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The Effect of placental extracts on acute infectious diseases

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THE EFFECT OF PLACENTAL EXTRACTS ON ACUTE INFECTIOUS DISEASES.

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INTRODUCTION

Earliest medical history reveals that the cause for immunity of the new born to certain disease processes has been diligently sought for, by workers of each historical period. Explanations have been forwarded as to why an immunity should exist for one disease and not for another; but as in so many cases these theories have fallen into discard or have lacked substantiating evidence to bear out the claims made for them.

The mechanism of the immunity has again had many different hypothesis, each, which again, has failed to produce necessary proof to uphold it.

During the past decade much experimental work has been done in an attempt to either prove or disprove these theories, and to determine the feasibility of producing this immunity by artificial means.

It is the purpose of this paper to evaluate the work which has been done, and if possible to determine in some measure the practical application of the results.

In discussing the effect of placental extracts upon acute infectious diseases, it would seem only proper to determine the mechanism of immunity in the human new born and perhaps show why there has been so much discrepancy in the past.

The general consensus of opinion seems to be that immunity in the new born takes place as a result of:-

1. Transfer of anti bodies via the colostrum or milk.
2. Transfer of anti bodies from the maternal blood through the placental barrier.
3. Tissue which grows very rapidly as in the early months of the infants life, shows resistance or immunity to disease processes.

In regard to colostrum as the main agent in producing immunity, we find differing opinions. However, Smith and Little(1) have shown that colostrum is the chief agent for transfering known anti bodies to the new born calf, and it also seems to have the important function of protecting the calf against a general invasion by organisms of the B Coli group which find their way into the Gastro-intestinal tract during the first few days of life. Calves who fail to obtain colostrum have a seventy-five per cent mortality as a

result of a generalized B Coli septicemia. While controls allowed to feed after birth for fifteen minutes only, escaped infection indicating that only a small amount is necessary for protection. Post mortem examinations were carefully performed in order to determine the exact cause of death and any questionable findings eliminated that particular calf in the results of the experiments. Later, these same authors(2) gathered a series of calves at birth. Cleansed them with warm soda water, dried them and then transported the calves in a heated truck to a barn one and a half miles from the dam, thus making certain that the calves received no colostrum.

Then blood was obtained from the jugular of the cow under aseptic conditions and injected into the calf. The protection afforded against B Coli infections was not quite so profound as when the calf was allowed to suck, but that there was evidence of immunity could not be questioned.

Famulener (3) corroborated this work with goats in a somewhat different type of experiment. A number of goats already pregnant were immunized to red cells. The kids born at a subsequent date did not have hemolysius in their serum, but depended upon colostrum

to get them.

Little and Orcutt (4) have shown that calves born of cows which are highly immune to B. abortus because of natural infections have no agglutinus in their serums at birth. After the ingestion of colostrum, agglutinus appear in the serum of the calf equal or surpassing the titer of the cow.

Howe (5) in performing chemical analysis upon the blood of the calf, has shown it to be lacking in both pseudo-globulin and englobulin. After ingestion, there is a striking rise of these substances as compared to control animals.

Studies were instituted by Lewis and Wells (6) who tried to apply the above findings to infants. They found the globulin content of six different specimens of cord blood to be normal in pseudo-globulin, but lacking in englobulin. After colostrum feeding, the deficiency was remedied. Lewis and Wells concluded that colostrum is of importance, since in their opinion englobulin is the predominant protein of colostrum and is unique in its association with anti bodies. Experiments which appear later in this paper would appear to disprove this conclusion.

Boyd has corroborated the work of Lewis and Wells. They found ten specimens of cord blood to be

PROTEIN CONTENT OF HUMAN BLOOD

ADULT	TOTAL NITROGEN	ENGLOBULIN NITROGEN	PSEUDO-GLOBULIN NITROGEN I	PSEUDO-GLOBULIN NITROGEN II	TOTAL GLOBULIN	ALBUMEN GLOBULIN
1.	1.379	0.254	0.363	0.070	0.687	0.540
2.	1.322	0.223	0.232	0.193	0.648	0.492
3.	1.340	0.236	0.193	0.122	0.551	0.532
4.	1.167	0.247	0.102	0.100	0.449	0.421
5.	1.423	0.280	0.158	0.109	0.547	0.408
New Born infant umbilical cord blood						
1.	0.689	0.006	0.122	0.079	0.207	0.445
2.	0.972	0.000	0.390	0.013	0.403	0.528
3.	0.788	0.009	0.170	0.090	0.269	0.443
4.	1.060	0.000	0.324	0.105	0.429	0.587
5.	0.854	0.000	-	-	0.383	-
6.	1.064	0.005	-	-	0.405	-
Infants with Colostrum						
3 weeks	0.983	0.206	0.222	0.087	0.515	
13 days	0.887	0.090	-	-	0.1407	
11 days	0.887	0.089	-	-	-	
Infants no Colostrum						
3 weeks	0.885	0.082	0.206	0.098	0.386	

Figure I. PROTEIN CONTENT OF HUMAN BLOOD.

absent in euglobulin fraction. After feeding colostrum, seven became normal and the remaining three were even more deficient. Figure I is taken from Lewis and Wells (6) and illustrates the blood chemistry of the proteins in the infant, and the changes in the chemistry after colostrum feedings.

The discrepancies of Lewis and Wells and Boyd on cord blood as compared to Howe on calves blood, is due to variations in placental permeability of the different species. Grosser (7) made a comprehensive study of placental histology of different animals, and has shown that the number of cell layers separating maternal and fetal circulation per species, is as follows in Figure 2. In analyzing the literature, striking conformity resulted, when cows, goats, horses and sheep were used. A similar correspondence, when human beings, rabbits, guinea pigs and mice were used. The majority of workers, such as, Ehrlich, Merkel, Staubli, Polano and Shumacher who worked on guinea pigs or man, agreed that the placenta was permeable to anti bodies. On the other hand, Roemer, Famulener, Little and Orcutt, etc., who worked on goats and cows, found the placenta to be impermeable.

Bourquin (8) conformed the permeability in placentas of guinea pigs and rabbits, to diphtheria

		MATERNAL				FETAL				
		Endo-thelium	Conn. tissue	Epi-thelium	Uterus cavum	Endo-thelium	Conn. tissue	Epi-thelium	Type	Remarks
NOMENCLATURE										
OLD Semi Placenta or Placenta oppositae	NEW Placentae epithelio chorealis	+	+	+	+	"	+	"	Pig	True adiciduate
	Placentae syndumo chorealis	+	+ partly	— mostly	—	"	+	"	Rumi-nants	Transition forms
Placentae verae or Placentae Conjugatae	Placentae endothelio chorealis	+	almost entirely	—	—	"	+	"	Carniv-ora	Placentae Zonariae (diciduate)
	Placentae haemo-chorealis	—	—	—	—	"	+	"	Rodentia Insect-ivora Apes Man	Placentae discordales (diciduate)

Figure 2. Histology of various placental types.

anti-toxin, hemolysis and agglutinus. Earlier investigators thought the placenta to be impervious to proteins, and hence to anti bodies, unless it had been injured by disease or by heterologous proteins, such as horse serum anti-toxin, used in the course of experiments. Thus, transmission of anti bodies or proteins would be pathologic and not physiologic. But Bourquin examined each placenta carefully for Gross lesions and found none. In addition to this, Staublé (9) found transmission was present in individuals, whose mothers had been immunized before they were ever pregnant.

Various workers have demonstrated more specifically, that in humans the placental barrier is not impervious to immune bodies. Since diphtheria anti-toxin so readily lends itself to this type of experiment, it is the one most commonly used. Fischl and Wundscheim (10) first demonstrated the presence of diphtheria anti-toxin in cord blood of a large percentage of cases.

VonGroer and Kassowitz (11) found that eighty-four per cent of their cases were identical with maternal blood; but the Schick reaction did not always coincide. There were no controls in this series, however.

Zingher (12) also compiled a series of cases in which the infants Schick reaction was compared with that of the mother. Twenty-three cases under three months of age were found to be identical in reactions, but since reactions of the cord blood was not tested, there is some question as to how important a role colostrum may have played.

Ruh and McClelland (13) in an extensive series of ninety five cases all less than seven days old, found the Schick test to correspond between infant and mother, except in four cases, where the mothers gave positive reactions and the babies a negative reaction, and in one the mother was negative while the baby was positive. Of the total series, seventy-five were negative and twenty were positive. Eight infants were tested before nursing and it was found that when the mother gave a negative reaction, the reaction of the infant was negative before the ingestion of colostrum.

Kuttner and Ratner (14) probably have the most convincing group of studies, due to the fact that they are complete and carefully controlled. Schicks were run on women from one to three weeks ante-partum. Controls of heated anti-toxin were also run. The readings were taken in forty-eight hours and again in

several days. Specimens of the infants cord blood were taken from positive and negative mothers alike, and comparative determinations were made on maternal and cord blood according to the Kellogg technique, which is as follows:-

First, a negative reaction must have at least 1/30 anti-toxin unit per c.c. of blood. The L +- dose of toxin is diluted in 30 c.c. of normal saline, so that each c.c. contains 1/30 the L +- dose. This toxin dilution is then combined with an equal amount of the serum to be tested, and then allowed to stand at room temperature for thirty minutes. Then 0.2 c.c. is injected intra-cutaneously, this representing 1/300 L +- dose in addition with what anti-toxin may be contained in 0.1 c.c. of the serum to be tested. Thus, if the patient's serum contains more than 1/30 unit per c.c., the amount of toxin present will be completely neutralized and no reaction will occur. But, if the patient's serum contains exactly 1/30 unit of anti-toxin per c.c., it means that 1/300 of a minimum lethal dose of toxin is left unneutralized, and a slight reddening will occur. If the patient's serum contains less than 1/30 unit of anti-toxin per c.c., reddening and induration later developing into a definite superficial necrosis results. Readings

are best made in forty-eight to seventy-two hours. Guinea pigs weighing from four hundred to five hundred grams give the most uniform results.

A series of thirty cases, with both positive and negative Schicks, were compared to see the degree of correspondence between infants and mothers. None of the mothers gave a history of having diphtheria. The Schick was found to correspond with the Kellogg, which was employed on the serums of both mothers and infants. Figure 3 gives results in table form. (14)

CASES 30	SCHICK REACTION ON MOTHERS		KELLOGG METHOD	
	Neg.	Pos.	Maternal Blood	Cord Blood
	19	11	11 -- * 19 -	11 -- 19 -

Figure 3. * -- indicates a positive skin reaction in the guinea pig, showing less than 1/30 anti-toxin unit per c.c. of serum tested. - indicates absence of reaction in the guinea pig, showing that the serum contained at least 1/30 anti-toxin unit per c.c. of serum tested.

The results of another series of fifty cases studied by the same authors to determine the correspondence of Schick reactions are found in Figure 4.

CASES	REACTION OF MOTHER & BABY - BOTH NEG.	REACTION OF MOTHER & BABY - BOTH POS.	POS. REACTION IN MOTHER. NEG. OR DOUBTFUL IN BABY.	NEG. REACTION IN MOTHER. POS. IN BABY.	PSEUDO-REACTION MOTHER ' BABY	
50	39	4	7 *	0	10	0

Figure 4. * Discrepancy in these seven cases is due to the greater amount of pressure required to give an intra-dermal test in the infant, and because the toxin diffuses more rapidly in the infants skin, thus permitting no reaction. (Kellogg technique proved this to be true.)

Some experimentors were of the belief that the placenta was permeable, but that the titer was less in the cord blood. This was disproven when accurate comparisons were made as to the anti-toxin content of maternal and cord blood. Figure 5.

After having completed the foregoing experiments, Kuttner and Ratner conducted further studies to show the effect of nursing upon the anti-body titer. Pflanz and Schmidt (15) had shown many years previously, that the anti-body titer of milk is always less than blood in individuals with a high titer. In fact, they found the anti-body titer of milk to be from 1/10 to 1/25 less than cord blood.

In these studies they have a series of cases to show the effect of colostrum, transitional milk and normal milk. The classification of these substances depended upon the following coiteria.

1. Colostrum:- Secretion forty-eight to sixty hours post partum. Shows many colostrum corpuscles and coagulates upon boiling.
2. Transitional Milk:- Twelve to eighty hours post partum. Does not coagulate upon boiling, but shows a fair number of colostrum corpuscles.

3. Milk:- Secretion is fourth day or more post partum. Does not coagulate on boiling, and has very few or no colostrum corpuscles.

The technique employed, was an injection of from one to eight c.c. of colostrum or milk, (depending upon the experiment being performed at that time), from women with a negative Schick, intraperitoneally in guinea pigs weighing from two hundred fifty to three hundred grams. At the same time, one c.c. of cord blood was injected into controls. Twenty-four hours later, one c.c. of dilute toxin, equivalent to from 1/15 L dose to 1/30 L dose (depending on the weight of the pig) was injected into the animal, death resulting in from forty-eight to seventy-two hours later. Necropsies were performed on all animals and any other pathological process found present, invalidated the pig in the results of the experiment. All pigs that survived were observed for one month. The lesions found were typical of diphtheria, such as, supra-renal congestion and enlargement, edema and necrosis at the site of injection.

The conclusions arrived at were, that the anti-toxic titer of infants serum is not raised by nursing.

CASE	DILUTION OF L -- DOSE IN 0.1 c.c. *	MATERNAL SERUM	CORD BLOOD SERUM	UNIT PER c.c. OF MATERNAL BLOOD	UNIT PER c.c. OF CORD BLOOD
1.	†1/100 1/150	- +	- +	1/10 +	1/10
2.	1/50 1/25	- +	- +	1/5	1/5
3.	1/50 1/25	- +	- +	1/5	1/5
4.	1/25 1/10	- +	- +	2/5	2/5
5.	1/15 1/10	- +	- +	2/3	2/3
6.	1/50 1/25	- +	- +	1/5	1/5
7.	1/150 1/100	- +	- +	1/15	1/15
8.	1/50 1/25	- +	- +	1/5	1/5
9.	1/50 1/25	- +	- +	1/5	1/5
10.	1/25 1/10	- +	- +	2/5	2/5
11.	1/15 1/10	- +	- +	2/3	2/3
12.	1/150 1/100	- +	- +	1/15	1/15
13.	1/15 1/10	- +	- +	2/3	2/3

Figure 5.* Actual amount of toxin expressed in fraction L + dose.

† See following page for complete interpretation.

+ calculations from these measurements are based on following reasoning: Kellogg has shown that 1/300 of minimal lethal dose of toxin is the smallest amount that will give a skin reaction, characterized by redness, some induration and subsiding without necrosis. The smallest amount that will produce necrosis is 1/40 of minimal lethal dose. Since only Schick negative cases were studied quantitatively, we knew that 0.1 c.c. of all these serums would neutralize at least 1/300 of the L + dose. In order to establish the limit of anti-toxin content in any particular specimen of blood, a constant amount of serum, 0.1 c.c. was combined with increasing fractions of L.+ dose. The amounts varied from 1/150 to 1/10 L + dose. The limits of the neutralizing action of each serum, were thus reached as shown in the chart. The minus sign indicating the largest amount of toxin, which gave no reaction on the guinea pig and the plus sign, a definitely positive reaction usually going on to necrosis. The amount of anti-toxin per c.c. was thus calculated as follows: In case 1, 0.1 c.c. of serum plus 1/100 L + dose was negative. The serum therefore, contained 1/10 or more anti-toxin

unit per c.c., but not much more since the same serum mixed with 1/50 L + dose, gave a positive reaction. It therefore, must have contained more than 1/10 or less than 1/6 unit of anti-toxin. More specific results are shown in Figures 6, 7, 8 and 9.

From the preceding experiments, it would seem safe to conclude that, in some animals colostrum is essential for life. In humans, however, we don't know whether agglutinus are permeable to the placenta, but experimentally, anti-toxins are. And since infants, whose mothers die at birth, seem to make out as well as other infants, agglutinus are also probably transmitted.

	COLOSTRUM						CORD BLOOD					CONTROL			
CASE	HOURS POST PARTUM	AMT. INJECT INTRA-PERITON-EALLY	PIG	WT. IN GMS.	1 c.c. TOXIN INJECT SUB. Q.	RESULT IN HRS.	AMT. INJECT INTRA-PERITON-EALLY	PIG	WT. IN GMS.	1 c.c. TOXIN INJECT SUB. Q.	RESULT	PIG	WT. IN GMS.	1 c.c. TOXIN INJECT. SUB. Q.	RESULT
1.	48	2	A591	275	1/15 L	Dead 48	1	A588	250	1/15 L	Disch.	A598	275	1/15 L	Dead 48 hrs.
2.	48	6	A590	275	1/15 L	" "									
	48	2	A677	280	1/15 L	" 72	1	A675	230	1/15 L	"	A688	240	1/15 L	" 72 "
3.	48	1	A499	275	1/15 L	" 48	1	A535	230	1/15 L	"	A689	240	1/15 L	" " "
	48	3	A686	270	1/15 L	" 72									
4.	48	1	694	315	1/10 L	" 96	1	692	285	1/15 L	"	685	295	1/10 L	" 48 "
	48	4	499	305	1/10 L	Disch.									
5.	70	1	677	315	1/10 L	Dead 48	1	680	275	1/10 L	Dead 96	686	295	1/10 L	" 72 "
	70	4	687	310	1/10 L	" "									
6.	66	2	186	275	1/15 L	" 72	1	691	240	1/15 L	Disch.	675	245	1/15 L	" " "
	66	2	695	250	1/15 L	" "						522	295	1/10 L	" 48 "
7.	53	8	529	375	1/10 L	" "	1	526	325	1/10 L	"	A535	335	1/10 L	" " "
8.	57	4	692	245	1/15 L	" "	1	676	235	1/15 L	Dead 10 days	693	240	1/15 L	" " "
9.	48	4	682	265	1/15 L	" "	1	681	255	1/15 L	Disch.	697	260	1/15 L	" 72 "
10.	56	6	A191	290	1/15 L	" 96	1	A186	275	1/15 L	Dead 8 days	281	280	1/15 L	" " "
11.	48	4	535	305	1/10 L	" 72	1	532	285	1/15 L	Disch.	538	295	1/10 L	" 48 "
12.	24	2	307	260	1/30 L	" 5 days	1	A465	260	1/20 L	"	282	260	1/30 L	" 7 days
12.	24	5	158	255	1/30 L	D" 72						A468	255	1/30 L	2 15 "

Figure 6. Results of protection tests with colostrum and cord blood.

	MILK						CONTROL			
CASE	DAYS POST PARTUM	AMT. INJECTED INTRA-PERI-TONEALLY	PIG	WT. IN GRAMS	1 c.c. TOXIN SUB Q.	RESULT	PIG	WT.	1 c.c. TOXIN SUB Q.	RESULT
1.	12	8	A492	365	1/10 L +	Death 48 hrs.	535	335	1/10 L +	Death 48 hrs.
2.	10	7	A536	260	1/15 L +	Death 72 hrs.	A524	270	1/15 L +	Death 72 hrs.
3.	11	10	A496	220	1/15 L +	Death 48 hrs.	500	280	1/15 L +	Death 96 hrs.
4.	4	6	A495	210	1/15 L +	Death 72 hrs.	A317	265	1/15 L +	Death 48 hrs.
5.	10	8	A507	345	1/10 L +	Death 72 hrs.	440	315	1/10 L +	Death 48 hrs.
6.	4	8	A552	370	1/10 L +	Death 72 hrs.	258	230	1/10 L +	-
7.	4	8	499	325	1/15 L +	Death 72 hrs.	A535	235	1/10 L +	Death 48 hrs.
8.	4.5	6	A166	220	1/15 L +	Death 48 hrs.	281	280	1/15 L +	Death 72 hrs.
	4.5	6	A440	220	1/15 L +		255	220	1/15 L +	Death 48 hrs.
9.	12	10	A499	220	1/15 L +	Death 5 days	571	290	1/15 L +	Death 72 hrs.

Figure 7. Results of protection from milk.

TRANSITIONAL MILK

CASES	AMT. INJECTED INTRA-PERITONEALLY	PIG	WEIGHT	1 c.c. TOXIN SUB Q.	RESULT
1.	7	A635	220	1/15 L --	
	5	A487	245	1/15 L --	
	5	225	225	1/15 L --	
2.	8	A496	275	1/15 L --	
3.	2	A587	275	1/15 L --	
	2	A634	275	1/15 L --	

Figure 8: Result of Protection test with Transitional Milk.

CASE	TOXIN DOSE IN 0.1 c.c.	SERUM FROM CORD BLOODS 0.1 c.c.	INFANTS SERUM	UNIT ANTI-TOXIN PER c.c. CORD BLOOD	UNIT ANTI-TOXIN PER c.c. INFANTS BLOOD
1.	1/50 L +	-	-	1/5	1/5
	1/25 L +	+	+	1/5	1/5
2.	1/5 L +	-	-	2 units or more	2 units or more
3.	1/25 L +	-	-	2/5 units or more	2/5 units or more
4.	1/50 L +	-	-		
	1/25 L +	-	+	2/5	1/5
5.	1/50 L +	-	-		
	1/25 L +	+	+	1/5	1/5

Figure 9: Results of tests on infants who nursed, taken six to ten days after birth.

The third possible source of infant immunity, is ascribed to some resistant factor in rapidly growing, near embryonic tissue. Work on this subject is exceedingly sparse, but that there is some justification of this theory, is not to be questioned.

Studies in vitro were made on several different types of tissues and their reactions to diphtheria toxin.

Wadsworth and Vories (16) recorded the absence of any inter-reaction between diphtheria toxin and brain tissue or leukocytes. The same results were obtained between leukocytes and tetanus toxin, but Wasserman and Takaki (17) found brain tissue to neutralize the tetanus toxin.

Wadsworth and Hoppe (18) experimented with mature and embryonic cardiac tissue and found both to have no binding power upon the toxin. Procedure and results are given in Figure 10. It was when embryonic cardiac tissue was cultured on media, that the startling ability to combine with toxin was first noted. One five hundredth of M.L.D. standard diphtheria toxin in one tenth of a c.c. of Lockes solution was placed on tissue culture medium with tissue growing on it for a period of forty-eight hours at thirty-seven and five tenths

TISSUE EMULSION (0.5 c.c. used)	DIPHTHERIA TOXIN DILUTION	EXPOSURE FOR NEUTRAL-IZATION	PREPARATION OF INOCULA	METHOD OF INOCULATION	RESULTS
0.1 gm. of embryonic guinea pig cardiac tissue in 0.5 c.c. Lockes Solution	1 M.L.D. of standard diph. toxin in 0.5 c.c. Lockes solution	3 hrs. at 37.5 C in the dark.	Centrifugalization, 3 min. at low speed. Supernatant fluid drawn into syringe with hypodermic needle. Sediment washed in 0.5 c.c. Lockes soln. centrifugalized & supernatant fluid drawn into same syringe.	Sub. Q.	Death in 91 hrs.
			Sediment washed in 0.1 c.c. Lockes soln. centrifugalized supernatant fluid drawn into unrinsed syringe.	Intra-Cutaneous	* No skin reaction
			Sediment suspended in 1 c.c. Lockes soln.	Sub. Q.	No reactions
0.1 gm. of mature guinea pig cardiac tissue in 0.5 c.c. Lockes Soln.	1 M.L.D. of standard diph. toxin in 0.5 c.c. of Lockes Soln.	4 hrs. at 37.5 C in the dark.	Centrifugalization, 3 min. at low speed. Supernatant fluid drawn into syringe with hypodermic needle. Sediment washed in 0.5 c.c. Lockes Soln. & supernatant fluid drawn into same syringe.	Sub. Q.	Death in 98 hrs.
			Sediment washed in 0.1 c.c. Lockes soln. centrifugalized, supernatant fluid drawn into unrinsed syringe.	Intra-cutaneous	* No skin reaction
Control	1 M.L.D. of standard diph. toxin in 0.5 c.c. Lockes Soln.		Syringe rinsed in 0.5 c.c. Lockes soln.	Sub. Q.	Death in 91 hrs.

* A typical Schick reaction results from 1/500 M.L.D. White guinea pigs were used 250 to 255 gms.

Figure 10. Effect of exposure with embryonic and mature cardiac tissue upon potency of diphtheria toxin. (18)

AMOUNT	DOSE IN LOCKES SOLUTION	EXPOSED FOR 48 HOURS AT 37.5 C.	SKIN REACTION
1/500 M.L.D. STANDARD Diph. Toxin	0.1	On tissue culture medium without tissue present.	Reaction like a Schick test.
1/500 M.L.D. standard Diph. Toxin	0.1	On tissue culture growing on culture medium.	No reaction
1/500 M.L.D. standard Diph. Toxin	0.1	Heated 100 C for three min. & cooled. One tissue culture medium without tissue present.	No reaction

250 gm. white guinea pig was used.

Figure 11. Effect of growing embryonic cardiac tissue upon potency of diphtheria toxin. Practical type of test. (18.

degrees centigrade. Washings from this were injected intra-dermally into a white, two hundred and fifty gram guinea pig. No reaction was observed, but controls gave a reaction similar to the Schick test, as seen in Figure 11.

McKhann and Coady (19) state that infants as a general rule, are not attacked by scarlet fever under eight years of age and rarely under three years. Diphtheria and poliomyelitis show the same tendency, but to a less striking degree. Measles are very rare in children under five months of age, even though they may be intimately exposed to it. New born babies may develope chicken pox, but they more commonly escape it. Mumps is rare, even up to two years of age.

Were lack of exposure the factor in the low incidence of these diseases in early infancy, certainly such a highly communicable condition as measles, should be more common than pertussis or pyogenic infections, yet, such is not the cases. Pertussis is frequently encountered in young infants, and the incidence of streptococcal and staphylococcal infections is notoriously common. Certain types of infections, for example, colon bacillus, sepsis and colon bacillus meningitis are encountered almost

exclusively in the very young.

Any and all of the infections to which the infant ordinarily appears immune, may occur in the first weeks of life, if the mother has also developed the infection or is demonstrably susceptible to it.

Thus, measles may occur in the new born infants of mothers who have never experienced the disease, and chicken pox has been observed at birth when the infection has been present in the mother. Therefore, it might be assumed that the immunity of the new born depends upon the immunity of the mother, passed on to the infant through placental or mammary transmission of anti bodies.

This assumption, however, requires certain exceptions, for well authenticated cases are recorded of infants resisting a disease, even though thoroughly exposed to the mother suffering from the infection. Occasionally, scarlet fever in the nursing mother is not followed by infection in the baby, and frequently vaccination reactions are entirely different in the mother and infant. It is in these cases that the theory of immunity in rapidly growing tissues may have its application.

The passage of antigen through the placenta with the active production of anti bodies by the

fetus is difficult to demonstrate, because active and passive immunity acquired during fetal life cannot readily be distinguished. However, data obtained from study of the responses of new born infants, are thought to have some bearing on the subject. Very young infants develope agglutinus poorly, following the injection of typhoid vaccine, and the response to protective injection of diphtheria toxin anti-toxin, is slight as compared with that of older children. Since in the new born the response to injection of antigen is often inadequate, the development of an active immunity by the fetus has somewhat been discounted.

On the other hand, the placental transmission in humans of anti bodies may be considered to be well established, and the factor of mammary transmission has been ruled out by examination of the antibody content of the cord blood, as compared with later examinations on the infants blood.

It is worthy to note that those anti bodies which have been demonstrated to pass human placenta, are antivirul or anti toxic in character. This is in keeping with clinical observations, that the virus diseases and the bactereal diseases in which symptoms are due to absorption of toxins, are the ones to which the new born infant is resistant. The

crux of the situation lies in the fact, that if the immunity of the infant is passive in character and is derived largely from the mother by transmission of anti bodies through the placenta, it is peculiar that it should persist for so many months, when one considers the short duration of artificial passive immunity induced by the injection of horse serum or human convalescent serum.

The consideration of the clinical and experimental evidence concerning immunity in infants to infectious diseases, led these authors (McKhann and Chu) (20) to undertake further studies on the subject. The demonstrable presence of anti bodies in the cord blood together with a knowledge of the possibilities of tissue immunity in embryonic or rapidly growing tissues, suggests the study of anti bodies in the placenta, and the possibilities of extracts of placentas which might be capable of increasing the protective reaction in infants and children to certain diseases. Where as the blood obtained from the umbilical cord represents fetal blood and should contain only anti bodies to which the placenta is permeable. The material which may be extracted from the placenta, represents fetal blood, maternal blood and probably extracts from the placental tissue itself.

It could then be assumed that not only anti bodies present in cord blood, would be present in extracts made from placenta, but also that anti bodies might be present to which the placenta is impermeable.

In preliminary tests, sterile salt solution extracts were prepared and were found capable of neutralizing diphtheria toxin, of blanching the rash of scarlet fever. The presence of diphtheria and scarlet anti bodies was not constant in individual placentas.

The method of procedure consisted of using only normal appearing placentas in individuals, who had no evidence of toxemia nor syphilis, lots varying from three to five in number were collected and the umbilical cords were tied so as to prevent loss of fetal blood contained in the placenta. Placentas were placed in sterile containers and repeatedly incised with a sterile amputation knife. For each placenta from one hundred to two hundred c.c. of two per cent sodium chloride solution was added. The tendency for placental blood to lyse was overcome by the use of hypertonic salt solution. The lot was allowed to stand ~~over night~~ in the refrigerator for forty-eight hours, then desiccated and centrifuged to free it from blood cells and debris.

Precipitation of the protein fraction was carried out, first by addition of a saturated solution of ammonium sulphate, in such amount as to bring the salt concentration up to thirty-three per cent saturation. The precipitate was removed by filtration through hardened paper (C. S & S, 575) on a Buchner filter. To the filtrate, saturated ammonium sulphate was added in sufficient quantity to bring the concentration up to fifty per cent. The precipitate that was formed by increasing the concentration was also collected by filtering. The two precipitates were separately dried between sheets of filter paper and immediately redisolved in salt solution. Each was placed in a cellophane bag and dialysed against running water for twelve hours, and then against physiological salt solution until the dialysate became free from sulphates. Dialysis for a longer time against water, resulted in precipitates in both fractions which could not be redisolved without marked alteration in hydrogen ion concentration of the material.

The precipitate that came down with thirty-three per cent ammonium sulphate, was hard to collect and negligible in amount. It was designated as fraction A rather than englobulen. While that

precipitate that came down with fifty per cent ammonium sulphate was designated as fraction B rather than pseudo-globulin. This terminology was adopted because of the presence of indeterminant amounts of tissue proteins in placental extracts, and secondly, because later studies show the separation of serum englobulin and pseudo-globulin is not accomplished by a single differential precipitation. The precipitate, however, may be dependably considered as globulin.

After dialysis, the solutions of extract were centrifuged, and then bottled.

Because of anti body loss with Berkfelds in sterilization, chemical methods were adapted.

Mercurophen was later used, as was merthiolate in concentrations from one to five thousand to one to eight thousand, for purposes of sterilization. Extracts were rendered sterile in a few hours, and in tests conducted five days later, they were still pure.

Three to eight c.c. was injected into three hundred gram guinea pig, without evidence of infection or poisoning.

The possibility of hormonal influence was also investigated. It takes one hundred per cent

ammonium sulphate to precipitate sex hormones from urine. Tests were made on rabbits, and the ovaries displayed negative results.

Experimental results as tabulated by the Kellogg method, showed that fraction A was less potent than fraction B, and that chemically sterilized products were much more potent than filtered products.

Fraction A contained but little scarlet fever blanching stuff. Fraction B was often better than commercial products.

Although the amount of diphtheria anti-toxin in the extract is small, probably entirely inadequate to be of clinical value, the measurement of diphtheria anti-toxin could be made readily on animals, and thus the determinations of the diphtheria anti-toxin content of various lots gave some evidence of potency and degree of concentration attained. (19)

Tests of the diphtheria toxin neutralizing capacity revealed, that although there was some anti-toxin in the englobulin fraction, there was more in the pseudo-globulin. The separations of the globulin fractions was incomplete in the initial precipitations, so a purified englobulin fraction was prepared. This fraction contained almost none of the diphtheria

anté body. On the contrary the purified pseudo-globulin fraction was relatively potent.

Among the factors which influence the affectiveness of the serum in the prophylaxis or modification of measles are:-

1. Time of injection.

2. Dosage.

3. Potency of material. If the potency of material could be standardized, it should be possible to secure predictable results. According to Figure 12, by pooling placentas in large lots to minimize the variation in anté body content of individual placentas, and by standardizing the dosage of the preparation on basis of its protein content, it has been possible to prepare an extract of almost uniform potency, which promises to eliminate variability in results. By the use of potent preparations in adequate doses, we have evidence that measles may be prevented or its course modified by treatment of patients as late as ten days after exposure.

Additional studies in the effectiveness of placental extracts in measles are recorded by McKhann and Chu. (21) Figure 13. (19)

Forty susceptible children, in course of hospitalization for other causes, became more or

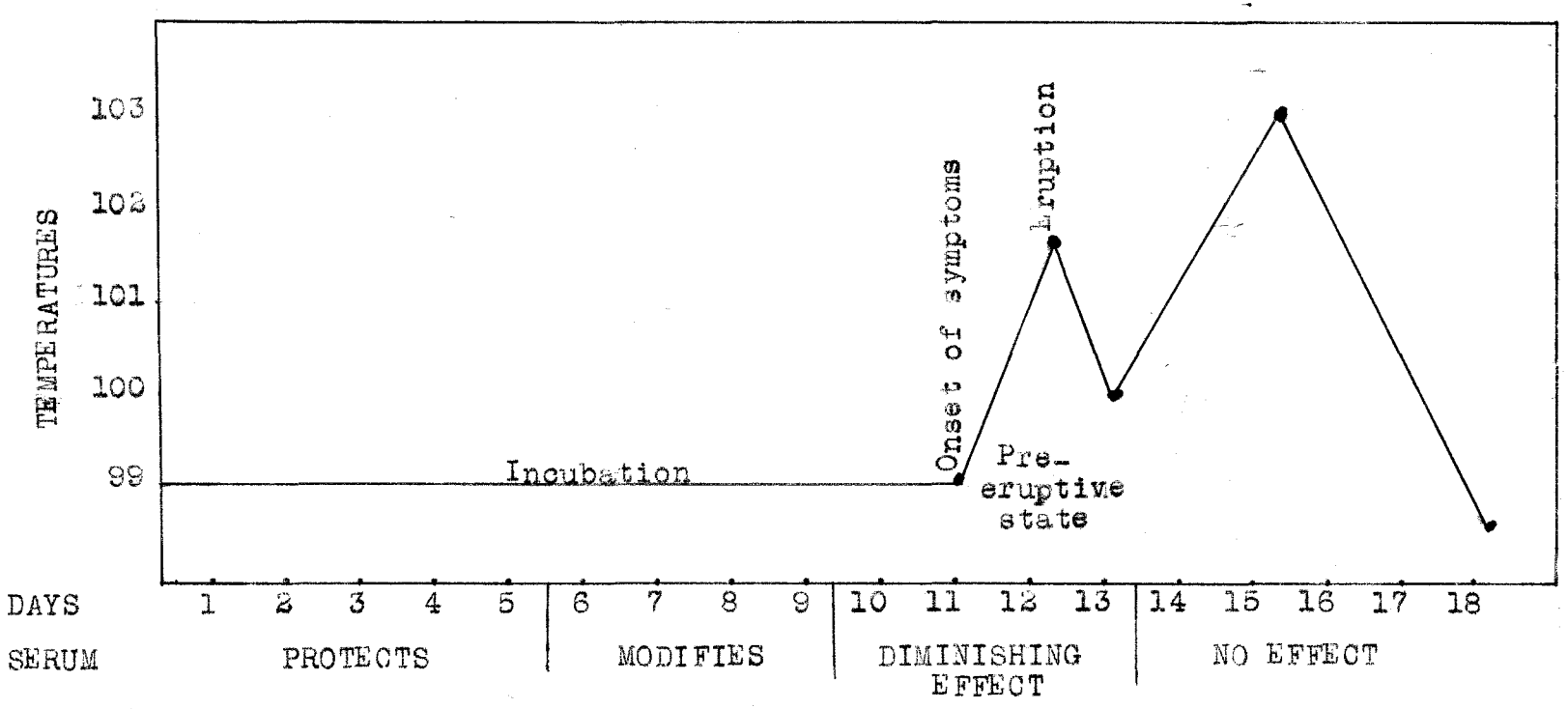

Figure 13 (19). Effectiveness of serum in prevention or modification of measles.

If the potency and dosage of serum are standardized, the time of injection becomes the factor determining the effectiveness of the treatment. The potency of different lots of convalescent serums of different adult bloods, varies considerably, so that in the dosages ordinarily used, predictable results cannot always be obtained. Pooled placental extracts in standardized dosages promise to overcome this difficulty.

	Complete Protection	Modification	Typical Measles
Placental extract to prevent the disease - 95 patients	91	4	0
Placenta extract to modify the disease - 8 patients.	5	3	0
Convalescent serum or adult blood to prevent disease - 33 patients.	29	4	0
No treatment - 2 patients.	0	0	2

Figure 13 (19) Prevention and modification of measles by placental extract.

less intimately exposed to measles and were given placental extract. Twenty other children in the hospital likewise, were susceptible to the disease and exposed at the same time, received adult immune serum, convalescent serum or no serum. The remaining three children who received placental extract were exposed at home to a sister who had the disease. The children in the hospital became exposed in small groups, so a separate consideration of each group is desirable.

Group I.

Ten children ranging in age from eighteen months to six years, were intimately exposed for one day to a child suffering from measles. The contact with the patient suffering from measles was so intimate, that the transmission of the disease would seem certain. Seven of the ten children received placental extract in dosages, varying from four to ten c.c. before the fourth day after exposure. While the remaining three children received thirty c.c. each of adult whole blood, on the third day after exposure. In six of seven children who received placental extract, measles did not develope, while in one on the seventeenth day after exposure, a mild modified type of the disease developed. In the three

who received whole blood, a modified form of the disease developed.

Group II.

Six children from three to ten years became intimately exposed to measles. Each received an intramuscular injection of five c.c. of placental exfract, in four instances, as late as the fifth day after exposure. Measles developed in none of the six children. However, in another child on the ward, similarly exposed, who was thought to have had the measles and who therefore received no prophylactic treatment, typical severe measles developed, indicating the probable thorough exposure of the potents in this group.

Group III.

Two patients, one and three years of age, were exposed for three days to a patient in the catarrhal and early errupture stages of measles. On fourth day after exposure and again on the sixth day, each of these children received an injection of three c.c. of placental extract. Measles developed in neither child. In a third child, however, who did not receive placental extract or other prophylactic serum, typical severe measles developed.

Group IV.

The twelve children in this group were on one of the wards of the Convalescent Home of the Children's Hospital, where contact among the patients is intimate. Previous experience indicated, that measles would sweep through the entire group of susceptible children unless the disease could be stamped out promptly. Six of these children ranging from two to nine years received five c.c. each of placental extract. On the fourth day of exposure to measles, the remaining six children of similar ages received four c.c. each of convalescent serum. None of the twelve children contracted measles.

Group V.

Eleven children ranging from three to nine years were exposed for two days to a child in the catarrhal stage of measles. Seven of the children received six c.c. each of placental extract on the fifth day after the last exposure (seven days after beginning of exposure). Five of these children did not contract measles; in one the modified disease developed.

Group VI.

Seven children between ten months and two years of age came in contact with a child in catarrhal

stage of measles. Six of these seven received placental extract intra-muscularly on third and fourth day after exposure. The remaining child, twenty months of age, was quite ill with tuberculosis and was given ten c.c. of extract. None of these seven children contracted measles.

Group VII..

Nine children non immune to measles, ranging from four to nine years in age, were exposed for three days to a patient in the catarrhal and early erruptive stages of measles. Four of these children received six c.c. each of placental extract on the fifth day, after first exposure. The remaining five children received thirty-three c.c. each of whole adult blood on fifth day, after first exposure. None of these children contracted measles.

The three children who were exposed to measles at home are of especial interest. Two of the children, aged five and seven years, were intimately exposed to their sister, who had measles. A modified form of disease was desired in these children, so administering of placental extract was delayed until the eight day after the beginning of the exposure, in an effort to obtain the modified form of the disease. One child received five c.c. of extract intra-muscularly; the

other received six c.c. In both children mild modified measles developed. The third child treated at home was also in intimate contact with the sister, who had measles. The extract in six c.c. dosage was given on the eighth day after beginning exposure, in an effort to obtain the modified form of the disease. In this patient no measles developed in spite of the intimate contact with the sister who had the disease, and dispite the delayed administration of the extract.

In no instance did the intra-muscular injection of placental extract appear to be without effect in either prevention or modification of measles in non immune contacts, and in no instance was there a general or local reaction or evidence of local infection following the injection of placental extract.

In these cases, the effective dosage of extract was slightly higher than the usual dosage of convalescent serum. Further studies on the protein content of the extract indicate that the standardization of the preparation with development of uniform potency and dosage may be reasonably expected.

Similar studies were conducted by Toomey and August in an effort to determine the efficiency of placental extracts upon scarlet fever, as compared with that of convalescent serum. The extract was

prepared in much the same way as has already been described.

The convalescent scarlet fever serum was obtained at least twenty-one days after the acute illness had begun and was likewise inactivated.

When serum was extracted by ether, it was washed three times with ether and then filtered. When it was filtered, a Berkfeld filter no. "W" was used and when it was catalyzed, one drop of normal serum was used as the catalyzing agent.

The only patients who received injections were those with good macular rashes. Injections were not made into patients whose rashes had been present for more than forty-eight hours. Most of them were done within twenty-four hours of the exanthem. In each case .5 c.c. was injected, unless otherwise noted.

For convenience, reactions were read as follows: + indicates 1 cm. of blanching; + + 3 cm. blanching + + + 4 cm. blanching; + + + + 5 cm. blanching.

Two hundred and twenty seven placental serums were tested. Of this number one hundred one, or seventy-nine per cent caused definite blanching of the rash of scarlet fever.

The blanching powers was not affected by either extraction nor filtration.

Certain placental serums blanched as well as convalescent serums. Figure 14.

Extracts had practically the same degree and incidence of blanching as the corresponding maternal serum, and usually gave better blanching results than scarlet fever anti-toxins used as controls in same experiment. Figures 15 and 16.

Placental serum was sufficiently anti-toxic to neutralize Dick toxin in vitro, so that when a freshly prepared mixture of equal parts of placental serum and scarlet fever toxin was injected into a susceptible person, (one with a positive reaction to Dick test,) a negative reaction to Dick test was obtained.

Often convalescent serum and usually scarlet fever anti-toxin were not as potent in this regard.

PATIENT	BLANCHING POWER OF PLACENTAL SERUM	BLANCHING POWER OF CONVALESCENT SCARLET FEVER SERUM
L. G.	+ + +	+ + +
W. J.	+ + +	+ + +
Z. D.	+ +	+ +
M. G.	+ + + +	+
A. S.	+	+
M. C.	+	+
M. F.	+	Neg.
M. F.	+	+
L. E.	+ +	Neg.
L. E.	+ +	Neg.

Figure 14. Relative blanching powers of convalescent serum and placental serum. Each patient received an injection of different placental serum. (22)

PATIENT	PLACENTAL EXTRACT	MATERNAL SERUM	ANTI-TOXIN
H. J.	±	±	Neg.
J. S.	±	±	Neg.
L. G.	±	±	±
W. C.	+	+	Neg.
S. W.	Neg.	Neg.	Neg.
J. N.	+	Neg.	Neg.
L. G.	Neg.	Neg.	Neg.
D. M.	+	+	Neg.
L. P.	±	±	Neg.
O. W.	+	+	Neg.
P. D.	+	±	Neg.
P. T.	+	±	Neg.
M.MM.	Neg.	Neg.	Neg.
M. J.	±	±	Neg.
R. O.	Neg.	Neg.	Neg.
M. E.	Neg.	Neg.	Neg.
B. M.	+	±	Neg.
L. H.	±	Neg.	Neg.
A. M.	+	+	Neg.
M. P.	+	Neg.	Neg.
T. C.	+	+	Neg.

Figure 15. (22) Comparisons of reactions between placental serum and maternal serum; anti-toxin used as a control. Degree of reaction is not detailed in this experiment. The reactions are read as being only negative or positive. Each patient received injections of placental and maternal serum from a different mother.

PATIENT	PLACENTAL SERUM			SCARLET FEVER ANTI-TOXIN		
	BLANCHING	NEG.	REDNESS	BLANCHING	NEG.	REDNESS
H. L.	+ + + +					+ + + +
B. L.	+ + + +				Yes	
E. L.	+ + + +					+ + + +
S. P.	+ + + +					+ +
C. D.		Yes			Yes	
V. H.		Yes			Yes	
L. S.		Yes			Yes	
J. W.	+ + + +				Yes	
L. M.		Yes		+ + + +		
R. M.	+ + + +			+ + + +		
G. I.	+ + + +				Yes	
G. G.	+ + + +			+ + + +		
R. O.		Yes			Yes	
F. D.	+ + + +			+ + + +		
H. K.		Yes			Yes	
E. W.		Yes			Yes	

Figure 16. (22) Comparison of reactions between scarlet fever anti-toxins and placental serum.

Despite the fact that there is considerable uncertainty about the immunologic aspects of scarlet fever and the relation of the Dick toxin to it, it seems fairly definitely established, as the result of the work of the Dicks and others, that most individuals exhibiting a positive Dick test are susceptible to scarlet fever. And that the test may be regarded as a quantitative as well as a qualitative measure of the degree of susceptibility to the disease.

It was thought by Dr. Ross (23), therefore, that some light might be shed on the action of placental extract, if one were to administer it to a group of children who reacted positively to the Dick test, and follow this administration by repeated Dick tests at frequent intervals, considering any variation on size of reaction as an indication of changed levels of immunity to scarlet fever.

The study was undertaken on fifteen children being treated for orthopedic conditions in the Shriners Hospital for crippled children, who were found to react positively to the Dick toxin as prepared by the Connaught Laboratories, Toronto. None of these patients had had scarlet fever. Their ages varied from fifteen months to fourteen and one half years. The placental extract was administered

intra-muscularly in single doses of five c.c. to ten c.c.

In most cases there were complaints of considerable discomfort at the site of injection for several hours. In no instance was there fever or other evidence of systemic reaction.

The tests were read in twenty-four hours and carefully measured by Dr. Ross. In the event of oval reactions, the mean of the length and breadth was considered as the diameter. The controls consisted of five children, with positive Dick tests, chosen at random. To these no extract was given.

Figure 17 shows graphically the effect of the extract on the Dick tests of these children. All the children who received the extract, showed a tendency to become almost, or definitely Dick negative in a few days, with a tendency to become Dick positive again in a varying period. Eight of the fifteen reached or fell below the level. Five c.m. generally considered the borderline between positive and negative Dick tests.

The amount of material administered, and the age of the patient have no relationship to the rapidity of fall in the curves, the level to which they returned or the rate of return.

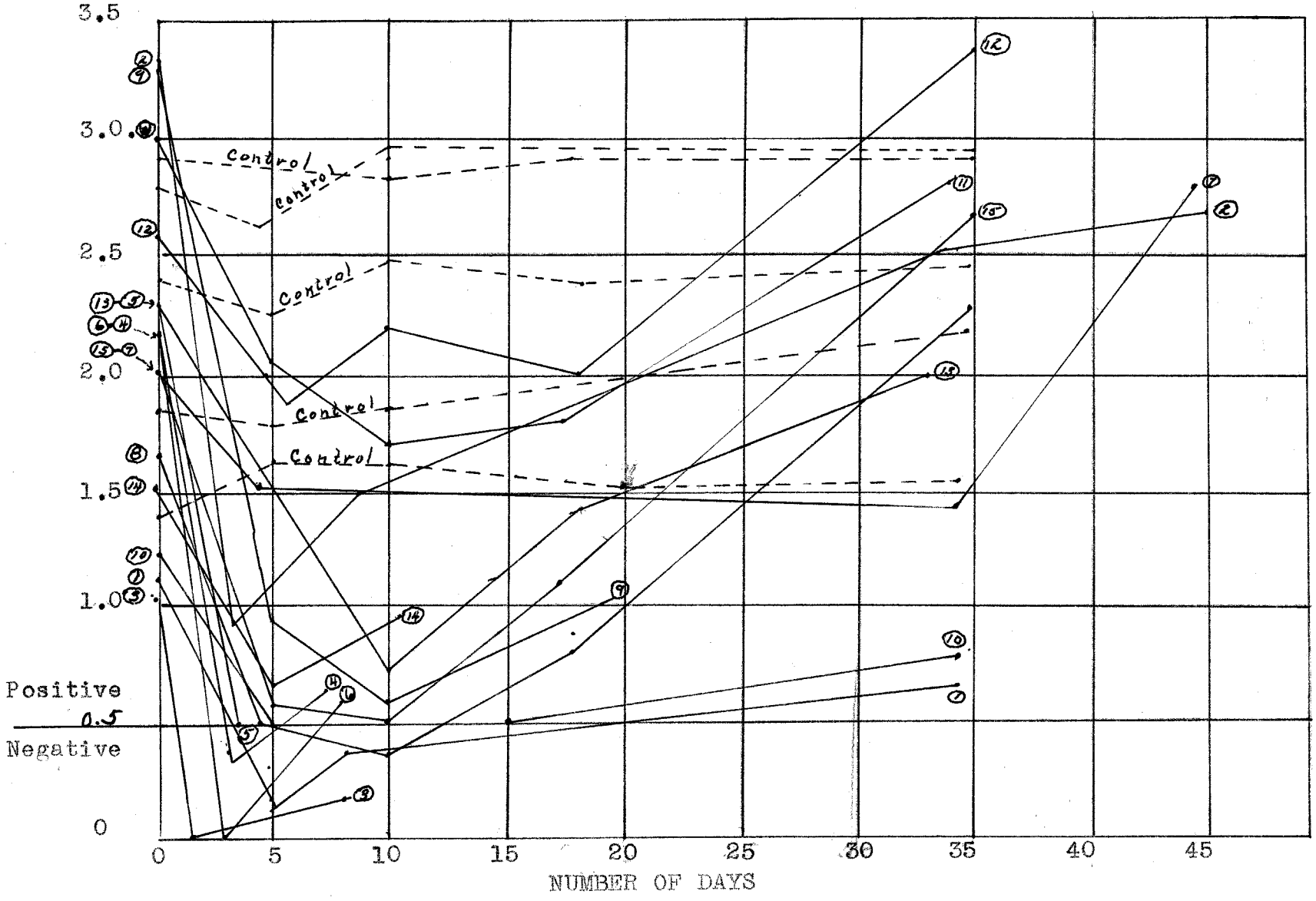

Figure 17. Chart showing variations in size of Dick tests, made at intervals after administration of placental extract to fifteen Dick positive children.

SUMMARY AND CONCLUSIONS

Through persistant efforts of scientific workers it has become an established fact that the new born's immunity is due to one of three, or a combination of all the following factors.

1. Transmission of immunity from the mother to the infant via the colostrum.

2. Transmission of immunity from the mother to the fetus through the placental barrier.

3. Immunity in the infant because rapidly growing or near embryonic tissue is resistant to disease processes.

The first theory was a controversial matter for years. One group using one type of animal found it to be impermeable. Another group using a different species of animal found it to be permeable. The fallacy in the controversy was found to be in the histological differences in the placentas of animals according to their position in the phylogenetic scale. Both groups were correct. In the human, however, experimental studies have shown that the placenta is permeable to immune substances and that colostrum has no effect on anti-

body titer as contrasted to some species of animals.

Another factor which enters into the picture but which cannot be properly evaluated, because of insufficient knowledge, at the present time, is the ability of rapidly growing tissue to neutralize toxins.

The crux of the situation is that the immunity applies only to the diseases caused by viruses or by bacteria whose toxins produce the disease. Another problem is that if the immunity aquired from the mother is passive in character and lasts for the length of time that it does, why should immunity produced by identical artificial means be so short lived.

The feasability of using placental extracts is based on the rationale that it not only contains anti bodies from the maternal blood and fetal blood but also from the placenta which is an embryonic type of structure itself.

Results of extracts when clinically used may be summarized as follows:

1. There were no deleterious effects or reactions from the use of the serum.

2. The extract has an ability to prevent or modify measles, that is superior to whole adult blood

and equal if not superior to convalescent serum.

3. It has the ability to render Dick positive individuals negative for a number of days.

4. Its blanching effects upon a scarlet fever eruption is superior to both convalescent serum and commercial anti toxins.

Placental extracts present a convenient and less troublesome method than hither to fore known way of preventing or modifying measles and scarlet fever. Uniform results will be brought about by careful standardization of the product, known doseages and proper time of administration.

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