control; this animal received the smallest dose (0.1 cc.) similarly to one of the supposed immune litter. The results are shown in the following protocol:

TABLE II.

Rabbit No.	Weight	Dose	Results
	gm.	cc.	
300	600	0.1	No effect, gained 250 gm. in 7 days.
202	625	0.5	Marked oedema; died on 7th day.
235	700	2.5	Marked oedema; died on 3rd day.
209	735	10.0	Died during 1st day.
197*	1200	0.1	Marked oedema; died on 3rd day.

* Normal control.

Rabbit No. 300 was finally killed November 2, 1928, by a blow on the head. It then weighed 2600 gm. and was normal in every way. All of the other animals that died showed the characteristic lesions of infection by *B. sordellii, i. e.,* extensive subcutaneous oedema, with a little congestion but no emphysema.

The normal control rabbit, though much larger than the immune rabbits, showed approximately the same development of lesions and died in about the same time as the immune test rabbit (No. 235) which received 25 times as large a dose of the culture. The results clearly indicated that immunity to *B. sordellii* may be transmitted from female rabbits to their offspring, but whether by placental transmission or by lactation is, of course, impossible to state from this experiment.

4525

The Buffered Diluent for Schick Toxin.

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The instability of diphtheria toxin in diluted state has always presented obstacles to the preparation of outfits for the Schick test. It has been the custom to supply a small amount of undiluted toxin (or toxin in a small amount of glycerin) in one container and the required amount of saline diluent in another container, which required the mixing of toxin and diluent by the physician at the time of performing the Schick tests. A ready-made dilution of toxin which

would retain its toxicity under the varying conditions to which Schick outfits are usually subjected would make for lower cost of manufacture, greater convenience and greater accuracy of the test itself.

A buffered diluent described by Glenny, Pope and Waddington[1] might make such a procedure feasible. They found that most diphtheria toxins diluted in the buffer maintained their toxicity for 6 weeks at room temperature and for 6 months or more at 0° C. We began a series of experiments to check their results, using a toxin which was being employed routinely in Schick test outfits.

The toxin was prepared from veal infusion made sugar free with *B. coli,* containing 2% Difco Proteose Peptone, 0.5% sodium chloride and having a pH of 7.4 after autoclaving 30 minutes at 120° C. The "Park-Williams No. 8" strain was used and the toxin harvested after incubation at 35° C. for 5 days. The toxin was 6 years old at the time of the experiments and had an M.L.D. of 0.015 cc. Two M.L.D. plus 20% excess was accurately measured into capillary tubes and the contents diluted in 10 cc. vials of isotonic salt solution containing 0.4% phenol or in 10 cc. vials of the buffered diluent (pH 8.1) prepared according to the Glenny, Pope and Waddington directions.

The diluent is a 1.5% aqueous solution of a mixture of 57 gm. crystal borax, 84 gm. boric acid, 99 gm. sodium chloride. This solution is stable on autoclaving and has a final pH from 8.0 to 8.4.

Our toxin diluted with this buffer solution showed little loss of toxicity when left at 37.5° C. for 24 hours. This test is said to indicate that the toxin would maintain its toxicity for 6 weeks at room temperature. An attempt was made to approximate the conditions which Schick toxin undergoes in actual use. Vials of toxin diluted (a) with isotonic salt solution, or (b) with the buffer solution were sent to 2 district health officers to be carried with them on their usual rounds for 2 months. A third set was placed in a car which was driven every day and another was left at room temperature in the laboratory. Some sets were placed at 7° C. and tested at monthly intervals while others were placed at 33° C. The vials after being returned from the district health officers remained at laboratory temperature. Toxicity was determined by injecting 4 cc. and 5 cc. from each vial subcutaneously into 250 gm. guinea pigs. These injections represented approximately 1.0 and 1.25 M.L.D. respectively. The results are given in the table.

[1] Glenny, A. T., Pope, C. G., and Waddington, Hilda, *J. Path. and Bact.,* 1928, xxxi, 133.

TABLE I.

Conditions to which diluted toxin was submitted	Diluted in Buffered Diluent			Diluted in Salt Solution		
	5 cc. pigs	4 cc. pigs	Remarks	5 cc. pigs	4 cc. pigs	Remarks
At time of dilution	3, 3, 3	4, 4, 4				See test at 7° C. 2-6 months below
37.5° for 24 hr.	6, 5, 3	4, 7, 4		S, S, S	S, S, S	No local reaction
In car and office during Nov., Dec. and Jan. (10 wks.)	3, 5, 6, 4	4, 4, 3, 4		S, S, S, S	S, S, S, S	"
Same as above plus 4 months at room temperature	4, 9	S, S,	Maximum induration	S, S	S, S	"
In car for 10 weeks, driven about 1000 miles	3	4		S	S	"
Same as above plus 4 months at room temperature	5	8		S	8	Not toxic death
Room temperature for 10 wks.	3	3		S	S	No local reaction
33° C. 10 wks.	S	S	Maximum induration	S	S	"
7° C. 2 months	2	3		2	4	
7° C. 6 months	2	3		3	4	

Integers represent days survival of pigs after injection.
S = survival.

The results agree closely with those of Glenny, Pope and Waddington even though the time at room temperature was extended a month (they, however, report one toxin which retained full strength at room temperature for 14 weeks). In the present experiments there was no apparent deterioration of the diphtheria toxin diluted with unbuffered saline in 6 months at 7° C., although Glenny *et al* state that reduction to half strength occurs in 2 months at 0° C. When kept at 37.5° C. for 24 hours the toxin diluted with saline lost practically all of its toxicity while that diluted with the buffered solution was only slightly affected.

The tests on the companion sets exposed to the conditions which the average Schick outfit would have to meet showed that for a period of 10 weeks the buffered diluted toxin maintained its toxicity while the saline diluted toxin was destroyed.

The belief that toxins which retain their toxicity for 24 hours at 37.5° C. will retain it for 6 weeks at room temperature has been confirmed. It seems certain that those buffered toxin dilutions

which stand up under this 24 hour test at 37.5° C. would be entirely satisfactory for distribution in Schick outfits.

Similar experiments have been undertaken with several of our toxins and toxins from 7 other laboratories in order to ascertain if the diluent is adapted to a sufficiently large number of toxins to warrant adopting the routine dispensing of toxin ready-diluted for Schick tests. The results of these experiments will be published in full later.

4526

Concentration of Amino-Acids, Urea, and Total Non-Protein Nitrogen in Foetal and Maternal Blood of Rabbit During Pregnancy.

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It has long been recognized that there are histological changes in the placenta at various stages of pregnancy. There has been little investigation, however, of the possibility that these morphological alterations are linked with coincident changes in the concentration of various metabolites, such as glucose, urea and amino-acids in the blood which bathes the foetal and maternal surfaces of the placental boundary. In recent observations, however, it has been found that the concentration of blood sugar[1] and hemoglobin[2] is strikingly different on the foetal and maternal sides of the placental barrier in the rabbit at the beginning of the last third of the gestation period; on the other hand, at term, the blood sugar and hemoglobin are found to approach much more nearly an equal concentration in the foetal and maternal blood. It is obvious that data derived from observations at term may fail to give a complete understanding of the regulatory activity of the placenta. If the previous observations in the human are viewed in this light, as for instance, the careful description by Plass[3] of the concentration of urea, amino-acids, and total non-protein nitrogen in foetal and maternal blood at parturition, and the studies of Slemons,[4] it is evident that the data are limited to observations at a single stage of pregnancy.

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[1] Snyder, F. F., and Hoskins, F. M., *Anat. Rec.*, 1928, xxxviii, 28.

[2] Zeidberg, L. D., unpublished observations from this laboratory.

[3] Plass, E. D., *Johns Hopkins Hosp. Bull.*, 1925, xxxvi, 393.

[4] Slemons, J. M., 1919, "The Nutrition of the Foetus."