LIX. ON THE ALLEGED ACTION OF X-RAYS UPON CHOLESTEROL.

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THE experiments described here are part of a study of the chemical changes produced in known constituents of cells by radiations of short wave-length. The first substance chosen for investigation was cholesterol, for the reason that it is present in practically all tissues, and changes are known to be produced in it by radiations of longer wave-length (ultra-violet light). It was hoped that the investigations would throw some light upon the changes in the tissues which lead up to X-ray cancer.

EXPERIMENTS WITH X-RAYS.

In the greater number of experiments a solution of cholesterol was placed in a glass dish 5 cm. deep and 22 cm. in diameter which was then closed, to exclude nitrogen oxides present in the X-ray room, by parchment sealed down with adhesive strapping. The dish was placed 3 or 4 cm. below the unshielded X-ray tube; the whole of the dish then lay within the cone of rays. The tube used in these experiments was a deep therapy Coolidge tube with tungsten target, activated from a coil. The current through the tube was 4 m.A. The voltages indicated in Table I were estimated by means of a sphere-gap (10 cm. diameter spheres) and checked at intervals by the spectrograph. No filter was used. The distance from the anticathode to the surface of the solution was 14 cm. The dish and tube were kept cool by an air-fan, and the solutions were exposed to X-rays for times ranging from 30 to 90 minutes; with potentials of from 100,000 to 200,000 volts (Table I).

When a thin layer (*e.g.* 9 mm.) of a dilute solution of cholesterol in chloroform or carbon tetrachloride is exposed to penetrating X-rays (200 k.v.) for an hour, the residue on evaporation of the solvent is found to be no longer white and crystalline as in a control sample, but to consist of an amorphous gummy mass of greenish colour and of considerably lowered melting-point (50–100° as against 146° in the original cholesterol). At first sight this result seems to reveal a striking direct effect of the rays upon the cholesterol molecule, and this conclusion has actually been drawn by one observer [Roffo, 1925; see below]. But later experiments showed that the change observed in the cholesterol is secondary to decomposition brought about by the radiation in certain chlorine-containing solvents, namely chloroform and carbon

tetrachloride; if benzene be used as solvent no such change occurs (Table I), and again no change was observed when a gum-saline emulsion of cholesterol was irradiated.

Table I.

Action of X-rays upon solutions of cholesterol.

Solvent	Cholesterol grams in 100 cc.	Depth of solu- tion mm.	Time of ex- posure minutes	Kilo- volts *	Melting-point		Lowering of melting- point °C.
					Irradiated ° C.	Control ° C.	
Chloroform	0.02	170	60	200	125-130	143-145	16
"	"	"	"	100	140-143	141-144	1
"	"	120	"	200	125-130	143-145	16
"	"	45	"	200	100-115	141.5-145.5	35
"	"	"	"	100	137-141	144-146	6
"	"	9.5	"	200	60-90	139-142.5	66
"	0.05	"	"	"	57-76	141-144	77
"	0.075	"	90	"	60-72	141-144	77
"	0.075	9	90	"	65-71	143-145	76
"	0.075	9	90	"	59-65	—	c. 80
"	0.05	4	30	"	100-115	140-144	34
"	0.044	"	"	175	107	—	c. 35
"	0.044	"	"	"	55	—	c. 87
Chloroform in com- pletely filled tubes	0.06	"	60	200	83-110	—	c. 45
Chloroform in current of N	0.06	5	60	200	106-113	—	c. 32
Carbon tetrachloride	0.02	170	60	200	124-131	144-145.5	17
"	0.025	5.5	30	"	70-90	140-143	62
"	"	4	30	"	60-75		74
"	0.05	4	60	"	60-80	139-143	71
Monochlorobenzene	0.08	7	90	200	133-138	142-146	8
Benzene	0.02	6	60	200	143-144.5	143-145	0
"	0.05	3	30	200	138-144	—	c. 2
"	0.02	6	60	100	139-142	143-145	3
Gum-saline emulsion	0.02	9	90	200	138-142	—	c. 2

Action of radium upon solutions of cholesterol.

Chloroform	0.1	9	2 days	50 mg. RaSO ₄ in Pt tube	140-143	145-146.5	4
"	"	"	16 days	"	125-133	144-146	16
"	0.1	"	16 days	40 mg. RaSO ₄ in glass tube	50-70	140-144	82
Benzene	0.1	"	2 days	50 mg. RaSO ₄ in Pt tube	144-145	143-145	0
"	"	"	18 days	"	140-142	142-144	2

* The wave length of the shortest X-rays present is inversely proportional to the voltage used. 200 k.v. gives "hard" rays (*i.e.* more penetrating), 100 k.v. "soft" rays (*i.e.* less penetrating).

Details of technique.

All the solvents used were purified by drying and fractionation. The change in the cholesterol, in a suitable solvent, is increased by reducing the depth of the solution exposed; thus in chloroform the melting-point is reduced to 125-130° when the depth is 170 mm., and to 65-70° when the depth is 9 mm., the controls recovered from non-irradiated solutions in each case melting at 143-145°. It must be remembered, however, that the dose of rays received by the solution is considerably greater in a shallow vessel, as the

scattered rays from the bottom of the vessel then penetrate the whole depth of the solution. The minimum time of exposure necessary to produce any change was not investigated. It is evident that the effect depends upon the use of high voltages, for in comparable experiments (see Table I, chloroform) a potential of 200 k.v. gave a melting-point of 100–115°, while 100 k.v. gave a melting-point of 137–141°, which is but very little lower than that of the control (144–145°).

Roffo [1925] has described this conversion of cholesterol in chloroform solution by X-rays into an amorphous greenish material, but he makes the statement that this change occurs also when benzene is used as solvent, which is in complete contradiction to the observations reported in the present paper. Now the results given in Table I above show that no such change occurred in a benzene solution; the irradiated cholesterol was recovered with no change in melting-point (143–145° in the irradiated, and 143–145° in the control, sample). Further experiments showed that the amorphous product recovered from an irradiated solution in chloroform or carbon tetrachloride could not be a simple decomposition product of cholesterol¹. The product was found to possess, in addition to the very low melting-point shown by many examples in Table I, the following properties: (1) a gain in weight of not less than 5 % over that of the cholesterol taken, (2) a chlorine content of from 3.8 to 4.9% (estimations by Bacon's method [1909]). Although, in view of the great difficulty of removing the chlorine-containing solvent, caution is needed in drawing such a conclusion, there seems to be very little doubt that a chlorinated derivative of cholesterol is formed. As soon as the presence of chlorine in the product was detected, a sample of chloroform irradiated in an atmosphere of nitrogen was tested with potassium iodide and starch and was found to contain free chlorine. Hence the conditions required for chlorination are present². The absence of change in a benzene solution, and in a watery emulsion, seems then to be due to the absence of organically combined chlorine. In order to investigate further the importance of a halogen element, a chlorine compound of a different type, namely monochlorobenzene, was used in one experiment as solvent. The lowering of melting-point was very slight (to 133–138° in comparison with 142–146°); hence the extensive change, giving melting-points from 60 to 100°, is not brought about by all organic chlorine compounds.

The possibility was considered that the effect was due to secondary radiations from the chlorine atom. To test this, a benzene solution of cholesterol

¹ In attempting to purify the gummy material, great difficulty was experienced in removing the last traces of solvent. It was found best to evaporate to apparent dryness *in vacuo*, then to redissolve in ether and again evaporate *in vacuo*, and to repeat this solution in ether and evaporation five times; it was hoped that in this way the ether would carry off the last traces of the less volatile solvent. In some experiments, which gave similar results, the removal of solvent was carried out *in vacuo* at 100°.

² The boiling-point of the chloroform used was found to be lowered by about 1° after irradiation. This is in disagreement with the results of Roffo.

was poured on to a layer of solid calcium chloride, and irradiated, but no significant change in melting-point was produced. The change is not dependent upon oxidation in a solution exposed to air, for the same lowering of melting-point is observed (1) when a chloroform solution is irradiated in completely filled corked tubes and (2) when a chloroform solution is saturated with hydrogen or nitrogen and exposed to a current of the gas throughout the period of irradiation (Table I).

Further evidence of the change brought about in cholesterol in chloroform solution is afforded by (1) a change in the rotatory power from -46.3° to -21.15° (green Hg line), (2) a diminution in the iodine value (Wijs) from 163, an abnormally high value such as is given by cholesterol, to 102.

Before the experiments upon the irradiation of chloroform alone had shown that the change in cholesterol was due to chlorination, experiments had been begun in which the amorphous product was tested upon animals for cancer-producing power. A solution in xylene of the material was painted twice weekly on the interscapular region of two mice, and on the ear of a rabbit, for 200 days, but no tumours were produced. Roffo and Correa [1924] have described a large decrease in the cholesterol content of tumours (rat sarcoma) exposed to X-rays. Any such decomposition of cholesterol *in vivo* can hardly be due to the process, dependent upon chlorination, which occurs in certain solvents *in vitro*. Experiments upon the cholesterol content of whole animals and of separate tissues before and after irradiation are now in progress in this Institute.

EXPERIMENTS WITH RADIUM.

A tube of glass or platinum 0.5 mm. thick containing radium sulphate was suspended in a glass tube containing about 20 cc. of cholesterol solution, so that a thickness of about 9 mm. of solution surrounded the tube. (For details, see Table I.) The cholesterol was recovered practically unaltered from solution in benzene. With a chloroform solution, radium in a platinum tube produced practically no change ($140-143^\circ$) in two days, and a distinct change ($125-133^\circ$) in 16 days; when a glass tube, which permits β -radiation in addition to γ -rays to pass, was used, a product exactly similar to that produced by X-rays at high voltage was obtained, namely an amorphous material melting at $50-70^\circ$. Radiations from radium have therefore the same action as have X-rays upon chlorine-containing solvents. Kailan [1917] had shown that chlorine is produced by the action of radium on chloroform and carbon tetrachloride, but I was not aware of his work when the experiments described here were carried out.

The decomposition of these chlorine compounds appears to depend upon rays of wave-length less than about $0.1 \text{ \AA}$ because (1) these would be produced by a voltage of 200 k.v. but not by 100 k.v.; as shown in Table I this voltage produces a large change in cholesterol (m.p. 55°) while with a voltage of 100,000 volts there is no change in the melting-point: and (2) the screening

action of the platinum tube is most effective upon the longer γ -rays, and the enclosure of radium in a platinum tube greatly diminishes the effect upon cholesterol (Table I).

Some months after the experiment reported in this paper had been completed, Dognon [1926] published some qualitative observations which confirm in every way the importance of the chemical nature of the solvent, and are in direct contradiction with the findings of Roffo. Dognon employed the Liebermann test as a criterion of change in irradiated solutions of cholesterol and found (1) that the test rapidly became negative when the solvent was carbon tetrachloride, chloroform, or bromoform, and (2) that no change occurred in benzene, carbon disulphide and monochlorobenzene. The additional negative result with another chlorine-free solvent, namely carbon disulphide, is a valuable confirmation of the dependence of the change upon a halogen.

SUMMARY.

X-rays have been alleged to decompose cholesterol when in solution *in vitro* in various organic solvents, and it has been implied that similar changes occur *in vivo* when tumours are exposed to X-rays. The experiments described above show that X-rays act upon cholesterol only in certain chlorine-containing solvents (chloroform and carbon tetrachloride); in benzene no change is produced, and in monochlorobenzene the change is very slight. The decomposition of cholesterol in chloroform and carbon tetrachloride appears to depend upon a liberation of chlorine, the rays thus acting upon these solvents and not directly upon the cholesterol. Experiments with radium gave confirmatory results with regard to action on the solvent. In view of the dependence of the change upon chlorine-containing solvents it seems unlikely that this decomposition of cholesterol can occur in irradiated tissues.

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