THE ACTIVATION OF CHOLESTEROL BY RADIATION

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It was recently reported by A. H. Roffo and A. E. Roffo (16) that cholesterol after irradiation with sunlight or ultra-violet light is capable of blackening a photographic plate placed at a short distance from it. This effect was attributed to an ultra-violet phosphorescence of which spectra were recorded. Cholesterol irradiated for 240 hours with ultra-violet light and subsequently exposed for 72 hours before the slit of a high aperture spectrograph was said to yield two characteristic lines at 3533 Å and 3572 Å respectively.

In view of the remarkable character of this spectrum and the importance attached to the phenomenon by Roffo in relation to the production of skin cancer by ultra-violet light, experiments were undertaken to investigate further the nature of this "phosphorescence." In the course of our investigations we found that a fairly large literature already existed on the subject, a number of the phenomena subsequently observed by us having been established by previous workers. A general review of the subject was therefore thought of interest as in many cases recent workers appear to have overlooked earlier experiments.¹

PHOTOGRAPHIC BLACKENING

It has previously been observed by Šťíteský (22), Hamano (5), and Hugounenq (10, 11), in addition to Roffo, that cholesterol after irradiation with ultra-violet light is capable of blackening a photographic plate in its vicinity. Hamano (6) and Šťíteský (22) obtained the corresponding effect with X rays. We have verified the phenomenon with different types of exciting radiation, as follows:

(a) Ultra-violet and Visible Light: Commercial cholesterol (Glaxo) was purified through the dibromide and recrystallized from alcohol.² A fixed quantity (0.1 gm.) of the purified cholesterol was spread on the bottom of a glass dish, 2.6 cm. in diameter and 2.0 cm. deep, and irradiated from above by a quartz mercury arc (Thermal Syndicate type, 200 volts d.c., 2.5 amps. running current) at a distance of 40 cm. The time of irradiation in different experiments varied from 30 minutes to 600 hours.

The irradiated cholesterol was then exposed to a photographic plate (Ilford Process) laid across the top of the dish with the emulsion side downwards, the experiment being carried out inside a light-tight box in a dark room. Careful control experiments were carried out, unirradiated cholesterol, irradiated and unirradiated glass and quartz vessels being similarly exposed to photographic

¹ Since the communication of this paper an account of experimental work leading to similar conclusions has been published by Stavely and Bergmann: Am. J. Cancer 30: 749, 1937.
² We wish to thank Mr. F. L. Warren for the purification of the cholesterol used by us.

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plates. The unirradiated glass and quartz vessels were inactive, but the glass and quartz dishes after irradiation affected the plate in a narrow ring where actual contact with the emulsion occurred. Fresh cholesterol had no effect on the plate during a 10-day exposure, even if previously left in diffuse daylight in the laboratory for 15 days.

Irradiated cholesterol produces a fairly uniform blackening over the whole projected area of the dish. We have found that the blackening depends upon (a) the time of irradiation of the cholesterol and (b) the time of exposure to the plate. Table I shows the results of the experiments to determine the variation of blackening with amount of incident radiation, the subsequent exposure to the photographic plate being kept constant at 94 hours. The photographic density \( D = \log_{10} \frac{I_o}{I} \) was measured with a simple photometer embodying a copper-copper oxide cell.

<table>
<thead>
<tr>
<th>Time of irradiation by mercury arc</th>
<th>Density of plate: ( D = \log_{10} \frac{I_o}{I} ) Exposure constant at 94 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 hours</td>
<td>0.017</td>
</tr>
<tr>
<td>4 hours</td>
<td>0.048</td>
</tr>
<tr>
<td>6 hours</td>
<td>0.085</td>
</tr>
<tr>
<td>18 hours</td>
<td>0.106</td>
</tr>
<tr>
<td>42 hours</td>
<td>0.152</td>
</tr>
<tr>
<td>90 hours</td>
<td>0.159</td>
</tr>
<tr>
<td>193 hours</td>
<td>0.195</td>
</tr>
<tr>
<td>435 hours</td>
<td>0.199</td>
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</tbody>
</table>

We have also found that cholesterol irradiated with ultra-violet light is capable of blackening a plate even after an interval of 13 days (72 hours exposure), though the "activity" decreases with the interval after irradiation.

Experiments have been carried out in which cholesterol was irradiated through a sheet of glass suspended above the dish but intercepting the incident light, thus cutting out radiation of wavelength less than about 3200 Å. Twenty-four hours' irradiation in air sufficed to give a definite blackening \( D = 0.049 \), though this was much less than would have resulted from irradiation with the complete mercury vapour spectrum (see Table I).

Control experiments were carried out in the neighbourhood of the arc with the cholesterol shielded from the direct radiation, but no blackening effect was observed. Thus any possible chemical action due to ozone in the neighbourhood of the lamp during the times of irradiation used by us may be regarded as eliminated.

In further attempts to detect possible phosphorescence we have irradiated cholesterol for 245 hours and then exposed it before the slit of a spectrograph of high aperture for 97 hours but not the slightest evidence of a phosphorescence spectrum has been obtained.

(b) X rays: Further specimens of cholesterol (0.1 gm.) were irradiated with X rays (Metallix tube; 150 kv.; 0.3 mm. Cu; f.s.d. 7 cm.), two doses being employed in different experiments. Cholesterol which had received a dose of \( 10^9 \) international roentgens \( (r) \) blackened the plate as previously, but the photographic density \( D = 0.027 \) was less than that obtained with 4 hours' ultra-violet irradiation under our standard conditions. In a later ex-
experiment a dose of $10^9$ r was administered, but the blackening obtained ($D = 0.030$) was very little changed. In both experiments the time of exposure to the plate was 71 hours.

(c) *Beta and Gamma Rays*: A solution of 0.1 gm. of cholesterol in 8.5 c.c. alcohol was irradiated with beta and gamma rays from a monel tube (0.2 mm. wall; 20 mm. active length) containing 35 mg. radium element. This was suspended for 7 days in the solution contained in a quartz test tube. It was noted that the solution fluoresced under the action of the rays, and a spectrum of the light was obtained by exposure to a Raman quartz spectrograph (Hilger F/4) during irradiation.

A general fluorescence was observed between 2200 Å and 5000 Å having a maximum intensity at about 4000–4300 Å. Since a similar fluorescence is shown by a quartz tube containing pure ethyl alcohol subjected to the radiations from the 35 mg. radium needle, and is also shown to a less extent by the quartz tube itself, this fluorescence cannot be wholly attributable to the presence of the cholesterol.

The activation experiment was repeated using solid cholesterol on which two 5 mg. radium needles (0.2 mm. monel wall; active length 10 mm.) were laid for 18 days. The solid also emitted a faint fluorescence under the action of the beta and gamma rays.

Both of these specimens were tested for their effect on a photographic plate. The irradiated solid was found to be active ($D = 0.057$), but the solution gave entirely negative results, both specimens being exposed for 95 hours.

*Effect of Filters*: In order to ascertain the nature of the action on the photographic plate our ultra-violet light experiments were repeated, interposing a glass seal 1.72 mm. thick between the irradiated cholesterol and the plate. Preliminary spectroscopic work showed that the limit of transmission of the glass lay at about 3200 Å. It was found that the "phosphorescence" was completely cut off by the glass.

This is in agreement with Roffo's experimental results, but if the cholesterol were emitting radiation of the wavelength quoted by him (3522 Å and 3572 Å), it would be expected to pass through the glass quite easily. We next used as a filter a quartz plate 2.31 mm. thick, again tested spectroscopically and found to transmit easily to 2000 Å. The quartz is as effective as glass in preventing the photographic blackening and makes its explanation in terms of ultra-violet phosphorescence untenable unless the wavelength of the emitted radiation is below the transmission limit of quartz.

In a further experiment cellophane, 0.02 mm. thick, replaced the glass or quartz. This filter was found to reduce somewhat the action on the plate, though undoubted blackening has been observed even with a double thickness of cellophane.

A number of other workers have found similar filtration effects (Stříteský, 22; Vollmer, 25; Beck, 1; Hugouenq, 11); both quartz and glass usually being found impervious. Though Roffo (16) states that the effect is transmitted through quartz, his published photographs are inconclusive. Stříteský (22) noted that the blackening effect of the X-irradiated cholesterol was similarly eliminated by glass, quartz, rock salt, gelatine and paper.
Ionisation: It was reported by Roffo (17) that air in the neighbourhood of heavily irradiated cholesterol is ionized. His experiments were carried out with a commercial X-ray dosimeter adapted for the purpose. The sensitivity was of the order of 10^{-10} amp. We have carried out similar experiments with more sensitive apparatus, employing two types of instruments: (a) a standard alpha ray electroscope and (b) an apparatus dependent upon the use of a Lindemann electrometer (sensitivity 200 div/volt) coupled to a large ionisation chamber inside which the cholesterol was placed. The latter instrument is certainly capable of measuring 10^{-14} amp.

In spite of several attempts we have been unable to detect any ionisation in the vicinity of specimens of cholesterol irradiated with ultra-violet light for 24 and 550 hours respectively.

Similarly Huguenq (11), using an instrument capable of measuring 10^{-13} amp., found no ionisation in the vicinity of irradiated cholesterol. In view of the very small ionisation produced by ultra-violet light in air, it would, in fact, require a very powerful source of ultra-violet phosphorescence to produce ionisation of the order which could be detected in Roffo’s experiments.

Shadow Experiments: If the photographic blackening were due to electromagnetic radiation emitted by cholesterol, it would be expected that a sharp shadow would be cast by an object placed between the source and the photographic plate. We have carried out numerous experiments with various geometrical arrangements of source and object, but no such effect has been observed. The conditions were carefully studied and as a sufficiently small "point" source is difficult to obtain while at the same time securing appreciable blackening, experiments were carried out with a "line source" of irradiated cholesterol above which, at a distance of 45 mm., a brass wire 1.6 mm. diameter was fixed. Although the wire was accurately parallel to the line source, there was no evidence of a shadow, but a diffuse general blackening was observed on a plate at 13 mm. above the wire. Cholesterol was also arranged in a definite pattern in the dish and somewhat more intense blackening was noticed immediately above it during a normal length of exposure, but after prolonged exposure the blackening became almost uniform. The variation of blackening at different points on the plate was certainly less than expected from inverse square law calculations.

Evidence of Chemical Nature of Active Agent

Viewed as a whole the experimental evidence so far obtained does not support the theory that the blackening is due to ultra-violet phosphorescence, but points rather to the production of some effective chemical agent in the cholesterol during irradiation. Experimental work to test this theory was therefore commenced.

Specimens of cholesterol were placed in a dish fitted with a brass lid containing a quartz or glass window. This container was sealed with picein and could then be evacuated to a pressure of approximately 0.2 mm. Hg by means of a rotary oil pump. After irradiation under reduced pressure for 58 hours the cholesterol was exposed to a photographic plate in vacuo for 76 hours. A very slight blackening (D ≈ 0.007) resulted, whether the chole-
terol had been irradiated through a window of quartz or glass. Control experiments in which the cholesterol was irradiated in air at normal pressure and exposed in vacuo gave considerably more blackening ($D = 0.048$). The residual activity in vacuo may be due to difficulty in removing traces of oxygen from the cholesterol.

Previous workers have found similar results. No photographic effect was observed after ultra-violet irradiation of cholesterol in nitrogen (Roffo, 18), in hydrogen, or in vacuo (Roffo, 18; Hamano, 5), or in CO$_2$ (Stříteský, 22). Hamano (6) similarly found no effect from cholesterol irradiated with x-rays in an atmosphere of CO$_2$, while both Stříteský (22) and Roffo (18) observed that the substitution of oxygen for atmospheric air increased the ultra-violet activation.

It was shown by Stříteský (22) that air passed through a vessel containing irradiated cholesterol would itself blacken a photographic plate, evidently indicative of the transference of an active gas from the site of irradiation.

![Figure 1](image)

The possibility of the diffusion of the blackening agent was tested by us in a series of experiments similar to those of Hugounenq (11), using a small glass vessel having an orifice at some distance from the irradiated cholesterol and of the shape shown in Figs. 1a and b.

Cholesterol irradiated in the standard dish for 10 or more hours was transferred to this vessel and a photographic plate placed over the orifice at A. Long exposures were necessary to obtain any effect on the plate, but distinct blackening was observed after 113 hours. The results of experiments with varying relative positions of the aperture and cholesterol were somewhat conflicting, on some occasions greater blackening being obtained when the aperture was lowest (Fig. 1a), and in others when the relative positions of cholesterol and aperture (Fig. 1b) were reversed. There is no doubt, however, that the active agent can diffuse around the bent neck of the vessel to the plate.

**Effects of Heating**

A considerable rise in temperature of the cholesterol is observed during the irradiation with ultra-violet light, a mercury thermometer placed near the cholesterol reaching in our experiments $40-45^\circ$ C. as a maximum. As other workers (Stříteský, 22; Hugounenq, 11; Henning, 9) had observed that the blackening is increased when the irradiation is carried out at higher temperatures, we have performed experiments on the effects of heating alone. Specimens of cholesterol were heated in a small electric furnace in the dark to temperatures of 65, 95, 127, 164 and 195$^\circ$ C. each for a period of 17 hours.
and were subsequently exposed for 144 hours to photographic plates. No blackening was observed with those specimens kept below the melting point of cholesterol (148° C.), but those heated to 165° C. and 194° C. produced definite blackenings which were nevertheless much smaller than would have been observed after ultra-violet irradiation of corresponding duration. It is concluded, therefore, that the rise of temperature of the cholesterol during the ultra-violet irradiation is of itself not sufficient to produce a photographic action.

The activity of melted specimens of cholesterol has been previously observed by Stříteský (22) and Roffo (18).

**Chemical Theory of "Activation"**

It will be seen that the photographic blackening effects obtained by us may be interpreted in terms of the production of an active gas by the interaction of atmospheric oxygen and cholesterol under the stimulus of radiation. Phenomena of this kind are well known under the general term "Russell effect." Russell (20) showed that a large number of substances, such as metals, terpenes, certain essential and vegetable oils, etc., will affect a photographic plate and that this effect may be transmitted through various filters (gelatine, celluloid, india rubber, etc.) but not through quartz, glass, or mica. Russell also demonstrated that the blackening is due to the liberation of hydrogen peroxide formed by the action of atmospheric air on unstable peroxides or ozonides to which the active substances give rise when in contact with oxygen. Various other workers have also shown that H₂O₂, in common with a number of other liquids and gases, will blacken a photographic plate (Eder, 3; Precht, 14; Machu, 13).

We therefore repeated many of our experiments, replacing the irradiated cholesterol by a small piece of filter paper soaked in a solution of H₂O₂, and found that intense blackening is easily obtained. If the concentration of H₂O₂ is too high, "reversal" or "solarisation" is observed. For instance, a normal blackening was obtained with 0.02 c.c. of a 1/10 dilution of 20 volume H₂O₂ solution, and "reversal" when 0.15 c.c. of the same solution was employed. We have observed the same effects through cellophane and black paper. There is some doubt whether similar blackening may be caused by ozone, the evidence on the whole being against its photographic activity (Richarz, 15; Dony-Hénault, 2; Uhrig, 23; Gunckell, 4; Villard, 24; Schaum and Braun, 21; Eder, 3).

We have noticed definite changes in the cholesterol after irradiation, as for instance a yellow colouration under the action of ultra-violet light (but not from X rays up to the limit of dosage reached, 10⁶ r); depression of melting point from 148° to 105–120° C.; changes in solubility in alcohol, lard, and other solvents; loss in weight. A number of these effects have been previously observed in other investigations (Roffo, 18; Stříteský, 22). The presence of an aldehyde and a peroxide in irradiated cholesterol has been reported by Hugounenq (11) and Stříteský (22), and changes in the iodine and acetyl numbers of cholesterol have been noted by the latter.

The formation of an ozonide in cholesterol on irradiation with ultra-violet
light would furnish a further example of a well known type of reaction in unsaturated compounds. It is exemplified in olive oil, cod-liver oil, butter, oleic acid, camphor, triolein, terpenes, indol and scatol, etc. (Stříteský, 22; Kohl, 12; Haxthausen, 8; Hamano, 5; Beck, 1; Vollmer, 25; Hugounenq, 10, 11; Henning, 9; Russell, 20), all of which will reproduce the photographic blackening phenomenon. Ozonides of oleic acid and of a number of other unsaturated compounds have been prepared, notably by Harries (7). We have ourselves noted that after irradiation the more unsaturated 7-dehydrocholesterol causes considerably more blackening than cholesterol itself ($D = 0.3139$ in 4 hours under standard conditions, whereas for cholesterol $D = 0.0479$).

**Absorption Spectra**

Further evidence of chemical change has been obtained by a study of the absorption spectra of cholesterol solutions prepared from non-irradiated, irradiated, and heated samples. The results are preliminary, but it appears that on irradiation of cholesterol with ultra-violet light a substance is formed having an absorption maximum at about 2350 Å. Cholesterol heated above its melting point has a diminished solubility in alcohol, while irradiated specimens appear to have an increased solubility in that medium. An ethereal solution of the alcohol-insoluble fraction of a specimen heated to 195° C. for 17 hours in air, in the dark, shows a band at about 2550 Å. The alcoholic solution of the heated specimen has the band at 2350 Å and a "step-out" at 2600 Å, probably due to the presence of a small quantity of the ether-soluble component. The spectroscopic examination of irradiated and heated cholesterol specimens is being continued.

Roffo (19) has published absorption spectra from which he concludes that the solutions of irradiated or heated cholesterol "present absorption bands in the ultra-violet part, which correspond to the substances containing phenanthrene nuclei such as are observed in the cancer-producing bodies, derivatives of coal tar" (p. 452). The published spectra are purely qualitative, however. They are obtained with a discontinuous source of ultra-violet light which makes photometric measurements impossible, and do not appear to justify the conclusions drawn.

**Conclusions**

The nature of the change in cholesterol on irradiation has not yet been fully elucidated. We have found no evidence that the photographic blackening due to irradiated cholesterol is due to an electromagnetic radiation emitted as phosphorescence, but the facts are most readily understood if cholesterol forms an unstable ozonide, with the subsequent production of $\text{H}_2\text{O}_2$, which is the immediate active agent on the photographic plate.

In view of the presence of cholesterol in the skin, and the undoubted production of skin tumours as a result of irradiation with ultra-violet light and X rays, further work on the chemical changes in cholesterol consequent on irradiation, as well as a search for possible carcinogenic products, is in progress.

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3 We are indebted to Dr. C. L. Hewett for the preparation of this compound.
REFERENCE