CHOLESTEROL AS CARCINOGEN

I. Sarcoma Induction by Cholesterol in a Sensitive Strain of Mice

II. Croton Oil a Complete Carcinogen

I. HIEGER

From the Chester Beatty Research Institute, Royal Cancer Hospital, London, S.W.3

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I. SARCOMA INDUCTION BY CHOLESTEROL IN A SENSITIVE STRAIN OF MICE

Earlier papers in this series (see e.g. Hieger, 1959) have presented experimental results showing that :

(a) the carcinogenic factor in the unsaponifiable fraction of human tissue (liver, lung, kidney, muscle) is cholesterol ;

(b) commercial cholesterol injected subcutaneously induces sarcoma in mice and stringent purification of such cholesterol does not impair its potency ;

(c) different strains of mice and even different batches of the same pure strain show gross differences of susceptibility to carcinogenesis by cholesterol ;

(d) the solvents used for administering the cholesterol (e.g. olive oil) have a very low sarcoma-inducing potency (5 tumours in 1122 mice).

The first part of this paper deals with an explanation of (c), i.e. the differences in susceptibility to cholesterol.

The table shown below (Table I) summarises the results of experiments carried out on cholesterol as carcinogen subsequent to those described in the 1959 paper.

Where the "Code" (laboratory labelling) of an experiment carries an asterisk(*), the mice were housed in a room where hydrocarbon-carcinogens (e.g. benzopyrene) are used (in order to test the effects of contamination). All other experiments were carried out in a room free from hydrocarbon-carcinogens. It is obvious from the results that atmospheric contamination by powerful carcinogens does not carry any risk to experiments where cholesterol is being tested.

The experiments are entered in the table in the order in which they were carried out, that is to say, Experiment 1 was started then 2, then 3, etc. This arrangement is useful in demonstrating that no appreciable part of the carcinogenic potency of the active preparations can be attributed to possible variations in the composition of successive batches of the vehicle (olive oil) which is a commercial product, and therefore different samples (hospital dispensary quality) might well have differences in composition owing to seasonal variations or to geographical changes in the source of the olive crop. For example, Experiment 27 gave a high yield of tumours but Experiment 28 a very low yield ; since these

experiments were started within a few weeks of each other the composition of the oil can hardly be invoked to explain differences of carcinogenic potency.

Unless otherwise stated the concentration of cholesterol was 9 per cent in olive oil ; in the first few experiments the preparations were made by dissolving 1 g. of cholesterol in 10 g. olive oil on the water bath, and 0·2 c.c. of the hot solution was injected in the mice subcutaneously three times at intervals of about three weeks. In the later, and great majority of the experiments, the preparations were made rather differently : 1 g. cholesterol and a little olive oil were thoroughly stirred into a paste with a glass rod, then the remainder of the 10 g. of olive oil was added and stirred well, the whole heated on the water bath till solution and rapidly cooled with stirring. A paste was thus obtained which had been heated for only a few minutes and could be injected cold through the usual No. 1 needle.

The results of the experiments in Table I show that :

(a) under the conditions existing in our laboratory in the last few years, mice of C_{57}, $C_{57} \times C_3H$, Swiss S, BALB/C and Stock strains when injected thrice with 0·2 c.c. of a 9 per cent suspension of commercial or pure cholesterol have shown, at the site of injection, an incidence of sarcoma which ranged between about 5 and 0 per cent ;

(b) the incidence is sometimes increased by enlarging the dose of cholesterol ; by giving the first injection when the mice are only 1 day old ; by crowding a number of mice in a large cage instead of housing them as is usual 5 to a cage ; in one or two experiments by substituting lard for olive oil ; or by adding egg powder to the diet :

(c) the yield of sarcomas is much more effectively increased by using mice of the Buffalo and the BRO strains, or by adding a small proportion of croton oil to the cholesterol preparation ; in the most productive experiment (No. 45), sarcomas developed in 16 Buffalo mice out of an initial lot of 50, of which 38 lived more than 1 year, the yield being thus more than 40 per cent ;

(d) in BRO mice sarcoma will develop at the site of injection of croton oil (0·4 per cent) in olive oil.

Discussion

The obvious conclusion to be drawn from Table I is that the genetic nature of the mouse is the decisive factor in determining susceptibility to carcinogenesis by cholesterol, or by croton oil ; but it is also necessary to explain why some batches of stock mice (i.e. mice of unknown ancestry, originally obtained from a dealer a decade or two ago and bred in the laboratory without regard to relationship at mating) and of C_{57} strain mice can sometimes develop sarcoma in over 10 per cent of their number on injection with cholesterol, while other batches do so in only 2 per cent or are even resistant (0 per cent).

As a first step it might be suggested that the requirements for susceptibility to carcinogenesis by cholesterol, or by threshold doses of carcinogenic hydrocarbons (see Hieger, 1959, for a development of this theme) depend upon slight alterations in the genetic controlling system, alterations so slight that they can be found in different batches of what are alleged to be pure line strains of mice.

The studies of Dunn, Heston and Deringer (1956) on the incidence of spontaneous sarcoma in different groups of pure line mice suckled by foster-mothers of

TABLE I.—*Sarcoma Induction in Mice by Cholesterol*

Experiment	Code	Preparation C = cholesterol Comm. = commercial	Strain of mice	Number of mice and survivors (months) Initial	After 12 m.	After 18 m.	After 24 m.	Sarcomas	% Incidence on initial number	Calculated on survivors at 1 year (%)	
1	374 C	Pure C in olive oil (9%)	Stock	60	34	14	0	1	2	3	In all experiments, unless otherwise stated, each mouse received 3 injections of 0·2 c.c. of a 9% solution of C.
2	376 E	Pure C in olive oil (9%)	Stock	63	40	21	4	0	—	—	—
3	Fenu*	Comm. C in olive oil	C_{57}	536	—	—	—	12	2·2	—	—
4	Janu*	Comm. C in sesame oil	C_{57}	49	36	28	9	0	—	—	—
5	Sibo*	Comm. C in olive oil + tristearin (12%)	Stock	50	35	14	3	0	—	—	—
6	Peti*	Comm. C in olive oil	$C_3H \times C_{57}$	65	62	49	30	1	2	2	—
7	Xozu*	Comm. C in olive oil + oleic acid 9%	Stock / Swiss S	50	32	2	2	0	—	—	—
8	Zave*	Acetone-crystallised C in lard (13%)	Stock / Swiss	50	41	18	6	1	2	2·5	—
9	415 R	Acetone-crystallised C in lard (13%)	Stock / Swiss	50	32	23	6	1	2	3	—
10	Bewi*	Acetone-crystallised C in lard (13%)	Stock / C_{57}	71	29	11	6	3	4	10	(5 injections of 0·3 c.c. each. Egg powder in diet.)
11	Dixo*	Acetone-crystallised C in lard (13%)	Stock / C_{57}	50	25	14	8	4	8	16	(3 injections of 0·75 c.c).
12	377 F	Pure C in olive oil (30%)	Stock	47	33	22	3	0	—	—	—
13	381 J	Pure C in olive oil 9%	C_{57} / Stock	67	47	38	8	1	1·5	2	-
14	382 K	Pure C in sesame oil + 20% stearic acid	C_{57} / Stock	59	38	19	7	0	—	—	—
15	383 L	Pure C in olive oil	C_{57} / Stock	68	53	29	19	2	3	4	(Mice also had 4 injections oestrone 70γ.)
16	384 M	Pure C in olive oil	C_{57}	53	43	32	8	3	6	7	—
17	385 N	Comm. C in olive oil	C_{57}	69	56	26	21	3	4	6	Egg powder in dt as in Experiment 10.
18	387 P	Pure C in olive oil	C_{57}	80	67	60	28	1	1·3	1·5	Young mice < 1 month of age.
19	388 Q	Pure C in olive oil	C_{57} / Swiss S	50	37	23	21	3	6	10	Young mice, 1 day old.

20	389 R	Pure C in olive oil	Swiss S	51	31	11	5	1	2	3	—	
21	395 X 396 Y	Comm. C in olive oil suspension 25%	Swiss $C_3H \times C_{57}$	63	54	42	32	1	2	—	—	
22	397 Z	Comm. C in olive oil 15%	C_{57} Stock $C_3H \times C_{57}$	61	52	37	23	3	5	6	Mice were injected 6 times	
23	401 D	Pure C in olive oil	Stock C_{57}	50	25	13	6	0	—	—	—	
24	402 E	Comm. C in olive oil 15%	Stock C_{57}	88	72	22	0	1	1·3	—	Castrated mice.	
25	403 F	Comm. C in glyceryl-mono-oleate, 25%	Stock C_{57}	54	34	22	7	1	2	—	—	
26	405 H 410 M	Comm. C in olive oil	Stock C_{57}	80	44	36	17	5	6	12	A "crowding" experiment, 20 mice per cage.	
27	407 J	Comm. C in olive oil	Buffalo	51	36	32	7	12	24	33	—	
28	409 L	Comm. C in olive oil	Stock $C_3H \times C_{57}$	52	28	19	7	1	2	4	Mice had 300 r γ-irradiation.	
29	411 N	Comm. C in olive oil + tristearin	Stock Swiss S	50	28	19	4	1	2	4	—	
30	412 O	Comm. C in olive oil	Stock Swiss S BRO C_{57}	47	30	12	1	4	8	13	Mice when 1 day old received 0·01 c.c. turpentine.	
31	415 R	C (crystallised from acetone) in lard, 15%	Stock BRO Swiss S	50	30	23	6	4	8	13	—	
32	416 S	Comm. C in olive oil, 9% + 0·4% croton —oil	C_{57} Stock Swiss S BRO	60	52	24	4	9	15	17	5 of the 9 sarcomas appeared in the 15 BRO mice of which 11 lived > 1 year.	
33	418 U	Comm. C in olive oil	LAC	62	38	28	7	0	—	—	—	
34	419 V	C (crystallised from acetone) in olive oil	C_{57}	50	29	22	8	0	—	—	Each mouse had 6 injections.	
35	421 X	Comm. C in olive oil	BALB/C	43	31	22	14	2	5	—	—	
36	425 B	Comm. C in olive oil	101	32	16	3	1	0	—	—	—	
37	427 D	Pure C in olive oil	Buffalo	63	44	24	0	7	11	21	—	
38	429 F	C (crystallised from acetone) in olive oil	BRO	65	46	40	—	2	4	—	—	
39	430 G	C (crystallised from acetone) in olive oil + 0·4% croton oil	Stock BRO C_{57} 101	80	34	23	—	7	9	21	5 of the 7 sarcomas appeared in the 35 BRO mice, 23 of which lived > 1 year.	
40	Migo*	C (crystallised from acetone) in olive oil + 0·4% croton oil	Stock C_{57} BRO	61	34	—	—	3	5	10	—	

TABLE I.—*Sarcoma Induction in Mice by Cholesterol*—(continued)

Experiment	Code	Preparation C = cholesterol Comm. = commercial	Strain of mice	Number of mice and survivors (months) Initial	After 12 m.	After 18 m.	After 24 m.	Sarcomas	% Incidence on initial number	Calculated on survivors at 1 year (%)	
41	431 H	NO cholesterol, 0·4% croton oil in olive oil	Stock BRO C_{57} 101	71	47	33	—	5	7	10	4 of the 5 sarcomas were in the 35 BRO mice.
42	433 J	Pure C in olive oil	BRO	50	46	31	—	3	6	6	—
43	434 K	Pure C in olive oil	Buffalo	54	46	—	—	9	17	20	—
44	435 L	C crystallised from acetone, in olive oil	BRO	50	40	—	—	9	18	22	—
45	437 N	Comm. C in olive oil	Buffalo	50	38	—	—	16	32	40	Latent periods (months) 6, 6½, 7, 7, 7½, 9, 9, 10, 10, 10, 10½, 10½, 10½, 12½, 13, 14.
46	428 E	NO cholesterol, control on olive oil alone	Buffalo	60	41	19	—	0	0	0	Control test on solvent.

different genetic make-up (see Hieger, 1959) suggest that either the liability to sarcoma is mediated by an agent in milk on Bittner-mammary-factor lines, or the nature of the diet in early life determines susceptibility ; and similar considerations may apply to the action of cholesterol.

Work of Bischoff

Bischoff and co-workers (Bischoff *et al.*, 1955) reported that some oxidation derivatives of cholesterol can induce sarcoma in Buffalo mice, that a hydroperoxide is the most potent (60 per cent of sarcomas), but that pure cholesterol is ineffective. The results with hydroperoxide have not been confirmed by the author ; in his experiments the hydroperoxide is hardly active in Buffalo mice but has induced 5 sarcomas in 50 C_{57} mice (Hieger, 1959) ; cholesterol, and particularly commercial cholesterol, is highly carcinogenic in Buffalo mice and less so in C_{57} mice.

II. CROTON OIL A COMPLETE CARCINOGEN

In order to see if the irritant properties of croton oil (silica, turpentine, desoxycholic acid, oleic acid and other agents had been tried in previous experiments) might act as an enhancing or co-carcinogenic agent, a small proportion of the oil (0·4 per cent) was added to the cholesterol solution in olive oil (9 per cent) before injecting into a batch of 60 mice (Experiment 32) consisting of animals from C_{57}, Swiss S, BRO and Stock strains ; 9 sarcomas developed, 5 of these in the BRO group (15 mice). A similar result appeared in a repeat of this test (Experiment 39). As a control test, a similar series (Experiment 41) was injected with 0·4 per cent croton oil in olive oil (three injections of 0·2 c.c.) *without* cholesterol ; 5 sarcomas were obtained, 4 of these in the BRO group of 35 mice, 30 of which lived over 1 year.

One has to conclude that croton oil is a complete carcinogen, to which BRO mice are specially sensitive.

SUMMARY

1. Mice of the Buffalo strain are specially sensitive to sarcoma induction by the subcutaneous injection of cholesterol in olive oil (three injections of 0·2 c.c. of a 9 per cent suspension) ; in the most productive experiment using commercial cholesterol, sarcoma developed in 16 of a series of 50 mice, 38 of which lived more than 1 year. In a test using highly purified cholesterol, sarcoma appeared in 9 Buffalo mice out of 54 injected.

2. Croton oil in olive oil (0·4 per cent) produced sarcoma at the site of injection in 4 BRO mice out of 35 injected.

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