use of the statistical experimental design are unquestionably more accurate than if no design had been utilized.

In the bioassay procedure described a standard method of burning (contact) and a standard laboratory animal (guinea pig) have been chosen. Additional studies will be required to indicate whether the same burn on other animals requires the same enzymes or, conversely, whether different types of burns on the same laboratory animal are as readily debrided.

Summary. A semi-quantitative bioassay has been described for determining the ability of proteolytic enzymes to debride third degree burn eschars on guinea pigs. With the aid of statistical designs, body weight and site of application were established as the 2 most important experimental variables. Trypsin, a highly purified mold enzyme, and 2 proteinase preparations from *C. histolyticum* have been compared for their ability to debride contact burns. pHisoHex was found to inhibit the 0-22% proteinases.

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Deposition of Cholesterol and Cholestanol in Experimental Rabbit Atherosclerosis.* (26542)

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When cholesterol-C^{14} (prepared biosynthetically from acetate-1-C^{14}) and cholesterol-H^3 (prepared by the Wilzbach method) were fed to rabbits(1), a striking discrepancy was observed in the counts of cholesterol samples isolated from tissues like aortae, livers or small intestines. Those isolated from cholesterol-H^3 fed animals showed a much lower radioactivity transfer than those from cholesterol-C^{14} fed rabbits. Since the C^{14} and H^3 cholesterols both appeared to be radiochemically homogeneous (having been subjected to bromination and various chromatographic procedures respectively) it was suggested that the difference in behavior between these 2 labelled cholesterols might have been the result of a peripheral oxidation of cholesterol during its stay in the body, eliminating H^3 atoms from the molecule. Shortly after these experiments were finished, a report of findings by Nystrom and Sunko(2) about the addition of tritium to the double bond of cholesterol when Wilzbach's method of tritiation(3) was used, raised the question whether cholestanol-H^3 was a contaminant in our previous cholesterol-H^3 sample, despite the purification technics we had used. Since cholestanol-H^3 might be metabolized differently from cholesterol-H^3 and might be responsible for the differences of radioactivity observed in earlier experiments, it was decided to repeat the earlier feeding experiments using cholesterol-H^3 purified in such a way as to eliminate all cholestanol-H^3 formed during tritiation. Special work showed that it is possible to eliminate completely cholestanol-H^3 by repeated bro-

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mination and debromination of cholesterol-H^3.

To test the assumption that cholestanol-H^3 present in the cholesterol-H^3 fed was the cause of the discrepancy observed in the earlier experiments, pure cholestanol-H^3 was prepared by hydrogenation of cholesterol-H^3 with non-radioactive hydrogen and fed to rabbits in a separate experiment.

Methods. Cholesterol-C^{14} was obtained from rats after injection of sodium acetate-1-C^{14} and cholesterol-H^3 was prepared by treatment of highly purified cholesterol or its acetate with tritium according to Wilzbach, stabilized by boiling with alkali in methanol and then after appropriate dilution with pure cholesterol purified completely by bromination and debromination and recrystallization from methanol-ether(4). These procedures were repeated until the isolated pure cholesterol-H^3 fractions showed the same constant count as the corresponding fractions from the bromination mother liquors. Four such brominations were required. These procedures will be described in a later report.

Experiment 1. Five male and 5 female New Zealand white rabbits weighing 3-4 lb were fed for 12 weeks 900 mg per day and per animal of commercial cholesterol (Wilson), mixed with their normal food. Two of the animals then received, for 6 days instead of the commercial material, 900 mg cholesterol-C^{14}. The other 8 were fed 900 mg pure cholesterol-H^3 for 6 days in addition to their normal rations. On the day after the last feeding of radioactive material all animals were killed and blood taken for a serum cholesterol determination. The aortae, livers and small intestines were combined for each group and cholesterol isolated as reported before(5). All animals showed gross atherosclerotic lesions and their serum cholesterol varied from 300 to 1300 mg%.

Experiment 2. Four male and 3 female New Zealand white rabbits weighing 3-4 lb were fed each for 6 weeks per day 900 mg commercial cholesterol. During the seventh week they received daily a mixture of 810 mg nonradioactive pure cholesterol and 90 mg pure cholestanol-H^3 which according to a Liebermann - Burchard determination contained 0.35% cholesterol-H^3. In these 900 mg of mixture cholestanol-H^3 made nearly 10% of the total. The count of this cholestanol-H^3 was 3.8×10^4 per min per mg while the count of the cholesterol-H^3 was only 1.44×10^2 per min per mg. The rabbits were killed after 6 daily feedings. They showed atherosclerotic lesions and their blood serum cholesterols were from 276 to 600 mg%. Aortae, livers and gastrointestinal tracts were treated as in the other series.

Results are presented in Tables I and II. Contrary to the previous results, in Experiment I (Table I) the dilution in the tissues was essentially the same for C^{14} or H^3 cholesterol, showing that both types of cholesterol, as should be expected, were deposited in the same manner. Appropriate calculation shows that about 0.06 and 0.02 mg respectively of cholesterol were laid down in the aorta per day per animal and per gram of aorta tissue, a value of the same magnitude as found in the earlier experiments (0.03 mg). It is evident therefore that the disappearance of radioactivity reported earlier(1) was not caused by a peripheral oxidation, but by the presence of a higher than cholesterol-H^3 counting companion which was deposited in the tissues only in very small amounts if at all. That the cholesterol-H^3 used in the present investigation was free from cholestanol-H^3 suggests strongly that the higher counting companion in earlier experiments was cholestanol-H^3. Experiment 2 confirms this explanation (Table II). The mixture fed here contained the bulk of radioactivity in the form of cholestanol-H^3, but only a very small part of this radioactivity appears in the cholesterol isolated from the tissues when the dilution figures are compared with the dilution in Table I. It is evident that what is laid down is mainly cholesterol but as observed earlier (6) cholestanol is definitely also deposited in the tissues. It is also clear from the dilution figures that deposition of cholesterol and cholestanol is quite different for different tissues. The lesser dilution of the fed material in the small intestine shows that considerably more of both substances is retained there than in

TABLE I. Feeding of Cholesterol-C^{14} and Cholesterol-H^3 to Rabbits.

	2 rabbits fed cholesterol-C^{14} with 3734 counts/min./mg				8 rabbits fed cholesterol-H^3 with 16408 counts/min./mg			
		Cholesterol-C^{14} isolated				Cholesterol-H^3 isolated		
	mg cholesterol/g wet tissue	Count per min./mg	Dilution*	mg cholesterol-C^{14} laid down per day/rabbit/g wet tissue	mg cholesterol/g wet tissue	Count per min./mg	Dilution*	mg cholesterol-H^3 laid down per day/rabbit/g wet tissue
Aorta	3.8	663	5.6	.056	3.6	3586	4.6	.018
Liver	3.7	1196	3.1	.01	3.3	5956	2.8	.033
Small intestine	1.5	1544	2.4	.049	2.8	7485	2.2	.031

* Dilution gives the relation between counts of cholesterol fed, divided by counts of cholesterol isolated from tissues, both counted in a scintillation counter. Cholesterol-C^{14} samples were also counted with an end window counter on which the starting material had 244 counts/min./mg. Figures obtained for the dilution in this case were 4.9, 2.8 and 2.2, identical with the figures in the cholesterol-H^3 experiment.

TABLE II. Feeding a Mixture of Cholesterol-H^3 and Cholestanol-H^3.

7 rabbits fed: Cholesterol-H^3 with 1.44×10^2 counts/min./mg
Cholestanol-H^3 with 3.8×10^4 counts/min./mg

		Sterol-H^3 isolated*				
	mg sterol isolated per g wet tissue	Counts per min./mg	Dilution total of sterol-H^3	Dilution calculated† for Cholesterol-H^3	Dilution calculated† for Cholestanol-H^3	mg sterol-H^3 laid down per day/rabbit/g wet tissue
Aorta	2.1	980	388	5	400	.014
Liver	5.4	1008	377	3	396	.039
Small intestine	2.2	3600	105	2	11	.046

* Total sterol isolated from digitonide precipitation of extracts from tissues.
† Figures for dilution of cholesterol-H^3 were taken from Table I and used to calculate dilution for cholestanol-H^3.

the aortae and livers. This phenomenon is evidently connected with the passage of these substances through the intestinal wall.

Summary. Contrary to the original findings it is established that cholesterol-C^{14} and cholesterol-H^3 are deposited in tissues of rabbits in exactly the same way. The reason for the discrepancy in deposition of the two substances in earlier experiments was found in the presence of chemically negligible traces of cholestanol-H^3 which had then not been eliminated from cholesterol-H^3 fed. Deposition is different for different tissues and that of cholestanol is only a very small fraction of that for cholesterol.

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