against from 2 to 10 minimum lethal doses, a sign that the protection afforded by sulfanilamide against the hemorrhage factor parallels the protection afforded against the lethal factor. In protecting against lethality, sulfanilamide nullified the effect of 2.6 mg (2 M.L.D.) to 13 mg (10 M.L.D.).

However, in protecting against hemorrhage, sulfanilamide suppressed the effect of no more than 0.006 mg (about 1 M.H.D.) to 0.1 mg (about 16 M.H.D.). The ratio of M.L.D. to M.H.D. is about 1 : 200, obtained by dividing 1.3 mg (1 M.L.D.) by 0.006 mg (1 M.H.D.). Considering that only one two-hundredth of a lethal dose of the relatively unpurified toxin is required for tumor hemorrhage, an inhibition of, say, 90% of one M.L.D. of the toxin should leave a residual toxicity equivalent to 20 minimum hemorrhage doses. As seen in the table, animals may understandably be protected against the lethal effect of the toxin and yet undergo tumor hemorrhage. This observation constitutes further evidence that the vascular bed of the tumor, whose breakdown is responsible for the hemorrhage, is many times more susceptible to the toxin than is the organism as a whole to the lethal action of the toxin. Shear,[3] using a highly purified bacterial toxin, has reported that the hemorrhage-lethality ratio may be as high as 1 : 5000 if lethality is measured with non-tumor-bearing mice, and 1 : 500 if lethality is measured with tumor-bearing mice.

The mode of action of sulfanilamide in respect to protection against lethality and tumor-hemorrhage is as yet unknown. It is interesting that Andervont and Shimkin[4] found that ascorbic acid affords protection against toxin-induced tumor hemorrhage. It appears difficult to decide, however, whether this effect is mediated by raising the threshold of capillary resistance, or whether ascorbic acid (or sulfanilamide) may participate in a specific detoxication directed against the bacterial endotoxin.

In view of the previous conclusion[2] that the hemorrhage factor is a component of the O antigens of most gram negative bacteria, and in line with the evidence that sulfanilamide inhibits both the lethal effect and the tumor hemorrhage effect of these antigens, it appears reasonable from this parallelism in action that both tumor-hemorrhage and lethality are expressions of a common chemical factor characteristic of the O antigens.

[3] Shear, H. J., *Cancer Research*, 1941, **1**, 731.

[4] Andervont, H. B., and Shimkin, M. B., *Am. J. Cancer*, 1939, **36**, 451.

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Effect of Electrolytes upon Kahn Precipitates from Human and Animal Sera.

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The problems created by the existence of falsely positive serologic reactions have been a source of difficulty since the advent of the Wassermann test.[1] Kahn[2] reported the first satisfactory differential test; this was based upon specific temperature differences.

We became interested in determining whether or not reactions in human and animal sera could be distinguished by the effect of electrolytes. Animal sera were used in these studies because they give general biologic reactions which are apparently of the same type as those found in human falsely positive serologic reactions.[2,3]

[1] Wassermann, A., Neisser, A., and Bruck, C., *Deutsch med. Wchnschr.*, 1906, **32**, 745.

[2] Kahn, R. L., *Arch. Derm. and Syph.*, 1940, **41**, 817.

[3] Eagle, H., *Laboratory Diagnosis of Syphilis*, Chap. XVIII, C. V. Mosby Co., 1937.

While studying the effect of NaCl solutions upon the Kahn precipitates from animal sera, it was observed that the precipitate resulting from the reaction of Kahn antigen and horse serum was soluble in 4% NaCl solution. At the same salt concentration, precipitates from syphilitic human sera remain insoluble. The effect of other concentrations of NaCl (ranging from 0% to 30%) upon horse, human, guinea pig, hog, cow, sheep, and chicken sera were investigated. All sera were diluted to give approximately two plus reactions in the standard Kahn test by a method previously described.[4] Verification tests[2] were made upon each serum to check the specificity of the reaction. All sera gave the expected reactions (*i.e.*, either syphilitic, general biologic, or negative type) with this test. Controls were also made using the same quantities of serum and salt solution. However, an artificial antigen containing the same quantities of alcohol and saline as in the original antigen was used. Control reactions were completely negative except in the chicken sera.

It was found that each species of animal serum investigated gave characteristic aggregation and dispersion patterns of the Kahn precipitate. These were zonal in character. The most important differences in the behavior of the precipitates were as follows:

Sera from presumably non-syphilitic persons giving negative reactions in the standard Kahn test showed a complete lack of immediate precipitation. However, after standing overnight, strong precipitation occurred in the salt range from 0.0% to 0.3% similar to the results of Dunlop and Sugden,[5] and weak precipitation from 6% to 14% NaCl similar to Mackie and Anderson.[6] The reactions in other zones were completely negative. Syphilitic sera, however, gave maximal precipitation between 2% and 8% NaCl and practically no precipitation between 20% and 25% NaCl; the reactions in the rest of the zones were moderate.

Although guinea pig sera give no reactions in the usual serologic tests,[3] an immediate precipitation occurred at low salt concentrations (0.0% to 0.3% NaCl).

The Kahn precipitates from horse sera were dispersed and almost completely dissolved when concentrations between 2% and 6% NaCl were employed. The reactions in the other zones were moderate or strongly positive.

Hog, cow, and sheep sera gave almost identical reactions with this method. There was considerable variation in the results with individual sera. In general, however, strong reactions were obtained at low salt concentrations up to 0.3% There was a gradual weakening of precipitation until approximately 10 to 14% NaCl concentrations were reached. Very weak or negative reactions were then obtained. Moderate precipitation was again evident at about 27% salt concentration. The zone of negativity occurred at a much higher salt concentration in this group than in horse sera.

Chicken sera gave atypically coarse flocculation and very dense precipitation at high salt concentrations (25% to 30%). The controls gave results similar to the reactions with Kahn antigen. These precipitates were qualitatively and quantitatively different from the precipitates in the mammalian sera tested.

This is the first instance, as far as we are aware, in which the principle of differential electrolyte concentration has been used to distinguish false reactions in serodiagnostic tests from syphilitic reactions.* Aside from its bearing upon serologic tests, this method may be another means of distinguishing between the sera of some animal species and also determining additional biological rela-

[4] Green, M. N., and Forster, G. F., *Am. J. Syph., Gon. and Ven. Dis.*, 1942, **26**, 632.

[5] Dunlop, E. M., and Sugden, S., *J. Path. Bact.*, 1934, **39**, 149.

[6] Mackie, T. J., and Anderson, C. G., *J. Path. Bact.*, 1937, **44**, 603.

* Since this work was completed, Kahn[7] has announced a new practical verification test for distinguishing human syphilitic and human falsely reacting sera by a differential electrolyte concentration method applied to both serum and antigen suspension.

[7] Kahn, R. L., *Univ. Hosp. Bull.*, Ann Arbor, 1942, **8**, 45.

tionships between animal sera. Further studies are in progress in this field.

It was also observed that salt concentrations between 2% and 8% NaCl stabilized the Kahn reaction obtained with syphilitic sera by preventing fading. The sensitivity of these reactions was increased from 2 to 4 times that of the standard Kahn reaction in which 0.9% NaCl is used. The practical application of this finding is being further investigated.†

† We desire to express our appreciation to Dr. R. L. Kahn, University of Michigan, and Dr. J. Zichis of this laboratory, for their interest and criticism throughout the course of this investigation; also to Dr. E. J. Czarnetzky of Wilson and Company for his cooperation in furnishing many of the animal sera used in this study.

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Selective Filtration of Vitamin C by the Placenta.

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That the concentration of vitamin C is higher in cord than in maternal blood has been established.[1] The mechanism responsible for this phenomenon has been under dispute. Giroud[2] and his coworkers attributed it to the ability of the fetus to synthesize vitamin C. Others held that the depressing effects of exercise and sweating of labor[3] and of anesthesia, lead to the loss of vitamin C by the maternal organism. Manahan and Eastman[4] have established that this discrepancy can be explained by the selective action of the placenta.

We are presenting herein studies of 7 patients observed in the ante-natal clinic and the obstetrical ward of the Bonne Bay Cottage Hospital of Norris Point, Newfoundland. These patients were encountered in the course of a nutritional survey, a comprehensive report of which is in preparation.

Method. Blood plasma vitamin C determinations were carried out on nonfasting bloods, following the technic of Farmer and Abt.[5] Dietary histories were obtained to establish types of food ingested though no attempt was made to estimate caloric intake. Maternal vitamin C status was established before delivery by repeated blood plasma determinations. In 5 instances a sample of blood was obtained just prior to delivery. In 6 of the 7 patients, blood samples were also taken within 6 hr after parturition. Blood was collected from the umbilical stump in each instance at the time of delivery. All readings were made in triplicate.

Results. In all 7 instances, the vitamin C levels in the cord blood plasma were higher than in the maternal blood. As noted in Table I, patients No. 1 and 3, who had been receiving vitamin C therapy and whose blood levels were in the zone indicating saturation, showed proportional increase in vitamin C levels of the cord. Although patient No. 2 had been on a diet low in vitamin C, the cord blood was at the level of saturation. Patients No. 4 and 5 had been on diets in which the vitamin C content was negligible. The readings of the cord blood levels of patient No. 4 suggest the possibility of latent scurvy at birth, contrary to the implication of Braestrup.[1] Patient No. 5 was given one gram

1 Baestrup, P. W., *Acta Pediat.*, 1937, **2**, 328.

2 Giroud, A., *Compt. Rend. Soc. de Biol.*, 1936, **121**, 1062.

3 Smith, S., *The Vitamins*, Am. Med. Assn., 1939, **21**, 389.

4 Manahan, C. P., and Eastman, N. J., *Johns Hopkins Hosp. Bull.*, 1938, **62**, 478.

5 Farmer, C. J., and Abt, A. F., PROC. SOC. EXP. BIOL. AND MED., 1936, **34**, 146.