substance in placental tissue itself was found. In spectrum, or selectivity of action against various species, bactericidal effects of each placental preparation were similar to those of the maternal and neonatal serums from the same patient. Bactericidal activity was relatively independent of concentrations of properdin, lysozyme, and complement, but depended on presence of a heat-stable specific factor, which may be regarded as antibody, and a heat-labile non-specific factor, probably bactericidal complement. In all these respects antibacterial activity of the placenta resembles that of normal mammalian serums (20). Thus, this activity of the placenta appears to be derived from the blood, which forms a large fraction of the bulk of the organ as it is delivered.

Summary. 1. Serum was obtained from umbilical cords of healthy newborn babies and from their mothers just after delivery. Liquid preparations of placentas were produced by freezing, thawing, comminution and centrifugation. When tested against 7 species of bacteria, sera and placenta showed bactericidal activity, particularly against Gram negative forms. Placenta and cord serum were active against the same organisms as maternal serum from same patient. 2. Maternal and cord sera and placentas contained lysozyme, complement, and properdin. Placenta and cord serum showed a higher concentration of lysozyme than maternal serum. Placental preparations contained less complement and properdin than serums. 3. Heating at 56°C for 30 minutes caused complete loss of bactericidal activity in placental preparations. Absorption with killed bacteria usually caused loss of bactericidal activity, generally against the absorbing organism only, and this specific bactericidal activity could be restored by addition of heated placenta. 4. Placenta contains antibacterial substances of blood but seems to have little or no detectable bactericidal substance of its own. Apparently it protects the fetus from bacterial infection largely by transmitting protective substances from mother and mechanically impeding access of bacteria to the fetus.

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Cushing's Syndrome V. Chemical Inhibition of 11β-Hydroxylating Enzyme Systems in Hyperplastic and Neoplastic Adrenal Glands.* (25793)

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The clinical distinction between adrenal hyperplasia and adrenal tumor as the cause for Cushing's syndrome has been based in part upon tests which inhibit ACTH secretion (1), and also upon response of the adrenal glands to stimulation with exogenous ACTH (2-4). In general, adrenal tumors are neither suppressed by exogenous steroids nor stimulated with ACTH, whereas hyperplastic glands usually hyper-respond to stimulation with ACTH and are suppressed by administration of exogenous corticosteroids. However, these tests do not permit absolute distinction between the two pathological states (5-7). Su 4885, (2-methyl-1, 2-bis-(3-pyridyl)-1-propanone),‡ when administered in proper dosage to the human subject, selectively causes inhibition of 11β-hydroxylation within the adrenal cortex. This substance has been extensively studied in the normal individual and in patients with adrenal and pituitary dysfunction(7,8). Differences in response to Su 4885 have been noted to occur between patients with adrenal tumors and those with bilateral hyperplasia(8). The purpose of this investigation has been to define the action of Su 4885 upon the biosynthetic mechanisms within the adrenal gland in Cushing's syndrome when due to either bilateral adrenal hyperplasia or neoplasia.

Methods and materials. In all, 7 patients with documented Cushing's syndrome were studied. Two patients were shown to have adrenal carcinoma and 5 to have bilateral adrenal hyperplasia. The urinary ketogenic steroids were determined by a modification of the Norymberski technic(9). The methods for extraction, separation and identification of the various corticosteroid metabolites have been reported(10). In brief, urinary steroids after extraction were separated by chromatography on $Bush_1$ and $Bush_5$ systems. Eluted steroid fractions were quantitated by a modified Porter-Silber reaction. The tetrahydro S§ metabolite was identified by melting point determinations and infrared spectra, together with similarities in Rf values to a standard on several different chromatographic systems. Twenty-four-hour urine collections were obtained for several days before administration of the Su 4885 compound and for several days thereafter. 750 mg of Su 4885 were administered every 4 hours for 6 doses except in the case of St. who received a total of 200 mg in this period of time.

Results. Adrenal hyperplasia. Administration of Su 4885 over a one day period to 5 patients with bilateral adrenal hyperplasia and Cushing's syndrome resulted in a marked increase in urinary ketogenic steroids (Table I). In 4 of these 5 patients, maximal rise occurred the day following administration of Su 4885. In the fifth patient, Gu., maximal increase was observed on day of administration of the compound.

The results of identification and quantitation of the various hydrocortisone and com-

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§ 3α, 17α,21-triol, pregnan-20-one.