hypothesis advanced by Fine and his collaborators(3). These investigators postulated that the lethal effects produced by antibiotics administered by gavage to animals in which the RES had been blocked are attributable to relase of endotoxin from the normal intestinal flora. It has been shown that injections of endotoxin induce increased properdin titers after an initial decline(4). It is not unlikely, therefore, that in the present study the antibiotics caused increased quantities of endotoxin to be released into the intestinal tract. This in turn would, after passage through the mucosa, produce the observed elevation of the properdin titer. Antibiotics probably exert effects other than their bactericidal activities, however. For example, it has been claimed that antibiotics abrogate the depressant action of endotoxin upon the RES(11). Thus, it is conceivable that the observed changes in properdin might be ascribable to some side-action of the antibiotics.

In Group II, the increase in properdin titers resulting from treatment with milk of magnesia alone is also difficult to explain. The milk of magnesia may also have released increased quantities of endotoxin, or it may have affected mucosal permeability to endotoxin, or it may have acted by some entirely different mechanism.

Conclusion. In dogs subjected to a standard hemorrhagic hypotension, survival rate was significantly increased by prior treatment with antibiotics. This regimen eliminated typical *E. coli,* and markedly reduced the Gram-negative fecal flora. Seven of 8 of these survivors had significantly increased properdin titers following antibiotic administration. In a group of dogs treated with milk of magnesia, though the survival rate was not increased, the survivors all had significantly increased properdin titers. The pre-treatment properdin titers were not significantly related to subsequent survival from standard hemorrhagic hypotension.

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Received March 20, 1961. P.S.E.B.M., 1961, v107.

Transfer of Water Across the Perfused Umbilical Cord.* (26707)

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An appreciable proportion of the water exchange of the amniotic fluid takes place through the intermedium of the fetus(1). The mechanism and site of this exchange is unknown. Since micturition and deglutition cannot account for the magnitude of this exchange, a transfer across the umbilical cord and possibly the fetal surface of the placenta has been suggested as an alternative explana-

* This investigation was supported by a grant-in-aid from Assn. for the Aid of Crippled Children, New York.

TABLE I. Composition of Solution Representing the Amniotic Fluid Compartment.

	g/100 ml
Sodium chloride	.620
Potassium "	.037
Sodium bicarbonate	.168
" phosphate (buffer)	.100
Urea	.027
Glucose	.060
Human serum albumin	1.200

tion(2). It is conceivable that water, electrolytes and metabolic products may pass through Wharton's jelly at a sufficiently rapid rate to account for part of the turnover of the constituents of the amniotic fluid.

To test this hypothesis preliminary experiments were designed and carried out wherein the transfer rate of water was measured by means of radioactive tracers. Sections of human cord were perfused with Group O, Rh negative blood under a pulsating flow while the organ was immersed in a solution, the composition of which approximated that of amniotic fluid. The preparation represents a closed 2-compartment system in steady state, suited for a mathematical interpretation of tracer curves.

Experimental. Fresh human cords were obtained from the delivery floor. The cords were clamped while still pulsating in order to insure distention of the arteries which facilitated the subsequent cannulation. They were placed in warm Ringer's solution and the vessels on one end cannulated within less than 10 minutes after delivery. The clamps were removed and blood drained to remove all clots. The arteries were distended with a few ml of heparinized Group O blood and cannulas inserted in the opposite ends. The 2 arteries were then connected to the vein in such fashion that the direction of flow in arteries and veins was in an opposite direction. The open arterial ends were connected to a "roller pump" and the vein connected with an oxygenating system described by Nyberg, Westin and Enhorning(3). The oxygenated blood was then fed into the pump, completing the cycle. Total volume of circulating blood was 480 ml.

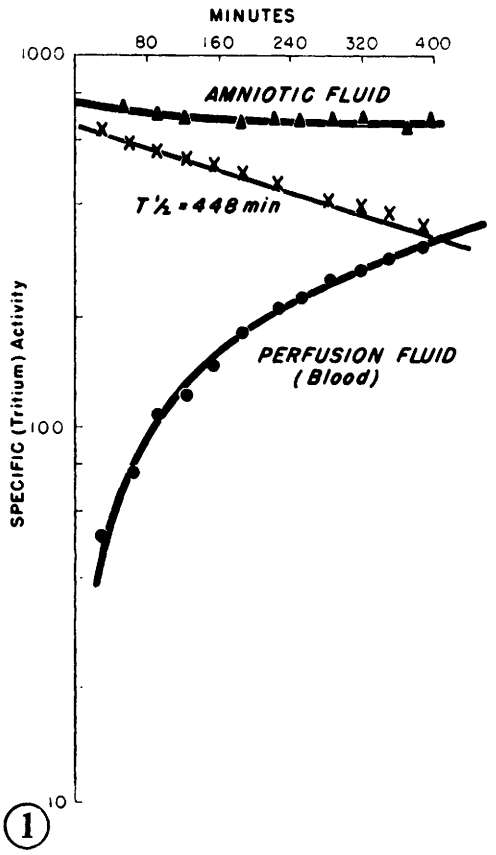
The system was briefly tested for leaks and the cord immersed in a synthetic amniotic fluid, the composition of which is given in Table I. The container was completely filled with fluid (5100 ml), sealed with silicone grease and tightly closed with numerous set screws. Provisions were also made for continuous pressure measurements, thermostatic control, removal of samples and agitation without disturbing the continuity of the system.

The perfusion was then started by setting the pump and oxygenator in motion and balancing their output. Because of the limitation of the oxygenation system, perfusion rate was set at 180 ml/min with a pulse rate of 162 beats per minute. The system was tested for at least one hour during which time equilibrium was established. Three to 4 millicuries of tritiated water were then injected into the blood or amniotic fluid compartment and samples withdrawn at frequent intervals. The samples were collected in melting point tubes and sealed. The tritium analyses were performed by subliming one drop of blood or amniotic fluid into the conversion apparatus of Graff and Rittenberg (4) and radioactivity determined according to Glasscock(5) using Bernstein-Ballantine counters in the proportional region. The results, expressed as specific activity, were then plotted on a semi-log scale and the curves analyzed by standard methods. Two examples are reproduced in Fig. 1 and 2 where the conditions were identical (length of cord, flow rate, volume). In the former (Fig. 1) the tracer was injected into the blood compartment, in the latter (Fig. 2) the amniotic fluid served as the primary compartment.

Calculation of transfer rates. The specific activity curves reproduced can be expressed by exponential equations of the form:

$$\text{Amount of tracer in the ith compartment at time t} = A_{1j}\, e^{-a_j t} + A_{eq.}$$

Subtracting the amount of tracer at equilibrium ($A_{eq.}$) from each experimentally determined point, a straight line is obtained on a semi-log plot, the slope of which determines the value of a. For the curves reproduced in Fig. 1, the value of a is 0.00154 min^{-1}, or a $t_{1/2}$ of 448 minutes. If total amount of tracer injected into the primary compart-



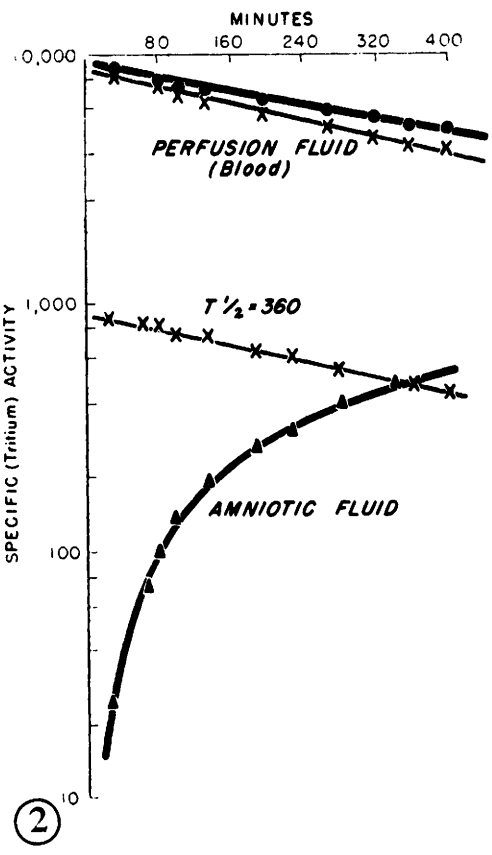


FIG. 1. Specific tritium activity (counts/min./mM × 10^2) in amniotic (▲) and perfusion fluid (●) as a function of time. Tracer was inj. into amniotic fluid compartment. Subtracting equilibrium value from each experimental point gives the straight line (×) which determines $t_{½}$.

FIG. 2. Specific tritium activity (counts/min./mM × 10^2) in amniotic (▲) and perfusion fluid (●) as a function of time in min. Conditions were identical to those described in Fig. 1, but tracer was inj. in perfusion fluid.

ment is 1, the fraction remaining in this compartment declines and reaches an equilibrium value of 0.914. The fraction of tracer in the amniotic fluid (AF) at any time t is then:

$$(\mathrm{HTO})_{\mathrm{AF}} = 0.086\, e^{-0.00154\, t} + 0.914 \ldots\ldots (2)$$

Simultaneously, the amount of tracer in the secondary compartment (perfusion fluid) rises and the fraction of tracer present at equilibrium will be 0.086 of total amount injected. The experimental curve for the perfusion fluid (PF) in Fig. 1 can be described by the equation:

$$(\mathrm{HTO})_{\mathrm{PF}} = 0.086\, e^{-0.00154\, t} + 0.086 \ldots\ldots (3)$$

Equations (2) and (3) uniquely define this system. The transfer rates can be calculated according to Berman and Schoenfeld (6), using the matrix notation:

$$[l] = [\mathrm{A}]\,[a]\,[\mathrm{A}]^{-1}$$

$$\text{or, } \begin{bmatrix} l_{11} & l_{12} \\ l_{21} & l_{22} \end{bmatrix} = \begin{bmatrix} 0.086 & 0.914 \\ -0.086 & 0.086 \end{bmatrix} \begin{bmatrix} 0.00154 & 0 \\ 0 & 0 \end{bmatrix} \begin{bmatrix} 1 & -10.62 \\ 1 & 1 \end{bmatrix}$$

$$= \begin{bmatrix} 1.32 & -14.06 \\ -1.32 & 14.06 \end{bmatrix} \times 10^{-4}$$

l_{21} is the fraction transferred from compartment 1 (amniotic fluid) to compartment 2 (perfusion fluid) and l_{12} the transfer in the reverse direction. To obtain the amount transferred per unit of time the l's must be multiplied by their respective compartmental

size

$$(1.32)(10^{-4})(5100) = (14.06)(10^{-4})(480)$$
$$= 0.675 \text{ ml/min.}$$

or an exchange rate of 40.5 ml/hr. A calculation performed in an analoguos manner using the data of Fig. 2 where the tracer was injected into the perfusion fluid, gives an exchange rate of 50.5 ml/hr.

Discussion. That various organic and inorganic substances can traverse the cord has been shown by Fehling(7) and Runge, Baur and Hartmann(8). When the vein of an isolated segment of human cord was perfused with acacia-thyrode solution containing various organic and inorganic dyes, these dyes appeared in the surrounding fluid within 20 to 30 minutes. Perfusion of the arteries failed to produce the same effect. Both authors considered but could not prove that large quantities of water are exchanged in a similar manner. Fehling (l.c.) also made an unsuccessful attempt to correlate the length of the cord with the amount of amniotic fluid.

The appearance of the radiotracer in the secondary compartment and its disappearance from the primary compartment constitutes adequate evidence that an exchange of water takes place. In the present perfusion system the only possible site of this exchange is the surface of the cord, *i.e.,* across vessel walls, Wharton's jelly and umbilical amnion. The amount of water exchanged is appreciable and under the conditions prevailing *in utero* this may well account for one-half of the water turnover of the amniotic fluid.

The length of the cord, *i.e.,* the surface available for the exchange, pressure differentials and rate of perfusion but not the volume of the amniotic fluid will be a function of the transfer rate. In the present experiments, these factors were kept constant (except for a slight difference in the actual length of the vessels but not the length of the cord) to obtain comparable data.

Of fundamental importance for determination of these transfer rates was the existence of a steady state within this system during the period over which measurements were made. By purely mechanical means a steady state can not be enforced in this system unless the organ remains in its normal healthy state. Utilizing the experience of others(3,9) only Group O, Rh negative blood was used as perfusion fluid, to prevent edema of the cord and transient or progressive changes in the size of the vessels. Under constant oxygen and carbon dioxide tension such perfusions can be carried on indefinitely(9).

The present preparation falls short of a direct *in utero* analogy in several respects. These are probably of quantitative rather than qualitative significance. Arterial and venous blood passing through the cord should differ in oxygen and carbon dioxide content. Perfusion rate should be more than twice that achieved in the present experiments, and finally, the theoretic interpretation does not take into account the fact that the effective size of the fetal compartment must be smaller than the volume of blood actually used.

In these preliminary experiments the primary objective was to keep the conditions constant and to demonstrate the existence of an exchange. More refined studies will clarify the relation to other factors.

Summary. 1. Fresh human umbilical cords 20 cm in length were perfused with heparinized Group O blood while immersed in a solution the composition of which was similar to amniotic fluid. 2. Tritiated water was added to the perfusion fluid (blood) or the surrounding bath and the change in specific tritium activity determined as a function of time. 3. Rate of exchange of water between the 2 compartments was calculated to be 40.5 and 50.5 ml of water per hour. 4. Evidence is presented that the umbilical vessels and Wharton's jelly play a significant role in the water exchange of the amniotic fluid.

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Received March 20, 1961. P.S.E.B.M., 1961, v107.

Hemagglutination and Hemagglutination-Inhibition with Coxsackie B Viruses. (26708)

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Coxsackie B virus infections are common occurrences in man and are responsible for several distinct syndromes, such as aseptic meningitis and pleurodynia, as well as for febrile illnesses which cannot be readily differentiated from those caused by other infectious agents. Although these viruses can usually be isolated from the throat or anal specimens of patients by the use of primate tissue cultures or suckling mice, these procedures are relatively cumbersome and are still beyond the scope of most clinical laboratories. The report that Coxsackie B type 3 virus agglutinated human erythrocytes(1), and the recent finding in this laboratory that certain strains of Coxsackie B virus types 1 and 5 also possessed this property, suggested that it might be possible to develop a hemagglutination-inhibition (HI) test which would be of use in diagnosis of human infections with these viruses. This report will describe such an HI procedure and the results obtained with it in a study of 25 children infected with Coxsackie B virus types 3, 4, and 5.

Materials and methods. The hemagglutinating strain of Coxsackie B virus type 3 was obtained from Dr. M. Goldfield. The hemagglutinating strains of Coxsackie B virus types 1 and 5 were found in this laboratory among a number of previously unidentified enteroviruses isolated in Toluca, Mexico and kindly made available to us by Dr. A. B. Sabin.

The materials and methods employed in hemagglutination and hemagglutination-inhibition procedures were the same as those used with reoviruses(2) unless otherwise noted. Rabbit antisera were prepared by an immunization schedule described previously (3). Neutralization tests with Coxsackie B virus type 4 were carried out in rhesus kidney cell cultures as described previously(3) using the "Powers" strain of this virus(4).

The human sera employed were from institutionalized nursery children who were under observation in a longitudinal study(5).

Results. The hemagglutinating properties of Coxsackie B virus types 1 and 5 were discovered during an investigation in which unidentified enteroviruses were systematically screened for their ability to agglutinate human erythrocytes. When a hemagglutinating virus could not be typed by HI as a serotype known to possess this property, additional identification technics were used until the agent was identified or was recognized as a new serotype. The identity of the type 1 and 5 strains was established by complement-fixation(6), then confirmed by HI using rabbit antisera. The strain of Coxsackie B type 3 virus described by Goldfield *et al.* is the only hemagglutinating strain of this type which we have encountered. A number of other Coxsackie B type 1 and 5 strains had been tested previously for hemagglutination with negative results.

An investigation of the conditions under which Coxsackie B virus types 1, 3, and 5 hemagglutinated revealed that optimum conditions for each of the 3 types were a sedimentation temperature of 37°C using eryth-