nective tissue directly exposed to it, and the result was a cyst containing thin fluid with keratin suspended in it. The cyst had walls that were bare except for a papillomatous mound projecting into its interior, or a cauliflower, sometimes low and broad-based but often pedunculated and not infrequently almost entirely filling the cyst cavity. All gradations between A and C tumors occurred, and their cells did not differ significantly in aspect. Those of both growths underwent carcinomatous change about equally often. As a rule the A papillomas extended more rapidly on the walls of the enlarging cysts than did the cancers arising from them, and these latter could be left behind by a judicious selection of grafts at the next transfer. The C papillomas on the other hand failed to outstrip the cancers and hence were soon supplanted by them.

Dr. James S. Henderson of this laboratory has generously permitted a report here on the character of 12 epidermal papillomas he has recently propagated. Four were induced by tarring the backs of C mice and 8 by the repeated application of 20-methylcholanthrene in benzene or Crabtree's medium(3). All proved on transplantation to be of the kind just described and those of the A and C types were equally frequent.

Accident, infection of the grafts, or some host influence may have determined the failure of the papillomas that gave rise to no tumors after transfer. The many that succeeded have yielded growths that enlarged at much the same moderate pace,—a surprising fact in view of the activity that some papillomas manifest when situated on the skin and the indolence of others. Evidently local cutaneous conditions have much to do with this difference.

Conclusions. The transplantation of numerous epidermal papillomas induced by tar or 20-methylcholanthrene has shown them to be due to neoplastic changes of a single kind. Their wide diversity of form and behavior, when situated on the skin, seems referable in the main to differing capabilities of their cells. The great majority of them, indeed perhaps all, are transplantable in series, when favoring sites are provided them.

1. Rous, P., Allen, R. A., *J. Exp. Med.*, 1958, v107, 63.

2. Mider, G. B., Morton, J. J., *Am. J. Path.*, 1939, v15, 299.

3. Crabtree, H. G., *J. Path. and Bact.*, 1940, v51, 299.

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Experiments on Cholesterol Atherosclerosis in Rabbits.* (25136)

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Duff(1) suggested that atherosclerotic lesions may be due to impurities when rabbits are fed cholesterol over a long period. Recently Altschul showed that heated cholesterol (2) or dried and heated egg yolk(3) were more effective than cholesterol itself in obtaining atherosclerotic damage in rabbits, guinea pigs or hamsters, suggesting that companions of cholesterol or products formed during heating were responsible. Nevertheless, most investigations on cholesterol atherosclerosis were made with commercially available, so-called pure cholesterol† which contained small amounts of impurities; (cf. 4). Experi-

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† Anitschkow and Chalatow(5) and Wacker and Hueck(6) reported feeding "pure" cholesterol ("reines Cholesterin") to rabbits. Study of original papers suggests that they really used commercial cholesterol. Their work, therefore, is not as conclusive as appears from Kritchevsky's book(7).

ments therefore seemed advisable with carefully purified cholesterol and with impurities contained in commercial, so-called pure cholesterol. Usual methods do not completely purify cholesterol. Only conversion to 5,6-dibromocholestanol-3 and subsequent debromination gave pure material(8). Later, however, L. L. Smith(9) found that even chromatographically pure 5,6-dibromocholestanol-3 gave partially autoxidized cholesterol. In the present work these difficulties have been overcome.

Methods. Preparation of pure cholesterol. From 250-300 g batches of commercial cholesterol (Wilson) the oxalic acid complex was prepared in ethylacetate(10) and after its decomposition the cholesterol was dried in air. Every day 15 g of this cholesterol were brominated for final purification according to Fieser(11), but the appropriately washed last ether solution was diluted with about two-thirds its volume of 95% ethanol and the solvents distilled off *in vacuo*. When cholesterol started to crystallize the flask was cooled in ice. The crystals were filtered with suction and washed with ice-cold ethanol. As soon as most of the solvent was sucked off, a rubber dam was applied and suction continued for one-half hour. Ten to 12 g pure cholesterol were obtained, with about 7-8% moisture (ethanol). This figure was used to calculate amount for feeding. When 1.5 g of a C^{14}-cholesterol of 1300 c/min/mg were treated in exactly the same way a reverse phase chromatogram on kerosinized paper(12) showed only one peak of radioactivity. A similarly treated sample gave only one single peak on a silica gel column. *Isolation of impurities from commercial cholesterol.* 200 lb of commercial cholesterol (Wilson) were purified through the courtesy of Schering Corp., Bloomfield, N. J., by treatment with anhydrous oxalic acid in hexane. The mother liquors were distilled leaving impurities as a brown residue (about 2% of starting material). Three hundred g of this were treated in ethylacetate with 60 g anhydrous oxalic acid, the complex discarded and the mother liquor boiled in a large beaker with water until ethylacetate was eliminated. The air-dry residue contained 14% cholesterol-like substances, determined as digitonides. It was used together with commercial cholesterol to feed Group IV rabbits. For Group III rabbits, which received impurities alone, the insoluble residue was dissolved in 95% ethanol and precipitated with digitonin, the mother liquor evaporated *in vacuo* and last traces of water eliminated azeotropically with benzene. The dry residue was taken up in pentane, filtered from the insoluble and evaporated to dryness. The soft air-dried residue still contained 7.8% digitonin precipitable material. Two to 3 months old Dutch belted, New Zealand and Chinchilla rabbits were so arranged that each of 5 groups contained all 3 types of animals and members of both sexes. Ten animals were started in each group, but some died of respiratory infections (Table I). Every morning each animal was fed 50 g rabbit chow with 5% cotton seed oil and the test substance. When this was eaten, normal rabbit chow, hay, carrots and lettuce were given. Test substances were fed for 12 weeks, omitting Sundays. Each rabbit in Group I received/day 900 mg of purified cholesterol, Group II got 900 mg of commercial cholesterol (Wilson)/day and Group III had 200 mg of impurities/day containing 15.6 mg of digitonin precipitable material. Group IV received 200 mg of impurities/day, containing 28 mg digitonin-precipitable substance (calculated as cholesterol) plus 872 mg of commercial cholesterol. In this mixture impurities amounted to about 16%. Group V were control animals which received only 5% cotton seed oil with their daily diet. For Groups II-IV the cholesterol and/or its impurities were dissolved in ether, but to exclude ether peroxides the purified cholesterol for Group I was dissolved in pentane before added to the chow. From the combined aortae of each group, cholesterol was isolated after hydrolysis. For serum cholesterol determinations 5 ml blood were withdrawn from each rabbit by heart puncture. In Groups I and II the method of Kingsley and Schaffert(13) was used, but unfortunately no smaller aliquots were made and the values obtained are therefore too low and cannot be statistically evaluated. For Groups III-V the procedure of Abell *et al.*(14) was employed. *Histological procedures.* Animals were killed by cervical

TABLE I. Effects of Purified and of Commercial Cholesterol and Its Impurities on Serum and Aorta Cholesterol and the Degree of Atherosclerotic Lesions.

Group No.	Material added to normal diet	Total mg cholesterol fed/animal /day	No. of animals		Cholesterol in 100 ml serum		mg/g wet aorta	No. of animals with atherosclerotic lesions				
			At start	At end	mg	Mean ± S.E.		5+	4+	3+	2+	+	—
I	Purified cholesterol	900	10	10	488*		5.0	5	2	2	1	0	
II	Commercial cholesterol	"	"	5	<488*		6.4		2	3	3	2	
				5	>455	455 ± 0							
III	Impurities	15.6	"	9	25– 258	100 ± 28.4	.9						9
IV	Commercial cholesterol + impurities	872 28	"	6	400–4000	1947 ±557	5.4		1		1	4	
V	Controls	0	"	7	14– 55	24 ± 5.4	.9						7

* Precise values not determined. However, in 40 animals receiving 900 mg commercial cholesterol/day for 12 wk in other experiments, a mean was found of 804 mg (120-1980, ± 72).

fracture and tissues fixed as usual for sectioning. Hematoxylin-eosin was used for staining. Sections of aorta, tongue, heart, kidney, adrenal, liver, spleen and eye were coded for grading based on nature, frequency and intensity of atherosclerotic lesions in each section. From this the grade of damage for each animal was compiled. The strongest alterations were assigned as 5+, absence of damage as –, with 4+, 3+, 2+, + as intermediates.

Results. Table I shows that in Groups I, II and IV feeding of large amounts of cholesterol increased serum cholesterol and that cholesterol laid down in the aortae was increased about 5 to 6 times. Feeding a large amount of impurities from commercial cholesterol in Group III did not influence aorta cholesterol, but the small amount of digitonin precipitable substance left in the impurities increased serum values to about 4 times the normal.

The last columns of Table I present histological investigation of the tissues. Extensive damage occurred in Groups I and II, but highly purified cholesterol caused more severe lesions in Group I. Animals in Group III, fed a large amount of impurities had normal aorta cholesterol and showed no lesions although their serum cholesterol was somewhat higher than normal. A single animal had some calcification of the aortic media. Animals in Group IV which received the same total amount of cholesterol as those of Groups I or II but simultaneously a large amount of the same impurities as Group III had high aorta cholesterol and serum cholesterols at least as high as in Groups I and II, but damage from atherosclerotic lesions in their tissues was considerably less. Appropriate calculation shows that the differences between control and experimental groups for serum cholesterol and for the atherosclerotic lesions are statistically significant. It appears therefore that natural impurities of cholesterol include substances which counteract the effect of pure cholesterol in producing atherosclerotic lesions.

Little is known about the chemical nature of these substances. Cholesterol, made mainly from spinal cords of cattle and from cattle or sheep brain, is probably deposited from blood and should therefore contain companions simi-

lar to those isolated from blood and other tissues. Such investigations by Hardegger, Ruzicka and Tagmann(15) showed that the substances from non-saponifiable extractives of aorta, serum, liver and other tissues are either oxidation products of cholesterol or derivatives of those. This is understandable, considering experiments of Borgstroem and Wintersteiner(16) which show how readily cholesterol can be oxidized by air under conditions similar to the situation of cholesterol in blood, where it is exposed to dissolved oxygen in the presence of solubilizing substances and buffers at a slightly basic pH. More recent contributions to the problem of companions of cholesterol have been made by Fieser(11) and Schwenk *et al.*(17). The impurities may include unknown substances other than steroids. The natural companions of cholesterol, contained in the material used in this work, are evidently different from substances formed when cholesterol is heated(2,3). Such substances enhance the atherosclerotic effect of cholesterol, but the same author(12,18) has found that *in vitro* oxidation of cholesterol decreases its atherogenicity.

Summary. Feeding of highly purified cholesterol to rabbits for 12 weeks resulted in high serum cholesterol and atherosclerotic lesions, which appear more severe than those obtained with commercial cholesterol. This latter contained about 2% impurities isolated from mother liquors after treatment of commercial cholesterol with anhydrous oxalic acid in organic solvents. Such impurities did not produce atherosclerotic lesions when fed to rabbits, but when given together with cholesterol caused less extensive lesions than those obtained with pure or commercial cholesterol.

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1. Duff, G. L., *Am. Arch. Path.,* 1934, v20, 81, 259.
2. Altschul, R., *Selected Studies on Arteriosclerosis.* Ch. C Thomas, Springfield, Ill., 1950, 135.
3. ———, *Am. Heart J.,* 1950, v40, 401.
4. Cook, R. P., Editor, *Cholesterol.,* Acad. Press Publ., N. Y., 1958, 21, 76.
5. Anitschkow, N., Chalatow, S., *Zentralbl. Allg. Path. pathol. Anatomie,* 1913, v24, 1.
6. Wacker, I., Hueck, W., *Muench. Med. Woch.,* 1913, v60, 2079.
7. Kritschevsky, D., *Cholesterol,* John Wiley Sons, N. Y., 1958, 143.
8. Schwenk, E., Werthessen, N. T., *Arch. Biochem. and Biophys.,* 1952, v40, 334.
9. Smith, L. L., *J. Am. Chem. Soc.,* 1954, v76, 3232.
10. Miescher, K., Kaegi, H., *Helv. Chim. Acta.,* 1941, v24, 986.
11. Fieser, L. F., *J. Am. Chem. Soc.,* 1953, v75, 5421.
12. Alexander, G. J., Schwenk, E., *Arch. Biochem. and Biophys.,* 1957, v66, 381.
13. Kingsley, G., Schaffert, R., *Biochem. J.,* 1949, v180, 315.
14. Abell, L. L., Leny, B. B., Brodie, B. B., Kendall, F. E., *J. Biol. Chem.,* 1952, v195, 357.
15. Hardegger, E., Ruzicka, L., Tagmann, E., *Helv. Chim. Acta,* 1953, v26, 2205.
16. Borgstroem, S., Wintersteiner, O., *J. Biol. Chem.,* 1941, v141, 597; 1942, v145, 309.
17. Schwenk, E., Alexander, G. J., Fish, C. A., Stoudt, T. H., *Fed. Proc.,* 1955, v14, 752.
18. Altschul, R., *J. Am. Med. Assn.,* 1958, v168, 822.

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In vitro Absorption of Serotonin by Thrombocytes of Rheumatoid Arthritic and Non-Arthritic Individuals.* (25137)

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It was noted(1) that the 5-hydroxytryptamine (5-HT, serotonin) content/unit of circulating blood thrombocytes was decreased in patients with rheumatoid arthritis or other inflammatory states, as compared to patients with no evidence of inflammation. Since Spector and Willoughby(2) have shown that 5-HT

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