than the 15th day of pregnancy.

Summary. A sensitive biologic test for monkey chorionic gonadotrophin (MCG) has been developed based upon the augmentation of MCG either by human eunuch gonadotrophin or human postmenopausal gonadotrophin, using uterine weight of immature rats as the end point. With this method MCG was detected as early as 12 days postmating. The duration of MCG excretion in urine was from day 12 to day 38. The amount excreted reached a maximum between days 18 and 28. The maximum levels were between 205 IU and 288 IU of HCG per 24 hours.

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Effect of Fat and Cholesterol Ingestion Upon Formation and Storage of Lipids and Cholesterol from Acetate-1-C^{14}.* (32089)

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A diet containing large amounts of polyunsaturated fatty acids (PUFA) as a replacement for one with an excess of saturated fatty acids appears to be associated with lowered blood lipid and cholesterol levels(1,2). Several theories have been advanced as possible explanations as to why PUFA ingestion may under certain conditions lower blood serum lipid and cholesterol levels. Bloomfield(3) proposed that the serum cholesterol level was decreased by an altered partition of it between the blood and liver with an increase in cholesterol of the liver. Another theory involved the suggestion that the lowered cholesterol may be the result of a decreased cholesterol synthesis induced by the metabolism of PUFA as a result of an increased efficiency of system involved(1). The report that extrahepatic tissues such as intestinal tissue may synthesize cholesterol(4) suggests that tissues other than the liver may play some role in effecting blood cholesterol levels, although the liver is believed to be the major source of blood cholesterol(5,6).

This investigation was undertaken in an attempt to learn more about the effect of cholesterol ingestion on the effect of PUFA intake upon the formation of total lipids and cholesterol in various tissues of rats. The storage in these tissues of lipid and cholesterol as well as their activities resulting from their formation during an interval after an injection of labeled acetate were determined. Such information should indicate whether there was a difference in the action of saturated and unsaturated fatty acids upon the disposition of cholesterol in the tissues after ingesting a cholesterolemic diet.

Materials and methods. Eight male albino rats weighing about 160 g had been maintained on commercial rat pellets prior to initiation of this experiment. First 4 rats were fed a diet of ground rat pellets‡ enriched with 20% lard, 1% cholesterol and 0.5% sodium taurocholate. The remaining 4 rats were fed a similar diet except 20% lard was replaced by 20% corn oil. All rats were fed 10 days. Be-

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‡ Uncle Johnny's BSD Laboratory Animal Diet, containing not less than 26.0% protein, 5% fat, was prepared by Uncle Johnny Mills, Houston, Texas.

fore sacrificing, rats were fasted for 24 hours and again refed same diets *ad libitum* for another 24 hours. Then 2 rats, one on each diet and about the same weight, were injected intraperitoneally with 50 μc of sodium acetate-l-C^{14}, and were sacrificed 3 hours later by collecting as much blood as possible after ether anesthesia. This procedure was continued until all 8 rats had been utilized.

Liver and fatty tissues were removed and chilled immediately. After flushing well with cold saline, intestinal slices were prepared from the middle part of the small intestine. Approximately duplicate 0.5 g samples of all these tissues were weighed, digested with 30% KOH and after acidification, the lipids extracted with chloroform. The serum lipids were extracted according to the method of Sperry and Webb(7). Tissue extracts were evaporated under nitrogen and the lipids weighed. Lipid was dissolved in acetone-alcohol (1-1) mixture and one aliquot was taken for determining the total lipid activity. Another aliquot was used to precipitate the cholesterol as the digitonide and finally to determine the cholesterol activity and cholesterol content(7). Later the cholesterol values were verified by TLC determinations for activity measurements and it was observed that similar results were obtained by both methods. In all cases, the radioactivities of the lipids and cholesterols were determined after the addition of 15 ml of scintillation fluid mixture(8) by the use of a Beckman scintillation counter. Counts were corrected for background and for quenching when necessary.

Results. Total concentrations of tissue lipids and cholesterol and activity of each resulting from injected labeled acetate in 8 rats were determined (Table I and II). Blood levels of labeled lipid and cholesterol as well as total cholesterol were about 27% lower in rats ingesting the diet containing the polyunsaturated fatty acids (PUFA) instead of the more saturated ones. More total lipid appears to be stored in the liver and epididymal fat tissue in rats on PUFA diet while similar amounts of lipid in intestinal and mesenteric fat tissues were present in rats on both diets (Table I). When the diets contained the unsaturated fat, the livers had a higher choles-

TABLE I. Tissue Content of Lipid and Cholesterol of Rats on Diets Containing 20% Lard or Corn Oil and 1% Cholesterol.

Tissue*	Tissue lipid content			Tissue cholesterol content		
	La†	CO	Dif.‡ %	La	CO	Dif. %
	mg/g tissue			mg/g tissue		
B				.68 ± .05	.50 ± .03	−26
L	145.3 ± 7.1	170.3 ± 12.9	+17	32.8 ± 3.1	46.2 ± 2.8	+40
IS	44.4 ± 3.1	45.2 ± 1.1	—	6.0 ± .4	4.7 ± .7	−22
EF	53.6 ± 1.5	61.5 ± 2.8	+15	1.0 ± .03	.6 ± .2	−40
MF	51.3 ± 4.0	54.7 ± 3.1	—	1.2 ± .6	1.1 ± .4	—

* B = blood, L = liver, IS = intestinal slices, EF and MF = epididymal fat pads and mesenteric fat, respectively. All blood values given per ml.
† La and CO indicate results with standard errors from 4 rats fed lard and 4 fed corn oil.
‡ Dif. % column was obtained by CO-La/La × 100 per cent differences in 2 groups.

TABLE II. Effect of Diet on Tissue Lipid and Cholesterol Activities after Injection of Labeled Acetate.*

Tissue*	Tissue lipid activity			Tissue cholesterol activity		
	La†	CO	Dif.‡ %	La	CO	Dif. %
	cpm/0.1g tissue			cpm/0.1g tissue		
B	871 ± 81	622 ± 49	−29	441 ± 53	324 ± 20	−27
L	10,981 ± 384	10,500 ± 349	—	238 ± 43	105 ± 14	−56
IS	22,760 ± 481	23,350 ± 460	+11	2,833 ± 44	2,141 ± 57	−25
EF	32,720 ± 476	38,434 ± 428	+17	252 ± 27	121 ± 8	−52
MF	90,511 ± 510	81,050 ± 558	−10	437 ± 65	260 ± 23	−41

* See footnotes of Table I.

terol content while the intestinal and epididymal fat tissues had less cholesterol.

There were similar amounts of labeled lipid in the livers of rats on both diets but slightly larger amounts were found in the intestinal tissue and epididymal fat pads, less in the mesenteric fat of the rats fed corn oil (Table II). The higher concentration of cholesterol in the livers of rats fed corn oil was associated with a decrease in the activity of the cholesterol in that tissue as well as in the intestinal tissue and less storage in the fatty tissues than in the same tissues of lard fed rats. The specific radioactivities of the lipids, as cpm/mg of tissue lipid or cholesterol, were calculated and listed in Table III.

Discussion. Preliminary results after a 10 day feeding of similar diets without cholesterol suggested an increased synthesis of cholesterol by the liver when an unsaturated fat as corn oil was fed and thus gave no indication of an inhibition of liver cholesterol formation by the amount of it normally stored during that period. The data for this period obtained in an unpublished study was, for lard 23.9 ± 1.0 mg cholesterol per gram of liver tissue as compared with 26.9 ± 0.6 for corn oil or only a 13% increase in cholesterol content. The synthesis of cholesterol from labeled acetate was 65.9% greater in the latter by radioactivity measurement. Hence a cholesterolemic diet was employed in this study in an attempt to determine the effect of an increased storage of cholesterol in the tissues. This diet with PUFA was effective in producing the same inhibition of cholesterol synthesis found in normal cholesterol metabolism as a result of the presence of excess sterol(9). The decreased formation of cholesterol appears to depend on its accumulation in the liver tissue.

The results obtained would not be affected by dietary constituents other than the fats included since otherwise they were the same in both diets. Similar results were obtained when safflower was substituted for corn oil as a source of the PUFA. Neither could the food intake be a factor since there was appoximately a 40% gain in body weight in both dietary groups of rats. Hence diets did not have a detrimental effect on growth or food intake.

The decrease in the specific activities in the liver cholesterol of rats on the PUFA diet (Table III) are also in line with the reported inhibition of cholesterol synthesis by an increase in liver sterol although a part of this decrease must be accounted for by the increased liver cholesterol content initially. The results suggest a significant lowering of cholesterol specific activities in the fatty tissue, however, the very low cholesterol content of such tissues must be considered in determining the importance of this finding.

Apparently this altered partition of cholesterol between liver and blood with an increased liver content(3) is possible because a very efficient mechanism exists in rat liver which allows the rat to store cholesterol for long periods without evidence of harm to the animal(10). Thus reduced blood cholesterol levels are associated with PUFA ingestion whether cholesterol is or is not included in the diet. It remains to be established whether a reduced synthesis and perhaps preparation for entrance into the blood along with an increased metabolism of cholesterol play a part in the reduced blood cholesterol levels of rats fed PUFA and cholesterol. This effect would be supported by an apparent decrease in cholesterol formation in the intestinal tissue and possibly a decrease of its presence in the

TABLE III. Lipid and Cholesterol Specific Activities after Labeled Acetate Injection.*

Tissue	Lipid activity			Cholesterol activity		
	La†	CO	Dif.‡ %	La	CO	Dif. %
	cpm/mg lipid			cpm/mg cholesterol		
L	756 ± 52	617 ± 28	−18	73 ± 4	23 ± 3	−68
IS	5,126 ± 256	5,608 ± 236	+ 9	4,721 ± 66	4,555 ± 50	—
EF	6,173 ± 711	6,104 ± 129	—	2,520 ± 28	2,016 ± 29	−20
MF	17,643 ± 603	14,817 ± 738	−16	3,642 ± 137	2,364 ± 23	−35

* See footnotes of Table I.

fatty tissues. The quantitative significance of these findings remains to be evaluated.

Summary. Rats were fed a cholesterolemic diet which contained either 20% lard or corn oil. The blood lipid and cholesterol levels were appreciably lower in the rats fed diets containing an excess of PUFA. The livers of these rats had a higher cholesterol content but equal lipid and appreciably lower cholesterol activities than the group ingesting saturated fatty acids. Results suggest that a decreased rate of cholesterol synthesis occurs only after sufficient cholesterol has accumulated in the liver to inhibit its formation. The greater activities in the lipids of the intestinal and fatty tissue apparently were not promoting higher blood lipid levels. The activities of the cholesterol of both intestinal and fatty tissues were lower in the PUFA fed rats. Thus a decreased synthesis of cholesterol occurs in the liver as well as the intestinal tissue along with a slightly decreased content in the fatty tissues of rats fed a cholesterolemic diet containing large amounts of PUFA.

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Irradiated and Preserved Leukocytes in Mixed Leukocyte Cultures.* (32090)

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After several days' incubation, cultures of mixed leukocytes from 2 individuals contain immature mononuclear cells which probably are derived from small lymphocytes. These cells incorporate thymidine into DNA, and measurements of H^3-thymidine uptake have been used to quantitate the blastogenic reaction in mixed leukocyte cultures(1,2). The disadvantage of the mixed leukocyte reaction has been that the reaction is not "one-way". Various attempts have been made to develop a technique of unidirectional measurement. Red blood cells and platelets were ineffective (1). Frozen and thawed leukocytes have been used by some workers(3,4,5), but we and others(6-9) have failed to demonstrate consistent blastogenesis with frozen and thawed cells. We have reported that X-irradiated cells could be used for unidirectional stimulation in mixed leukocytes cultures(9). Homologous macrophages(10) and cells treated with mitomycin C(11) or nitrogen mustard(12) have been used to achieve one-way stimulation.

Techniques for low temperature storage of peripheral blood leukocytes have been published by Ashwood-Smith(13), Cavins(14), and Pegg(15). We decided to study the possible application of these techniques to the

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