*DYNAMICS OF CHOLESTEROL METABOLISM, I. FACTORS REGULATING TOTAL STEROL BIOSYNTHESIS AND ACCUMULATION IN THE RAT**

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During the course of steroid balance studies in rats, it was observed that total animal sterol synthesis was nearly proportional to caloric intake and appeared independent of several experimental dietary changes. Total animal sterol synthesis was also noted to be independent of dietary sterol levels at the lower levels found in ordinary laboratory diets, implying that cholesterol feed-back inhibition of its own synthesis[1] may not be significant under such dietary conditions. These findings form the basis of this report.

Methods.—Animals and diets: Data was collected from 38 male Wistar rats over a 15 week period. This included a 2 or 3 week control period, during which animals were fed laboratory chow, and a 12 week experimental dietary period. The animals were divided into six groups, each group named according to its principal diet. Diets A, E, and F had a dietary base of natural products blended to a laboratory chow. Diets B, C, and D had a synthetic dietary base of casein, sucrose, and cellulose. Caloric equivalents were calculated from manufacturer's specifications. The experimental diet contents are detailed in Table 1. Caloric intake among the groups was variable since differences in appetite, diet acceptance, and diet absorption were observed. Diet C was modified 4 times before it was satisfactorily accepted. These differences in caloric intake were reflected in growth (Fig. 1). Animals were chloroformed during the 15th week, and tissues were processed immediately or frozen. Carcasses were weighed, minced, diluted with water to 1,000 gm, and blended mechanically. Weighed aliquots were taken for analysis.

Fecal sampling: Feces were collected on aluminum screening and analyzed weekly. Fecal pellets were air-dried for 3 days after being separated manually from spilled food and hair. This type of collection was used because total fecal steroids, independent of minor oxidative changes,[2] were being analyzed. The dried pellets were ground to a fine uniform powder in a mechanical blendor and two gm were taken for analysis.

TABLE 1

SUMMARY OF EXPERIMENTAL DIETS

Name of diet	Basic Ingredient Source	%	Added Drug or Food Name	%	Dietary Composition (per cent by weight) Protein	Carbohydrate	Fat	Calories per 100 gm	Dietary Sterol mg/gm Total sterol	Cholesterol
A	RM*	100	None	—	24.3	50.0	4.15	335	1.06	0.23
B	NBC-1†	100	None	—	20.4	57.0	2.44	332	0.06	0.06
C-1	NBC-2‡	100	None	—	20.0	38.0	30.0	502	0.04	0.04
C-2	Diet C-1	67	NBC-3§	33	21.7	47.0	20.0	455	0.05	0.05
C-3	Diet C-2	50	Diet B	50	21.0	52.0	11.2	393	0.06	0.06
C-4	Diet C-3	50	Diet B	50	20.7	54.5	6.8	362	0.06	0.06
D	Diet B	98	Nicotinic acid	2	20.0	56.0	2.40	326**	0.06	0.06
E	Diet A	80	Lard	20	19.2	40.0	23.2	446	0.98	0.22
F	Diet A	80	Stearic acid	20	19.2	40.0	23.2	446	0.96	0.21

* Rockland mouse diet, A. E. Staley Mfg. Co., Decatur, Illinois. Fatty acid content measured 36.8 mg/gm and showed by gas chromatographic analysis; 14:0—1.6%; 16:0—20.0%; 16:1—2.4%; 18:0—7.3%; 18:1—31.8%; 18:2—34.4%; 18:3—2.3%.

† Nutritional Biochemical Co. (NBC), Cleveland, Ohio. "Fat-free" test diet with essential fat added as "methyl linoleate—75%." Gas chromatographic analysis of this fat source showed the following fatty acid content: 16:0—7.1%; 18:0—2.9%; 18:1—13.0%; and 18:2—77.0%.

‡ NBC "fat-free" test diet modified to contain 20% protein and 30% "methyl linoleate—75," gift of Nutritional Biochemical Co.

§ NBC "cholesterol-free" test diet.

** Assumes no caloric value for nicotinic acid.

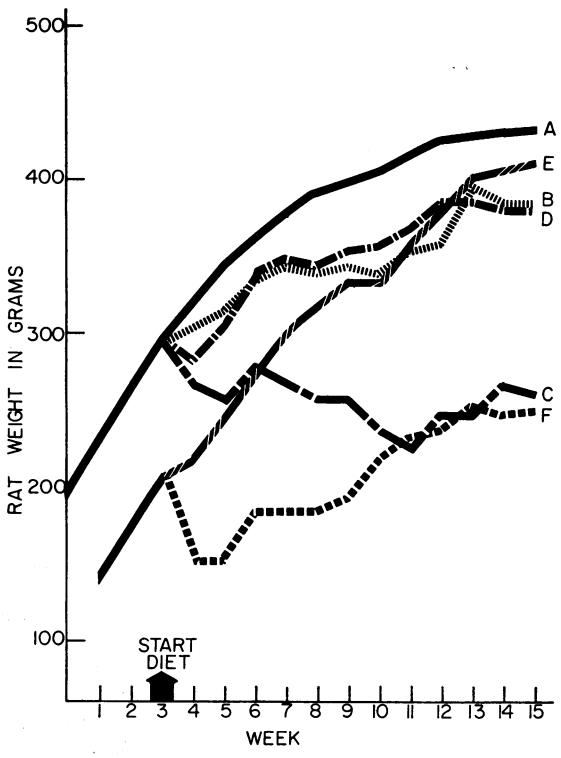

FIG. 1.—Average growth curves for each of the 6 groups of rats. Experimental diets were begun in the fourth week (arrow).

Extractions and analyses: Carcass aliquots and samples of diets were extracted with acetone-ethanol (1:1).[3] After hydrolysis and digitonin precipitation[4] they were analyzed for total fast- and slow-acting sterols.[5] Dietary sterols were also analyzed by gas-liquid chromatography.[6] Feces were extracted from 50 vol acetone:alcohol, 1:1, V/V, refluxed at ebullient temperature for 3 hr. Aliquots of the lipid extracts were reduced under nitrogen to 2–5 ml and saponified for 1 hr over steam in 10 ml of 80% ethanol and 1 *N* potassium hydroxide. Then 20 ml distilled water was added to each sample, and nonsaponifiable lipids were extracted with hexane. Six hexane extractions were necessary for uniform and reproducible results.[7] The nonsaponifiables were washed with distilled water, dried over sodium sulfate, and filtered. The filtrate contained "total fecal sterols." The aqueous residual was acidified with 8 *N* sulfuric acid to pH 1. Acidic lipids were extracted with three ether extractions. The ether extract was washed with saturated sodium chloride until neutral, dried over sodium sulfate, and filtered. The extract contained "total fecal bile acids."

Analysis of fecal sterols and bile acids: "Total fecal sterols" were analyzed as fast- and slow-acting sterols by the Liebermann-Burchard color method of Moore and Baumann.[5] During the first and second weeks and from the tenth to twelfth week selected samples of feces were analyzed by 4 different methods on hexane and/or ether extracted nonsaponifiables (Table 2). The four methods were: (1) the Liebermann-Burchard color method (LB);[5] (2) gas-liquid chromatography (GLC);[6,8] (3) gravimetric analysis of sterol digitonides; and (4) LB color method on precipitated digitonides. The consistent agreement of the LB color reaction from hexane extracted material with isotopic data (see below) and with data obtained by the more laborious methods of gas chromatography and gravimetric analysis of precipitated digitonides made it the method of choice for sterol determinations. "Total fecal bile acids" denotes results obtained by application of the fluorimetric method of Levin *et al.*[9] to the acidic lipid extract described above.

Isotope studies: Isotopic data following injection of purified[10] cholesterol 4-C^{14} corroborated chemical data. During the tenth week, three animals from groups A, B, D, E, and F were in-

TABLE 2

COMPARATIVE RESULTS OF TOTAL FECAL STEROLS IN RAT FECES DETERMINED BY 4 METHODS

Diet	Week	No. of samples	Non-saponifiables extracted with:	Total Sterols as Mg per 2 Gm Rat Feces by; L-B color	Gas chromatography	Digitonin ppt. (gravimetric)	Digitonin ppt. (L-B color)
A	2	3	Hexane	6.7	6.9	6.7	3.1
A	2	3	Ether	8.7	6.5	7.2	3.8
B	10–12	7	Hexane	4.3	3.6	4.8	2.6
C	10–12	9	Hexane	5.9	6.8	7.7	4.0
D	10–12	9	Hexane	5.6	5.4	6.0	3.4
E	10–12	6	Hexane	7.9	7.7	9.8	5.7
F	10–12	6	Hexane	4.7	4.4	6.0	2.8

jected intraperitoneally with 1.76 × 10^6 cpm of tracer cholesterol 4-C^{14} in 0.5 ml 1% bovine serum albumin in 0.85% saline solution. Radioactivity in fecal bile acid and sterol fractions was analyzed weekly. Isotopic fecal bile acid:sterol ratios were compared with chemical ratios as a check on the chemical methods.

Calculation of sterol synthesis by the balance method: Total body sterol synthesis was calculated from sterol intake and output (as fecal sterols and bile acids) and changes in total body sterol content. It was assumed that significant sterol degradation does not occur. Total body sterols were calculated at the beginning of the dietary study by analysis of 4 control rats and at the end by analysis of the subject animals. Changes in body sterol were assumed to be a straight line function with respect to time (Fig. 2). While this assumption may not be strictly correct, it can be seen from Table 3 that the weekly changes assumed for body sterol content were small compared to total animal sterol synthesis. The equation for calculating the sterol synthesis data is $S_T = \Delta s + Fs + Fba - Ds$, where S_T is the total sterol synthesized (column 9), Δs is the change in total body sterol (column 6), Fs is total fecal sterol (column 7), Fba is total fecal bile acids (column 8), and Ds is dietary sterol (column 5).

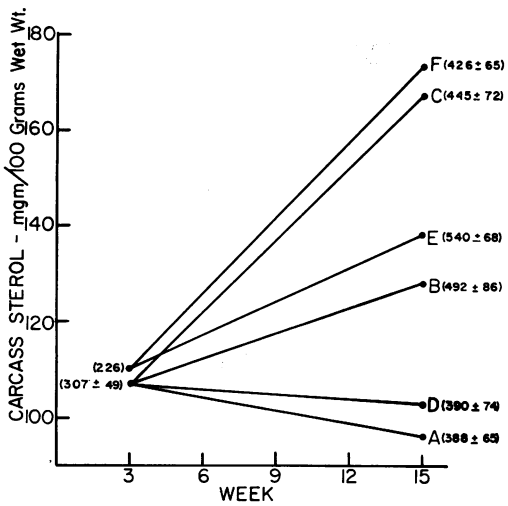

FIG. 2.—Changes in carcass sterol concentration from weeks 4–15. Figures in parentheses are averages of total carcass sterol for each group at time of sacrifice.

Results.—The most significant results of this study are summarized in Table 3 which represents a 7 week steady state period. The term "steady state" means a steady dietary state during which dietary intake and sterol biosynthesis were relatively level within each group.

Sterol synthesis in the normal 400 gm rat fed laboratory chow during the steady state period varied 183–238 mg per rat per week. Total sterol synthesis was roughly proportional to caloric intake within all groups. Figure 3 is a plot of the 72 individual mean weekly values for each group during the 12 week experimental diet period. Figure 4, a plot of the average values measured during the steady state period, is based upon 40 average values which show a slope of 0.40 mg sterol synthesis per calorie and a correlation coefficient of 0.77.

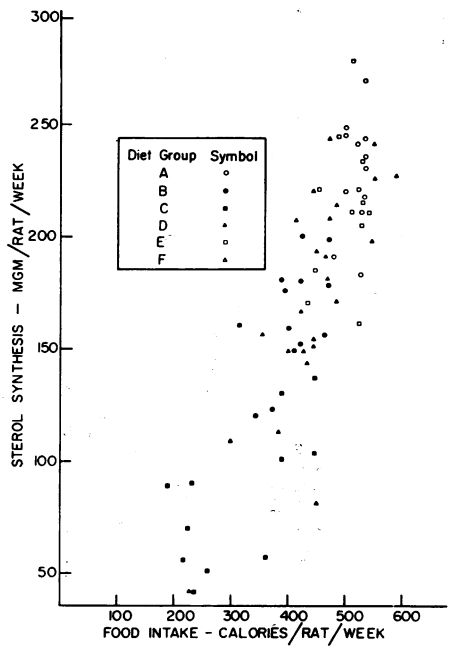

FIG. 3.—Spot diagram of weekly average values of sterol synthesis and caloric intake during entire experimental dietary period (weeks 4–15).

Sterol synthesis and dietary sterol: It might be assumed from published data con-

TABLE 3

SUMMARY OF STEADY STATE BALANCE DATA
(ACCUMULATED FROM 6TH THROUGH 12TH WEEK)*

1	2	3	4	5	6	7	8	9	10
Diet	No. animals	Average weight in grams†	Caloric intake calories/rat/week	Total sterol intake mg/rat/week§	Increase in body sterol mg/rat/week	Total fecal sterol mgm/rat/week	Total fecal bile acid mgm/rat/week	—Total sterol synthesis— mgm/rat/ week	mgm/ calorie
A	4	396	524 (13.2)‡	168	9	158 (16.4)	221 (16.5)	220 (28.4)	0.42
B	4	345	402 (28.6)	7	13	45 (6.4)	103 (13.8)	156 (26.5)	0.39
C	7	253	261 (62.6)	4	11	41 (9.2)	39 (9.6)	87 (40.8)	0.33
D	8	356	440 (43.7)	8	9	56 (6.7)	114 (11.0)	171 (22.8)	0.39
E	5	325	526 (10.2)	115	28	121 (12.3)	187 (19.4)	221 (33.2)	0.42
F	6	227	542 (37.3)**	135	32	130 (5.1)	200 (17.8)	228 (26.1)	0.38

* Diet F data tabulated for 8th through 12th week.
† Mean animal weights at start of diets, i.e., end of week 3; diets A–D 297 gm: diets E & F, 198 gm.
‡ Figures in parentheses represent standard deviations of the means.
§ Gas chromatographic analysis showed the dietary sterol of diets A, E, and F included 22% cholesterol, and diets B, C, and D 100% cholesterol.
** This figure derived by assuming *absorption* of stearic acid is 66% as indicated by fecal stearic acid titration data.

cerning cholesterol feed-back inhibition of its own synthesis[1, 11–14] that an animal normally fed a diet containing some cholesterol would increase its sterol synthetic capacity if that diet were changed to one containing less cholesterol. At the low levels of dietary cholesterol concentration in these experiments,[14a] this was not the case. For example, Group B animals had consistently lower rates of sterol synthesis than did Group A. The differences were determined by dietary factors other than cholesterol.

Accumulation of sterol in carcass: Concentration of sterol in carcass is seen in Figure 2 which assumes carcass sterol concentration is representative of the whole animal, and changes in carcass sterol are linear with respect to time. Changes in body sterol in mg per rat per week (Column 6, Table 3) were interpolations from Figure 2. General features noted from these data are: (1) Animals which lost weight and failed to grow (diets C and F) had higher concentrations of sterols in carcass than controls. (2) The variations in acceptance, absorption, and excretion between animals on different diets must be considered when comparisons are made. (3) Dietary bulk, or fecal residue, appears to be inversely related to sterol accumulation. Carcasses from low bulk, low residue, synthetic diet B animals had generally higher sterol concentrations and total body sterol accumulations than did control animals on laboratory chow (diet A). When chow-based diets A and E are compared, there are similar and striking differences. Both groups ate and grew at comparable rates throughout the study. The average weekly dry weight of feces for groups A and E was 46.5 and 30.0 gm, respectively. Diet E, the well-tolerated diet rich in saturated fat and yielding less fecal bulk, led to both increased concentration of carcass sterol and increased total carcass sterol, although it contained less sterol than diet A, and the animals which ate it synthesized sterol at the same rate as did those with diet A. It can be seen from Table 3, columns 7 and 8, that the diet A animals consistently excreted 10 to

20 per cent more fecal sterols and bile acids than did the animals fed diet E. Other groups are more complicated to compare because of disparities of growth and caloric intake. The Group C animals accumulated almost as much total sterol (Fig. 2) as did their better fed controls (Group B), despite poor diet acceptance and a highly unsaturated diet. A major factor involved appeared to be the small amount of fecal residue and steroids.

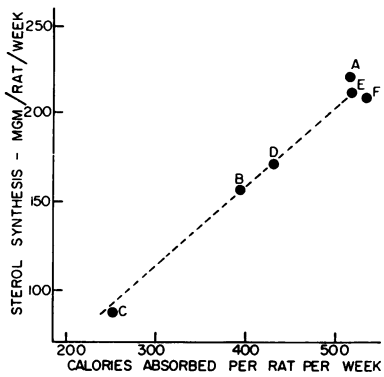

FIG. 4.—Relationship of caloric intake to total animal sterol synthesis during steady state period (weeks 6–12). Values for each group are averages taken from columns 4 and 9 of Table 4.

Figure 5 is a plot of feces weight versus total fecal bile acids during the steady state period. Bile acid excretion was closely related to the weight of fecal residue. The same was true of fecal sterol excretion when the synthetic and laboratory chow diets were compared (Table 3). These data are consistent with the observation that carcass sterol accumulation is inversely related to fecal bulk. A determining factor in carcass sterol accumulation lies in the ability of the animal to excrete its own sterol either as sterol or as bile acid. It would seem from these data that dietary bulk is a major medium for transporting steroids from the animal body.

Isotope studies: Isotopic data following injection of cholesterol 4-C^{14} corroborated conclusions from the chemical balances. The radioactivity of excreted fecal products paralleled closely and confirmed the chemical results. Table 4 shows results for a period when all animals were in a steady state, and illustrates the similarity between chemical and isotopic data. The fecal bile acid to sterol ratio was higher for synthetic diets B and D than for laboratory chow diets A, E, and F. The consistency of these ratios was borne out by analysis of the chemical figures from weeks 4–14.

Discussion.—This work confirms several lines of investigation by others. Previous workers have shown *in vitro*[15] and *in vivo*[16] that fasting diminished cholesterol biosynthesis and that the cholesterol biosynthetic pathway is energy-dependent.[3a, 17] It is not surprising that sterol synthesis is calorie-dependent. The mature animal in a steady state of sterol balance accounts for his entire sterol synthesis by G. I. tract turnover (excluding urinary and skin losses). It seems possible that fecal steroid excretion somehow affects sterol synthesis. The rate of removal of steroids from the body by fecal residues correlates with the rate of body sterol synthesis. It is as if an inhibitor of sterol synthesis were being removed in the feces, and parallels the work of Thompson and Vars,[18,19] and Eriksson[20] who showed that bile

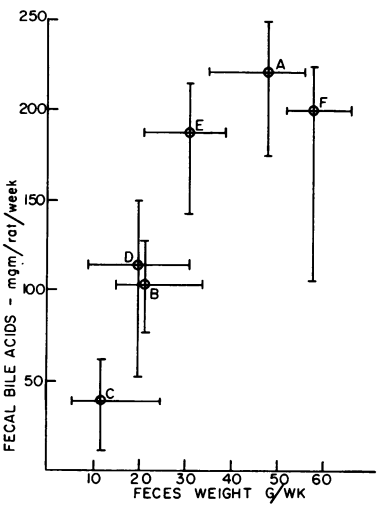

FIG. 5.—Relationship between total fecal bile acids and air-dried feces weight during steady state period. Vertical and horizontal lines give range of values for each point.

TABLE 4

COMPARISON OF BILE ACID TO STEROL RATIOS DERIVED FROM CHEMICAL AND ISOTOPIC DATA FOR RATS ON VARIOUS DIETS*

Diet	Bile acid:sterol ratios (Weeks 11 and 12)		
	Chemical	C^{14}	Average
A	1.44	1.32	1.38
B	2.41	2.14	2.27
D	1.90	1.82	1.86
E	1.58	1.54	1.56
F	1.55	1.56	1.56

* Figures are averages of simultaneous chemical and radioactivity determinations of six animal weeks for each diet during weeks 11 and 12 of the study.

acid synthesis increased 10-fold in bile fistula rats. The animal with a high residue diet acts similarly to the bile fistula animal, and it might be said that it has an internal form of bile fistula. Portman and Murphy[21] had similar results when they emphasized the effect of diet on the excretion of fecal metabolites of cholesterol.

Bile acids are known to influence cholesterol synthesis[22,23] and it might be speculated that it is the bile acids being removed in the feces that are the normal physiological inhibitors of cholesterol biosynthesis. It is probable that bile acids which inhibit sterol biosynthesis must be unconjugated, since bile duct ligation, followed by elevations in serum *conjugated* bile acids, leads to increased rates of cholesterol biosynthesis.[24] One may question whether cholesterol itself is truly the inhibitor of its own biosynthesis as demonstrated by feeding experiments. Another explanation might be that some cholesterol in transit through the gut is modified to a steroid acidic product which inhibits cholesterol biosynthesis.

From these ideas an over-all concept of body sterol metabolism in terms of biosynthesis, accumulation, and excretion can be envisaged. Total animal sterol biosynthesis is regulated by three physiological factors: (1) *caloric intake* which provides the necessary energy for sterol synthesis; (2) *fecal steroid excretion* which is postulated to regulate sterol biosynthesis by influencing the reabsorption of unconjugated bile acids. These acids are postulated to be prime physiological inhibitors of sterol biosynthesis. Fecal steroid excretion is in turn proportional to fecal residue or bulk; (3) *dietary cholesterol* which when fed in higher concentrations (approaching the 0.5 per cent level) also regulates sterol biosynthesis, although the mechanism may be similar to that controlled by fecal steroid excretion.

Total sterol accumulation in an animal fed a calorically adequate, low cholesterol diet is primarily regulated by fecal steroid excretion, and this is proportional to fecal residue. Although increased fecal steroid excretion promotes higher rates of endogenous sterol biosynthesis, the dominant effect observed is lesser body sterol accumulation. Similarly, when fecal steroid excretion is reduced by a low residue diet and total animal sterol biosynthesis falls, body sterol accumulation nevertheless increases. For these reasons, animal sterol accumulation appears to be less dependent upon the rate of total animal sterol biosynthesis than upon the differential rates between absorption of exogenous sterol and excretion of steroid in dietary residue. This concept is entirely consistent with the work of Steiner *et al.*[25] and Manalo *et al.*[26] in humans who have challenged the concept that the diminished hepatic cholesterol biosynthesis found after cholesterol feeding can adequately compensate for increased absorption of excess dietary cholesterol.

The rates of total sterol synthesis presented are similar to those found by others when the specific dietary conditions of individual studies are noted. Pihl *et al.*[27]

estimated that the half time of carcass sterol turnover was 31–32 days in rats fed 321 calories per week. Gould and Cook[3b] estimated that the rate of synthesis in a 300 gm rat would be 14 mg per day or 98 mg per week. These values fall within the spread observed in Figure 3. Lindstedt and Norman,[28] feeding rats white bread and water, arrived at a slightly lower figure for total sterol synthesis of 9–12 mg per 300 gm rat, but the caloric intake was not recorded.

Nicotinic acid (Group D) was not found to influence significantly total sterol synthesis accumulation, or excretion in these studies.

Summary.—Balance studies in adult male rats have shown that total animal sterol synthesis was proportional to caloric intake. Total body sterol biosynthesis was independent of variations in cholesterol feeding levels when these levels remained in the range of 0.006–0.023 per cent by weight. Fecal dietary residue, as measured by air-dried weight, was also related to total animal sterol synthesis and inversely related to the accumulation of sterol in the animal carcass. A cholesterol-accumulating, or atherogenic, diet is one which is high in calories, well refined, and low in fecal residue. Such a diet would contain a high fat content to provide plentiful calories with minimal residue and optimum conditions for cholesterol absorption and reabsorption.

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* Definition of terms to be used in this paper: *Sterol*—a neutral lipid of the cyclopentanophenanthrene series; *Bile acid*—an acidic lipid of the same series; *Steroid*—inclusive term for sterols and bile acids; *Sterol synthesis*—the creation of a sterol or bile acid from a nonsteroid precursor, presumably acetate. Bile acid synthesis is included in the term sterol synthesis, since cholesterol is generally accepted as an intermediate.

[1] Gould, R. G., and C. B. Taylor, *Fed. Proc.*, **9**, 179 (1950).

[2] Wintersteiner, O., and S. Bergström, *J. Biol. Chem.*, **137**, 785 (1941).

[3] Gould, R. G., and R. P. Cook, in *Cholesterol, Chemistry, Biochemistry, and Pathology*, ed. R. P. Cook (New York: Academic Press, 1958), p. 484.

[3a] *Ibid.*, p. 211.

[3b] *Ibid.*, p. 258.

[4] Sperry, W. M., and M. Webb, *J. Biol. Chem.*, **187**, 97 (1950).

[5] Moore, P. R., and C. A. Baumann, *J. Biol. Chem.*, **195**, 615 (1952).

[6] Bloomfield, D. K., *Anal. Chem.*, **34**, 737 (1962).

[7] Bloomfield, D. K., unpublished data.

[8] Bloomfield, D. K., *J. Chromatography*, **9**, 411 (1962).

[9] Levin, S. J., J. L. Irvin, and C. G. Johnston, *Anal. Chem.*, **33**, 856 (1961).

[10] Danielsson, H., *Biochim. Biophys. Acta*, **27**, 401 (1958).

[11] Alfin-Slater, R. B., M. C. Schotz, F. Shimoda, and H. J. Deuel, Jr., *J. Biol. Chem.*, **195**, 311 (1952).

[12] Tomkins, G. M., H. Sheppard, and I. L. Chaikoff, *J. Biol. Chem.*, **201**, 137 (1953).

[13] Langdon, R. G., and K. Bloch, *J. Biol. Chem.* **202**, 77 (1953).

[14] Siperstein, M. D., and M. J. Guest, *J. Clin. Invest.*, **39**, 642 (1961).

[14a] Diet A contained 1.06 mg total sterol per gm of diet. Of this, 22%, 0.23 mg per gm diet, was cholesterol as determined by gas chromatographic analysis. This dietary cholesterol level of 0.023% by weight must be compared with experiments showing cholesterol feed-back inhibition where cholesterol has been fed at levels of 0.5–5%[1,11–14] or 20–200 times the level present in

ordinary laboratory chow. Diet B, supposedly cholesterol-free, contained 0.06 mg cholesterol per gm of diet, or about one fourth the cholesterol content of diet A.

[15] Tomkins, G. M., and I. L. Chaikoff, *J. Biol. Chem.*, **196**, 569 (1952).

[16] Van Bruggen, J. T., T. T. Hutchens, C. K. Claycomb, W. J. Cathey, and E. S. West, *J. Biol. Chem.*, **209**, 635 (1954).

[17] Bucher, N. L. R., and J. McGarrahan, *J. Biol. Chem.*, **222**, 1 (1956).

[18] Thompson, J. C., and H. M. Vars, *Proc. Soc. Exptl. Biol. Med.*, **83**, 246 (1953).

[19] Thompson, J. C., and H. M. Vars, *Am. J. Physiol.*, **179**, 405 (1954).

[20] Eriksson, S., *Proc. Soc. Exptl. Biol. Med.*, **94**, 578 (1957).

[21] Portman, O. W., and P. Murphy, *Arch. Biochem. Biophys.*, **76**, 367 (1958).

[22] Bergström, S., and H. Danielsson, *Acta Physiol. Scand.*, **43**, 1 (1958).

[23] Beher, W. T., and G. D. Baker, *Fed. Proc.*, **21**, 298 (1962).

[24] Frantz, I. D., Jr., and B. T. Hinkelman, *J. Exptl. Med.*, **101**, 225 (1955).

[25] Steiner, A., E. J. Howard, and S. Akgun, *J. Am. Med. Assoc.*, **181**, 186 (1962).

[26] Manalo, P., C. B. Taylor, C. Fetcho, and E. Barber, *Circulation*, **26**, 661 (1962).

[27] Pihl, A., K. Bloch, and H. S. Anker, *J. Biol. Chem.*, **183**, 441 (1950).

[28] Lindstedt, S., and A. Norman, *Acta Physiol. Scand.*, **38**, 121 (1956).

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*ON THE CONSTRUCTION OF CERTAIN LIAPUNOV FUNCTIONS**

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This note is a brief account of a longer paper which will be published elsewhere.[1]

Let $r(x,y)$ be a function of class C' neighboring (0,0) with $r(0,0) = 0$, and consider the differential equation

$$\ddot{x} = r(x,\dot{x}). \tag{1}$$

With (1) we associate the system

$$\begin{aligned} \dot{x} &= y, \\ \dot{y} &= r(x,y). \end{aligned} \tag{2}$$

The system (2) is said to be *regular* near (0,0) if $r_y(0,0) \neq 0$.

LEMMA. *If the system* (2) *is regular and has the origin as an isolated singular point, the function* $V(x,y)$ *defined by the equation*

$$2V(x,y) = y^2 - 2\int_0^x r(x,0)dx$$

is a "Liapunov" function which determines the stability or instability of the origin.

Specifically, this function V may be employed in conjunction with well-known theorems of Liapunov, Cetaev, and LaSalle-Lefschetz to determine the nature of the critical point at (0,0) [see ref. 2].

THEOREM 1. *If the origin is an isolated critical point of a regular system* (2), *this point is (locally) asymptotically stable if*

$$r_y(0,0) < 0 \text{ and } xr(x,0) < 0 \ (x \neq 0).$$

In all other regular cases it is unstable.