fulfilled under the conditions of this study. In the 5 experiments in which coronary flow was measured, there was no effect in 3 cases, a slight decrease in flow in one, and a slight increase in one. The dose in the last case was below the C.E.D.

Summary. A new cardiac drug, a dimethylxanthine genate, has been studied in the completely isolated cat heart. It has been found to exhibit marked cardiotonic properties, and low toxicity. It exerts no appreciable coronary dilator effect on the completely isolated heart.

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Antibody Response of Persons with Pellagra, Beriberi and Riboflavin Deficiency.*

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During the past two decades, extensive animal experimentation has shown that in certain instances a direct relationship exists between the severity of an induced infection and the nutritional state of the animal. In a large group of persons with naturally occurring pellagra, beriberi, and riboflavin deficiency, Riddle, Spies and Hudson[1] found a depression of the blood's bactericidal power for streptococci and staphylococci, and demonstrated a characteristic flora of hemolytic strains of these organisms from oral and ocular lesions in riboflavin deficiency. The present report is concerned with observations on the relationship between these deficiency states in human beings and the response of their immune systems to antigenic stimulation.

Materials and Methods. Fifty patients were selected from the Nutrition Clinic of the Hillman Hospital, Birmingham, Alabama, using the following criteria:

1. A history indicating that their economic status and food-habits made it unlikely that they would or could change their diet significantly during the 5-month period of observation from May to Sep-

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1 Riddle, Jackson W., Spies, Tom D., and Hudson, N. Paul, PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 361.

tember. 2. Some evidence of nutritional failure. The patients were classified as to the type of disease and the degree of severity. Group I included 17 patients, 7 of whom had symptoms of mild deficiency disease, and 10 undernourished patients who presented no clinical signs of a deficiency at the time of the study, but who had previously exhibited these symptoms. Group II included 23 patients who had diagnostic lesions of moderately severe deficiency disease. Group III included 8 patients with clinical evidence of rather severe pellagra.

3. An absence of *Bacterium tularense* agglutinins as evidenced by negative reactions of the serum prior to injection of antigen. Suspensions of an avirulent strain of the tularense organism—No. 38 Francis—grown on gluco-cystine blood-agar and washed with physiological saline were prepared at weekly intervals; 0.5 cc was added to 1 cc of serum-dilutions ranging from 1:10 to 1:640.

4. An absence of *Brucella melitensis* agglutinins shown by negative reactions of the serum. (This was to eliminate the possibility of cross-reaction with the tularense antigen.)

Each patient was injected intramuscularly with a formalin-killed detoxified suspension of several strains of *B. tularense,* which was prepared and standardized by Mr. William Hesselbrock in the laboratory of Dr. Lee Foshay, University of Cincinnati. The injections were given on 3 successive days to avoid the reactions that injections of the tularense organism sometimes produce when given at longer intervals. On completion of the injections, agglutinative tests of the sera were done at weekly intervals. The brief interval between injections insured responses of a primary nature allowing for observation of the fall in titer over a relatively short period. At monthly intervals agglutinin-nitrogen determinations were made according to the method of Heidelberger and Kabat.[2] The bacteria for these determinations were grown on gluco-cystine blood-agar, washed with physiological saline and preserved with 0.01% merthiolate. The suspension contained 0.525 mg nitrogen per cc. One cc was added to 0.5 cc of serum and the mixture was incubated at 37°C for 2 hours. After overnight chilling, the sediment was packed and washed and the Kjeldahl determinations were made.

Our antigens and technics were identical with those used by Foshay and Hesselbrock in a series of persons with no clinical evidence of deficiency disease. These investigators have kindly allowed the use of their unpublished results to compare with our findings in persons with nutritional deficiencies.

[2] Heidelberger, Michael, and Kabat, Elvin A., *J. Exp. Med.*, 1934, **60**, 643.

One of our associates who had received the Foshay suspension two months earlier furnished serum for a positive control.

Observations. In the control group, the agglutinative titer after injection ranged from 1:20 to 1:1280 with a large majority between 1:80 and 1:160. The length of time required for this response to appear varied from 12 to 40 days after the initial stimulation. While the period of titer-maintenance varied, agglutination could be detected in various dilutions a year after vaccination in 50% of these persons. The positive control serum was constant at 1:80 throughout the period of study.

In our patients with deficiency-disease, the time required for a response to appear varied from 9 to 30 days following the first injection of the suspension. The period over which the titer was maintained deserves special note, since in all cases it was significantly less than in normal persons. In no instance was there a positive reaction after 64 days and in some of the severe cases the agglutinins disappeared in as short a time as 7 to 10 days from the time of the highest response. In the more severe cases the maintenance-time was lessened in accordance with the severity of the deficiency.

The maximal titer obtained varied in the 3 groups. In Group I they ranged from 1:40 to 1:320/640 and were well within the normal range reported by Foshay.[3] In Group II the general response was from 1:20 to 1:40, and fell somewhat below the normal variation. In Group III 2 of the patients had titers of 1:20, while the remaining cases in this group had a peak of only 1:10.

The series of agglutinin-nitrogen determinations suggested a direct proportion between the nitrogen and the titers above 1:10. Fall in titer was accompanied by a corresponding decrease in detectable antibody-nitrogen.

Summary. Response to antigenic stimulation with *B. tularense* in persons with pellagra, beriberi, and riboflavin-deficiency was less than in normal persons. In general, the resulting agglutinative titers were less in proportion to the severity of the deficiency, and persons with the greatest deficiency had less ability to maintain their titers. There was a quantitative relation between the higher titers and the amount of agglutinin-nitrogen.

[3] Foshay, Lee, unpublished observations.