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A rapid procedure for testing the integrity of the maternal-fetal barrier in spontaneously delivered placentas

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The study of the maternal-fetal passage of drugs and other alien substances, particularly those of potentially hazardous substances of the occupational environment (with quantitative determinations in the blood of the fetal side of the placental vascular system) is an important consideration of perinatal medicine. Therefore, numerous authors (reviews by LEMTIS [18], GAGEL [5], and KIRCHNER [10]) have attempted with their contributions to solve the difficult problem of the physiologically correct perfusion of the placenta. In spite of remarkable advances in the field, the bilateral long-term in vitro perfusion of the human placenta has not yet been accomplished satisfactorily. In part this is due to the failure of systematically studying the potential interferences occurring during perfusion with blood. On the other hand, investigators may have been impeded in their study by the uncertainty as to whether the available placenta was injured or intact. Over several years we have developed in collaboration with GAGEL and following the experimental designs of KRANTZ and coworkers [14, 15, 4, 8, 9, 13] NESBITT et al. [21], as well as HAMRIN et al. [7] a modern apparatus equipped for the bilateral long-term perfusion of spontaneously delivered human placentas [20], stressing the study of both the most favorable experimental conditions as well as possible interfering factors (see also KIRCHNER and LEMTIS [11, 12]. During these experiments we designed a test which enables us to determine before the initiation of

Curriculum vitae

HORST GÜNTHER LEMTIS was born in 1923. He studied Medicine and Science at the University of Hamburg and Kiel and obtained his M. D. degree in 1954 at Kiel. Following his residency in Obstetrics and Gynecology at the Universities of Marburg and Göttingen, he was appointed to the senior staff of the Second Obstetric-Gynecology Service of the Free University, Berlin, in 1963.

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a lengthy perfusion experiment within a few minutes whether the maternal-fetal barrier of the experimental placenta is intact.

1. Methods

We started with the basic assumption that with a defective maternal-fetal barrier any dye present in the fetal vascular system should be transmitted very rapidly into the maternal placental circulation because of the pressure gradient from 80–90 mm Hg in the fetal-placental to 10 to 20 mm Hg in the maternal intervillous capillary system [23, 1, 3]. For this purpose, every ex-

perimental placenta was placed in the "artificial" uterus of our experimental apparatus [20]. The fetal vessels were filled with warm normal saline solution and 2 ml aliquots of a 0.2% solution of **Evans Blue** were injected at 2 minute intervals into the fetal placental circulation. The possible transplacental passage of the dye was **registered photo-electrically** at the location of the main drainage ("Collecting vein") of the maternal blood from the placenta in the artificial uterus. To this end our previously described experimental design was augmented by a universal oximeter. The dye concentration of the circulating fluid was measured with an ear electrode by the transmission principle. The resulting dye dilution curve was recorded simultaneously with the perfusion pressure and the minute volume continuously throughout the entire experiment on a compensation linear recorder. The choice of various sensitivities allowed the recording of even minute changes in the variables. A continuously adjustable bridge circuit allowed the adjustment of the units of measure of the apparatus to the circulating fluid of the individual experiments. BLEYL [2] had demonstrated that the fetal-maternal barrier is always penetrated after a substantial increase of the perfusion pressure. This fact was

of great importance for our experiments. Therefore, it was attempted to determine the limit of intravascular fetal placental pressure at which fetal maternal passage of a dye occurs. Since according to the findings of GAUER [6] pressure and minute volume are directly dependent on each other analogous to OHM's law, we increased the intravascular pressure by increasing the volume simultaneously with the EVANS Blue injections at 2 minute intervals.

2. Material

The placentas used for establishing the technique had been delivered spontaneously from healthy mothers after normal pregnancies and showed no gross injuries. Each placenta was prepared for the experiment immediately after birth.

3. Results

Tab. I demonstrates the correlation between the passage of dye from the fetal into the maternal placental circulation and perfusion pressure and thus simultaneously from the volume. Damaged placentas had a passage of EVANS Blue at the initial pressure of 50 mm Hg. Thus the barrier between the two vascular systems in these

Tab. I. Correlation between fetal-maternal passage of EVANS Blue and fetal-placental intravascular pressure. The increase in pressure was accomplished by increasing the minute volume. The passage of dye occurred at bold face values.

Placenta no.	Pressure increase (mm Hg)	50	60	70	80	90	100	120	140	160	Remarks
1	Increase of minute volume (ml/min)	30	35	40	45	70	75	95	120	150	
2		30	45	55	60	75	120	125	140	145	
3		25	30	35	50	60	85	90	105	135	
4		20	25	35	45	50	80	115	130	150	
5		35	40	45	70	90	125	130	145	160	
6		30	35	40	65	80	95	100	115	120	placenta defective
7		25	35	50	55	60	80	95	110	125	placenta defective
8		40	45	65	70	85	100	115	130	145	
9		30	40	45	50	70	75	90	110	135	
10		30	35	40	60	80	90	100	125	140	placenta defective
11		30	35	40	80	95	100	115	140	155	
12		25	30	40	50	60	80	100	110	120	
13		20	30	45	55	70	100	105	120	135	
14		25	30	40	50	65	70	95	105	130	

placentas was defect. In intact placentas the passage of the dye occurred only after a pressure rise to 100–160 mm Hg (see Tab. II). Figs. 1 and 2 demonstrate the difference between a placenta with intact and one with defective maternal-fetal barrier.

Tab. II. Pressure and minute volumes at which fetal-maternal dye passage occurred in intact placentas. $S(x)$ = sum of all measurements; \bar{x} = arithmetic mean.

	Pressure (mm Hg)	Minute volume ml/min
$S(x)$	1300	1165
\bar{x}	118.2	105.9
median	120	105
80% range	100–140	80–120

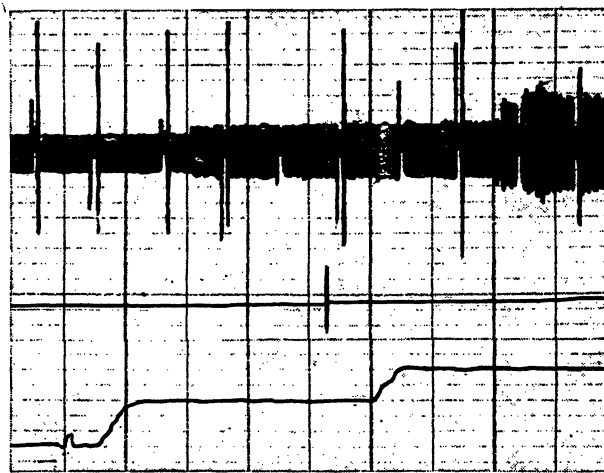


Fig. 1. Intact placenta. Dye dilution curve of EVANS Blue (below) which has been injected at two-minute intervals into the fetal placental vascular system simultaneously increasing the intravascular pressure. Above: the recording of intravascular pressure, middle: registration of minute volume.

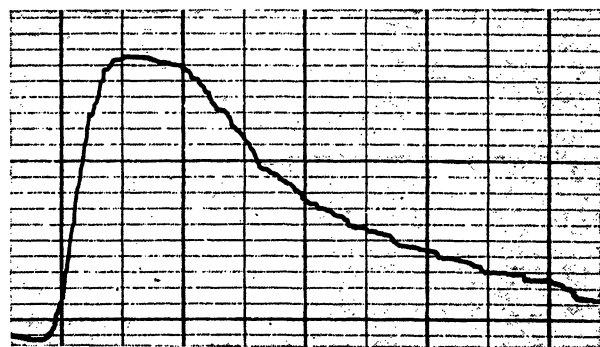


Fig. 2. Placenta with defective maternal-fetal barrier. Dye dilution curve following injection of EVANS Blue into the fetal placental vascular system recorded photo-electrically at the "main vein" of the maternal placental circulation.

4. Discussion

In the great majority of the placentas with intact maternal-fetal barrier which we have examined, the passage of Evans Blue from the fetal into the maternal circulation occurred only with unphysiologic high intravascular pressures at a mean of 120 mm Hg (see Tabs. I and II). Since both vascular systems were closed, the injected dye remained constantly in the fetal placental circulation with pressures under 100 mm Hg. Because the oximeter was not recalibrated to zero following the injections of EVANS Blue at 2 minute intervals, the curve must demonstrate a step-wise rise (Fig. 1).

Completely different results were found in placentas with a defective maternal-fetal barrier. Passage of dye from the fetal into the maternal circulation occurred already at the lowest initial pressures at the beginning of the experiments. The graphic depiction of the extinction curve shows a typical dye dilution curve (Fig. 2). Because the curves of an intact and a defective placenta are distinctly different from each other and the brief experiment can be carried out very quickly, it is ideally suited for testing the maternal-fetal barrier for its integrity. With the aid of this test we were able to demonstrate [19] that contrary to some assumptions only 30% of the spontaneously delivered placentas have a defective maternal-fetal barrier.

As demonstrated in Tabs. I and II the pressure limit at which even placentas with intact maternal-fetal barrier will always have a dye passage from the fetal into the maternal placental circulation ranges from 100–160 mm Hg. Possibly each placenta has its own threshold regarding this "opening pressure". Apparently, in each placenta stomata in the area of the maternal-fetal barrier are opened when the individual "maximal pressure" is reached. These gaps in the capillary walls in the fetal-placental vascular system have been demonstrated by PANIGEL [22] after the existence of stomata had been suspected by us since the experiments of BLEYL [2]. This author had utilized the wash-out technique described by us in 1952 [16] and 1955 [17] and had successfully accomplished a complete exsanguination of placentas if following the introduction of glass

cannulas into the umbilical arteries and closure of the umbilical vein, the placentas were perfused with pressures of over 100 mm Hg. Thus he had unknowingly proven that with sufficient, i. e.

abnormally high, intravascular pressure, all components of blood may pass through certain gaps in the capillary walls of the fetal vascular system which are not normally open.

Summary

In the course of several years we developed in collaboration with GAGEL based on three American models a modern apparatus for the bilateral long-term perfusion of normally born human placentas, stressing the study of favorable experimental conditions as well as that of possible interfering factors. Because the bilateral long-term perfusion of placentas with blood is costly, it should be ascertained that a planned experiment is worthwhile (i. e. that the experimental placenta is intact). Thus far no practical experimental design had been described. The authors have now developed a test allowing to determine within

a few minutes before the initiation of a lengthy experiment whether the maternal-fetal barrier is defective. To this end the fetal vascular system of the placenta placed in the "artificial" uterus is filled with warm normal saline solution to which Evans Blue is added. With a small photo-electric attachment at the "collecting vein" of the maternal circulation similarly filled with normal saline it can be determined immediately whether the placenta in question is damaged. Normally the maternal-fetal barrier is not passed by EVANS Blue. The test detected even minute defects.

Keywords: Diaplacental passage, dye passage (transplacental), maternal-fetal barrier, penetration, permeability, placental passage, placental perfusion, rapid placental test.

Zusammenfassung

Ein Schnelltest zur Prüfung der Mutter-Kind-Schranke spontan geborener Placenten auf Unversehrtheit.

Im Verlaufe mehrerer Jahre wurde gemeinsam mit GAGEL in Anlehnung an 3 amerikanische Modelle eine mit modernsten Geräten ausgestattete Anlage zur beidseitigen Dauerperfusion geborener menschlicher Placenten entwickelt, wobei auf das Studium der günstigsten Versuchsbedingungen sowie der möglichen Störfaktoren besonderer Wert gelegt wurde. — Da die doppelseitige Dauerperfusion von Placenten mit Blut sehr kostspielig ist, muß gesichert sein, daß ein geplantes Experiment auch lohnt, d. h. daß der zu untersuchende Mutterkuchen intakt ist. Hierzu gab es bisher noch keine in der Praxis bewährte Versuchsanordnung. Die Autoren entwickelten jetzt einen Test, der es gestattet, vor Beginn eines großen

Experiments innerhalb weniger Minuten festzustellen, ob die Mutter-Kind-Schranke defekt ist. Dazu wird der fetale Gefäßapparat der in den „künstlichen“ Uterus eingebrachten Plazenta lediglich mit temperierter physiologischer Kochsalzlösung aufgefüllt, der Evans Blue zugesetzt wird. Mittels einer kleinen photoelektrischen Vorrichtung läßt sich dann an der „Sammelvene“ des ebenfalls mit Kochsalzlösung beschickten mütterlichen Kreislaufs der Plazenta sofort feststellen, ob der Farbstoff aus den fetoplazentaren Strombahnen übergetreten ist, d. h. ob der betreffende Mutterkuchen verletzt ist. Normalerweise wird die Mutter-Kind-Schranke vom EVANS Blue nicht passiert. Mit dem Test werden selbst kleinste Defekte erfaßt.

Schlüsselwörter: Diaplacentare Passage, Farbstoffpassage (transplacentare), Mutter-Kind-Schranke, Penetration, Permeabilität, Plazentapassage, Plazentaperfusion, Plazenta-Schnelltest.

Résumé

Un test rapide pour l'examen de l'état de la barrière mère-foetus dans les placentas humains nés spontanément.

Après plusieurs années d'études réalisées avec le concours de GAGEL et s'appuyant sur trois modèles américains, les auteurs du présent article ont mis au point un système doté d'appareils ultra-modernes pour perfusions continuées ambilatérales de placentas humains nés. Un soin particulier a été porté sur l'étude des conditions optimales d'expérimentation et des facteurs de troubles éventuels. La perfusion placentaire continue ambilatérale avec du sang étant très coûteuse, il faut lui garantir le

maximum de chance de succès, c. à. d. s'assurer au préalable que le placenta à examiner est intact. Or, il n'existait aucun test sûr jusqu'à ce que les auteurs de cet article aient mis au point une méthode qui permet de détecter en quelques minutes avant l'intervention une défection éventuelle de la barrière mère-foetus. Pour cela, le système vasculaire foetal du placenta placé dans l'utérus «artificiel» est rempli d'une solution saline physiologique tempérée, additionnée de Bleu d'Evans. Au moyen d'un petit dispositif photo-électrique fixé sur la «veine principale» de la circulation maternelle du placenta remplie aussi de solution saline physiologique, il est alors possible d'ob-

server immédiatement si le colorant a traversé la barrière placentaire entre les circulations maternelle et foetale, c. à d. si le placenta maternel concerné comporte des lésions. Normalement en effet, le Bleu d'EVANS ne franchit

pas cette barrière entre la mère et le fœtus. Le test permet d'enregistrer même les plus petites lésions de la paroi des capillaires du fœtus.

Mots-clés: Barrière mère-fœtus, passage de colorant (transplacentaire), passage diaplacentaire, passage placentaire, pénétration, perfusion placentaire, perméabilité, test placentaire rapide.

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This work is dedicated to
Professor HÖRMANN on the occasion
of his sixtieth birthday.

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