Summary

In Acatenango, Guatemala studies were made of the dietetic intakes and requirements of groups of families, in the following way: 40 families were studied for exactly one day and a different group of 10 families was studied for exactly one week. This paper considers what information can be gotten from the caloric data by removing the "effect of requirement", points out important differences in the two types of studies, and shows how the "one-week method" was improperly performed, but indicates how this data was able at least partially to be salvaged. In particular, if it is important to see an "effect" of family, then it is not satisfactory to perform the one-day method, and if the seven-day method is used, the intakes must be recorded day-by-day and not just averaged over a week, lest the effects of family and of day be lost.

It is demonstrated that the effect of family is extremely important in Acatenango and that the effect of day of the week on caloric consumption is of very little importance.

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The relationship between diet and composition of bladder bile in mice

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With 5 tables

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A diet without added fat induces abundant formation of cholesterol gallstones in young hamsters when the carbohydrate is furnished entirely or mainly in the form of glucose, whereas the tendency to production of gallstones is nil or very slight when the carbohydrate is furnished entirely in the form of rice starch. The different influence of the two carbohydrates with respect to gallstone formation in hamsters is paralleled by a difference in the composition of the bladder bile of that species; the ratio between lipid-soluble

phosphorus and cholesterol and especially the ratio between bile acids and cholesterol being higher when the dietary carbohydrate is rice starch than when it is glucose (1).

Rearing of young mice on a fat-free diet with glucose as carbohydrate did not induce formation of gallstones, and analyses of the bladder bile from mice on such a diet showed that the ratio between bile acids and cholesterol was somewhat higher, and the ratio between lipid-soluble phosphorus and cholesterol much higher than previously found for hamsters reared on fat-free glucose diet. (2).

Nevertheless, it could be thought that also in mice a diet containing rice starch instead of glucose would yield higher values for the above-mentioned ratios than does the glucose diet.

The present study served to test this possibility. The results clearly showed that the rice starch diet had no such effect.

Thus, the markedly different influence of the two carbohydrates on the composition of bladder bile of hamsters is not a general principle applying to all species of rodents but rather a feature peculiar to hamsters.

Experimental

The mice were young from our stock colony of white mice (Strain NMRI), 33–39 days of age at the beginning of the experimental feeding. They were housed in metal cages with wire screen bottom and given the experimental diets indicated in table 1. Diet and water were available *ad libitum* during the feeding period which lasted from 42 to 45 days. At the end of the feeding period the mice were fasted over night, and the following morning allowed to run in a rotatable cylindrical cage for about 2 hours before being killed with chloroform. Laparotomy was immediately performed; a glass tube ending in a capillary was inserted into the gallbladder and bile drawn into the tube by suction. In most cases it was necessary to collect bile from several (2–7) animals into the tube in order to get

Table 1. Diets

	No. 1 g	No. 2 g	No. 3 g
Casein, crude[1])	20.0	20.0	20.0
Glucose	74.3	62.3	
Rice starch		12.0	74.3
Salt mixture[2])	5.0	5.0	5.0
Vitamin mixture[3])	0.5	0.5	0.5
Choline chloride	0.2	0.2	0.2
	100.0	100.0	100.0

[1]) "Dairinex", from Dansk Mejeri Industri & Export Kompagni, Stege, Denmark.

[2]) McCollum & Simmonds no. 185 (E. V. McCollum & N. Simmonds, J. Biol. Chem. **33**, 55–89, 1918).

[3]) The vitamin mixture used in the paper by Dam, Prange and Christensen, Z. Ernährungswiss. **6**, 97–106 (1965).

samples sufficient for each set of analyses (cholesterol, lipid-soluble phosphorus and bile acids). Usually, the gallbladder of the females contained more bile than the gallbladder of the males. The pooled bile (25–40 mg, weighed in the tube) was blown out of the tube, and suitable quantities speedily measured by means of Carlsberg pipettes (constriction pipettes), 5 microliter being used for determination of cholesterol and lipid-soluble phosphorus, 3 microliter for determination of taurine-conjugated bile acids. All determinations were carried out in duplo.

The samples to be used for determination of cholesterol and lipid-soluble phosphorus were extracted with 300 microliter of a mixture of absolute ethanol and acetone (1:1) in a small (4.5 ml) centrifuge tube with polyethylene stopper in a water bath at 70–80 °C for 5 minutes. After violent shaking in a shaking apparatus (Microid Flask Shaker, Griffin & George, Ltd., England) and centrifugation, the supernatant was transferred into a 2 ml measuring flask by a Carlsberg pipette. The residue was reextracted twice with 100 microliter of the ethanol acetone mixture and filled up to 2 ml in the measuring flask.

For determination of *lipid-soluble phosphorus*, 400 microliter of the content of the measuring flask (corresponding to 1 microliter bile) were transferred to a Pyrex test tube, evaporated in a stream of nitrogen and incinerated with 50 microliter perchloric acid (70–72%) over a micro-burner for about 5 minutes, until the brown color had disappeared. Thereafter the content of phosphorus was determined by means of ammonium molybdate and amino-naphthol-sulfonate according to the method of W. M. SPERRY (3) using one tenth of the quantity of reagent indicated in the description of this method. After 10 minutes at room temperature, the absorbance at 330 nm was read in a Beckman Spectrophotometer (in semimicro-cuvettes) against the reagents plus water. A solution of sodium phosphate in water containing 0.204 microgram P in 300 microliter served as standard.

For determination of *cholesterol*, the rest of the content of the measuring flask (1.6 ml), corresponding to 4 microliter bile, was transferred quantitatively into a centrifuge tube and evaporated at 70 °C in a stream of nitrogen; 500 microliter chloroform was added and the mixture shaken violently in the shaking apparatus. After centrifugation, the supernatant was transferred into a conical centrifuge tube, and the residue reextracted 3 times with 200 microliter chloroform. The combined extracts were evaporated under nitrogen, taken up in chloroform and chromatographed (ascending) on paper as described by HANSEN & DAM (4). Cholesterol was determined in the eluate by the method of ZLATKIS as modified by HANSEN & DAM (4).

Taurine-conjugated cholic acid and taurine-conjugated chenodeoxy + deoxycholic acids were determined by ascending chromatography on Whatman 3 MM paper using 70% formic acid as the stationary phase and isoamylacetate:heptane (85:15) as the mobile phase as described by SJÖVALL (5).

No attempt was made to determine trace amounts of other taurine-conjugated bile acids occuring in mouse bile.

Paper chromatography of several 3-microliter samples for glycine-conjugated bile acids according to SJÖVALL (6) gave no visible spots. Since DANIELSON & KAZUNO (7) have found that in the mouse, bile acids are present in bile exclusively as taurine-conjugates further attempts to detect glycine-conjugated bile acids were not made.

Neither was p_H of the bile determined, since in our previous studies with hamsters (1) the p_H of the bladder bile was essentially the same whether the dietary carbohydrate was glucose or rice starch. A few determinations of p_H of bladder bile of mice on glucose diet (2) had given values of 7.4 to 7.8, i. e. essentially the same values as those found for hamster bladder bile.

Results and discussion

None of the mice in any of the groups had gallstones, but in several cases small gelatinous lumps were observed in the bladder bile, especially in bile from mice reared on the two diets containing glucose.

The results of the bile analyses are presented in tables 2, 3 and 4.

Table 2. Data for bladder bile of mice reared on diets containing 74.3% glucose and no added fat*)

Bile sample No.	Sex of animals	Number of animals contributing to bile sample	Duration of feeding, days	Cholesterol mMol/l	Lipid-soluble P mMol/l	TC mMol/l	TCD + TD mMol/l	T mMol/l	Molar ratios: Di / Tri	Molar ratios: T / Cholesterol	Molar ratios: Lipid-sol. P / Cholesterol	Molar ratios: T / Lipid-sol. P
1	m	5	42	6.3	39.5	78.0	9.9	87.9	0.13	14.0	6.3	2.2
2	m	5	42	5.7	44.2	78.9	11.7	90.6	0.15	15.9	7.8	2.0
3	m	7	44	3.6	29.0	56.2	10.6	66.8	0.19	18.6	8.1	2.3
4	m	2	43	3.2	36.9	53.9	10.8	64.7	0.20	20.2	11.3	1.8
5	m	5	43	4.7	37.7	55.5	29.0	84.5	0.52	18.0	8.0	2.2
6	m	5	43	3.3	17.9	34.3	6.7	41.0	0.19	12.4	5.4	2.3
									0.23^{6} $\pm$ 0.06	$16.5^{6} \pm 1.2$	$7.8^{6} \pm 0.8$	2.1 ± 0.1
7	f	2	43	3.5	42.3	55.5	9.1	64.6	0.16	18.4	12.1	1.5
8	f	1	43	4.7	43.2	77.4	8.6	86.0	0.11	18.3	9.2	2.0
9	f	3	43	3.7	42.9	85.3	4.4	89.7	0.05	24.2	11.6	2.1
10	f	2	43	5.7	51.6	61.3	10.3	71.6	0.17	12.6	9.1	1.4
11	f	3	43	7.2	44.2	59.9	6.2	66.1	0.10	9.2	6.1	1.5
12	f	3	45	6.8	53.0	87.1	16.3	103.4	0.19	15.2	7.8	2.0
									0.13^{6} $\pm$ 0.02	$16.3^{6} \pm 2.1$	$9.3^{6} \pm 0.9$	$1.8^{6} \pm 0.1$

*) Abbreviations: m = males;
f = females;
TC = taurocholic acid;
TCD + TD = taurochenodeoxycholic acid + taurodeoxycholic acid;
T = TC + TCD + TD

Table 3. Data for bladder bile of mice reared on a diet containing 62.3% glucose, 12% rice starch and no added fat

Bile sample No.	Sex of animals	Number of animals contributing to bile sample	Duration of feeding, days	Cholesterol mMol/l	Lipid-soluble P mMol/l	TC mMol/l	TCD + TD mMol/l	T mMol/l	Molar ratios: Di / Tri	Molar ratios: T / Cholesterol	Molar ratios: Lipid-sol. P / Cholesterol	Molar ratios: T / Lipid-sol. P
13	m	5	42	5.6	37.9	78.5	17.0	95.5	0.22	17.1	6.7	2.5
14	m	7	42	5.7	41.6	65.7	12.5	78.2	0.19	13.7	7.3	1.9
15	m	1	44	2.9	32.7	59.7	6.0	65.7	0.10	22.7	11.3	2.0
16	m	4	44	4.0	36.1	63.6	9.6	73.2	0.15	18.3	9.0	2.0
17	m	4	43	5.1	36.8	63.2	23.5	86.7	0.37	17.0	7.2	2.4
18	m	6	43	5.2	34.2	62.4	20.5	82.9	0.33	15.9	6.6	2.4
19	m	4	49	3.8	25.9	48.7	7.5	56.2	0.15	14.8	6.8	2.2
									0.22[7] ± 0.04	17.1[7] ± 1.1	7.8[7] ± 0.7	2.2[7] ± 0.1
20	f	2	43	7.6	48.1	72.5	12.0	84.5	0.17	11.1	6.3	1.8
21	f	1	43	4.2	39.4	89.4	15.9	105.3	0.18	25.1	9.4	2.7
22	f	4	43	6.4	35.5	78.7	6.7	85.4	0.09	13.3	5.5	2.4
23	f	2	43	4.6	50.7	53.6	8.1	61.7	0.15	13.4	11.0	1.2
24	f	2	43	5.9	60.4	83.6	11.2	94.8	0.13	16.1	10.2	1.6
25	f	4	45	5.5	47.0	63.2	9.4	72.6	0.15	13.2	8.5	1.5
									0.15[6] ± 0.01	15.4[6] ± 2.1	8.5[6] ± 0.9	1.9[6] ± 0.2

Table 4. Data for bladder bile of mice reared on diets containing 74,3% rice starch and no added fat

Bile sample No.	Sex of animals	Number of animals contributing to bile sample	Duration of feeding, days	Cholesterol mMol/l	Lipid-soluble P mMol/l	TC mMol/l	TCD + TD mMol/l	T mMol/l	Molar ratios: Di / Tri	Molar ratios: T / Cholesterol	Molar ratios: Lipid-sol. P / Cholesterol	Molar ratios: T / Lipid-sol. P
26	m	6	42	4.9	30.8	42.5	14.7	57.2	0.35	11.7	6.3	1.9
27	m	3	42	4.9	35.8	56.0	9.7	65.7	0.17	13.4	7.3	1.8
28	m	3	42	4.1	24.0	54.3	8.9	53.2	0.16	15.4	5.9	2.6
29	m	4	44	4.2	21.2	45.6	9.6	55.2	0.21	13.1	5.0	2.6
30	m	6	43	2.9	19.9	33.9	11.4	45.3	0.34	15.6	6.9	2.3
31	m	3	43	6.2	26.9	62.2	7.5	69.7	0.12	11.2	4.3	1.1
32	m	3	43	4.0	21.4	40.9	12.6	53.5	0.31	13.4	5.4	2.5
33	m	4	49	4.6	28.2	56.6	17.3	73.9	0.31	16.1	6.1	2.6
									$0.25^{8} \pm 0.03$	$13.7^{8} \pm 0.6$	$5.9^{8} \pm 0.4$	$2.2^{8} \pm 0.2$
34	f	2	43	5.7	36.6	48.5	9.7	58.2	0.20	10.2	6.4	1.6
35	f	1	43	6.0	36.3	44.2	5.4	49.6	0.12	8.3	6.1	1.4
36	f	2	43	6.6	33.5	82.4	10.6	93.0	0.13	14.1	5.1	2.8
37	f	4	43	5.5	35.8	70.2	9.1	79.3	0.13	14.4	6.5	2.2
38	f	4	45	5.3	36.8	80.1	15.0	95.1	0.19	17.9	6.9	2.6
39	f	2	45	3.4	29.0	50.4	13.1	63.5	0.26	18.7	8.5	2.2
									$0.17^{6} \pm 0.02$	$13.9^{6} \pm 1.7$	$6.6^{6} \pm 0.6$	$2.1^{6} \pm 0.2$

Evidently, the diet containing 74.3% rice starch has not produced higher values for the ratios taurine-conjugated bile acids/cholesterol and lipid-soluble phosphorus/cholesterol than has the diet containing 74.3% glucose or the diet containing 62.3% glucose plus 12% rice starch. On the contrary, the values for these ratios tend to be slightly lower for the animals on the rice starch diet than for the animals on the two diets containing glucose.

Since the content of taurine-conjugated bile acids in mouse bile is equal to or almost equal to the total content of bile acids, it is of interest to notice that the values for the ratio taurine-conjugated bile acids/cholesterol found for mice on glucose diet in the present study are not much higher than the values for the ratio "total bile acids"/cholesterol previously found for hamsters on glucose diet (1). The values for the concentration of cholesterol in bladder bile of animals on glucose diet are not much different for the two species, and previously, no difference was found with respect to p_H. Therefore, the present findings strengthen the view that the non-appearance of cholesterol gallstones in mice on glucose diet depends upon the relatively high ratios between lipid-soluble phosphorus and cholesterol, provided that differences with respect to other factors such as the individual bile acids or components not determined to not play a decisive role.

Since the above-mentioned conclusions with respect to the composition of the bile could be reached on the basis of the data presented in tables 3, 4 and 5, we did not analyze the bile from all the mice raised on the diets (about 80 in each group).

The mice grew fairly well on all three diets, fastest on the diet containing 62.3% glucose and 12% rice starch, slowest on the diet containing 74.3% glucose (table 5). Gross signs of deficiency of essential fatty acids were no observed within the experimental period.

In experiments with hamsters (8), it was noticed that on rice starch diets, the feces were sometimes light colored indicating that undigested starch had passed through the intestinal tract. Later unpublished observations showed that the weight of the feces dried *in vacuo* over P_2O_5) from 3 male and 3 female hamsters during 10 days equalled 2–6% of the diet consumed when the carbohydrate was glucose, but from 10–16% when the carbohydrate was rice starch, paralleling a degree of digestion of the rice starch varying from 98.5 to 87.5%.

A similar difference with respect to appearance and weight of the feces could not be found for mice. The color of the feces (collected from 7 males and 7 females in each group through 8 days from the 33rd day of experimental feeding) was brown to black, and the weight after drying *in vacuo* over P_2O_5 varied between 2.2 and 2.8 per cent of the diet consumed irrespective of whether the dietary carbohydrate was glucose or rice starch.

It is not possible at present to answer the question whether the marked difference in bulk of the feces from hamsters on glucose diet and hamsters on rice starch diet is in some way related to the observed difference with respect to composition of bladder bile from hamsters on these two diets, and conversely, whether the absence of a similar marked difference in bulk of the feces from mice on glucose diet and on rice starch diet, respectively, is related to the absence of a corresponding difference with respect to the composition of bladder bile from mice on the diets in question.

Table 5. Weight and weight gain during six weeks of mice raised on the three diets. Standard deviations is indicated by ±

Dietary carbohydrate	Sex	All animals raised on the three diets: Number of mice	Weight at start g	Weight after 6 weeks g	Weight gain during 6 weeks g	Animals from which bile was collected: Number of mice	Weight at start g	Weight after 6 weeks g	Weight gain during 6 weeks g
74.3%	m	40	22.4±0.8	33.8±0.7	11.4±1.1	29	23.1±0.8	34.7±0.7	11.6±1.1
glucose	f	40	21.1±0.6	31.5±0.5	10.4±0.7	14	20.9±1.3	31.2±0.9	10.3±1.6
62.3% glucose	m	39	22.7±0.7	36.4±0.8	13.7±1.1	31	22.9±0.8	37.3±0.3	14.4±0.9
+ 12% rice starch	f	42	20.8±0.6	33.4±0.5	12.6±0.7	15	21.7±0.9	34.4±0.6	12.7±1.1
74.3% rice	m	40	22.3±0.7	34.8±0.7	12.5±1.0	32	21.8±0.8	34.3±0.7	12.5±1.0
starch	f	41	20.6±0.7	32.6±0.7	12.0±1.0	15	21.4±1.5	33.3±1.3	11.9±2.0

Summary

Three groups of young white mice (strain NMRI) were reared on artificial diets without added fat, consisting of casein 20%, carbohydrate 74.3%, salt mixture 5%, vitamin mixture 0.5%, and choline chloride 0.2%. Group 1 received the carbohydrate entirely in the form of glucose, group 2 received the carbohydrate in the form of 62.3% glucose + 12% rice starch, whereas group 3 received the carbohydrate entirely in the form of rice starch.

After having received the diets for 42–45 days the mice were killed and their bladder bile analyzed quantitatively with respect to cholesterol, lipid-soluble phosphorus and taurine-conjugated bile acids. The object being to determine whether in mice the diet in which all the carbohydrate is rice starch produces higher ratios between bile acids and cholesterol and between lipid-soluble phosphorus and cholesterol than do the two other diets. No evidence for such an effect of the rice starch diet was found. The average values of the above-mentioned ratios were even slightly lower for the animals in group 3 than for the animals in groups 1 and 2. Therefore, the previously found markedly different effect of the two carbohydrates, rice starch and glucose, on the composition of bladder bile of hamsters does not represent a general principle applying to all species of rodents.

Zusammenfassung

Drei Gruppen von jungen Mäusen (Strain NMRI) wurden mit künstlichen Nahrungen ohne Fettzusatz gefüttert. Die Nahrungen bestanden aus Casein 20%, Kohlenhydrat 74,3%, Salzgemisch 5%, Vitamingemisch 0,5% und Cholinchlorid 0,2%. Gruppe 1 erhielt das Kohlenhydrat gänzlich in der Form von Glucose, Gruppe 2 erhielt das Kohlenhydrat in der Form von 62,3% Glucose + 12% Reisstärke, während Gruppe 3 das Kohlenhydrat gänzlich in der Form von Reisstärke erhielt.

Nach einer Fütterungszeit von 42 bis 45 Tagen wurden die Mäuse getötet, und ihre Blasengalle auf Cholesterin, lipid-löslichen Phosphor und taurin-konjugierte Gallensäuren quantitativ analysiert, um festzustellen, ob bei dieser Tierart die Nahrung, in welcher das Kohlenhydrat gänzlich aus Reisstärke besteht, höhere Werte der Verhältnisse zwischen Gallensäuren und Cholesterin und zwischen lipid-löslichem Phosphor und Cholesterin herbeiführt, als die beiden anderen Nahrungen. Die Ergebnisse zeigten, daß dies nicht der Fall ist. Die Mittelwerte jedes der erwähnten Verhältnisse waren eher ein wenig niedriger für Gruppe 3 als für Gruppe 1 und Gruppe 2. Die früher gefundene Tatsache, daß bei jungen Hamstern, die Nahrung, in welcher das Kohlenhydrat gänzlich aus Reisstärke besteht, zu höheren Werten der erwähnten Verhältnisse führt als die Nahrung, in welcher das Kohlenhydrat aus Glucose besteht, ist somit nicht als ein für alle Arten von Nagern allgemein geltendes Prinzip aufzufassen.

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Aus dem Institut für Tierernährung der Universität Hohenheim
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Über Selengehalte pflanzlicher, tierischer und anderer Stoffe*)

1. Mitteilung: Selengehalte in Futtermitteln

Von W. OELSCHLÄGER und K. H. MENKE

Mit 2 Tabellen

(Eingegangen am 25. September 1968)

Im Jahre 1957 ist Selen, das seit 1842 biologisch lediglich wegen seiner Giftwirkung Beachtung fand, zu einem für die Ernährungsphysiologie und die praktische Tierhaltung sehr interessanten Element geworden, als SCHWARZ und FOLTZ (1) in Selen ein lebenswichtiges Spurenelement für höhere Tiere erkannt haben.

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