Summary. 1) Rates of inactivation of tissue-culture-derived, foot-and-mouth disease virus, type A, strain 119 (FMDV-A119), at various pH levels and temperatures and by formaldehyde were determined. Ranges of pH and temperature investigated were 2.0 through 10.0 and 4°C through 61°C, respectively: formaldehyde was employed at 0.009%. The results are interpretable by first-order kinetics. However, at pH 5 and 6 and also at 55° and 61°C small fractions of the virus population had much lower first-order inactivation rates than the bulk of the virus. Possibilities concerning the nature of the fractions with higher resistance are discussed. 2) Rates of inactivation at various pH levels were determined at 4°C. Below pH 4 the virus was totally destroyed within a few seconds. At pH 5 and 6 infectivity was lost at a rate of about 90% per second and minute, respectively, until only one-millionth of the virus remained. This residual virus was very stable to further inactivation. At pH 6.5 and 10, 90% of the virus was inactivated every 14 hours. The virus showed marked stability only at pH 7 and 7.5, losing little infectivity during a 5-week period. At pH 8 and 9, a 90% reduction of infectivity occurred within a 3- and a 1-week period, respectively. 3) Rates of thermal inactivation were determined at pH 7.5. The time intervals required for the inactivation of 90% of the virus existing at any time were as follows: 18 weeks at 4°; 11 days at 20°; 21 hours at 37°; 7 hours at 43°; 1 hour at 49°; 20 seconds at 55° to a survival of 0.001, 7 minutes thereafter; and 3 seconds at 61°C to a survival of 0.00001, 11 minutes thereafter. Activation energies calculated for loss of infectivity below and above 43°C were 27,200 and 120,600 calories per mole of FMDV, respectively. 4) Virus treated with formaldehyde at a concentration of 0.009% was inactivated at a rate of 90% per day of storage at 4°C.

1. Bachrach, H. L., Hess, W. R., and Callis, J. J., *Science*, 1955, v122, 1269.

2. Youngner, J. S., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 202.

3. Dulbecco, R., and Vogt, M., *J. Exp. Med.*, 1954, v99, 167.

4. Reed, L. J., and Muench, H., *Am. J. Hyg.*, 1938, v27, 493.

5. Michaelis, L., *Biochem. Z.*, 1931, v234, 139.

6. Bachrach, H. L., and Schwerdt, C. E., *J. Immunol.*, 1952, v69, 551.

7. Glasstone, S., *Textbook of Physical Chemistry*, 2nd ed., D. Van Nostrand Co., N. Y., 1946, p1088.

8. Lauffer, M. A., and Price, W. C., *J. Biol. Chem.*, 1941, v138, 1.

Received March 4, 1957. P.S.E.B.M., 1957, v95.

Weekly Variations in Serum Cholesterol Levels of Monkeys. (23149)

DAVID KRITCHEVSKY AND R. F. J. MCCANDLESS. (Introduced by H. R. Cox)
Viral and Rickettsial Section, Research Division, Am. Cyanamid Co., Pearl River, N. Y.

Since the original reports of Peterson(1,2) on the hypocholesterolemic effect of soy sterols on cholesterol-fed chickens, many papers have appeared concerning the effect of soy sterols and b-sitosterol in lowering serum cholesterol levels in cholesterol-fed rabbits (3) and cholesterol-eating humans(4,5,6). The latter observation has not been confirmed in all instances(7). It has been suggested that the soy sterols exert their effect by interfering with the absorption of dietary cholesterol(4,8). In experiments using cholesterol-C^{14} interference was inferred by Chaikoff(9) and Kritchevsky(10) but not by Rosenman (11). The question of what influence soy sterols have on reabsorption of cholesterol has not been answered. As an effort in this direction we fed b-sitosterol to monkeys on a diet that was essentially cholesterol-free. Feedings were carried out daily for 6 weeks, with the animals being observed for two weeks before and 6 weeks after the feeding period. We observed no inordinate changes in the serum cholesterol levels either before or after the

TABLE I. Serum Cholesterol Levels (mg %) in Monkeys before, during and after Sitosterol Administration.

Monkey No.	Weeks 1	2*	3	4	5	6	7	8†	9	10	11	12	13	14
A	141	114	128	140	127	111	140	111	lost	145	110	107	131	118
B	132	161	175	186	181	132	149	130	144	143	127	144	155	119
C	122	137	115	130	117	153	141	137	121	214	124	132	131	124
D	86	90	95	128	104	95	109	118	111	131	148	127	145	117
E	146	159	184	157	129	137	168	127	143	257	137	137	152	149
F	lost	113	122	134	95	84	107	96	98	92	127	98	123	98

* Animals on 1 g sitosterol daily after this bleeding.
† Animals off sitosterol after this bleeding.

feeding experiment, but did see swings in the weekly cholesterol levels which resemble those reported in humans by Wilkinson(12), and which have also been noted by Fahrenbach.*

Methods. Six male Cynomolgus monkeys weighing 4-6 lbs were used. Each animal was bled weekly and the serum cholesterol levels determined in duplicate by the method of Trinder(13). This was done for 14 weeks, as follows: for 2 weeks before sitosterol feeding, during the 6 weeks of feeding, and for 6 weeks thereafter. Feedings of sitosterol were daily, by stomach tube, and one gram was delivered in each case. The basic diet of the monkeys consisted of Monkey Wafers (Dietrich and Gambrill, Inc., Frederick, Md.) containing soy flour, wheat germ, soy bean oil meal, dry skim milk, alfalfa meal, sucrose, bone meal, salt and vitamins. The wafers were augmented with fruit.

Results. The protocol for the feeding of sitosterol to human beings involves amounts ranging from 9 to 24 g daily(14) given in divided doses before each meal. In the monkey experiment, the gram of sitosterol was administered just before the animal's single daily feeding. This dosage for a 4-6 lb monkey compares well with that fed to patients.

TABLE II. Average Serum Cholesterol Levels (mg %) in Monkeys during and after Sitosterol Feeding.

Monkey No.	Sitosterol feeding*	Range	Post-sitosterol*	Range
A	126	111-140	122	107-145
B	159	130-186	139	119-155
C	132	115-153	141	121-214
D	108	95-128	130	111-148
E	150	127-184	165	137-257
F	106	84-134	106	92-127

* Period of observation—6 wk.

* Unpublished data.

Two things became evident from the data presented in Tables I and II. First, sitosterol feeding to normocholesterolemic animals on a diet devoid of cholesterol does not change serum cholesterol levels appreciably. This finding could support the belief that the mode of action of sitosterol is by interference with the absorption of dietary cholesterol. The second observation is that normal variations in cholesterol levels from week to week are so great as to obscure changes that might be brought about by the sitosterol. Wilkinson (12) has argued that in view of these wide swings, great care must be taken in establishing base levels for serum cholesterol in experiments designed to measure any outside effects. More data, covering longer time intervals, must be accumulated to evaluate overall cholesterol variations.

Summary. Six monkeys maintained on a diet essentially free of cholesterol were fed 1 g sitosterol daily for 6 weeks, and serum cholesterol levels were assayed before, during and after the feeding period. The data present no clear-cut evidence that the sitosterol lowered the level of serum cholesterol. The animals all exhibited wide swings in weekly cholesterol levels not related to sitosterol intake.

1. Peterson, D. W., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 143.

2. Peterson, D. W., Nichols, C. W., and Shneour, E. A., *J. Nutrition,* 1952, v47, 57.

3. Pollak, O. J., *Circulation,* 1953, v7, 696.

4. ———, *ibid.,* 1953, v7, 702.

5. Best, M. M., Duncan, C. H., Van Loon, E. J., and Wathen, J. D., *ibid.,* 1954, v10, 201.

6. Joyner, C. R., and Kuo, P. T., *Am. J. Med. Sci.*, 1955, v230, 636.

7. Wilkinson, C. F., Boyle, E., Jackson, R. S., and Benjamin, M. R., *Metabolism*, 1955, v4, 302.

8. Davis, W. W., *Trans. N. Y. Acad. Sci.*, 1955, v18, 123.

9. Hernandez, H. H., Peterson, D. W., Chaikoff, I. L., and Dauben, W. G., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 498.

10. Burke, K. A., McCandless, R. F. J. and Kritchevsky, D., *ibid.*, 1954, v87, 87.

11. Rosenman, R. H., Byers, S. O., and Friedman, M., *Circulation Research*, 1954, v2, 160.

12. Levere, A. H., Bozian, R. C., Jackson, R. S., and Wilkinson, C. F., *Circulation*, 1955, v12, 483.

13. Trinder, P., *Analyst*, 1952, v77, 321.

14. Shipley, R. E., *Trans. N. Y. Acad. Sci.*, 1955, v18, 111.

Received March 11, 1957. P.S.E.B.M., 1957, v95.

Insulinase-Inhibitory Action of Metabolic Derivatives of L-Tryptophan.* (23150)

I. ARTHUR MIRSKY, DANIEL DIENGOTT AND GLADYS PERISUTTI
Department of Clinical Science, School of Medicine, University of Pittsburgh.

A decrease in blood sugar concentration of normal rats is produced by a number of metabolic derivatives of L-tryptophan administered by stomach tube(1). Since these compounds are not effective in the severely diabetic alloxanized rat, the hypoglycemic response produced in the normal animal cannot be due to either a decrease in availability of some hyperglycemic agent or to an acute hepatotoxic action and inhibition of enzyme systems involved in glycogenolysis. Insulin dependency of the hypoglycemic response, however, may result from either an increase in secretion of insulin or a decrease in destruction of endogenous insulin consequent to an inhibition of insulinase. Accordingly, the influence of various metabolic products of L-tryptophan on the activity of insulinase *in vitro* and *in vivo* was determined.

Methods. Extracts of livers from fed, male Carworth rats were prepared as described previously(2). The incubation mixture consisted of 1 ml of extract plus 1 ml of Sörensen's M/15 phosphate buffer(3) containing 20 units of a mixture of I^{131} labeled and unlabeled insulin as well as various concentrations of the metabolic products of L-tryptophan. As a control, the same mixture was incubated without the L-tryptophan derivatives. At the end of 30 minutes of incubation at 37°C and pH 7.8, 2 ml of 10% trichloracetic acid was added to the incubation mixture and the quantity of insulin degraded was computed as previously described(2) from the percentage of total radioactivity which appeared in the trichloracetic acid filtrate. The metabolic products of L-tryptophan† used, were indole-3-acetic acid, anthranilic acid, kynurenic acid, picolinic acid, quinolinic acid, nicotinic acid, nicotinamide, nicotinuric acid, 5-hydroxytryptophan, 5-hydroxytryptamine creatinine sulfate, 5-hydroxyindoleacetic acid, indole and skatole. These compounds were dissolved or suspended in 0.5% sodium bicarbonate and adjusted to pH 7.8. To determine the effect of the various compounds on insulinase activity of the intact animal, groups of 10 fed male mice were given a subcutaneous injection of 0.004 mM of the L-tryptophan derivatives in 0.05 ml/gram body weight. Immediately thereafter the mice were given a subcutaneous injection of 1 ml of 5.5% solution of glucose. One hour later,

* Aided in part by grants from Upjohn Co. and Foundations' Fund for Research in Psychiatry.

Presented in part before Am. Chem. Soc., Dallas, Texas, Apr. 9, 1956.

† We are indebted to Dr. M. E. Speeter of the Upjohn Co., to Dr. S. Udenfriend for samples of 5-hydroxyindoleacetic acid, and to Dr. R. K. Richards of Abbott Laboratories for samples of 5-hydroxytryptamine creatinine sulfate. The other compounds used were purchased from various commercial sources.