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A Note on the Interrelationship of Deficiency Diseases and Resistance to Infection.*

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Clinical recognition that deficiency diseases predispose to infections and, conversely, that infectious diseases predispose to vitamin deficiencies[1] suggested to us the need for a special investigation concerning this interrelationship. Although the general studies along this line are still in progress, the following observations seem sufficiently well defined to warrant publication.

Materials and Methods. The present studies were made on 150 selected cases of clinical pellagra, riboflavin deficiency and beriberi, along with other associated deficiency diseases. Before, during and after treatment, the bacterial flora of the lesions associated with the various vitamin deficiencies was investigated, venous blood for complement titrations was drawn, and the bactericidal power of the whole blood for *Staphylococcus aureus, Streptococcus viridans,* and *Neisseria intracellularis* was measured.

Smears from lesions and ulcers were examined and the organisms obtained by swabbing these loci were cultured and identified.

Blood for complement titrations was drawn aseptically in 3-5 cc amounts, allowed to clot on a slant in a sterile tube, and placed in the refrigerator for 18 to 24 hours. The serum was then diluted with physiological solution of sodium chloride in proportions of 1:30. Varying quantities of this dilution of complement serum decreasing from 1.0 cc to 0.1 cc, in units of 0.1 cc, were used in the titration, along with 2 units of a standardized anti-sheep hemolysin, a 2% suspension of sheep red corpuscles, and the amount of physiological saline required to bring the contents of each tube to a volume of 2.5 cc. The titrations were read after being incubated for one hour in a water bath of 38°C.

Using the Solis-Cohen technic,[2] the bactericidal power of the

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1 Spies, T. D., *Pellagra* (Section on Deficiency Diseases), A Textbook of Medicine, Edited by R. L. Cecil, W. B. Saunders Company, 1940.

whole blood for *Staphylococcus aureus, Streptococcus viridans,* and *Neisseria intracellularis* was determined. Twenty-four-hour cultures in 20% serum dextrose broth were used in the following concentrations: undiluted, 1:5, 1:25, 1:125, 1:525; sterile 20% serum dextrose broth was used as the diluent.

Observations and Discussion. In all cases Gram-positive cocci with hemolytic properties were the dominant organisms in the lesions in the angles of the mouth associated with a deficiency of riboflavin. Eighty percent of the cases yielded pure or nearly pure cultures of hemolytic strains of *Staphylococcus aureus,* and in the remaining 20% *Streptococcus hemolyticus* predominated. Striking zones of hemolysis, frequently having a radius of 4.0 to 5.0 mm, were produced by the various strains of *Staphylococcus aureus.* Following the administration of riboflavin, or substances containing it, the lesions healed rapidly[3-7] and the organisms were no longer demonstrable. *Streptococcus hemolyticus* or hemolytic strains of *Staphylococcus aureus* dominated the bacterial flora of the noses and throats of these same patients.[8]

In association with Dr. K. W. Ascher,[9] the authors have cultured hemolytic strains of *Staphylococcus aureus* from the bacterial flora of the eye, in 14 of 30 cases of conjunctivitis occurring in vitamin deficient patients. Likewise, *Corynebacterium xerosis* in large numbers was found in nearly all of the cases, and in a pure state in the spots of Bitot, which occurred in 5 of the 30 patients.

Observers[5, 10] have found masses of Vincent's organisms in the ulcerations, so frequently seen in persons with dietary deficiency diseases, of the tongue, gums, or buccal mucosa. In addition to the heavy permeation by Vincent's organisms, 64% of these ulcerations yielded pure or nearly pure cultures of *Streptococcus hemolyticus.* In the remaining 36% of the cases, *Staphylococcus aureus,* most of the strains of which were hemolytic, was found to be the dominant organism. Within 48 hours following treatment with such therapeutic agents as nicotinic acid, cozymase, muscle adenylic acid, yeast adenylic acid, or substances, such as yeast or liver extracts, which

[2] Heist, G. D., Solis-Cohen, S., and Solis-Cohen, M., *J. Immunol.,* 1918, **3**, 261.

[3] Sebrell, W. H., and Butler, R. E., *Public Health Rep.,* 1938, **53**, 22.

[4] Sebrell, W. H., and Butler, R. E., *Public Health Rep.,* 1939, **54**, 2121.

[5] Spies, T. D., Bean, W. B., and Ashe, W. F., *Ann. Int. Med.,* 1939, **12**, 1830.

[6] Spies, T. D., Vilter, R. W., and Ashe, W. F., *J. Am. Med. Assn.,* 1939, **113**, 931.

[7] Vilter, R. W., Vilter, S. P., and Spies, T. D., *J. Am. Med. Assn.,* 1939, **112**, 420.

[8] Riddle, J. W., Spies, T. D., and Hudson, N. P., unpublished observations.

[9] Riddle, J. W., Ascher, K. W., Spies, T. D., and Hudson, N. P., unpublished observations.

[10] Spies, T. D., and Cooper, C., *International Clinics,* 1938, Vol. 4, Series 47.

are rich in these factors, these ulcerated areas decreased in size, healed,[11] and the organisms disappeared.

From three hundred and seventy-five complement titrations, the following observations and interpretations have been made: the acutely deficient persons studied, with notably few exceptions, have a surprisingly low amount of complement. In persons with advanced deficiency disease, hemolysis was barely detectable in the tube containing the largest amount of complement serum (1.0 cc), in contrast to the average normal complement titre which produced a one plus hemolysis in the tube containing 0.4 cc of 1:30 complement serum. Following the administration of therapeutic agents, the complement titre increased along with the clinical improvement of the patient to a normal level within one to 5 days. The blood from subclinical and mild pellagrins contained either a normal or slightly subnormal titre which increased following treatment for the deficiency.

We find a pronounced depression in the bactericidal power of the whole blood for *Staphylococcus aureus* in the patients suffering more acutely from a deficiency of the Vitamin B complex. The blood of subclinical and mild pellagrins shows only a slight variation below that of our normals in its staphylococcidal power. The titres of sensitizing antibodies for *Streptococcus viridans* and *Neisseria intracellularis* were little affected by the presence of a Vitamin B complex deficiency. Findlay and MacLean[12] noted that rats, when maintained on a "Vitamin B free diet", exhibited a drop in the bactericidal power of the whole blood for *Staphylococcus aureus,* while the titres of natural antibodies for other bacteria were apparently uninfluenced by this deficiency.

Summary and Conclusions. 1. Our observations in 150 patients from a region of Alabama in which deficiency diseases are endemic show a relationship between these diseases and the resistance to, and presence of, infections with *Staphylococcus aureus* and *Streptococcus hemolyticus.* 2. The lesions at the corners of the mouth, characteristic of riboflavin deficiency, contained pure or nearly pure cultures of hemolytic strains of *Staphylococcus aureus* in 80% of the cases, and in the remaining 20%, *Streptococcus hemolyticus* predominated. Following the oral or intravenous administration of riboflavin or substances rich in it, the fissures healed rapidly and the organisms were no longer demonstrable. 3. When the bacterial flora of the conjunctival sacs were studied in cases of dietary deficiency disease and associated conjunctivitis, hemolytic strains of *Staphylococcus*

[11] Spies, T. D., Vilter, R. W., and Bean, W. B., unpublished observations.

[12] Findlay, G. M., and MacLean, I. S., *J. Biochem.*, 1925, **19**, 63.

aureus were found to predominate in 14 of the 30 cases. Smears and cultures demonstrated the presence of *Corynebacterium xerosis* in a pure state in all of the spots of Bitot which occurred in 5 cases. 4. In addition to masses of Vincent's organisms, 64% of the ulcerations of the tongue, gums, or buccal mucosa yielded *Streptococcus hemolyticus,* and the remaining 36% contained hemolytic strains of *Staphylococcus aureus.* Following specific therapy with anti-pellagric substances, the bacterial flora of these ulcerations, including the Vincent's organisms, promptly disappeared.

5. A low complement titre exists in acutely deficient patients, and in the subclinical and mild cases the titre is slightly subnormal or normal. Following clinical improvement the complement titre increases. 6. In the whole blood of acutely deficient patients there is a distinct depression in the bactericidal power for *Staphylococcus aureus,* whereas, only a slight diminution in staphylococcidal power was observed in the blood of subclinical and mild cases of vitamin deficiency.

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Attempts to Produce Poliomyelitis in Eastern Cotton Rats with Flexner's M. V. Strain.*

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Forty-five cotton rats were injected with 9 strains of poliomyelitis virus (Flexner M. V., Flexner W. E., Toomey, Philadelphia, Kramer, Harmon, Howitt, Trask, W. E., Australian). The doses injected were the same as those used by Armstrong[1] to produce poliomyelitis, *i. e.,* 0.06 cc intranasally, 0.06 cc intracerebrally and 0.5 cc subcutaneously of a 10% cord brain suspension. After 6 weeks of observation, 11 animals had died—2 after the injection of Harmon's and one each after the injection of the other strains, except Toomey's and Trask's. The cords and brains of those animals that died were made up into 10% suspensions in saline and injected into other animals. None of the latter either died or developed paralysis even after 4 months of observation.

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1 Armstrong, C., *Public Health Rep.,* 1939, **54**, 1719.