dose of water and then repeat the serum electrolyte examinations. If the water is not required in the pre-operative state it will rapidly be excreted, but if required it will be retained and the dilution effect described will take place.

Summary

If water is lost in excess of extracellular electrolyte a state of hypertonic dehydration may be induced.

In this condition the concentration of extracellular electrolytes may be normal or even above normal when the total quantity of electrolyte is decreased.

In the post-operative phase oedema may result if water is given in excess.

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EARLY IMMUNIZATION AGAINST WHOOPING-COUGH

BY

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Whooping-cough has rightly been described as one of the most prevalent and certainly the most lethal of acute specific infections of childhood (Parish, 1952): it caused 9,401 deaths in England and Wales in the ten years preceding 1951, 50% of which were in babies under 1 year old. American workers noted that approximately half their deaths from pertussis occurred in infants who had not yet reached the accepted age for immunization (Fox and Snartemo, 1949). It is also generally agreed that the younger the victim the greater is the severity of the disease and its attendant complications and sequelae.

Although the prophylactic uses of pertussis vaccine had previously been demonstrated (Madsen, 1925; Kendrick and Eldering, 1936; Sauer, 1933), immunization at an early age did not yield what appeared to be favourable results till 1947 (Sako, 1947). It was observed that infants under 3 months of age developed agglutinins as satisfactorily as those in any age group, and that 75% of infants under 6 months developed agglutinins.

Di Sant' Agnese (1949) recommended active immunization against pertussis as soon as possible after birth. In his series inoculations of combined pertussis, diphtheria, and tetanus antigen were completed at nine weeks after birth, and at 13 weeks 60% of these babies had pertussis agglutinin levels which were considered to be protective. It should be stated, however, that agglutinin titres are not accepted by all workers as an index of protective function.

Some investigators suggested that the variable response to early immunization might depend on the type of vaccine used; it was also thought that a more prolonged stimulus was required in young babies, and that this was provided by alum-precipitated vaccine (Sako, 1947). Disappointing results in young infants followed the use of fluid (suspended) vaccine (Sauer, 1941) and many more

febrile reactions were observed with it. Howard (1950), in a progress report, noted the alum-precipitated vaccine to have fewer side-effects, but it has been stated that severe or serious reactions occur with it because it causes more local trauma than the other types (Cockburn, 1951).

In the M.R.C. trial (1951) good clinical results were obtained with all five types of vaccine used, but great variability was disclosed. Suggested reasons for this included: (a) the selection of strains; (b) the medium on which the organisms for vaccine were grown; (c) the methods of harvesting the growth; or (d) the nature of killing and preserving agents.

With the foregoing facts in mind, we proceeded to investigate the results of immunizing babies at an early age with two different sorts of vaccine, and, further, we felt it would be of interest to observe whether there was an appreciable antibody level in babies at birth, and whether the naturally occurring antibody bore any relationship to the one produced by immunization.

Methods and Materials

All sera were tested against six antigenic suspensions. One was kindly supplied by Glaxo Laboratories Ltd., and consisted of a formolized suspension of two strains of *H. pertussis* containing $2,000 \times 10^6$ organisms per ml. in saline. The remaining five suspensions, of approximately the same number of organisms, were prepared from strains grown according to the technique described by Lacey (1953, personal communication). These were *H. pertussis* H5 and *H. paraptussis* 15 grown in both X and C modes, and *H. pertussis* H16 in the X mode only.

A high-titre rabbit serum prepared against the Glaxo antigenic suspension was used as a positive control.

The material for immunization was obtained as a commercial vaccine in two forms—an aluminium phosphate adsorbed preparation and a simple suspension. These were labelled A and B respectively for the purpose of the investigation.

Titration Technique.—Tests were made in 0.5 by 5 cm. tubes. Doubling dilutions of serum were made in saline, starting at a titre of 1:8 and concluding at 1:512. An equal volume of antigen was added to each dilution, and the tubes after mixing were placed in a water-bath at 37° C. for two hours. The final readings were made after allowing the tubes to stand overnight at room temperature. Only definite agglutination was read as positive, faint granularity and similar reactions being disregarded. Figures were recorded as serum dilutions; that is, the final dilutions were in fact double these. Dry, clean tubes were used to collect cord blood at delivery and maternal venous blood shortly after. Blood from the babies at 6 months of age was obtained by heel pricks and collected in Wright's capsules. Sera were separated and stored at -20° C. until tested.

Selection of Cases.—Cases were selected by choosing at random mothers who came to the antenatal clinics between certain fixed dates; these had the nature of the experiment explained to them, and 90 volunteers were divided into three groups of 30 each. Their case records were labelled A, B, and C. Group A babies received aluminium-adsorbed vaccine, Group B had suspended vaccine, and Group C had no immunization before 6 months and served as a control group: they were offered immunization at 6 months.

The basic plan of the investigation consisted in determination of the antibody level against the strains of *H. pertussis* and *H. paraptussis* described above, in mothers and babies at delivery, and in the babies at 6 months. Unfortunately there were defections. Some mothers changed their minds and refused immunization after their babies had been delivered, and some babies were excluded because of illness or congenital defects. There were also a few cases with incomplete injection records.

Immunization.—Babies in both groups were given three injections. Group A babies received 0.5 ml. of the aluminium-adsorbed vaccine intramuscularly at 8 to 14 days of age, 6 weeks, and 3 months. Group B babies received 1 ml. of suspended vaccine at the same intervals. No severe or untoward reactions to the injections were reported.

Results

Of 78 maternal sera tested 18 contained an antibody, whereas of the 76 baby sera tested 36 contained an antibody. However, in just under half these babies the cord titre was 1:8 or a weak 1:16 only, so it seems that the corresponding maternal antibody may have been too weak for demonstration at 1:8.

Where there was a demonstrable maternal antibody there was always a corresponding antibody in the cord blood, with one exception, and in this case the maternal and cord antibodies were different and both of low titre. The titre in the cord blood was usually one or two tubes higher. The most frequently occurring antibody in both cord and maternal blood was the one against the paraptussis strain (XPA15) grown in the X mode by Lacey's method (Tables I and II).

TABLE I.—Number of Cord and Maternal Sera which Contained Antibodies to One or More of the Antigenic Test Strains

	Cord	Maternal
Total No. of sera tested	76	78
No. containing antibodies	36	18
To commercial antigen	2	3
" paraptussis strain XPA15	28	12
" pertussis XH16 " CPA15	—	—
" " XH5	—	—
" " CH5	1	—
* both commercial antigen and XPA15	4	3
" pertussis XH16 and XPA15	1	—

* The titre to XPA15 was higher in all but one case.

The antibody produced in response to immunization with the commercial vaccine was specific for it. The immunized babies who had developed titres to the commercial vaccine at 6 months had lost their natural antibody by that time, with the exception of two in whom an antibody to the commercial antigen was present at birth. One of these also showed an antibody to the XPA15 strain, and the titre of this had fallen considerably at 6 months. In all the remaining babies which had a passive immunity at birth, the antibody was not detectable at 6 months. One baby that had been immunized with vaccine A was reported by its mother to have had a mild attack of whooping-cough which was treated by her own doctor, and in this child the titre at 9 months had risen from 1:32 to 1:128 for the commercial antigen. The naturally occurring antibody at birth was a weak one against the XPA15 strain and was lost by this time.

All 15 babies immunized with the aluminium-adsorbed vaccine A developed a titre by 6 months, whereas only 3 of the 15 babies immunized with suspended vaccine B did

so. Further, the vaccine A titres at 6 months were considerably higher than the vaccine B titres—the highest titre observed with the former being 1:256, and with the latter a weak 1:32. In the baby whose birth antibody was one for the commercial antigen alone, the titre rose after immunization from 1:8 to 1:64, and in the baby whose birth antibodies occurred to both the commercial antigen and XPA15, the former was enhanced by immunization, while the latter diminished till only just detectable at 1:8. Vaccine A was used in both these cases.

Of the 18 mothers in whom a demonstrable agglutinin was found at the time of delivery, three had an antibody level equal to that of the cord blood, three had a higher level, and 10 had a level only half as high as that of the cord. Of the remaining two, one had a different antibody from the corresponding cord blood, and from the other no cord blood was received.

Discussion

Although complete records were obtained for the full inquiry in only 50 cases of the original 90, satisfactory numbers are available to provide some interesting conclusions.

Only 18 of the 78 mothers tested had a demonstrable antibody against the test suspensions used, and it seems reasonable to suppose that had many more different suspensions been available for testing them more maternal sera might have shown the presence of an antibody.

In all but the one case in which the cord and maternal antibodies were different, the antibody found in the maternal serum was also present in the baby's serum. In addition, there were 18 babies who showed a just detectable antibody in the cord blood when the mother's serum gave no results. It was presumed that in these cases the maternal antibody was weaker in titre than the first dilution (1:8) used in the titrations. In 10 out of 18 instances the antibody titre in the cord blood was at least twice that of the corresponding maternal blood, and in only three cases was the maternal titre higher than that of the cord blood. These findings are in agreement with previous observations in regard to other antibody systems: Barr *et al.* (1949) in diphtheria, and Murray *et al.* (1950, 1953) in staphylococcal antihæmolyisin and streptolysin.

All babies but one had lost their natural antibody (where this was not one against the commercial antigen) by the age of 6 months, and this would seem to suggest a reason for immunization before that age. It was noted by Mushulow *et al.* (1941), Lapin (1942), and Miller *et al.* (1943) that in young infants the height of the antibody titre was reached two to four months after completion of immunization, with a "plateau effect" lasting three years or more.

The presence of an antibody in the baby's blood at birth seemed to have no blanketing effect on immunization, titres being developed in immunized babies whether or not they already possessed an antibody. In the two cases in which the antibodies present corresponded to the commercial

TABLE II.—Incidence of Naturally Occurring Antibodies at Birth and at 6 Months, and Response to Immunization with Vaccine A and Vaccine B (50 Cases)

	Vaccine A (15 Cases)			Vaccine B (15 Cases)			Control C (20 Cases)		
	Birth	6 Months		Birth	6 Months		Birth	6 Months	
		Natural Antibody Still Present	Antibody to Commercial Strain		Natural Antibody Still Present	Antibody to Commercial Strain		Natural Antibody Still Present	Antibody to Commercial Strain
Commercial strain	1	1 Same case	1	0	—	—	0	—	—
XPA15	6	0	6	6	0	1	9	0	—
Commercial strain and XPA15	1	1*	1	2	0	0	1	0	—
Other strains as in Table I	0	—	0	0	—	0	0	—	—
Totals with antibody	8	2	8	8	0	1	10	0	—
No antibody at birth	7		7	7		2	10		
Total	15		15	15		3	20		—

* Titre to XPA15 considerably diminished.

strains being used for immunization, the titres rose from 1:8 to 1:64, and from 1:8 to 1:32. Howard (1950) noted that antibody titres were definitely associated with immunity as judged by clinical criteria; according to Miller and Ryan (1948) there was no susceptibility where there was a titre, though immunity could exist without there being a demonstrable titre; the attack rate varied inversely with the titre. Paschoud (1952), in mouse-protection experiments, observed that rabbit-immune sera with a high agglutinin titre were also highly effective in protective function, although McGovern (1950) stated that agglutinin value did not correlate closely with the degree of protection afforded (mouse tests). Mishulow *et al.* (1941) had observed that the titres of agglutinating antibodies did not parallel the occurrence of protective antibodies, and stated further that there was no evidence that the antibody titre was an index of resistance to the disease.

In the present investigation laboratory methods demonstrated a specific response to the commercial antigen only, there being no accompanying rise in the titres of the naturally occurring antibodies. Indeed, all but one of these had disappeared by the time the final tests were made at 6 months.

In view of the recognized existence of strain variation and related antigenic changes in *H. pertussis* (Bordet and Sleswyk, 1910; Bordet, 1912; Standfast, 1951; Lacey, 1951), and of the role of *H. paraptussis* in causing much "so-called whooping-cough" (Halpern and Halpern, 1948; Miller and Ryan, 1948), the production of a universally efficient vaccine seems to be a question still open to discussion. It has been noted by Ungar (1954) that pertussis vaccines do not protect laboratory animals against *H. paraptussis* infections.

By the test-tube methods described above, the aluminium-adsorbed vaccine appeared to elicit a better response than the plain suspension.

Summary

A brief review of work on pertussis immunization in babies is given.

Details of an investigation into the possibilities of early immunization, and the laboratory techniques used, are described.

Antibody levels at birth were found in about half the babies tested. All babies but one had lost their natural antibody by the age of 6 months. More than one type of natural antibody occurred in the series.

Appreciable agglutinin titres were obtained in all babies who received the aluminium-adsorbed pertussis vaccine. A poorer response was achieved with the suspended vaccine.

The presence of a naturally occurring antibody in the cord blood did not affect the production of an antibody in response to immunization.

Response to immunization as indicated by laboratory methods was specific for the commercial antigen.

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EVALUATION OF THE RAT OVARIAN HYPERAEMIA TEST FOR PREGNANCY DIAGNOSIS

BY

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Immature rats are more resistant than mice to toxic urines (Evans and Simpson, 1930), and after treatment with gonadotrophin their ovaries show easily recognizable hyperaemia (Zondek, 1926). This reaction was first utilized by Reiprich (1933, 1934) for purposes of a pregnancy test. Since that time the method has aroused considerable interest and numerous workers have reported on it favourably (Walker and Walker, 1938; Kelso, 1940; Frank and Berman, 1941; Salmon *et al.*, 1942a, 1942b; Kupperman *et al.*, 1943; Soman, 1944; Kaminester, 1944; Ramsey *et al.*, 1944; Kupperman and Greenblatt, 1944; Kline, 1944; Aschheim and Varangot, 1945; Sala *et al.*, 1945; Hinglais and Hinglais, 1947; Riddell, 1949). Positive results may be obtained as early as two hours after subcutaneous injection or even one hour on intravenous injection (Sturgis and Haour, 1951). Most workers, however, used longer periods ranging from 6 hours to 18 or 24 hours, and the latter apparently led to more accurate results (Zondek *et al.*, 1945).

Rakoff and Fried (1947) found that the administration of a pituitary extract increased the sensitivity of test animals to urinary chorionic gonadotrophin and suggested this as an improvement of the test (Fried and Rakoff, 1949, 1950). The use of the pituitary synergist has, however, proved unsatisfactory in the hands of other workers (Hoffman *et al.*, 1951). Farris (1944, 1946, 1950) was the first to draw attention to false-positive results: he stated that during the normal menstrual cycle erroneous results may be obtained for three or four days. These findings were disputed by Kupperman and Greenblatt (1946) and Bunde (1947), who found that in their series all the errors were false negatives. However, since then an increasing number of workers have reported false-positive findings in their control material (Behnken *et al.*, 1948; Fried, 1948; Riley *et al.*, 1948; Dubrausky, 1950; Kelsey *et al.*, 1950; Hoffman *et al.*, 1951). Furthermore, different authors used different techniques: the number of rats per test, the strain, the mode of inoculation (subcutaneous, intraperitoneal, intravenous) all varied as well as the total volume of urine injected. Readings were taken at 1, 2, 3, 6, 18, or 24 hours afterwards, and the criteria of accepting a positive result were also at variance. Many of the workers compared their results with the Aschheim-