THE INFLUENCE OF INTRAVENOUSLY ADMINISTERED SURFACE-ACTIVE AGENTS ON THE DEVELOPMENT OF EXPERIMENTAL ATHEROSCLEROSIS IN RABBITS*

By AARON KELLNER, M.D., JAMES W. CORRELL, M.D., AND ANTHONY T. LADD, M.D.

(*From the Department of Pathology, The New York Hospital—Cornell Medical Center, New York*)

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Marked hypercholesterolemia is regularly manifested by rabbits and certain other animals fed diets containing added cholesterol, and it is well known that such cholesterol-fed animals frequently develop atherosclerosis (1–3). Yet the mechanism whereby cholesterol feeding induces atherosclerosis remains undisclosed, and a number of facts have made it seem doubtful that hypercholesterolemia *per se* causes atherosclerosis, either natural or experimental (4, 5). The experiments now to be reported were undertaken to learn more about the relationship of elevated blood lipids, particularly the blood phospholipids, to the development of experimental atherosclerosis.

Our interest in the possible role of blood phospholipids in the pathogenesis of atherosclerosis originated from the observation that the serum cholesterol levels of cholesterol-fed rabbits with atherosclerosis were usually elevated in amount and in addition were regularly higher than were the serum phospholipid levels of the same animals (6, 7), this representing a reversal of the normal situation in the rabbit in which the serum phospholipid level is usually slightly higher than the cholesterol level (6, 8). Since the phospholipids had been shown by Boyd (9) to play an important part in maintaining the stability of the serum lipid emulsion—a fact subsequently confirmed and extended by Ahrens and Kunkel (10)—it seemed possible that the serum cholesterol in atherosclerotic animals, in addition to being markedly increased in amount, may also have been relatively unstable in view of the lack of a parallel increase in the phospholipid, and that this might be an important factor in the development of the experimental atherosclerosis. More recently we have found that significant increases in serum lipid content, and particularly notable increases in serum phospholipid levels, regularly follow the intravenous injection of two surface-active agents into rabbits on a normal cholesterol-free diet (11). We were led therefore to study in detail the effects of the intravenously administered sur-

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face-active agents on the development of atherosclerosis in cholesterol-fed rabbits and the relationship of increased blood phospholipids to the process.[1]

Materials and Methods

Market-bought rabbits of mixed breed, weighing between 2,050 and 4,150 gm., and equally divided between males and females, were used. All animals were fed a stock diet of Rockland rabbit pellets, to which powdered cholesterol or cholesterol and Tween 80[2] were added in amounts to be indicated further on. The animals fed cholesterol and those fed cholesterol together with Tween 80 had these substances added to their diets on alternate days three times a week. A weighed amount of cholesterol and a measured amount of Tween 80 were added to the pellets in individual food cups and thoroughly mixed. The same cup was used for each animal and was not emptied during the course of the experiment. On the intervening days plain pellets were added to the residue in the cup, so that practically all the cholesterol and Tween 80 administered to a particular rabbit was eventually consumed. Tween 80 was added to the oral cholesterol in some of these experiments because our experience with this substance had indicated that when fed to rabbits without added cholesterol it produced no significant change in blood lipids, but when given with cholesterol it enhanced the absorption of the cholesterol and resulted in more rapid and more marked elevation of blood cholesterol levels and the more certain development of atherosclerosis (12).

The surface-active agents injected intravenously were Tween 80 and Triton A20.[3] Solutions of these agents for intravenous use were prepared as previously described (11).

The rabbits were bled just prior to the start of each experiment and at periodic intervals thereafter, and the levels of blood lipids were determined by procedures described previously (11).

It was felt necessary to follow the weights of the animals very carefully because of the observations of Firstbrook that a decrease in body weight may interfere with the development of atherosclerosis in cholesterol-fed rabbits (13). Hence the animals were weighed at the start of each experiment and at intervals thereafter.

Complete postmortem examinations were performed on all animals. The heart and entire aorta were removed *en bloc*, and after fixation in 4 per cent formaldehyde, were stained with Sudan IV to accentuate the atherosclerotic areas, which regularly took on an orange-red color. The degree of atherosclerosis of the aorta visible in the gross was estimated on an arbitrary scale from 0 to ++++, where 0 represents absence of visible atherosclerosis; + represents minimal, just visible atherosclerotic streaks or plaques; ++++ represents extensive covering of most of the intimal surface of the aorta with numerous, coalescent, raised plaques; and grades ++ and +++ are intermediate between the latter two. Sections for microscopic study were taken from selected sites in those instances in which it was felt necessary to confirm the presence or absence of minimal atherosclerosis, and, in addition, from representative lesions of all grades of severity. The minimal lesions consisted for the most part of slightly raised intimal plaques containing clusters of foam cells; these plaques increased progressivey in thickness and extent in the more advanced lesions, and in those graded as ++++, were almost as thick as the media. In the latter lesions, too, the outlines of the foam cells frequently could not be seen, the cells appearing to be degenerated, and in a few such cases early hyalinization and calcification of the intima were evident.

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[1] For preliminary note see *Fed. Proc.*, 1949, **8**, 360.

[2] Polyoxyalkylene sorbitan monooleate, manufactured by the Atlas Powder Co., Wilmington.

[3] Arylalkylpolyether of phenol, manufactured by Rohm and Haas, Inc., Philadelphia.

The Effects on Cholesterol-Fed Rabbits of Repeated Intravenous Injections of Tween 80

An experiment was first done to learn the effects of intravenously administered Tween 80 on the blood cholesterol and phospholipid levels and on the development of atherosclerosis in cholesterol-fed rabbits.

Three groups of animals were included in this experiment. Group 1 consisted of seven control rabbits that were fed the normal diet to which 0.5 gm. of cholesterol was added three times weekly. Group 2 consisted of five rabbits that were maintained on a normal diet, without added cholesterol, and were given twice daily intravenous injections of 7.5 cc. of 20 per cent Tween 80. Group 3 consisted of six rabbits that were fed 0.5 gm. of cholesterol three times weekly, and were in addition given intravenous injections of Tween 80 in the same amounts as the animals of group 2. Injections were given daily at about 9:00 a.m. and again at about 4:30 p.m., except on Saturdays, when the afternoon injection was omitted, and on Sundays, when no injections were given. The duration of the experiment was 13 weeks. Samples of blood for lipid determination were taken every 3 weeks, on Monday mornings prior to the injection of Tween 80.

It will be noted that the rabbits of group 1 (cholesterol-fed but not injected with Tween 80—see Table I) manifested only very slight elevations of the serum cholesterol and serum phospholipid levels 3 weeks after the cholesterol feeding had been started. After 6 weeks there were moderate elevations of these levels in all animals in this group and the hyperlipemia persisted thereafter, it being noteworthy that the level of blood cholesterol eventually exceeded that of the phospholipid in every animal. Five of the seven animals had atherosclerosis when they were examined postmortem at the end of the experiment, this being slight in three instances and moderate in two.

The rabbits of group 2 (given Tween 80 intravenously but not fed cholesterol) manifested a marked hyperlipemia which differed from that of group 1 in several respects: it appeared more promptly, being quite marked 3 weeks after the experiment had been started; the lipid levels were considerably higher, the amounts of cholesterol and phospholipid in the serum of the animals in this group in almost every case greatly exceeding those present in the rabbits of group 1; furthermore, the hyperlipemia differed qualitatively in that the phospholipid was regularly greater in amount than was the cholesterol in the serum of these animals. None of the rabbits in group 2 exhibited atherosclerosis of the aorta when examined at the end of the experiment.

The rabbits of group 3 (cholesterol-fed and given Tween 80 intravenously) all manifested a hyperlipemia which again was marked after 3 weeks and remained so thereafter, the levels of cholesterol and of phospholipid being appreciably higher in nearly every animal than was observed in the rabbits of groups 1 and 2. It is noteworthy that the phospholipid levels were regularly higher than were the cholesterol levels, as was the case in the rabbits of group 2. The tendency of the blood lipid levels to decrease after the 9th week, present in all the animals of group 3 and in some of the animals of group 1, has been observed previously in the hyperlipemia that follows cholesterol feeding in rabbits and has been attributed to impairment of absorption of cholesterol due to extensive infiltration of lipids into the mucosa of the intestine (14). Only one of the four rabbits in group 3 that survived to the end of the experiment had atherosclerosis of the aorta, and that was very slight.

It is apparent from the findings of this experiment that little or no atherosclerosis developed in the Tween 80–injected rabbits of group 2 and in the cholesterol-fed and Tween 80–injected rabbits of group 3 though these animals

had more marked and more sustained hypercholesterolemia than did the cholesterol-fed control rabbits of group 1 (Table I). It is noteworthy that the amount of phospholipid in the blood of these Tween 80–injected animals regu-

TABLE I

Effect of Intravenous Tween 80 on Serum Lipids and on the Development of Atherosclerosis in Rabbits Fed High Cholesterol and Normal Diets

Experimental group	Rabbit No.	Weight		Level of serum lipids							Atherosclerosis of aorta‡
					Time, *wks.*					Mean*	
		Initial	Final		0	3	6	9	12		
		kg.	*kg.*								
1. Control rabbits given cholesterol diet, and no Tween 80 intravenously	1	2.05	3.75	Chol	47	35	138	119	129	**94**	0
				PL	77	55	100	93	97	**84**	
	2	2.85	3.78	Chol	47	50	116	123	284	**124**	0
				PL	103	53	127	97	267	**129**	
	3	4.15	4.80	Chol	28	88	216	260	294	**177**	+
				PL	40	60	107	100	163	**94**	
	4	3.88	4.48	Chol	100	110	247	423	530	**282**	+
				PL	143	93	153	200	337	**185**	
	5	2.90	3.38	Chol	48	65	265	250	212	**168**	+
				PL	83	88	148	120	123	**112**	
	6	2.83	3.48	Chol	47	135	223	284	138	**165**	++
				PL	65	88	123	158	103	**107**	
	7	2.30	4.23	Chol	68	123	341	329	239	**220**	++
				PL	95	108	145	133	138	**124**	
2. Experimental rabbits given normal diet, and Tween 80 intravenously twice daily	8	3.75	3.95	Chol	47	229	654	463	550	**389**	0
				PL	97	355	720	703	850	**545**	
	9	2.85	4.33	Chol	60	169	229	375	232	**213**	0
				PL	100	255	400	647	470	**374**	
	10	2.78	3.33	Chol	42	275	678	113	530	**328**	0
				PL	73	435	750	265	650	**435**	
	11	2.70	3.48	Chol	37	297	429	445	688	**379**	0
				PL	77	467	445	565	940	**499**	
	12	2.80	4.10	Chol	65	241	281	290	263	**228**	0
				PL	97	323	415	305	440	**316**	

TABLE I—*Concluded*

Experimental group	Rabbit No.	Weight			Level of serum lipids						Atherosclerosis of aorta‡
					Time, *wks.*					Mean*	
		Initial	Final		0	3	6	9	12		
		kg.	*kg.*								
3. Experimental rabbits given cholesterol diet and Tween 80 intravenously twice daily	13	2.73	3.20	Chol	39	582	731	957	675	**597**	0
				PL	83	445	818	1105	720	**634**	
	14	2.85	3.38	Chol	37	319	485	450	320	**322**	0
				PL	53	320	625	465	390	**371**	
	15	3.0	3.50	Chol	63	338	566	557	507	**406**	0
				PL	70	493	473	665	570	**454**	
	16	2.78	3.45	Chol	47	342	948	1080	645	**612**	+
				PL	95	335	1235	1240	960	**773**	
	17	3.23	3.33	Chol	43	285	488	435	†		
				PL	68	450	458	488			
	18	3.1	3.1	Chol	44	313	†				
				PL	85	400					

Group 1. Cholesterol 0.5 gm. 3 times weekly for 13 weeks.
Group 2. 7.5 cc. of 20 per cent Tween 80 intravenously twice daily for 13 weeks.
Group 3. Cholesterol 0.5 gm. 3 times weekly
7.5 cc. of 20 per cent Tween 80 intravenously twice daily } for 13 weeks.
0 = just prior to beginning of experiment.
Chol = total serum cholesterol, mg./100 cc.
PL = phospholipid expressed as lecithin, mg./100 cc.
† = died.
* The figures for mean cholesterol and mean phospholipid represent the arithmetical average of the respective lipid levels determined for each animal during the course of the experiment, including those obtained just prior to the start of the experiment. They are given here and in the succeeding tables primarily to facilitate comparison with the data of other workers.
‡ As determined on postmortem examination at the end of the 13th week.

larly exceeded that of the cholesterol. Consideration will be given further on to the implications of these observations. These findings have recently been confirmed by Payne and Duff, as indicated in a preliminary report (15).

Blood Lipid Levels and Atherosclerosis of the Aorta of Cholesterol-Fed Rabbits Injected Intravenously with Triton A20

In studies reported in an accompanying paper (11) it was shown that Triton A20 is more effective than Tween 80 in inducing hyperlipemia when given

intravenously to rabbits on a cholesterol-free diet, and that this surface-active agent gives rise to increases in blood phospholipid and blood cholesterol content that are greater and more enduring than are those resulting from Tween 80. It therefore seemed probable that more definitive observations might be made on the relationship between hyperlipemia and atherosclerosis in animals given this agent. Furthermore, since the cholesterol-fed control animals in the preceding experiment exhibited only slight to moderate degrees of atherosclerosis it was thought desirable in the experiment now to be described to attempt to accentuate the development of atherosclerosis by the addition of Tween 80 to the diet of all animals fed cholesterol, as had been done in previous work (12).

For the purposes of this experiment forty-one rabbits were divided into four groups, numbered 4, 5, 6, and 7. Group 4 consisted of eleven control animals that were fed the stock diet to which 1.0 gm. of cholesterol and 10 cc. of Tween 80 were added three times a week; these animals received no Triton A20 intravenously. Group 5 consisted of eleven rabbits that were maintained on the stock diet without added cholesterol or Tween 80, and that received twice weekly intravenous injections of 4 cc. of 12.5 per cent Triton A20. There were eleven rabbits in group 6 and eight in group 7; each animal received twice weekly intravenous injections of Triton A20 in the same dose given to group 5. In addition, the animals in group 6 had 0.5 gm. of cholesterol and 4 cc. of Tween 80, and those in group 7 had 1.0 gm. of cholesterol and 10 cc. of Tween 80, added to their stock diet three times a week. The duration of the feeding plus injection was 9 weeks in each case.

The observations on blood lipid levels and aortic atherosclerosis in these four groups of animals are summarized in Tables II, III, IV, and V. The control rabbits of group 4 (fed cholesterol and Tween 80 but not injected with Triton A20—see Table II) had marked elevations of blood cholesterol and phospholipid 2 weeks after the start of the experiment, and these persisted for the duration of the experiment. It is of considerable interest that in each animal the level of blood cholesterol exceeded that of the blood phospholipid at every determination following the initial one taken at the beginning of the experiment. When examined postmortem, every animal in this group had atherosclerosis of the aorta, and in five of them the atherosclerosis was marked.

The rabbits of group 5 (given Triton A20 intravenously but not fed cholesterol—see Table III) manifested sustained elevations of blood cholesterol and phospholipid levels throughout the course of the experiment, and these elevations were in general of the same order of magnitude as were those of the animals of group 4, with the important difference that in the rabbits of group 5 the levels of blood phospholipid in most instances exceeded that of the blood cholesterol. Six of the animals in this group had no atherosclerosis of the aorta at the end of the experiment, four had very slight lesions, and only one had extensive lesions. The aortic lesions in these animals were similar in distribution and morphology to those observed in the cholesterol-fed rabbits of group 4.

The rabbits of groups 6 and 7 (fed cholesterol admixed with Tween 80 and also given Triton A20 intravenously—see Tables IV and V) had sustained elevations of the blood cholesterol level that in general were slightly greater than those observed in the control animals of group 4. It is of interest that the blood phospholipid levels of these animals, while consistently elevated, were nevertheless in almost every instance somewhat lower than the corresponding cholesterol levels, though the disparity between cholesterol and phospholipid in these two groups of animals was considerably less than that noted in the case of the controls (Table II). Seven of the animals in these two groups had no atherosclerosis of the aorta at the end of the experiment and twelve animals had aortic lesions that were slight to moderate in severity

TABLE II

*Group 4. Serum Cholesterol and Phospholipid Levels and Atherosclerosis of the Aorta of Rabbits Fed a High Cholesterol Diet**

Rabbit No.	Weight		Level of serum lipids							Atherosclerosis of aorta‡
				Time, *wks.*					Mean	
	Initial	Final		0	2	4	6	8		
	kg.	*kg.*								
1	2.89	3.65	Chol	73	231	413	361	412	**298**	+
			PL	85	135	170	200	223	**163**	
2	3.90	3.78	Chol	50	311	1280	382	297	**464**	+
			PL	78	183	588	173	195	**243**	
3	2.83	3.43	Chol	134	775	883	597	412	**560**	+
			PL	108	353	328	348	243	**276**	
4	2.65	3.28	Chol	43	153	386	289	143	**203**	++
			PL	90	105	195	175	90	**131**	
5	2.48	3.13	Chol	59	398	487	760	341	**409**	++
			PL	103	175	303	463	240	**257**	
6	3.18	4.0	Chol	66	632	1058	1382	974	**822**	++
			PL	103	463	620	710	495	**478**	
7	2.18	3.50	Chol	66	426	873	1230	826	**684**	+++
			PL	98	243	585	833	508	**453**	
8	2.55	2.63	Chol	30	631	1295	1132	1079	**833**	+++
			PL	58	473	815	848	765	**592**	
9	2.68	3.0	Chol	37	329	545	774	339	**405**	++++
			PL	75	225	300	555	200	**271**	
10	2.50	2.98	Chol	84	469	719	822	539	**539**	++++
			PL	88	260	468	490	305	**322**	
11	3.10	3.08	Chol	48	637	1062	1067	849	**733**	++++
			PL	80	453	710	715	493	**490**	

0 = just prior to beginning of experiment.

Chol = total serum cholesterol, mg./100 cc.

PL = phospholipid expressed as lecithin, mg./100 cc.

* Cholesterol 1.0 gm. / Tween 80 10 cc. } 3 times weekly for 9 weeks.

‡ As determined postmortem at the end of the 9th week of the experiment. The same is true in the animals of Tables III, IV, and V to follow.

It is noteworthy that the Triton-injected animals with only a few exceptions gained weight during the course of the experiment, the observation serving to rule out weight loss as a possible cause for the failure of these animals to develop as much atherosclerosis as did the controls.

TABLE III

*Group 5. Serum Cholesterol and Phospholipid Levels and Atherosclerosis of the Aorta of Rabbits Fed a Normal Diet and Given Repeated Intravenous Injections of Triton A20**

Rabbit No.	Weight		Level of serum lipids						Atherosclerosis of aorta
				Time, *wks.*				Mean	
	Initial	Final		0	3	6	9		
	kg.	*kg.*							
1	2.70	2.88	Chol	40	293	351	574	**315**	0
			PL	53	323	353	668	**349**	
2	2.50	2.55	Chol	44	487	463	756	**438**	0
			PL	53	628	480	880	**510**	
3	2.43	2.63	Chol	68	379	556	825	**457**	0
			PL	70	438	700	1050	**564**	
4	2.83	3.18	Chol	105	370	567	715	**439**	0
			PL	55	393	488	748	**421**	
5	3.18	3.53	Chol	76	638	845	1090	**662**	0
			PL	75	475	765	1370	**671**	
6	3.13	2.68	Chol	122	1008	1640	2550	**1330**	0
			PL	83	1030	2410	2340	**1466**	
7	3.20	3.35	Chol	106	242	540	560	**362**	+
			PL	110	240	555	573	**370**	
8	2.68	2.88	Chol	116	262	910	577	**466**	+
			PL	105	270	868	625	**467**	
9	2.40	2.33	Chol	74	516	770	842	**551**	+
			PL	70	698	850	1130	**687**	
10	2.40	2.65	Chol	34	710	890	1025	**665**	+
			PL	43	598	1000	1330	**743**	
11	2.80	2.88	Chol	21	251	582	585	**360**	+++
			PL	43	245	478	513	**320**	

* 4 cc. of 12.5 per cent Triton A20 intravenously twice weekly for 9 weeks.

The findings of the experiment as a whole make it clear that the rabbits given Triton A20 intravenously, whether maintained on a normal diet (Table III)

TABLE IV

Group 6. Serum Cholesterol and Phospholipid Levels and Atherosclerosis of the Aorta of Rabbits Fed a High Cholesterol Diet and Given Repeated Intravenous Injections of Triton A20‡*

Rabbit No.	Weight		Level of serum lipids						Atherosclerosis of aorta
				Time, *wks.*				Mean	
	Initial	Final		0	3	6	9		
	kg.	*kg.*							
1	2.53	3.03	Chol	81	199	400	475	**289**	0
			PL	85	158	240	443	**232**	
2	2.70	2.63	Chol	42	493	610	1240	**599**	0
			PL	48	463	608	1143	**566**	
3	2.35	2.48	Chol	35	578	1040	1560	**804**	0
			PL	43	528	1050	1540	**790**	
4	3.00	2.88	Chol	78	1020	1670	1882	**1160**	0
			PL	60	930	1240	1560	**948**	
5	3.20	3.65	Chol	35	228	332	585	**295**	+
			PL	40	203	295	565	**275**	
6	2.56	2.88	Chol	60	236	570	415	**320**	+
			PL	83	218	365	425	**273**	
7	2.48	3.00	Chol	43	550	458	715	**442**	+
			PL	63	318	313	703	**349**	
8	2.93	3.05	Chol	36	254	575	617	**371**	++
			PL	60	250	395	460	**291**	
9	2.45	2.65	Chol	30	660	994	1180	**716**	++
			PL	48	548	885	1040	**630**	
10	2.53	2.73	Chol	55	755	1220	1170	**800**	++
			PL	65	645	1013	1260	**745**	
11	3.03	3.16	Chol	77	781	1010	1880	**937**	++
			PL	98	663	728	1520	**752**	

* Cholesterol 0.5 gm. / Tween 80 4 cc. } 3 times weekly for 9 weeks.

‡ 4 cc. of 12.5 per cent Triton A20 intravenously twice weekly for 9 weeks.

or on a diet containing added cholesterol (Tables IV and V), developed less atherosclerosis of the aorta than did the control animals that were fed cholesterol but were not given Triton A20 intravenously (Table II). It is noteworthy that this decrease in the incidence and severity of atherosclerosis in the Triton-

injected rabbits occurred despite the fact that these animsls had blood cholesterol levels that in general were as high as, and in many instances were far higher than, those of the controls. Furthermore, the lipemia manifested by the Triton-injected animals differed strikingly from that of the cholesterol-fed con-

TABLE V

Group 7. Serum Cholesterol and Phospholipid Levels and Atherosclerosis of the Aorta of Rabbits Fed a High Cholesterol Diet and Given Repeated Intravenous Injections of Triton A20‡*

Rabbit No.	Weight		Level of serum lipids						Atherosclerosis of aorta
				Time, *wks.*				Mean	
	Initial	Final		0	3	6	9		
	kg.	*kg.*							
1	2.88	3.23	Chol	78	495	430	650	**413**	0
			PL	65	373	365	495	**325**	
2	2.98	3.20	Chol	78	1010	794	792	**669**	0
			PL	95	913	785	758	**663**	
3	2.93	2.91	Chol	119	1295	1040	913	**842**	0
			PL	113	1058	1043	1215	**857**	
4	2.43	3.00	Chol	76	657	623	467	**456**	+
			PL	80	435	450	558	**381**	
5	3.14	3.55	Chol	103	929	585	500	**529**	+
			PL	108	678	520	453	**440**	
6	3.00	3.28	Chol	125	1355	962	682	**781**	+
			PL	158	840	668	495	**525**	
7	2.65	3.18	Chol	81	2025	1390	1158	**1163**	++
			PL	80	1445	1023	830	**845**	
8	2.35	3.21	Chol	65	313	373	382	**283**	++
			PL	70	278	330	263	**235**	

* Cholesterol 1.0 gm. / Tween 80 10 cc. } 3 times weekly for 9 weeks.

‡ 4 cc. of 12.5 per cent Triton A20 intravenously twice weekly for 9 weeks.

trols; for in the former (Tables III, IV, and V) the level of the blood phospholipids was usually elevated to about the same degree as was that of the blood cholesterol, whereas in the latter (Table II) there was regularly a marked disparity between the levels of the blood phospholipids and the blood cholesterol, these cholesterol-fed control animals in every instance having a high cholesterol

level and a relatively low phospholipid level. It is evident from this experiment that the level of the blood cholesterol was not the only factor involved in the pathogenesis of the atherosclerosis, and the data indicate further that the level of the blood phospholipids, particularly the relationship between the blood cholesterol and phospholipids, may be an important additional factor involved in this process.

The atherosclerosis manifested in the Triton-injected rabbits of group 5 (Table III) is of particular interest because hitherto this lesion has been produced experimentally in rabbits only by the addition of cholesterol to the diet or by the intravenous injection of cholesterol emulsions (16). The lesions observed in this experiment represent the first production of atherosclerosis in the rabbit without the introduction of exogenous cholesterol into the animal

Attempts to Accelerate the Regression of Experimental Atherosclerosis by Means of Intravenously Injected Tween 80

Spontaneous regression of experimentally produced atherosclerosis following the cessation of cholesterol feeding has been demonstrated in rabbits, chickens, and dogs (1, 17, 18); the process in the rabbit, however, is a very slow one and requires many months for appreciable changes to take place. In view of the mobilization of lipids brought about by the intravenous administration of Tween 80, the following experiment was designed to see whether the injection

TABLE VI

Attempts to Accelerate Resorption of Atherosclerosis in Rabbits by Means of Repeated Intravenous Injections of Tween 80

Group 8		Group 9		Group 10	
Rabbit No.	Atherosclerosis of aorta	Rabbit No.	Atherosclerosis of aorta	Rabbit No.	Atherosclerosis of aorta
1	+++	8	++++	16	++++
2	++	9	++++	17	++++
3	++	10	++	18	+++
4	++	11	+++	19	+
5	++	12	++++	20	+++
6	++++	13	+++	21	++
7	++	14	++	22	++
		15	+++	23	+++

Group 8. Cholesterol 1.0 gm. and Tween 80 10 cc. 3 times weekly for 8 weeks.

Group 9. Cholesterol 1.0 gm. and Tween 80 10 cc. 3 times weekly for 8 weeks. Normal diet for 8 weeks.

Group 10. Cholesterol 1.0 gm. and Tween 80 10 cc. 3 times weekly for 8 weeks. 7.5 cc. of 20 per cent Tween 80 intravenously twice daily for 8 weeks.

of this agent would accelerate the regression of previously induced cholesterol atherosclerosis in the rabbit.

Twenty-three rabbits were fed 1.0 gm. of cholesterol and 10 cc. of Tween 80 three times a week for 8 weeks. At this time seven rabbits were killed and the degree of atherosclerosis of the aorta determined (group 8). The remaining sixteen animals were maintained for 8 more weeks on the stock diet without added cholesterol or Tween 80 (groups 9 and 10), eight of these sixteen animals receiving in addition twice daily intravenous injections of 7.5 cc. of 20 per cent Tween 80 (group 10). At the end of the second 8 week period the animals were sacrificed and the degree of atherosclerosis of the aorta noted.

The findings of this experiment are recorded in Table VI. It is evident that there was no significant difference in the incidence and severity of atherosclerosis in the animals which received intravenous Tween 80 for 8 weeks as compared with those of the control groups which did not. It was therefore concluded that under the conditions of the experiment intravenously administered Tween 80 did not accelerate resorption of previously existing atherosclerosis in rabbits. These results are not unlike those of Duff (5) who has reported that alloxan diabetes retards the development of atherosclerosis in cholesterol-fed rabbits but does not accelerate resorption of the lesions.

DISCUSSION

In the experiments here described the incidence and severity of atherosclerosis were decreased in cholesterol-fed rabbits given repeated intravenous injections of the surface-active agents Tween 80 and Triton A20, as compared with those found in control rabbits fed the same cholesterol diet. It is noteworthy that atherosclerosis often failed to develop in the animals given the surface-active agents intravenously even when they exhibited a marked and sustained hypercholesterolemia. Whether the blood phospholipids, which were also greatly increased in the injected animals, were responsible for the decreased incidence of atherosclerosis, or whether this was due to the presence of the injected surface-active agents in the circulating fluids, or to other factors, cannot now be definitely stated. It seems probable that the surface-active agents were present in the blood of the injected animals throughout the course of the experiment, and the effect of these agents in stabilizing lipid emulsions is well known (19). On the other hand, it has been shown both *in vivo* and *in vitro* that elevated phospholipids exert an important stabilizing influence on the lipid emulsion in hyperlipemic blood (9, 10), and it is therefore conceivable that the elevation of phospholipid in the Tween 80- and Triton A20–injected animals played a role in the decreased incidence of atherosclerosis. This possibility is further strengthened by the observation that cholesterol-fed rabbits with atherosclerosis were found consistently to have relatively low blood phospholipid levels as compared to their cholesterol levels. A similar disparity between blood phospholipid and cholesterol has been reported in chickens in

which atherosclerosis had been produced by cholesterol feeding (20), and more recently, in human beings with clinical evidence of atherosclerosis (21), the findings suggesting that the decrease in blood phospholipid with respect to cholesterol, by altering the colloidal stability of the lipid emulsion, may be involved in the pathogenesis of experimental and naturally occurring atherosclerosis. It would thus seem desirable to study directly the role of phospholipids in the pathogenesis of atherosclerosis by selectively raising the blood phospholipid level in cholesterol-fed animals. But this is not feasible at present, for the addition of phospholipids or of phospholipid precursors such as choline and inositol to the diet of normal or cholesterol-fed rabbits has no appreciable effect on blood phospholipid levels (22), while the intravenous administration of phospholipid emulsions gives rise to only slight and transitory elevations of the blood phospholipid level (22, 23), and no procedure has yet been devised whereby sustained elevations of blood phospholipids can be produced without concomitant increases in blood cholesterol.

The fact has considerable interest that atherosclerosis developed in some of the animals on normal diets that were given repeated intravenous injections of Triton A20 (Table III). For it represents the production in the rabbit while on a cholesterol-free diet of atherosclerosis morphologically indistinguishable from that produced by cholesterol feeding, a phenomenon that has not been reported heretofore. Furthermore, the observation shows that the effect of intravenously injected Tween 80 and Triton A20 in hindering the development of atherosclerosis is not an absolute one, and it indicates that there may be other factors involved in the pathogenesis of atherosclerosis in addition to the levels of blood cholesterol and phospholipid. In this connection it should be noted that phospholipids are probably not the only physiological factors that influence the solubility of lipids and the stability of lipid emulsions in body fluids. For Cohn and his coworkers have demonstrated that in human plasma almost all the lipid is in combination with an alpha- and a beta-globulin, some 25 per cent as an alpha-lipoprotein and about 75 per cent as a beta-lipoprotein (24), and furthermore, it has been shown that this beta-lipoprotein is, under certain conditions, soluble in aqueous media despite the fact that it is composed largely of lipid (25). These lipoproteins probably play an important role in the solubility and colloidal stability of the blood lipids, and the relationship of these lipid-protein complexes to the development of atherosclerosis deserves further investigation.

SUMMARY

A study was made of the relationship of blood lipids to the development of experimental atherosclerosis.

Rabbits fed a diet containing cholesterol were found to develop hyperlipemia characterized by a great increase in blood cholesterol and a much lesser increase

in blood phospholipids; after several weeks they manifested conspicuous atherosclerosis of the aorta, as has often been observed by others. Comparable rabbits fed the same diets containing added cholesterol were given in addition repeated intravenous injections of the surface-active agents Tween 80 and Triton A20; these animals developed hyperlipemia which was characterized by a great increase in blood cholesterol and an equivalent or even greater increase in phospholipids, and they had much less atherosclerosis than did the control rabbits fed cholesterol alone.

In further experiments it was observed that repeated intravenous injections of Tween 80 did not result in resorption of previously induced atherosclerosis in rabbits.

The findings are discussed in relation to the pathogenesis of natural and experimental atherosclerosis.

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