Rape Oil and Cholesterol Metabolism in Different Species with Reference To Experimental Atherosclerosis.* (22900)

K. K. CARROLL (Introduced by R. L. Noble)
Collip Medical Research Laboratory, University of Western Ontario, London, Canada.

Numerous studies on experimental atherosclerosis have provided ample evidence of differences in metabolism of cholesterol in different species of animals(1,2).¶ It is possible to produce marked hypercholesteremia and atherosclerotic lesions by feeding cholesterol to rabbits and chickens, and similar though less marked changes can be induced in guinea pigs. These effects can also be produced in dogs by concomitant administration of antithyroid compounds(3) and in monkeys by restriction of the intake of sulfur-containing amino-acids(4). Other species, notably the rat, appear to be particularly resistant to experimental atherosclerosis, although it is possible to increase the cholesterol content of blood and liver of the rat by giving bile acids and anti-thyroid compounds in addition to cholesterol(5,6).

While the rat is very resistant to production of atherosclerosis, and stores much less cholesterol than the rabbit when this compound is fed in the diet, studies in our laboratory have shown that rats fed diets containing rape oil deposit cholesterol in the adrenals and in the liver(7). Similar results were obtained by feeding erucic acid, one of the component fatty acids of rape oil(8,9). These diets, however, have little effect on blood cholesterol levels and no atheroma have been observed in the arteries after periods of 6 months or longer. It seemed of interest, therefore, to determine whether rape oil diets would cause more widespread deposition of cholesterol and formation of atheroma in species known to be susceptible to experimental atherosclerosis. This paper contrasts the results of feeding rape oil and of feeding cholesterol to animals of several different species.

TABLE I. Control Diets.

	Protein	Fat	Fibre
	——— % ———		
Masters fox chow	20(min)	3.5(min)	5 (max)
Purina rabbit chow	16.45	4.06	7.93
Grow mash	15(min)	3 (min)	7.5(max)

Methods. The rats used in these experiments belonged to the Sprague-Dawley strain, the mice were Swiss strain obtained from Rockland Farms, N. Y., and the chickens were White Leghorns from the Ontario Veterinary College, Guelph, Ontario. The guinea pigs, rabbits, cats and dogs were obtained locally. The dogs in the control group and all cats were females. Otherwise, male animals were used. The control diet for rats and mice consisted of Masters fox chow. Rabbits and guinea pigs were fed Purina rabbit chow checkers with supplement of cabbage twice a week, and the chickens received a Grow Mash prepared by the United Cooperatives of Ontario. The approximate composition of these diets is shown in Table I. The experimental diets were prepared by adding either 2% of powdered cholesterol or 25% of rape oil† to the control diets. The dogs were fed ¾ lb of Masters fox chow mixed with ¼ lb of rape oil once daily, together with a supplement of meat. All diets were fed for 28 days with the exception that the dogs were fed for 24 days. Cholesterol was determined by the Sperry-Webb procedure(10). Lipid extracts were prepared from single adrenals or 4 g samples of liver by grinding in acetone-alcohol(1:1) and extracting at boiling point with several portions of the same solvent. The extracts were made to measured volumes,

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¶ Pilgeram, L. O., *Fed. Proc.*, 1955, v14, 728.

† Generous supplies of refined rape oil were donated by Yocum Faust, Ltd., London, Ontario.

filtered, and aliquots used for cholesterol determinations. In the experiment with mice, tissues from 2 animals were pooled for each determination. The coefficient of digestibility of rape oil was measured in rats, guinea pigs and rabbits by a method similar to that of Augur, Rollman and Deuel(11). Food consumption for animals on both control and rape oil diets was recorded for an 8-day period (4 days in guinea pig experiments). Combined feces for this period were collected and 10 g aliquots were ground and extracted overnight in Soxhlet with ether. The residue was acidified with 3 cc of concentrated hydrochloric acid and reextracted with ether. Total fat in feces was 105, 92 and 81 mg/g of stool in rats, guinea pigs and rabbits on control diets respectively, and these values were subtracted from amounts of fat found in feces on rape oil diets. Percentage of rape oil absorbed was calculated from the difference between amount ingested and corrected amount of fat excreted in feces.

Results. It is well-known that feeding diets containing cholesterol to rabbits results in hypercholesteremia and also leads to deposition of cholesterol in the liver, adrenal and other tissues(12,13). Such results were readily obtained in our experiments by feeding cholesterol as 2% of the diet for 28 days (Fig. 1). This diet produced less spectacular effects in guinea pigs but there were significant increases in cholesterol concentration ($P<0.02$ for plasma and $P<0.01$ for adrenal and liver). Cholesterol was not fed to chickens in the present experiments but Dauber and Katz(14) have shown that it increases blood cholesterol as in the rabbit. Their description of gross and microscopic appearance of tissues of treated chickens also suggests that lipids were being deposited in liver and perhaps in adrenals and other organs as well.

In species less susceptible to experimental atherosclerosis, feeding cholesterol has little effect. In our experiments, rats responded with a small increase in adrenal cholesterol and mice with some increase in liver cholesterol.

The results of feeding a diet containing

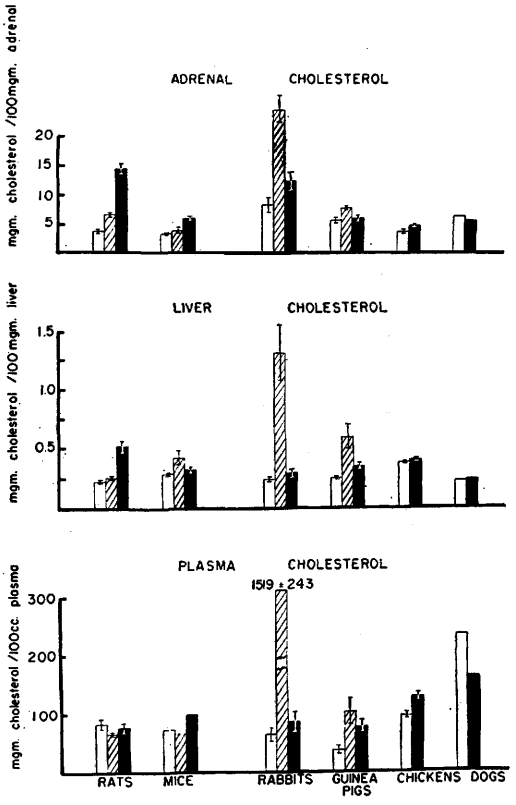

FIG. 1. Effects of different diets on plasma and tissue cholesterol concentrations. Open bars represent levels in animals on control diets; hatched bars represent levels on diets containing 2% cholesterol and shaded bars represent levels on diets containing 25% rape oil. Stand. error of mean is indicated in cases where it was determined.

25% of rape oil present a somewhat different picture. As had been shown previously, this regime caused a highly significant increase in adrenal and liver cholesterol in the rat ($P<0.001$), but no change in blood cholesterol. Adrenal cholesterol was also significantly increased in the mouse ($P<0.001$). (Insufficient determinations were made to test the significance of the increase in blood cholesterol). An attempt was also made to test the effect of rape oil on cats, another species which appears to be resistant to the production of experimental atherosclerosis. Results in this case were inconclusive because the cats refused to eat the rape oil diet and it was necessary to substitute a diet containing 10% of erucic acid, the active component of rape oil. Even this was poorly accepted and the

TABLE II. Effect of Diets on Body and Adrenal Weight.

	Control	Cholesterol	Rape oil	Control	Cholesterol	Rape oil	Control	Rape oil
	Rats			Mice			Dogs	
No. of animals	6	6	6	8	7	8	4	2
Initial wt (g)	84 ± 2.3	81 ± 1.0	84 ± 2.4	24 ± .5	24 ± .7	24 ± .8		17,400
Final wt (g)	191 ± 9.7	186 ± 9.7	162 ± 3.9	30 ± .4	29 ± 1.3	26 ± 1.2	11,200	19,600
Adrenal wt (mg)	31.7 ± 1.2	30.8 ± 1.6	38.7 ± 1.2	4.6 ± .2	4.2 ± .1	4.4 ± .3	835	1,631
	Rabbits			Guinea pigs			Chickens	
No. of animals	5	5	5	6	6	6	13	14
Initial wt (g)	2230 ± 170	2330 ± 36	2580 ± 19	361 ± 12	373 ± 17	419 ± 14	1690 ± 55	1520 ± 45
Final wt (g)	2690 ± 152	2890 ± 60	2890 ± 108	482 ± 11	503 ± 14	407 ± 11	2550 ± 55	1900 ± 60
Adrenal wt (mg)	168 ± 17	292 ± 24	198 ± 8.8	241 ± 28	257 ± 16	223 ± 12	182 ± 8.5	135 ± 7.6

animals lost weight during the experiment. However, after 24 days on the diet, 2 of the 3 cats had more than 10% cholesterol in the adrenals whereas the adrenals of control animals contained only 4% cholesterol.

The rape oil diet appeared to have little or no effect on the adrenal cholesterol of rabbits, guinea pigs, chickens or dogs. There was, however, a tendency towards hypercholesteremia in guinea pigs ($P<0.01$) and chickens ($P<0.001$), and liver cholesterol was increased in guinea pigs ($P<0.001$). Rabbits, which are most susceptible to cholesterol diets, showed hardly any change in blood or tissue cholesterol as a result of feeding rape oil. Values for adrenal, liver and plasma cholesterol concentration are shown in Fig. 1, while adrenal and body weights are listed in Table II. It may be noted that animals on rape oil diet did not grow as well as those on control and cholesterol diets.

It seemed possible that failure of rape oil to affect adrenal cholesterol in some species might result from poor digestibility of the oil in those species, and to test this the coefficient of digestibility of rape oil was measured in rats, guinea pigs and rabbits. The values of 58, 61 and 58 which were obtained in these 3 species respectively, indicate that digestibility is probably not a factor in explaining species differences. These values are low compared to the figure of 82 obtained by Deuel, Cheng and Morehouse for rats(15). However, these workers used a synthetic diet containing 15% of rape oil, while in our experiments the diet consisted of fox chow to which 25% by weight of rape oil had been added. Rape oil from different sources has also been shown to vary greatly in its content of erucic acid(16) and this may account for some of the difference in digestibility.

Such short term experiments as those described above do not preclude the possibility that a rape oil diet may cause cholesterol plaques in a species such as the rabbit in a longer term experiment. However, in one experiment in which 3 rabbits were fed 25% rape oil for 72 days there was no evidence of formation of plaques in the aorta or coronary arteries. In another experiment, one group

of 5 rabbits was fed 2% cholesterol and a second group was fed 2% cholesterol together with 20% rape oil for 125 days. At autopsy these groups showed no difference in number or size of plaques in the aorta‡ and there were no significant differences in adrenal, liver and blood cholesterols of the two groups.

Thus it appears that rather than having an enhanced effect on cholesterol levels, rape oil fails to produce any marked changes in species susceptible to experimental atherosclerosis. There is, in fact, some indication of a negative correlation between susceptibility to experimental atherosclerosis and ability to respond to rape oil with an increase in adrenal cholesterol levels. It is conceivable that both of these characteristics may be related to species differences in the metabolism of phospholipids like those observed by Pilgeram and Greenberg(17).

Summary. Rape oil caused a marked increase in adrenal cholesterol when fed to rats or mice but had little or no effect in rabbits, guinea pigs, chickens and dogs. This contrasts with results obtained by feeding cholesterol since dietary cholesterol tends to be deposited more readily in the latter four species. The differences in response to rape oil do not appear to be due to species differences in digestibility of rape oil.

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Changes in Serum Proteins During Growth of Malignant Cells *in vitro*.* (22901)

H. NAIM KENT AND GEORGE O. GEY
Department of Surgery, Johns Hopkins University School of Medicine, Baltimore, Md.

During periods of progressive growth of cells maintained as strains in continuous culture there is usually an initial adaptive growth followed by growth at near maximum rates and then a slowing down of growth but with the individual cells still active in fluid exchange, transport and storage. The products of these cellular activities may be expected to be reflected in the composition of the serum proteins when used as culture medium. The protein components appear to have importance in cellular responses not only because of their nutritional value but also in immune phenomena. Little is known how-

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