sentative of several experiments, and suggest that this antibiotic may inhibit the synthesis of pantothenate from *beta*-alanine.

Although it is probable that sufficient pantoyl lactone was synthesized by this organism, since growth proceeded normally when *beta*-alanine was the only addition to the medium, it was considered advisable to test the effect of streptomycin on the growth of this organism in the presence of both portions of the pantothenic acid molecule, as well as *beta*-alanine and pantothenic acid separately. Pantoyl lactone itself will not support the growth of this organism. The results of such experiments (Table I) suggest that the site of action of streptomycin in this system may be the inhibition of the coupling of *beta*-alanine and pantoyl lactone to form pantothenic acid. Although this conclusion appears reasonable on the basis of the growth studies presented, other possibilities exist. No definite conclusion can therefore be made until similar studies are carried out with resting cell suspensions or cell-free systems.

Summary. The results of studies on the growth response of *S. fragilis* to pantothenic acid and its precursors, *beta*-alanine and pantoyl lactone, in the presence of streptomycin, are interpreted to suggest that this antibiotic somehow interferes with the synthesis of pantothenate.

Received April 24, 1951. P.S.E.B.M., 1951, v77.

Acquired Resistance to the Shwartzman Phenomenon.* (18814)

LEIGHTON E. CLUFF AND IVAN L. BENNETT, JR. (Introduced by E. A. Stead, Jr.)

From the Department of Medicine, Duke University School of Medicine, Durham, N. C.

Although previous investigations of the effect of repeatedly eliciting the Shwartzman reaction had been somewhat contradictory, Beeson(1) showed clearly that if the reaction is produced at 2-day intervals, successive lesions diminish in intensity and animals become completely non-reactive. This state of resistance was abolished by the injection of thorotrast. The experiments reported here were designed to further elucidate this type of resistance to the Shwartzman phenomenon.

Experimental methods. White male rabbits of mixed breed weighing 2000 to 3000 g were used. The materials employed to elicit the reaction were the P-25 polysaccharide from *Serratia marcescens* (obtained from Dr. M. J. Shear) and meningococcus culture filtrate (prepared by Dr. B. Black-Schaffer). The dosage of P-25 was 0.05 mg in 0.5 ml physiologic saline intracutaneously for preparation and 0.1 mg in 1.0 ml intravenously. In some experiments these amounts were doubled, the injected volume remaining the same. Meningococcus filtrate was diluted 1:10 with physiologic saline; 0.2 ml of this material was used for skin preparation and 0.5 ml intravenously. With this dosage about 80% of animals tested showed typical hemorrhagic lesions. The intravenous "provocative" injection was given 24 hours after skin preparation and the resulting skin lesions were noted and recorded 24 hours later. The presence of hemorrhage was the only criterion used for recording the reaction as positive. In addition to the above materials, purified typhoid antigen (obtained from Dr. H. R. Morgan), which is also capable of eliciting the Shwartzman reaction was used. When multiple skin injections were given, the first was made in the right upper quadrant of the abdomen, the second in the left upper quadrant, the third on the right just caudal to the first, etc., alternating sides of the abdomen and moving the sites posteriorly. Hair was removed with electric clippers immediately prior to injection. For passive transfer experiments, blood was ob-

* This work was supported by the Anna H. Hanes Memorial Fund.

1. Beeson, P. B., PROC. SOC. EXP. BIOL. AND MED., 1947, v64, 146.

TABLE I. Animals 1-4 Given Repeated Preparatory and Provocative Injections Developed Non-Reactivity by Eighth Day While Control Animals 5-8 Had Typical Hemorrhagic Lesions When Retested on Tenth Day. After rest period of 21 days, all animals had positive reactions.

Day		1	2	3	4	5	6	7	8	9	10	rest	31	32
Animal	1	S	P+	S	P+	S	P+	S	P--	S	P—		S	P+
	2	S	P+	S	P+	S	P—	S	P—	S	P—		S	P+
	3	S	P+	S	P+	S	P--	S	P—	S	P--		S	P+
	4	S	P+	S	P+	S	P+	S	P—	S	P—		S	P+
	5	S	P+							S	P+		S	P+
	6	S	P+							S	P+		S	P+
	7	S	P+							S	P+		S	P+
	8	S	P+							S	P+		S	P+

S, .2 ml meningococcus filtrate intracut. P, .5 ml meningococcus filtrate intraven. +, skin hemorrhage. —, no hemorrhage.

tained by cardiac puncture, allowed to clot in sterile containers and after centrifugation serum was separated and stored at 4°C. The longest interval between bleeding and the use of a serum was 12 days.

Preliminary studies, an example of which is illustrated in Table I, confirmed Beeson's finding that animals subjected to repeated reactions on alternate days become non-reactive. It was found in this experiment that resistance disappeared after a rest period of 21-28 days.

Effect of repeated skin or intravenous injections only. To determine if resistance was dependent upon the development of typical skin lesions the following experiment was done. Sixteen animals, each of which had demonstrated a positive skin reaction with P-25, were divided into groups of 4 and treated as follows: Group A received repeated skin and intravenous injections of P-25 on alternate days; Group B received intravenous injections only on alternate days; Group C received skin injections only on alternate days; and Group D served as controls, remaining untreated. Group A developed lesions of diminishing severity and by the fourth set of injections (eighth day) failed to react. Groups B, C, and D were then retested. Lesions appeared only in the controls, animals of Group D. Animals of Groups A, B, and C recovered reactivity after a rest period of 21 days. Table II illustrates this study. This experiment was repeated using meningococcus filtrate with similar results, with the single exception that one of the animals given only intradermal injections showed skin hemorrhage when retested.

To evaluate the importance of the route of administration of the materials, 6 animals made resistant by repeated intradermal injections of meningococcal filtrate were retested as follows: 2 were prepared with 5 times the usual intracutaneous dose (0.2 ml of 1:2 dilution) and given the usual intravenous dose 24 hours later; 2 were prepared with the usual intracutaneous dose and given 5 times the usual intravenous dose (0.5 ml of 1:2 dilution) 24 hours later; and 2 were prepared with 5 times the usual skin dose and given 5 times the usual provocative dose 24 hours later. Positive reactions appeared in the 4 animals given the largest provocative injection; increasing the preparatory dose had no apparent effect in overcoming the resistance produced by repeated intracutaneous injections. Table III illustrates this experiment. Similar results were obtained in animals made resistant by repeated intravenous injections, namely, the non-reactivity could be overcome by employing large intravenous doses but not by increasing the intracutaneous injections. Finally, it was found that animals repeatedly given P-25 or meningococcus filtrate intravenously or intramuscularly in the dosage ordinarily employed for the skin injections developed resistance as promptly as animals given the full provocative dose.

Reactivity to heterologous materials. Four animals made resistant by repeated reactions to P-25 were tested with meningococcus filtrate; no lesions appeared. After a rest period of 21 days, all 4 rabbits exhibited large areas of skin hemorrhage when again tested with meningococcal filtrate. In a similar experi-

TABLE II. Development of Non-Reactivity in Animals Given Repeated Reactions (A1-4), Repeated Intraven. Injections (B1-4), or Repeated Intracut. Injections (C1-4) with Persistence of Reactivity in Untreated Controls (D1-4). All animals gave positive reactions when tested after 21 day rest period.

Day	1	2	3	4	5	6	7	8	9	10	rest	31	32
Animal A1	S	P+	S	P+	S	P+	S	P—	S	P—		S	P+
A2	S	P+	S	P+	S	P+	S	P—	S	P—		S	P+
A3	S	P+	S	P+	S	P+	S	P—	S	P—		S	P+
A4	S	P+	S	P+	S	P—	S	P—	S	P—		S	P+
B1	S	P+		P		P		P	S	P—		S	P+
B2	S	P+		P		P		P	S	P—		S	P+
B3	S	P+		P		P		P	S	P—		S	P+
B4	S	P+		P		P—		P	S	P—		S	P+
C1	S	P+	S		S		S		S	P—		S	P+
C2	S	P+	S		S		S		S	P—		S	P+
C3	S	P+	S		S		S		S	P—		S	P+
C4	S	P+	S		S		S		S	P—		S	P+
D1	S	P+							S	P+		S	P+
D2	S	P+							S	P+		S	P+
D3	S	P+							S	P+		S	P+
D4	S	P+							S	P+		S	P+

S, .05 mg P-25 in .5 ml physiologic saline intracut. P, .10 mg P-25 in 1.0 ml physiologic saline intraven. +, skin hemorrhage. —, no hemorrhage.

TABLE III. Development of Non-Reactivity with Repeated Elicitation of Shwartzman Reaction and Effect of Increasing Provocative Dose in Overcoming This Resistance. Increasing preparatory dose was ineffective in restoring reactivity.

Day	1	2	3	4	5	6	7	8	9	10	11	12
Animal 9	S	P+	S	P+	S	P+	S	P—	S	P—	SS	P—
10	S	P+	S	P+	S	P+	S	P—	S	P—	SS	P—
11	S	P+	S	P+	S	P—	S	P—	S	P—	S	PP+
12	S	P+	S	P+	S	P+	S	P+	S	P—	S	PP+
13	S	P+	S	P+	S	P+	S	P—	S	P—	SS	PP+
14	S	P+	S	P+	S	P+	S	P+	S	P—	SS	PP+

S, .2 ml 1:10 meningococcus filtrate intracut. P, .5 ml 1:10 meningococcus filtrate intraven. SS, .2 ml 1:2 meningococcus filtrate intracut. PP, .5 ml 1:2 meningococcus filtrate intraven. +, skin hemorrhage. —, no hemorrhage.

ment, animals rendered non-reactive to meningococcus filtrate failed to react to P-25; after a rest period of 24 days, however, typical lesions were elicited with the polysaccharide. Eight animals were given daily intravenous injections of increasing amounts of typhoid somatic antigen for 6 days (10, 20, 40, 100, 200, 400 μ). Four animals were tested on the eighth and ninth days with P-25 and 4 with meningococcus filtrate; no lesions appeared.

Failure of passive transfer by serum. Animals rendered non-reactive by repeated elicitation of the Shwartzman phenomenon at 2-day intervals were bled as soon as they failed to respond and attempts were made to transfer resistance passively to normal animals. In none of the experiments was the reaction abolished by serum transfer, nor was there any inactivation of the reacting material *in vitro* mixing with serum. These experiments may be summarized briefly. In each case, the serum was from an animal resistant to the reacting material used. 1. Three animals given 5.0 ml of serum intravenously before the preparatory injection reacted with typical skin lesions to P-25. Similarly 5.0 ml of serum given immediately before skin preparation failed to protect 3 animals against meningococcal filtrate. 2. The intravenous injection of 10.0 ml of serum 30 minutes before the intravenous injection of P-25 in 4 rabbits and meningococcus filtrate in 4 rabbits did not prevent the development of skin hemorrhage. 3. The potency of P-25 and of meningococcus filtrate as skin preparatory or intravenous provocative agents was unimpaired by mixture with serum, whether injected immediately after mixing or after incubation for 6

hours at 37°C. This experiment was performed twice, employing groups of 4 animals for each agent.

Summary and conclusions. 1. The resistance to the Shwartzman phenomenon which develops when the reaction is elicited at short intervals is transient, disappearing after a rest period of 21-28 days. 2. This resistance was found to be effective against Shwartzman active materials from heterologous bacterial species. 3. It was found that a transient state of non-reactivity could be produced by repeated inoculation of bacterial substances intracutaneously, intravenously or intramuscularly, evidence that the resistance is not dependent on the development of skin hemorrhage. 4. The resistance which develops by repetitive injections could be overcome by increasing the provocative intravenous dose but not by increasing the preparatory intracutaneous dose. 5. Attempts to transfer this resistance passively with homologous serum were unsuccessful.

Received May 17, 1951. P.S.E.B.M., 1951, v77.

Effect of Oral Thiamine Administration on Thiamine Content of the Stool.* (18815)

JOHN ESBEN KIRK AND MARGARET CHIEFFI. (Introduced by R. E. Shank.)
From the Division of Gerontology, Washington University School of Medicine, and the St. Louis City Infirmary Hospital, St. Louis, Mo.

The present investigation was undertaken with the purpose of establishing to what extent thiamine is lost in the stool in young and old individuals when this vitamin is given by mouth in therapeutic doses. Previous studies (1-5) have shown that subjects on an ordinary diet with a thiamine content of about 1.5 mg usually excrete between 0.4 and 1.0 mg of thiamine daily in the stool. According to Alexander and Landwehr(4) about half of the fecal thiamine exists inside the bodies of the bacteria, whereas the remaining half is filterable through a Seitz filter. Of the total thiamine in the stool 10-15% is present in a non-phosphorylated form, and 85-90% as cocarboxylase. As shown by Najjar and Holt(6) individuals on a thiamine-free diet will also excrete thiamine in their stools. That the thiamine found under these circumstances is derived from bacterial synthesis is suggested by the observation that treatment with succinyl-sulfathiazole will reduce the thiamine content to zero.

When thiamine is given by mouth in excess of the amount normally present in the diet a considerable part of the extra thiamine may be recovered from the stool. This increased excretion in the feces was first observed by Schultz and coworkers(1) and has later been confirmed by Youmans and his associates(2) and by Friedemann and his group(3). It was possible for Youmans to show that the large loss in the stool of administered thiamine also occurred in patients suffering from thiamine deficiency. In the studies by Friedemann and associates up to 30 mg of thiamine could be demonstrated in the stool with a daily thiamine supplementation of 40 mg, or, in other words, three-fourths of the thiamine

* Vitamin studies in middle-aged and old individuals VIII. Funds and material provided by Hoffmann-La Roche, Inc.

1. Schultz, A. S., Light, R. F., and Frey, C. N., PROC. SOC. EXP. BIOL. AND MED., 1938, v38, 404.

2. Youmans, J. B., Patton, E. W., Kennedy, A., Monroe, S. C., and Moore, B., *Trans. Am. Assn. Phys.*, 1940, v55, 141.

3. Friedemann, T. E., Kmieciak, T. C., Keegan, P. K., and Sheft, B. B., *Gastroenterology*, 1948, v11, 100.

4. Alexander, B., and Landwehr, G., *J. Clin. Invest.*, 1946, v25, 287.

5. Denko, C. W., Grundy, W. E., Porter, J. W., Berryman, G. H., Friedemann, T. E., and Youmans, J. B., *Arch. Biochem.*, 1946, v10, 33.

6. Najjar, V. A., and Holt, L. E., *J.A.M.A.*, 1943, v123, 683.