ence on sexual reflexes of recovery from spinal shock, the most revealing effect of withdrawal and administration of testosterone is seen in the reversal of mean number of responses for the two groups, after the eighth test, when group A was placed on testosterone and group B was taken off testosterone.

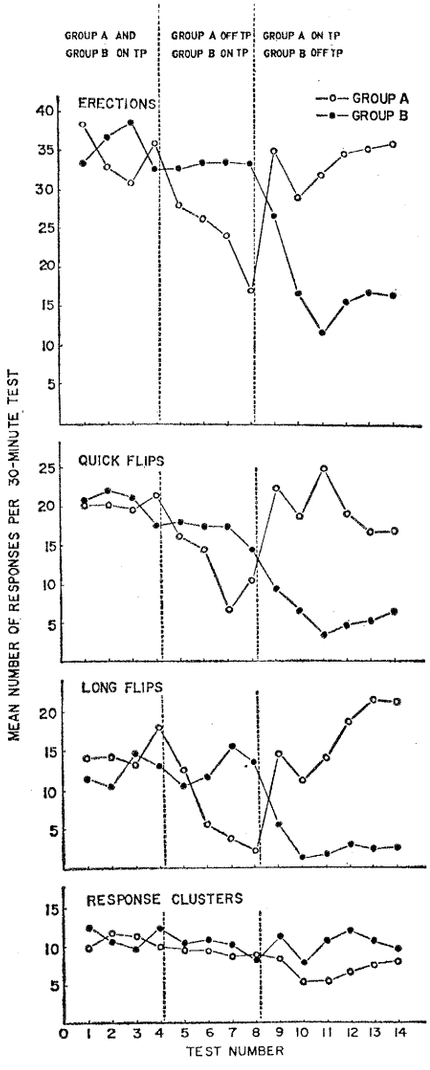

Fig. 2. Influence of withdrawal and administration of testosterone on number of erections, quick flips, long flips, and response clusters per test. Tests were conducted at 2-day intervals, and all animals were tested at 2-day intervals throughout the experiment. There were six rats with transected spinal cords in each group. When hormone withdrawal is indicated there was no injection given on the day of the last test which was conducted while the animals were on testosterone (TP). When readministration of hormone is indicated, the first injection was given 48 hours before the first test which was conducted while the animals were on testosterone. When it was withdrawn from rats in group B, the number of long flips per test fell to approximately two per test (individual range of zero to nine) on tests 10 through 14.

All animals showed the change characteristic of their respective groups, and the probability is less than .01 of this reversal occurring by chance. There was no appreciable effect on the number of response clusters per test (Fig. 2). Nor was there any detectable change in the latency to the first response cluster or in the intervals between response clusters which could be attributed to withdrawal or administration of testosterone. Thus there was a decline in the number of genital responses per cluster (and hence a decline in the duration of the cluster), but not in the timing mechanism controlling the onset of a response cluster.

It could be argued that the decline in sexual reflexes in the spinal animals following withdrawal of androgen is a reflection of a decreased sensitivity of genital sensory receptors, since it has been reported that genital papillae on the glans penis of the male rat decrease in size and number subsequent to castration (*6*). Two facts argue against this contention: (i) there is an occasional male rat which shows a complete ejaculatory pattern several months after castration (*2*); (ii) a complete mating response can be evoked in castrated male rats with hypothalamic implantation of testosterone in amounts too small to affect genital morphology (*7*).

The role that the sexual reflexes, which can be evoked from spinal rats, play in mating behavior of the intact male rat is uncertain. Assuming they have some function in copulation, it appears as though the decline of ejaculatory and, possibly, intromission behavior in the male rat following castration may be due to the influence of withdrawal of gonadal androgens on spinal neurons mediating the sexual reflexes. The more gradual decline in appetitive responses, such as investigation of the female genitalia, mounting, and pelvic thrusting, is probably a reflection of the effect of withdrawal of gonadal androgens on hypothalamic or other forebrain structures. This is suggested by studies which show an abolishment of mating behavior in male rats (which could not be attributed to impairment of gonadal androgen output) following hypothalamic lesions (*8*) and by a study by Davidson showing a resumption of mating activity in castrated male rats caused by the implantation of testosterone into the hypothalamus (*7*).

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4. Preliminary observations showed that in some male rats with transected spinal cords seminiferous tubules of the testicles undergo marked degeneration. Since there was some question whether intersitial cells (and hence androgen secretion) were also affected, all animals were castrated and administered a replacement dosage of androgen considered to be well above that required to maintain normal mating behavior of castrates. The daily dose of 0.2 mg of testosterone propionate is two to four times that estimated to be an adequate replacement dosage (*1*).
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Prevention of Induced Atherosclerosis by Peroxidase

Abstract. *Hepatocatalase peroxidase, an active peroxidase-oxidase subunit isolated from beef-liver catalase, prevents cholesterol deposition and aortic atherosclerosis in cholesterol-fed rabbits and has no apparent toxicity or undesirable side effects. No allergic or immunological reactions have been observed. The participation of this enzymatic subunit in homeostatic control mechanisms and its potential pharmacological value in the control of human atherosclerosis are suggested.*

The peroxidatic properties of catalase were recognized as early as 1936 by Keilin and Hartree (*1*). These authors postulated that "the physiological function of catalase would be mainly peroxidatic and only in exceptional cases a catalatic one" (*2*). However, in spite of the early recognition of its possible metabolic significance, little information on the peroxidatic activity of catalase has been gained over the years, owing to technical difficulties arising from the interfering action of its catalatic activity which is approximately 1000 times higher than its peroxidatic activity.

We have recently isolated a molecular subunit of beef hepatocatalase which exhibits high enzymatic activity as a peroxidase-oxidase and is essentially free of catalatic action (*3*). The hepatocatalase peroxidase subunit (HCP)

is a depolymerization product of hepatocatalase with an estimated molecular weight of 80,000 ± 12,000 and was obtained in the form of a stable, lyophilized, water-soluble powder after alkaline hydrolysis, dialysis, and lyophilization. The peroxidatic activity of the subunit was approximately 500 times higher than that of the intact catalase molecule. The availability of this preparation has made it possible to obtain basic biochemical information on the long-recognized but little-understood peroxidatic function of catalase.

The subunit HCP inhibits cholesterol biosynthesis from acetate and mevalonic acid by homogenates of rat liver (*3*). It also catalyzes aerobic oxidation of a number of hydrogen donors including reduced pyridine nucleotides, ascorbic acid, and reduced glutathione, all of which play a significant role in lipid metabolism. These oxidations are stimulated by thyroxine and phenolic estrogens (*3, 4*). In view of the well-documented hypocholesterolemic action of thyroxine and estrogens (*5*), these findings provided an interesting model for a homeostatic control mechanism of cholesterol metabolism mediated by an enzyme-hormone system, and suggested the desirability of investigating the effects of HCP in vivo.

Parenteral administration of HCP to normal rabbits and to rabbits with induced hypercholesterolemia and atherosclerosis causes a significant decrease of tissue cholesterol and of serum triglycerides, cholesterol, phospholipids, and β-lipoproteins with no evidence of toxicity or immunological reactions (*6, 7*).

We now describe the gross and histopathological evaluation of the aortas of cholesterol-fed rabbits with and without HCP treatment.

The HCP used in these studies was isolated from twice-crystallized beef-liver catalase prepared by the procedure of Tauber and Petit (*8*) and from commercial crystalline hepatocatalase (Sigma C-100). The procedure for its isolation, standard assay conditions, and definition of enzyme unit have been described (*3*). The peroxidase activity of these preparations was 40 unit/mg. Treatment with HCP was administered daily by intramuscular injection.

Twelve male white rabbits of comparable body weights were housed in individual cages and fed a cholesterol-supplemented diet (0.8 g of cholesterol added to the usual daily ration) for 15 weeks. The animals were fed a slightly decreased amount of food compared to their own norm, and all of the daily ration was therefore consumed.

Table 1. Gross and histopathological evaluation of aortic atherosclerotic lesions from four control and four HCP treated cholesterol-fed rabbits. C, Control group; T, HCP treated group; A_1, aortic arch; A_2, abdominal aorta; A_3, low abdominal aorta. Average values obtained for each section (A_1, A_2, A_3) and for total of the aortic intimas and medias from each group, standard deviation of the mean, and significance of difference of the mean (P) from treated (T) and control (C) rabbits are expressed.

Part	Fibrosis (0–3)		Degeneration of elastica (0–3)		Vessel wall (μ)		Plaques (% surface)		Fat content (0–3)		Mucopolysaccharides			
											Acid (0–3)		Neutral (0–3)	
	C	T	C	T	C	T	C	T	C	T	C	T	C	T
							Intima							
A_1	2.7 ± .43	2 ± .70	2.4 ± .69	1.9 ± .92	283 ± 103.7	226 ± 124	85 ± 25.9	77 ± 33.4			1.9 ± .78	1.6 ± .48	1.25 ± .82	1.25 ± .43
		$P < .005$		$P < .30$		$P < .40$		$P < .80$			$P < .40$		$P < 1$	
A_2	2.9 ± .33	1.4 ± .69	2.2 ± .55	1.2 ± .43	280 ± 50.9	128 ± 134.5	85 ± 16.6	17 ± 17.9			2.9 ± .33	1.6 ± 1.14	1.25 ± .43	1.25 ± .82
		$P < .001$		$P < .005$		$P < .02$		$P < .005$			$P < .02$		$P < 1$	
A_3	2.9 ± .33	1.4 ± 1.11	2.5 ± .5	1.1 ± .78	230 ± 90.3	94 ± 63.4	82 ± 8.3	30 ± 21.1			2.2 ± .83	1.6 ± .48	1.75 ± .43	0.75 ± .82
		$P < .005$		$P < .005$		$P < .005$		$P < .01$			$P < .20$		$P < .20$	
Total ($A_1 + A_2 + A_3$)														
	8.5 ± .50	4.8 ± 1.70	7.1 ± 1.05	4.2 ± 1.85	793 ± 124	448 ± 275	252 ± 22.8	125 ± 68	2.5 ± .5	1.25 ± .66	7 ± 1.65	4.8 ± 1.5	4.25 ± 1.29	3.25 ± 1.47
		$P < .001$		$P < .005$		$P < .025$		$P < .025$		$P < .005$	$P < .025$		$P < .50$	
							Media							
A_1	2 ± .5	2.1 ± .6	1.9 ± .6	1.4 ± .69	218 ± 25.5	338 ± 106.8					0.7 ± .66	1.37 ± .99	1.5 ± .66	0.5 ± .50
		$P < .80$		$P < .20$		$P < .02$					$P < .20$		$P < .05$	
A_2	2.1 ± .60	1.2 ± .66	1.6 ± .69	0.5 ± .70	241 ± 50.5	212 ± 18.4					1 ± .70	1.25 ± .82	1 ± .70	0.75 ± .43
		$P < .005$		$P < .01$		$P < .20$					$P < .60$		$P < .70$	
A_3	2 ± .5	0.9 ± .33	1.9 ± .60	1.4 ± .69	179 ± 45.5	173 ± 29.2					1.6 ± .48	1.37 ± .48	0.75 ± .48	0.25 ± .43
		$P < .001$		$P < .20$		$P < .80$					$P < .50$		$P < .25$	
Total ($A_1 + A_2 + A_3$)														
	6.1 ± .92	4.2 ± 1.39	5.4 ± .85	3.2 ± 1.78	638 ± 92.6	723 ± 125					3.3 ± .98	3.9 ± 1.24	3.25 ± .98	1.5 ± .36
		$P < .01$		$P < .01$		$P < .2$					$P < .50$		$P < .10$	

Throughout this period, six rabbits (treated group) received 80 units (2 mg per kilogram of body weight) of HCP dissolved in 1 ml of isotonic saline, and the remaining six (control group) received 2 mg of heat-inactivated peroxidase (30 minutes at 70°C) per kilogram of body weight. The rabbits were killed at the end of 15 weeks and two aortas from each group were chosen at random and removed for quantitative assay of cholesterol. The remaining eight aortas (four control and four treated) were evaluated independently by each of two pathologists (S.C.S. and R.W.), who had no information concerning the previous medication of the animal.

Each aorta was sampled in three places: arch (A_1), abdominal aorta (A_2), and low abdominal (A_3). Each specimen was analyzed grossly for accumulation of sudanophilic lipid in the intima and histopathologically for (i) proliferation of fibroelastic tissue and accumulation of mucopolysaccharides in the intima, and (ii) fraying or fragmentation of the elastica, mucopolysaccharide content, fibrosis, and thinning of the media.

These processes were separated by staining the paraffin sections differentially for collagen with the Masson trichrome method (*9*), for elastic tissue with the Verhoeff van Gieson technique (*10*), for acid mucopolysaccarides with colloidal iron (*11*), for neutral mucopolysaccharides with the periodic acid-Schiff technique (*12*), and the degree of changes were estimated.

Direct micrometric measurements were made of the thickness of the aortic intima and media. The percentage of aortic intima with plaques was estimated after the whole aortas were stained with Sudan IV, by comparing the red-stained areas in the original gross specimens and in color photographs of them. The relative degrees of histologic fibrosis, elastica degeneration, and mucopolysaccharide content were graded on a scale of 0 to 3+.

The four aortas randomly selected for quantitative cholesterol assay were homogenized in water, and the homogenates were dried to constant weight at reduced pressure. The dry powder was saponified with alcoholic KOH and extracted with petroleum ether; the ether extract was evaporated to dryness and taken up in a mixture of acetone and ethanol, and cholesterol was isolated as the digitonide and assayed colorimetrically (*13*).

Evaluation of the pathologic changes of the aortic intima and media of both control and HCP-treated cholesterol-fed rabbits is shown in Table 1. The generally accepted microscopic features of atherosclerosis, comprising proliferation of fibroelastic tissue and accumulation of lipids in the intima and fraying and fragmentation of the elastica with fibrosis and thinning in the media were significantly less pronounced in the aortas of treated rabbits.

Table 2. Toxicological studies on serum samples obtained from control and HCP treated rabbits at the end of 15-week experimental period. Average values from six control and six HCP treated rabbits and standard deviations of the mean are expressed.

Test (per 100 ml of serum)	Control	HCP-treated
SGOT (units)	34 ± 4.2	31 ± 5.3
Alkaline phosphatase (units)	9.2 ± 1.3	6.5 ± 1.2
Urea N (mg)	18 ± 3.1	15.2 ± 1.6
Creatinine (mg)	1.5 ± 0.3	1.2 ± 0.2
Glucose (mg)	99 ± 8.5	92 ± 7.8

Biochemical analyses of the cholesterol present in randomly selected aortas from two control rabbits and from two rabbits treated with HCP showed that the cholesterol content was considerably lower in the treated rabbits. Values for aortic cholesterol (mg per g of dry tissue) were: control rabbits, 75 and 64.3; HCP-treated rabbits, 23.5 and 16.5 (Fig. 1). These results agree with those from previous studies in which aortic cholesterol was significantly lower in HCP-treated rabbits (*7*).

No signs of toxicity or allergic reactions were apparent throughout the experimental period. The peroxidase subunit of catalase was well tolerated by rabbits, rats, and guinea pigs receiving as much as 1000 unit/kg daily over a 2-month period.

No significant differences have been observed when serum samples from control and experimental rabbits were analyzed for transaminase (SGOT) (*14*), alkaline phosphatase (*15*), urea nitrogen (*16*), creatinine (*17*), and glucose (*18*). All values were within normal limits (Table 2). Immunological studies, made at various stages of treatment, with the gel-diffusion autoradiographic technique (*19*) disclosed no evidence of HCP-reacting antibodies in the serums of treated rabbits.

As mentioned, previous studies had shown a significant decrease of tissue cholesterol and of triglycerides, phospholipids, cholesterol, and β-lipoproteins in the serums of rabbits treated with HCP (*6*, *7*). Our pathology studies demonstrate that HCP is also quite effective in reducing aortic atherosclerosis in cholesterol-fed rabbits.

Excessive circulating cholesterol has a natural tendency, as it penetrates the aortic wall, to initiate biochemical and later ultrastructural and histopathological alterations. The morphological end results are degeneration of the aortic elastic fibers and smooth muscle, with an increased deposition of collagen. Thickened fibrotic areas mingled with sudanophilic fat form gross, intimal plaques. The exact relations between these processes are unknown, but, in general, increased aortic acid mucopoly-

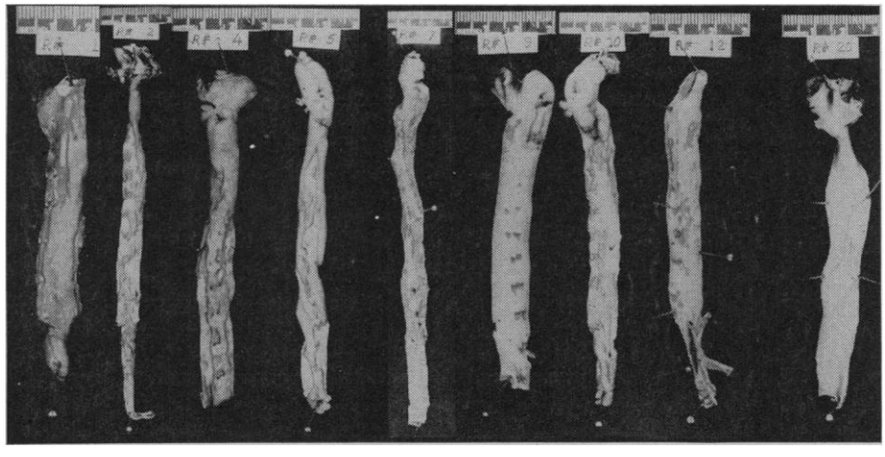

Fig. 1. Aortas from four control (Nos. 1, 2, 4, and 5) and four HCP-treated (Nos. 7, 9, 11, 12) cholesterol-fed rabbits. No. 20: Aorta from normal, untreated rabbit maintained on standard diet without added cholesterol (Sudan IV stain). Control animals show massive atheromatous lesions in contrast to moderate or minimum lesions in HCP-treated rabbits. The average serum cholesterol (mg per 100 ml of serum) when the animals were killed was 1637 ± 239 for the control group, and 398 ± 118 for the HCP-treated rabbits. Serum cholesterol in the normal, untreated rabbit (No. 20) was 41 mg per 100 ml of serum.

saccharides are observed in reactions to injury, and neutral mucopolysaccharides predominate in stabilized situations as observed in the repaired state.

Viewed in this way, the HCP treatment of cholesterol-fed rabbits protected them from aortic intimal and medial injury and its consequences to a significant degree, although under the experimental conditions of massive and prolonged cholesterol feeding it did not completely prevent cholesterol damage.

Although the biological mechanisms of this protective effect evidently involved are not yet clear, our studies, when considered with results from biochemical and toxicological studies (*6*, *7*), indicate the participation of a molecular subunit of hepatocatalase in the homeostatic control of lipid matabolism and suggest the potential pharmacological value of the isolated peroxidase subunit in the control of atherosclerosis.

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Prevention of Protein Denaturation during Exposure to Sterilization Temperatures

Abstract. *Firefly luciferase exposed to a temperature of 135°C for 36 hours retained up to 40 percent of its original activity. Prerequisites for heat stability were the use of a molecular sieve (Sephadex G-25 or Biogel P-300) and a high vacuum (5×10^{-4} mm-Hg). These studies present a possible solution to the problem of sterilization for exobiological experiments.*

The necessity of sterilizing components of planetary experiments by dry heat (135°C) presents a problem of some magnitude to experimenters concerned with systems, in which enzymes are involved, for the detection of life. The problem derives from the phenomenon of protein denaturation at elevated temperatures. Preliminary experiments which indicate a possible solution to this problem are described here.

Although denaturation of protein is still a phenomenon that has not yet been rigorously elucidated, there has emerged over the years a general concept. This denaturation involves changes in the secondary and tertiary configuration which are manifested by changes in solubility, chemical accessibility of certain functional groups, and in activity, if the protein is an enzyme. There are indications that denaturation proceeds in two stages, the first being an unfolding or swelling of peptide chains and the second consisting of a rupture of intramolecular bonds, that is, hydrogen and sulfur bonds, followed by a random re-formation of bonds both intra- and intermolecularly (*1*). This concept has led us to the hypothesis that the presence of a physical barrier between individual protein molecules may prevent random re-formation of secondary and tertiary bonding; thus, an isolated protein molecule, when exposed to stress that does not rupture primary structure (peptide linkage), would return to its original state after removal of stress. The probability of a return to the original molecular configuration is supported by the observation of Epstein *et al.* (*2*) that the most stable energy state of a protein is that corresponding to the original configuration. The validity of our hypothesis is possibly indicated by the experiments described below.

The model enzyme selected for these studies was firefly luciferase, which has a molecular weight of 100,000. Activity of this enzyme may be measured by the intensity of light emitted as the result of the addition of luciferin, adenosine triphosphate (ATP), and $MgSO_4$ (*3*).

The materials selected for their ability to provide intermolecular barriers were Sephadex G-25 and Biogel P-300. These compounds are molecular sieves that have the property of segregating individual molecules in their matrix structure. This matrix contains pores of controlled dimensions. Sephadex G-25 (Pharmacia Fine Chemicals, Inc.) is a cross-linked dextran gel with a pore size that corresponds to a protein with a molecular weight of approximately 5000; Biogel P-300 (Bio-Rad Laboratories), a polyacrylamide gel with pore size corresponding to a protein with a molecular weight of approximately 400,000. Both of these gels are chemically inert.

The experimental procedure was as follows: 2 ml of luciferase solution [containing partially purified luciferase, 1 mg of protein per milliliter; luciferin, 0.5 mg/ml; $MgSO_4$, 0.01*M*; and tris buffer (*p*H 7.4), 0.05*M*] was added to separate tubes of Sephadex G-25, Biogel P-300, dextran, bovine serum albumin, and diethylaminoethyl (DEAE) cellulose. These five mixtures were quick-frozen in liquid nitrogen and lyophilized for 24 hours. Then, in replicates of three, the following treatments were imposed: (i) storage at −80°C for 36 hours, (ii) exposure in air at 135°C for 36 hours, and (iii) exposure to 135°C for 36 hours with an initial chamber pressure of 5×10^{-4} mm-Hg which rose to 4×10^{-3} mm-Hg during heating. After these treatments samples were prepared for assay by the addition of 5 ml of distilled water. Activi-

Table 1. Retention of activity by firefly luciferase after heating.

Compound added	Retention of activity (%)		
	Unheated	Heat plus air	Heat with vacuum
Sephadex G-25	100	0	10
Biogel P-300	100	0	40
Dextran	100	0	0
Serum albumin	100	0	0
DEAE	100	0	0