

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 Schwartzman 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 Schwartzman active materials from heterologous bacterial species. 3. It was found that a transient state of non-reactivity could be produced by re-

peated 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.

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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.

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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., Kmiecik, 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.

given by mouth was lost in the stool.

Experimental. In the present investigation the thiamine content of the stool was determined in 12 younger individuals and in 21 old subjects, none of whom presented signs of gastrointestinal disease. The thiamine was determined by Hennessy and Cerecedo's(7) method in the modification of Friedemann and Kmiecik(8), which measures the total thiamine fluorometrically in the form of thiochrome. The prepared diet of the patients was found on analysis to contain an average of 1.5 mg thiamine daily. After the thiamine excretion in the stool on the unsupplemented diet had been determined, one tablet of 5 mg thiamine hydrochloride was given daily by mouth under supervision, and after a 4-week period of treatment the determination was repeated. Assays on the stool were finally made again 2 to 3 weeks after the discontinuation of the thiamine administration.

Results. The results of these studies are presented in Fig. 1, in which the values are expressed per 100 g of wet stool. It will be seen that the thiamine content of the stool both in the young and the old subjects increases considerably following the daily intake of 5 mg of thiamine, and that the excretion

returns to the original level after the thiamine supplementation has been discontinued. If the mean daily volume of the stool is estimated to 150 g it will be noted that an amount of thiamine corresponding to $\frac{1}{2}$ to $\frac{3}{4}$ of the dose given in tablet form is recovered in the stool. The lower excretion values observed in the aged patients are probably conditioned by a greater destruction of thiamine in the upper sections of the gastrointestinal tract.

In view of the rather low absorption of thiamine from the intestine the question arises if such low absorption may be the cause of development of moderate thiamine deficiency, occasionally observed on diets considered adequate with regard to thiamine content(9). In order to elucidate this problem a comparison was made of the fecal excretion of thiamine in 24 thiamine deficient and 20 non-deficient old patients receiving an unsupplemented diet with a thiamine content of 1.5 mg daily. The thiamine blood values of the deficient group were below 2.0 $\mu\text{g } \%$, of the normal group between 2.5 and 6.0 $\mu\text{g } \%$. The results of the stool assays showed only a slightly higher mean fecal thiamine excretion in the patients with low blood values (340 μg per 100 g wet stool, 1640 μg per 100 g dry stool) than in the individuals having a normal blood thiamine content (285 μg per 100 g wet stool, 1285 μg per 100 g dry stool).

It is important to point out from a therapeutic aspect that in spite of the large loss of thiamine in the stool observed during treatment with thiamine by mouth, the supplementation with 5 mg of thiamine hydrochloride daily to thiamine deficient old patients resulted in an increase of the blood thiamine values to a normal level. Thus it was found(10) in observations on 20 patients, that such treatment, maintained for 4 weeks, sufficed to elevate the mean blood thiamine concentration from 1.8 to 4.0 $\mu\text{g } \%$ and to cause a marked improvement of various deficiency signs present. Notwithstanding these therapeutic results it would seem advisable in

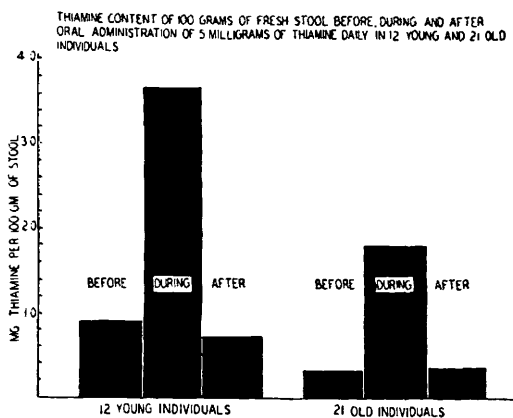


FIG. 1. The columns represent mg thiamine per 100 g of wet stool. The corresponding values expressed per 100 g of dry stool are 3.0, 9.2 and 4.3 mg respectively in the young individuals, and 1.2, 6.8 and 1.6 mg in the old subjects.

7. Hennessy, D. J., and Cerecedo, L. R., *J. Am. Chem. Soc.*, 1939, v61, 179.

8. Friedemann, T. E., and Kmiecik, T. C., *J. Lab. and Clin. Med.*, 1943, v28, 1262.

9. Kirk, J. E., and Chieffi, M., *J. Gerontol.*, 1950, v5, 236.

10. Chieffi, M., and Kirk, J. E., *J. Gerontol.*, 1950, v5, 326.

view of the large fecal thiamine loss encountered during oral thiamine treatment to administer the vitamin parenterally in those instances in which a rapid restoration of the blood thiamine level is desired.

It has not yet been established whether the excess thiamine observed in the stool after intake of commercial thiamine tablets is present in the bacteria or in the other fecal constituents; observations concerning this point would probably yield information of value. Thus if it can be shown that the excess thiamine in the stool under these conditions is non-filterable, this would suggest that the vitamin is incorporated in the bacteria and would presumably indicate that the intestinal bacteria had stolen the ingested thia-

mine. If on the other hand the thiamine can be recovered in the Seitz filtrate of the stool such finding would support the contention, recently proposed by Friedemann and his associates(3), that the intestine possesses a specific barrier against the absorption of thiamine.

Summary. The daily oral administration of 5 mg of thiamine hydrochloride in tablet form to 12 young and 21 old individuals was followed by an increase of the fecal thiamine excretion corresponding to one-half to three-fourths of the thiamine dose given. The fecal excretion of thiamine returned to the original level after the thiamine supplementation had been discontinued.

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Chromatographic Separation of Estrone, Estradiol and Estriol.* (18816)

JOSEPH F. NYC, DOROTHY M. MARON, JOSEPHINE B. GARST, AND HARRY B. FRIEDGOOD. (Introduced by S. Roberts.)

From the Departments of Physiological Chemistry and of Medicine, University of California at Los Angeles.

Chief among the outstanding problems in clinical endocrinology is that concerned with the separation and quantitative estimation of the 3 natural estrogens. With the publication of Boldingh's(1) method for the use of rubber columns for the chromatographic separation of fatty acids it occurred to us that this technic might be adaptable to the foregoing problem. This communication consists of a report on the successful adaptation of rubber columns to the chromatographic separation of the steroids in question.

Physical characteristics of the column. The chromatographic tubes are 8 mm internal diameter and at least 20 cm in length. A short tip of approximately 3 mm diameter at the base of the tube is equipped with a piece

of surgical rubber tubing $\frac{1}{8}$ inch I.D. The flow of liquid through this portion of the tube is controlled by a small screw clamp. *Preparation of the rubber.* Pulverized mealorub rubber† is extracted with acetone in a continuous extractor for 24 hours. Thereafter, the rubber is air dried, moistened with methanol and ground in a mortar. It is then transferred to a 20-mesh sieve which is immersed in methanol. The particles which pass through the sieve are washed with the latter solvent and stored under it until used for chromatography.

Preparation of the column. Rubber columns are prepared in duplicate. These consist of a test column on which the estrogens are to be chromatogrammed and a blank control column through which only the solvents will be passed. The blank column which is submitted simultaneously to the same chromatographic pro-

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1. Boldingh, J., *Rec. trav. chim.*, 1950, v69, 247.

† Vulcanized rubber powder obtained from Andresen Corp., Chicago.