possible foci of these organisms with or without resultant local symptomatic manifestations.

Ability of certain of these organisms to grow on hair should prove a point of differentiation. Determination of the growth factor will necessitate the separation of the hairs from the desquamated epithelial scales and the secretions of the scalp. This work is now in progress.

7232 P

Reduction of Methylene Blue by the Blood of Young Infants.

CARL H. SMITH.

From the Department of Pediatrics, Cornell University Medical College, and the New York Hospital.

The blood of infants shows significant alterations in the first few months of life. Most pronounced, perhaps, is the drop in the number of red blood cells and in hemoglobin. Another hitherto unrecognized feature apparently characteristic of this period is the rapidity with which the blood decolorizes methylene-blue.

The method employed in this investigation was as follows: A stock solution was prepared by dissolving 200 mg. of methylene blue* in 40 cc. of 3% sodium citrate* in doubly distilled water at pH 7.4 to 8.1. After filtration 0.07 cc. of this solution was dissolved in 50 cc. of sodium citrate of the same strength, giving a final concentration of methylene blue of approximately 1:140,000. This solution was left at least 24 hours in the ice-box before use. For each test 4 samples of freshly drawn blood was added to 0.1 cc. of the methylene blue solution. The quantities of blood with youngest subjects were .02, .03, .04, and .05 cc. respectively, and a range of .03 to .06 cc. in the older ones. The contents were thoroughly admixed and kept at 15°C. for 1 hour. The tubes were then centrifuged for 2 minutes. The extent of reduction was 0 when the dilute blue color was unchanged, to 4 plus when the dye was completely reduced to that of water.

This study included the blood of 238 healthy individuals, 152 under one year of age, the remainder ranged to 40 years. The results are summarized in Table I.

* Methylene Blue, U. S. P. Medicinal, National Aniline and Chemical Co. Sodium Citrate, Baker's Analyzed.

TABLE I.
Reduction of Methylene Blue by Blood of Young Infants in % Cases.

Age	No. Subjects	Degree of Decolorization 0	1	2	3	4	No. Observations
0-1 mo.	54	0.9	5.7	4.8	21.1	67.3	104
1-4 mos.	134	0.	1.4	4.3	7.3	86.8	205
4-8 mos.	105	33.8	19.0	16.5	13.2	17.3	121
8 mos.-1 yr.	63	59.7	22.2	12.5	5.5	0	72
1-40 yrs.	92	86.6	10.2	2.3	0.7	0	127

The reduction of methylene blue proceeded most rapidly and completely in the large majority of observations during the first 4 months of life. After this period, the reducing power was markedly diminished and resembled the blood of the older child and adult.

Tests were also carried out periodically with the blood of 3 young rabbits. The results corresponded with the human infant except that the reducing power of the animals disappeared between 2 and 3 months of age.

Following the initial readings (primary reduction phase) the tubes were placed in the ice-box (5-6° C.). Reduction of the dye proceeded with all bloods regardless of age, and in 24 hours reduction was usually complete. The more rapid rate of decolorization, however, occurred with the smallest quantities of blood in the young baby. The clearness of the supernatant layer of the young baby's blood always distinguishes it from that of older ages. After reduction had been completed in the ice-box, the clear supernatant fluid was replaced by 0.1 cc. of fresh methylene blue. After gentle shaking and sedimentation, reduction proceeded rapidly in the blood of the young infant and slowly or not at all in the blood of older individuals. Preliminary washing of the red cells by prolonged centrifugalization interfered with the reaction. The dye was not decolorized in an acid solution of a pH 6.3. No decolorization took place at 2°. The optimal temperature appeared to be 15° C. At 37° the reaction was less marked and often confused by hemolysis. At 5° decolorization persisted indefinitely. The blue color returned by exposure to higher temperatures.

The serum, plasma, platelets and white cells were eliminated as essential factors. The reduction of the dye bore no quantitative relationship to the amount of hemoglobin in the specimen or to the hematocrit volume. Directly after birth when the hemoglobin was highest the reduction of methylene blue was often much less marked than in the weeks that followed (Table I). That hemoglobin or some other portion of the erythrocyte appeared to be involved in the decolorization process, was indicated by oxidizing hemoglobin to

methemoglobin by a trace of sodium nitrite. This produced methemoglobin without laking the blood and interfered with the decolorization reaction. Age of the red cells as indicated by reticulation was also eliminated as a factor since reticulated cells are present in greatest number at birth.

Since methylene blue is converted into its leuco-derivative by reduction, restoration of the color should be accomplished by oxidation. The clear supernatant layer, however, could not be oxidized chemically or by vigorous shaking to the original blue color. On the other hand, the dye was associated with the cellular layer for a blue color developed in the supernatant layer, after the addition of dye-free sodium citrate to the centrifuged cells at 37° C.

These studies show that the red blood cells of the young infant and animal possess the property of reacting with methylene blue which is modified during growth. Many of the phases of this reaction suggest a relationship to the adsorption phenomenon.

7233 P

Bacterial Variation in Pneumococcus and Streptococcus Hemolyticus.

M. H. DAWSON. (Introduced by A. R. Dochez.)

From the Department of Medicine, College of Physicians and Surgeons, Columbia University, and the Presbyterian Hospital, New York City.

Arkwright[1] originally described 2 variant forms of members of the colon-typhoid-dysentery group which he designated by the terms S (smooth) and R (rough). Recently evidence has accumulated[2] to show that members of the colon-typhoid-dysentery group, as well as many other bacterial species, possess a third and distinct variant form, the M (mucoid). Organisms in the mucoid phase possess distinct capsules and form large mucoid colonies.

Griffith[3] described 2 variant forms of pneumococcus and, adopting Arkwright's terminology, designated them as S (smooth) and R (rough). A new variant form of pneumococcus was recently described by the author.[4] This new variant form was shown to be

[1] Arkwright, J. A., *J. Path. and Bact.*, 1921, **24**, 36.

[2] Hadley, P., personal communication.

[3] Griffith, F., *Rep. Pub. Health and Med. Subj., Ministry of Health*, No. 18, 1923, 1.

[4] Dawson, M. H., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 806.