strable rGG may be due in part to the shorter period of observation of NTS nephritis reported herein.

Summary. Clinical, biochemical, morphological and immunohistochemical characteristics of renal disease induced in rats with injections of anti-rat kidney rabbit serum (NTS) were similar in adult rats which were thymectomized at birth and in intact members of comparable age. The development of chronic glomerular lesions in thymectomized, NTS-treated rats indicates that progression of the disease is not necessarily dependent upon those immunologic functions related to thymic function, at least during the time interval studied.

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Effect of Butter Fat and Body Weight on Experimental Atherosclerosis in Rabbits.* (28858)

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The importance of diet as a prime factor in the etiology of atherosclerosis in man has been a subject of discussion and investigation for many years. Emphasis has been placed upon the cholesterol content and type of fat in the diet, although the role of body weight has also received some attention.

The literature on the correlation between dietary fat, serum lipids and atherosclerosis has been extensively reviewed by Wigand (17), Katz and Stamler(9) and others. Clinically, it has been demonstrated that significant and sustained reduction in serum cholesterol can be produced by placing the subjects on diets containing large amounts of vegetable fat(1,2,3,10,11,15). This effect has been uniformly attributed to the polyunsaturated character of vegetable oil. In experimental animals, there are conflicting reports concerning the effect of unsaturated and saturated fats. In some studies it has been shown that cholesterol-free diets high in saturated fats produce both hypercholesterolemia and experimental atheromatosis(17). In others, hyperlipemia is said to occur without atheromatosis(8), and in still others neither occurs(16).

There is also controversy as to the influence of body weight on atherosclerosis. For example, in man it has been demonstrated that advanced atherosclerosis was twice as common in the obese than in poorly nourished subjects(18). In rabbits it has been reported that the severity of atherosclerosis is directly proportional to high initial weight and rapid weight gain(4,5). However, the opposite effect has also been described(7).

In view of these controversies, we have attempted to evaluate the effect of butter fat and body weight on serum lipids and experimental atherogenesis in rabbits by feeding them cholesterol, butter and *ad libitum* stock diets in various amounts and combinations.

Material and methods. Two separate experiments were performed, using New Zealand white rabbits of known pedigree. The

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animals were individually housed in air-conditioned animal quarters. In Experiment I, 62 rabbits from 9 litters were divided into 4 groups, each group having at least one rabbit from each litter. In Experiment II, 32 rabbits from 6 litters were similarly divided into 3 groups. The number of animals in each group, type of diet given, and duration of each experiment are summarized in the Table.

Cholesterol in the diet was prepared by dissolving the designated amount in ether, then mixing the solution with a measured amount of Purina chow and allowing the ether to evaporate. This was given in the morning to insure complete consumption. The rest of the stock ration was offered after the rabbits had completely consumed the cholesterinized food. Butter in the food was prepared by mixing melted butter with a measured amount of stock diet.

In Exp. I, one-third of the animals in each group was sacrificed in 90 days, and a complete autopsy performed. The cholesterol and butter were then increased to 0.5 g each. At the end of 30 days another third of the animals was sacrificed. The remaining rabbits received 1.0 g of butter or cholesterol for 30 days more before sacrifice. In Exp. II, one animal from each group was sacrificed after 30 days and the remainder at the end of 62 days.

All animals were weighed and bled at 2-week intervals using the central artery of the ear. Ten ml of blood were collected for lipid determinations. Total lipids were determined by the method of Kunkel *et al*(14); total and free cholesterols according to Zak(20); and lipid phosphorus by a modified procedure of Youngburg and Youngburg(19) and that of Fiske and Subbarow(6). At autopsy all organs were weighed and examined and representative sections taken. The aortas were examined carefully and a visual grading of the extent of experimental atherosclerosis was done according to the methods outlined by Kobernick and Niwayama(12).

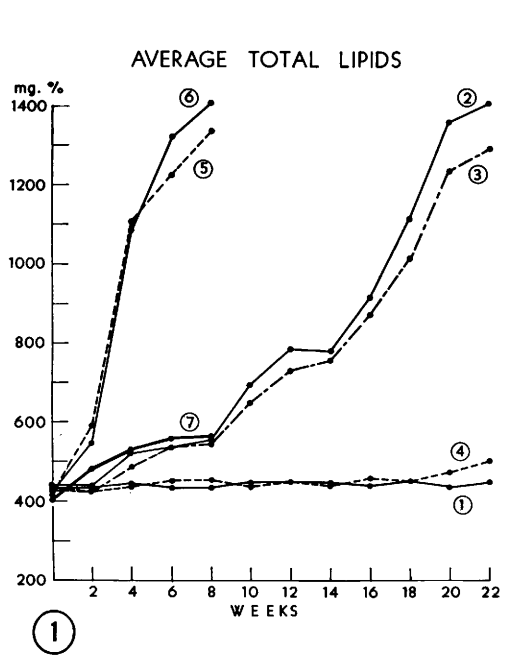
Results. Total serum lipids and lipid fractions were elevated in all animals fed cholesterol in their diet. Rate of increase corresponded rather closely with the daily dosage of cholesterol given (Fig. 1-4). Consequently, Groups 2 and 3 receiving 0.25 g of cholesterol daily required a longer period to achieve serum lipid levels similar to Groups 5 and 6 which received 1.0 g of cholesterol initially. The results were not influenced by greater intake of stock diet in Groups 2 and 5 (Table I).

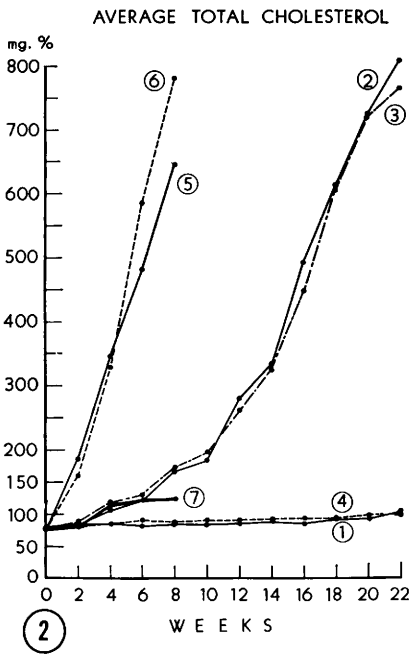
Administration of 0.25 of butter daily (Group 4) had no significant effect on the serum lipids of rabbits. When 10 g of butter per day were given (Group 7), there was a small but significant rise in all lipid fractions. At the end of the 8-week experiment, Group 6, receiving butter and cholesterol, achieved slightly higher levels of serum lipids than Group 5, which received *ad lib* stock diet and cholesterol. However, the differences are not significant by the "t" test.

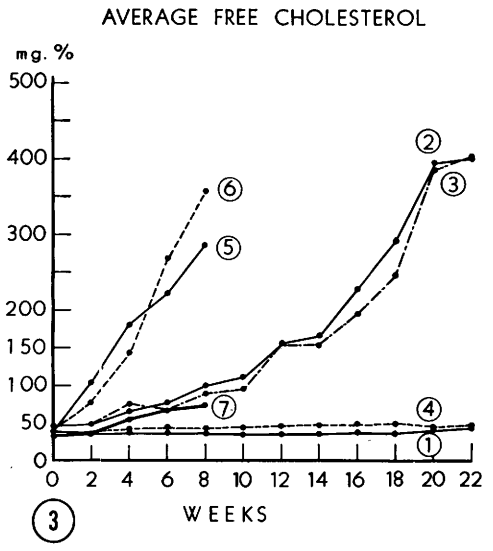
Experimental atherosclerosis of the aorta was absent in control animals (Group 1) and in animals fed butter alone (Groups 4 and 7). There is a positive correlation between severity of aortic lesions and degree of hyperlipemia, which in turn is directly related to total amount of cholesterol intake (table). This correlation is not constant in the individual rabbits. For example, in Group 6, animal No. 33 with a total cholesterol of 1100 mg% at the time of sacrifice showed one-plus (1+) acquired atherosclerosis while animal No. 34 with serum cholesterol of 750 mg% developed three-plus (3+) lesions. With the slightly higher serum lipid levels obtained in Group 6, the incidence and severity of atheromatosis (10/12 animals, 1+ to 3+) tend to be correspondingly greater than in Group 5 (6/11 animals, 1+ to 2+). No significant difference is observed between Groups 2 and 3 which consumed equal amounts of cholesterol but different amounts of stock diet (Table I).

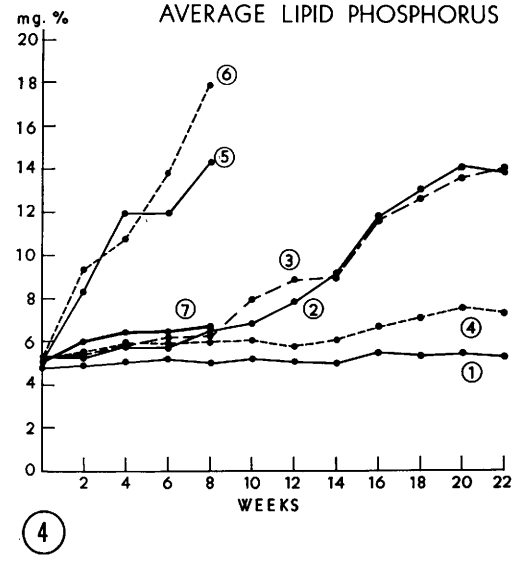
There is no significant difference in initial weight between any 2 groups in each experiment. Although the average weight gain tends to be slightly greater in the animals receiving *ad lib* diets (Groups 2 and 5), these values are not statistically significant.

Discussion. From this study it is evident that 0.25 g of butter per day had no effect on serum lipids or experimental atherogenesis in rabbits, whereas an equal amount of cho-









lesterol produced definite increases in serum lipids and atheromatous plaques in the aorta. We are not aware of any previous animal experiments in which such a small amount of fat was used. At the 10 g level a slight but unequivocal elevation of serum lipids above normal was observed ($p<.01$). The increase, however, was not as marked as those obtained by Wigand(17) using 8% butter in a 25 g/kg body weight ration (approximately 5 g butter per day), and by Hirsch and Nailor(8) who used 15-50 cc of cream containing 36% fat (roughly 5-15 g butter fat per day). It appears that the hyperlipemic effect in our animals cannot be attributed solely to the cholesterol in the butter (0.029 g in 10 g of butter), because in the same time interval comparable levels were achieved by 0.25 g of cholesterol daily (Groups 2 and 3) which is almost 9 times the amount in the

TABLE I.

Group	Diet	Days of exp	Total cholesterol intake (g)	Avg total daily food intake (g)	Avg initial wt (g)†	Avg wt gain (g)†	Incidence of atherosclerosis 0	1+	2+	3+	4+	Total No. animals	Total No. animals in group
1	100 g Purina chow (control)	150	0	100	2247 ±122	949 ±102	9	0	0	0	0	9	9
2*	a. .25 g cholesterol + *ad lib* Purina chow	90	22.5	124 ± 3†	2301 ± 70	1102 ± 83	6	1	0	0	0	7	18
	b. As (a), followed by 30 days of .5 g cholesterol + *ad lib* Purina chow	120	37.5				4	0	2	0	0	6	
	c. As (b), followed by 30 days of 1.0 g cholesterol + *ad lib* Purina chow	150	67.5				1	2	1	0	1	5	
3*	a. .25 g cholesterol + 100 g Purina chow	90	22.5	100.25	2292 ± 79	828 ± 67	5	0	0	0	0	5	17
	b. As (a), followed by 30 days of .5 g cholesterol + 100 g Purina chow	120	37.5				2	4	0	0	0	6	
	c. As (b), followed by 30 days of 1.0 g cholesterol + 100 g Purina chow	150	67.5				2	1	2	1	0	6	
4*	a. .25 g butter + 100 g Purina chow	90	.065	100.25	2319 ± 56	864 ± 72	6	0	0	0	0	6	18
	b. As (a), followed by 30 days of .5 g butter + 100 g Purina chow	120	.099				6	0	0	0	0	6	
	c. As (b), followed by 30 days of 1.0 g butter + 100 g Purina chow	150	.196				6	0	0	0	0	6	
5	1.0 g cholesterol + *ad lib* Purina chow	62	62.0	131 ± 5†	2503 ± 64	933 ±101	5	2	4	0	0	11	11
6	1.0 g cholesterol + 1.0 g butter + 98 g Purina chow	62	62.2	100	2520 ± 76	687 ± 70	2	4	3	3	0	12	12
7	10 g butter + 100 g Purina chow	62	1.80	110	2422 ± 83	699 ± 70	12	0	0	0	0	12	12

* See *Material and methods.*

† ± Standard error of mean.

butter. This suggests that a large part of the effect must have been due to the butter fat *per se*. Similarly, the greater incidence and severity of atherosclerosis in Group 6 than in Group 5 may be due to the butter (1.0 g daily) in the diet of the former. However, firm conclusions on this point would require additional experimentation.

Our present data show that, on the group level at least, severity of aortic lesions paralleled degree of hyperlipemia, which in turn is directly related to total amount of cholesterol ingested. Among individual animals, however, there was considerable variation, and this is consistent with previous findings reported from our laboratory(12,13).

The data on body weights are less conclusive. Initial weights are similar, and the increased amounts of daily ration consumed by Groups 2 and 5 did not produce significantly greater average weight gains. Since there are no differences in body weight, one cannot draw positive conclusions regarding the correlation between weight changes and atherosclerosis. We may state, however, that increased caloric intake in Groups 2 and 5 did not augment the cholesterol induced hyperlipemia nor increase the severity of atherosclerosis in those rabbits.

Summary and conclusions. 1. Rabbits were fed cholesterol, butter, and *ad libitum* Purina chow diets in various amounts and combinations to evaluate the effect of butter fat and body weight on serum lipids and experimental atherosclerosis. 2. 0.25 gram of butter per day had no effect on serum lipid levels nor did it produce aortic atheromata, whereas an equal amount of cholesterol produced definite increases in serum lipids and atheromatous plaques. 3. When 10 g of butter were given per day, there was a small but significant rise in all lipid fractions. 4. On the group level, a close correlation was found between severity of aortic lesions and degree of hyperlipemia, which in turn was related to total cholesterol intake. In individual animals considerable variation was encountered. 5. No differences were observed in initial weights or average weight gains. 6. Increased caloric intake in the form of *ad libitum* stock diets did not augment the cholesterol-induced hyperlipemia nor increase the severity of atherosclerosis in rabbits. 7. We may conclude from this study that saturated fats in the diet are not a major factor in the pathogenesis of experimental atherosclerosis in rabbits.

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