The Croonian Lectures

ON

RADIO-ACTIVITY AND CARCINOMA: AN EXPERIMENTAL INQUIRY.

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By W. S. LAZARUS-BARLOW, M.D., F.R.C.P.,

DIRECTOR OF THE CANCER RESEARCH LABORATORIES, THE
MIDDLESEX HOSPITAL.

[WITH SPECIAL PLATE.]

LECTURE I.

[AFTER thanking the College for the honour it had done him in inviting him to deliver the lectures, pointing out that the inquiry was still incomplete and that contributions to the entire research had been made by numerous workers in the Cancer Research Laboratories of the Middlesex Hospital, the lecturer proceeded as follows:]

The leading idea throughout the whole research has been the undoubted fact that workers with x rays are liable to suffer from carcinoma of the hand. It is unnecessary to labour this point in view of the conclusive evidence furnished by my former assistant, Mr. C. W. Rowntree, in his Hunterian Lecture before the Royal College of Surgeons of England on March 17th, 1909. From a careful examination of the microscopical sections shown by Mr. Rowntree, I am convinced that he is right in describing the x -ray condition which may follow on a somewhat severe and prolonged dermatitis occasioned by exposure to x rays as a squamous cell carcinoma.

Into the question of the relationship of the x rays to the carcinoma, whether it is specific and direct or non-specific and, so to speak, accidental, I do not propose to enter here. It will be more convenient to deal with the whole subject at the conclusion of the lectures. For the moment it will suffice to allow that exposure to x rays and carcinoma of the hand are closely related in certain cases.

The next idea of importance in connexion with the research is that for many years certain physical agents have been considered by the profession to be causally related to cancer of special sites. Thus clay pipes have been associated with cancer of the lip, paraffin and tar with cancer of the hands and arms of workers with these substances, soot with cancer of the scrotum in chimney sweeps, gall stones with cancer generally and particularly with cancer of the gall bladder. In addition, betel-nut chewing has been associated with cancer of the cheek, especially in Ceylon and the East, where the habit is common; use of the kangri has been associated with "kangri cancer" in Kashmir, while bilharziosis in Egypt has been held accountable for cancer of the bladder. Similarly it has been noted that cancer occasionally develops on old scars, on the floor of chronic ulcers, and on the site of lupus.

An approximation of these two ideas suggested the inquiry whether the clay pipes, the paraffin, tar, soot, etc., emit x rays or give other evidence of radio-active properties. If the answer to this question were in the positive, a single factor would be demonstrated to be present in a group of physical agents differing to the widest degree from one another, and the probability that each particular variety of cancer depended upon the physical agent usually inculpated would be strengthened, while presumption would be raised for the proposition that the common factor in all the physical agents—that is, their radio-activity—is causally related to the common factor in the pathological conditions to which they all are supposed to give rise—that is, the cancer.

It is remarkable that the cancer in all the cases that have been mentioned above, with the sole exception of that affecting the gall bladder, is squamous cell carcinoma. Moreover, the histological diagnosis is almost invariably to be made with great ease, Malpighian cells, prickle cells, and epithelial pearls being conspicuous. For this reason the present research has been confined to the group of carcinomata, meaning thereby squamous cell, columnar cell, and spheroidal cell, carcinomata as determined by microscopical examination.

The research, therefore, resolved itself into a determination whether clay pipes, etc., and carcinomatous material give evidence of radio-activity.

The two chief criteria of radio-activity relied on by the physicist are (1) the action of the substance in question upon a charged electroscope and (2) its action upon a photographic plate in the dark. Of these criteria action upon a photographic plate in the dark is well known to the medical profession in the production of skiagraphs, but it is upon the electrical properties that the physicist places more reliance.

Owing to the fact that the x -ray tube only emits gamma radiations, while such radio-active substances as radium, uranium, and thorium emit alpha particles, beta rays, and gamma rays, while they also are far more complex than their chemical names would suggest, it became necessary to consider these substances as well as the x rays. It is clear that a substance may belong to the radio-active group and yet fail to produce gamma radiations. Thus, "chemically pure" uranium oxide emits alpha particles and beta and gamma rays, but the physicist can separate a uranium oxide from this, which is indistinguishable in its chemical reactions, but differs electrically in emitting alpha particles alone. Such uranium is without effect upon a photographic plate, though it affects a charged electroscope.

In addition to their power of affecting a photographic plate in the dark, radio-active substances emitting gamma rays with their high velocity and penetrating power, or beta rays with their somewhat lower but still great velocity and penetrating power, have the property of affecting the photographic plate though separated from the film by such substances as aluminium, mica, etc. This property forms an important means of differentiating the action of a recognized radio-active substance, such as radium or thorium, from other substances which apparently fail to possess the power of acting through a screen. How far this differentiation between radio-active and non-radio active substances may be carried by this method will be discussed later.

Electrically, the recognized radio-active substances have the property of accelerating the discharge of an electroscope whatever the sign of the electricity with which its leaves are charged. This property depends upon the fact that the radio-active substance by the alpha particles (helium atoms charged +), beta rays (negative electrons) and gamma rays which it emits, ionizes the air in the electroscope, breaking the molecules up into their constituent atoms, each of which is electrically charged + or -. These charged atoms collide with the charged gold leaves, and such as are of opposite sign to the charge on the leaves neutralize a corresponding amount of electricity on the leaves. The greater the ionization of the air in a given time the more rapidly the charged leaves fall together, and the greater the radio-activity of the substance under investigation is said to be.

From what has been said it follows that the various substances dealt with in the present investigation call for consideration (1) as regards their action when exposed to a photographic plate in the dark, and (2) as regards their influence upon the rate of leak of an electroscope (a) charged + and (b) charged -. In addition, it is necessary to determine the same points in regard to non-carcinomatous substances, and, further, to determine the influence of radio-activity upon the division of cells by direct experiment.

In the present lecture I propose to show that certain animal tissues and other substances have the power of acting upon a photographic plate in the dark. In the second lecture I shall consider, as far as possible, the properties and nature of the substances in the tissues which possess this power. The third lecture will be devoted to a consideration of the electrical properties of the carcinomatous and non-carcinomatous substances; while the fourth lecture will deal with the influence of radio-activity upon cell division, and will include a short general survey of the bearings of the experiments.

THE SKOTOGRAPHIC ACTION OF CERTAIN ANIMAL TISSUES AND OTHER SUBSTANCES.*

If a sample of human liver be minced, dried in the hot-air oven at 100–110° C., reduced to a powder and some of

* I have ventured to use the word "skotograph" to signify the effect that is produced upon a photographic plate by certain substances in the dark (*skotos*, darkness), on the analogy of "photograph," "radio-graph," and "skiagraph." The effects to which attention is about to be directed are certainly not *photographs* in the ordinary sense of the word, neither are they *skiagraphs*, while to term them *radiographs* would be to assume the point at issue.

this powder be placed on a photographic film in [the dark and the plate and powder be kept in contact in complete darkness for a certain length of time, it will probably be found, on developing the plate, that the former situation of the powder is indicated by a more or less considerable, punctate, deposition of silver. Prior to development the photographic plate shows no alteration. Similarly, if a "pure" cholesterol gall stone be exposed to a photographic plate in the dark, subsequent development of the plate will probably show an intense deposition of silver which occupies a larger area than corresponds to the area of the calculus in contact with the plate (Figs. 1 and 2). Skotographic action is not manifested by all animal substances, for example, it is rarely produced by spleen or lung dried and powdered as above, while numerous experiments made with uric acid calculi, paraffin wax, and clay pipes have shown that these substances possess no skotographic power. Similarly certain varieties of micro-organism possess marked skotographic power, while others appear to be totally deficient.

It is unnecessary here to enter into the details of the method for obtaining skotographs, or the precautions to be taken in order to eliminate the action of light or of any outside influence of a recognized radio-active nature. These are all given elsewhere.¹ It must be stated, however, that the values assigned to the various plates showing skotographic action were determined by comparing them with the effect produced in a photographic plate by allowing x rays to act upon it through a Benoit's gauge. Since the gauge consists of aluminium graduated in thickness from 1 mm. to 12 mm. a graduated deposition of silver takes place on the plate. It was possible to graduate the degrees of silver deposition by this means from 0 to 6; and since the same skiagraph was used throughout, the values given for the various substances and tissues are comparable among themselves.

Tissues from Non-malignant Cases.

In all, tissues from 115 bodies of persons dying from some cause other than malignant disease were examined skotographically. In most instances the examination included samples of liver, kidney, spleen, and lung. The patients were of both sexes and of all ages; they were grouped for purposes of comparison into the following age-periods:

Under 2 years of age.	35 to 45 years.
2 to 20 years.	45 to 55 years; and
20 to 35 years.	Over 55 years of age.

This grouping of age-periods was made in order to institute a comparison with similar tissues derived from cases of carcinoma. In addition to the above, specimens of brain, pancreas, breast, liver of sheep and of ox, tissues of mouse, guinea-pig, and rabbit were examined skotographically, but no reference to the results will be made here.

The results of the investigation are summarized in the following table:

Table showing Mean Skotographic Values of Liver, Kidney, Spleen, and Lung in 115 Non-malignant Cases.

Age.	Males.				Females.			
	Liver.	Kidney.	Spleen.	Lung.	Liver.	Kidney.	Spleen.	Lung.
Under 2 years ... (10 males, 10 females)	1.7	2.3	0.8	0.9	3.1	1.6	0.9	1.1
2 to 20 years ... (9 males, 6 females)	2.1	2.4	0.6	0.4	3.2	2.8	0.4	0.6
21 to 35 years ... (12 males, 10 females)	3.0	2.6	1.1	0.75	3.7	2.3	0.5	0.6
36 to 45 years ... (9 males, 10 females)	2.7	1.6	0.3	0.0	3.4	3.9	0.9	0.5
46 to 55 years ... (10 males, 10 females)	3.3	1.4	0.5	0.0	4.2	2.7	0.8	0.8
Over 55 years ... (11 males, 8 females)	3.9	2.0	0.5	0.9	2.6	2.5	1.1	0.25
Means at all ages ... (61 males, 54 females)	2.8	2.1	0.6	0.6	3.4	2.8	0.8	0.6

From this table it appears that liver and kidney possess a far higher skotographic power than spleen or lung, and that the female tissue in each case had a greater skotographic power than the corresponding male tissue.

Owing to the large size of the liver and to the fact that it exerts the largest amount of skotographic effect, the figures for it may be examined more closely. It is seen

that the skotographic power of the liver in males increases steadily throughout life, with the exception of a fall at the age-period 36 to 45 years. In females this rise in skotographic power of the liver, including the fall at the age-period 36 to 45, is also seen. But whereas the values for the male reach their highest in the age-period "over 55 years," this same age-period in females witnesses a remarkable fall in skotographic power of the liver. Further reference to this point will be made in the last lecture.

The small mean values obtained for the spleen and lung are due to the fact that occasionally these organs exert a definite effect. Two-thirds of the total number of spleens examined skotographically, and two-thirds of the total number of lungs, were absolutely without skotographic power. On the other hand, only one-fifth of the total number of livers examined skotographically were without effect.

Carcinomatous Material.

The skotographic value of the primary mass has been determined in 17 cases of carcinoma; of secondary growths, 29 specimens from 24 cases have been examined; 11 of the cases were male, 30 female. Two primary and four secondary growths were without skotographic effect, or the effect was doubtful. The mean skotographic value of the male primary masses was 2.0, male secondary masses 2.5; that of the female primary masses was 3.6, female secondary masses 3.2. Hence the superiority of the skotographic value of female tissue over that of male tissue obtains also in the case of carcinoma (Figs. 3 and 4).

Tissues from Cases of Carcinoma.

The skotographic values of the liver, lung, kidney, and spleen have been determined in 72 cases of carcinoma, 41 of which showed important metastases, and 31 insignificant metastases or none at all. The cardinal facts that liver and kidney exert a greater skotographic power than lung and spleen, and that the skotographic power of a female tissue is greater than that of the corresponding male tissue, are shown in this class of case as in that in which there is no malignant disease. But it appears that the carcinomatous condition is not without effect upon the skotographic power of the various tissues. This is best seen by considering the case of the liver. The effect of carcinoma upon the skotographic power of the liver may be considered in two ways:

1. Livers, themselves free from metastasis, may be compared skotographically according as they are derived from cases in which the total mass of carcinomatous material in the body is great or is small.

2. A comparison may be made between the hepatic tissue of non-malignant cases and the hepatic tissue of carcinomatous cases in which the liver is the seat of numerous large metastases.

These two different methods of approaching the question agree in indicating that carcinoma augments the skotographic power of the liver in males and decreases it in females. In the following table are given the figures upon which this statement is founded.

Table to show the Influence of Carcinoma on the Skotographic Power of the Liver.

Age.	Males.				Females.			
	Non-malignant Cases.	Growth Present in Liver.	Much Growth in Body.	Little Growth in Body.	Non-malignant Cases.	Growth Present in Liver.	Much Growth in Body.	Little Growth in Body.
Under 35 ...	3.0	—	—	—	3.7	2.0	1.7	4.0
36-45 ...	2.7	3.5	2.75	—	3.4	3.5	3.7	4.25
46-55 ...	3.3	3.5	3.5	1.8	4.2	—	3.7	4.0
Over 55...	4.0	4.5	4.5	3.8	2.6	2.5	2.5	2.6

But consideration of the above table shows that the matter is not so simple. For, although male hepatic substance in the neighbourhood of liver metastases is more skotographic than non-malignant liver while the converse is true in the case of the female, and although male

hepatic substance where there is much growth in the body is more skotographic than when there is little while the converse is true in the case of the female, a small amount of carcinoma in the body appears to diminish the skotographic power of the male liver, while it augments that of the female liver. I do not attempt an explanation of these points; indeed, they need confirmation on a much larger number of cases. The regularity with which female liver and male liver act in opposite directions, in the face of what is apparently a single condition—that is, carcinoma—is, however, such that it suggests the existence of some sort of interaction between carcinoma and the skotographic property of the liver. On the other hand, we may be quite wrong in considering carcinoma as a single condition relative to the two sexes; it has already been shown that male and female carcinomatous materials differ in skotographic power.

The only condition in which a thoroughly satisfactory determination can be made of the influence of carcinomatous growth upon the skotographic power of the tissue in which it lies is that in which one of paired organs is the seat of metastasis. This is relatively so uncommon, and when it occurs the chance that the metastasis will be sufficiently large to influence the neighbouring tissue of the organ to any extent is so small that I have only obtained a single example. The case was one of carcinoma of the cervix, and one kidney was the seat of a fairly large number of metastases, while the other was free. The renal substance of the unaffected kidney had a skotographic power of 3, while the renal substance of the affected kidney was absolutely without effect upon the photographic plate. This case, therefore, conformed to the statement made above for the liver that, in the female, carcinoma leads to a diminution of the skotographic power of the tissue in which it lies.

Substances commonly supposed to be causally related to Carcinoma.

Numerous samples of clay pipe, soot, pitch, paraffin wax, metallic arsenic, arsenious oxide, betel nut, cholesterol gall stones, pigment gall stones, renal and vesical calculi, have been examined skotographically, the calculi, renal, biliary, and vesical, being made the subject of an extended research by Dr. Colwell.² Skotographic effect was exhibited by one sample of soot out of two examined, by betel nut on all of numerous occasions, by each of twenty-three specimens of cholesterol gall stone, more or less "pure," in three out of four samples of pigment gall stones examined, the effect being always very slight as compared with the action of the cholesterol calculi, and by thirty out of thirty-eight vesical calculi. Metallic arsenic and arsenious oxide produced effects upon the photographic plates, but inasmuch as the films showed alteration before development the action cannot be regarded as skotographic. On the other hand, none of nine specimens of clay pipe, of numerous samples of paraffin wax, of four samples of pitch from different localities, of several specimens of coal, yielded the slightest trace of skotographic action.

In the case of cholesterol gall stones the action on the plate extends widely beyond the area of the surface of the calculus in contact with the plate, but in the case of all the other substances the area of silver deposition was coextensive with the area of contact between substance and film. This is well seen in the skotographs of vesical calculi, in which rings of silver deposition alternate with rings of no deposition in such a way that a picture is formed of the surface of the calculus by which the skotograph was produced (Fig. 5). When the nucleus of a calculus, or certain laminae, or the entire calculus consists of "pure uric acid," the corresponding part of the film shows a complete absence of skotographic effect. As a rule, calculi or laminae of calculi consisting of ammonium urate, calcium oxalate, calcium phosphate, or ammonio-magnesium phosphate possessed skotographic power; but since instances to the contrary were found Dr. Colwell concluded that the chemical substances in question, *as such*, were not the effective agent. Similarly he found that purified cholesterol obtained from a gall stone which produced a profound effect upon a photographic plate was itself entirely devoid of skotographic power.

Skotographs of betel nut are of especial interest in connexion with the observation made by Dr. Russell, F.R.S.,³ that almost all varieties of wood affect a photo-

graphic plate when placed in contact with it under conditions of total darkness—that is, possess skotographic powers. This power is possessed by the winter and spring woods in different degrees, so that a skotograph of a piece of wood reproduces a picture of the grain of the wood in the same way as a skotograph of a vesical calculus reproduces the appearance of the cut surface of the calculus. Similarly a skotograph of a bisected betel nut shows the peculiar markings of the nut in all their detail (Fig. 6).

LECTURE II.

THE NATURE OF SKOTOGRAPHIC ACTION.

RUSSELL'S researches upon the effect of various woods upon a photographic plate in the dark led him to the conclusion that the deposition of silver is occasioned by traces of hydrogen peroxide formed owing to oxidation of the terpenes and other resinous substances in the wood in the presence of moisture. This strictly chemical action, albeit produced by traces of hydrogen peroxide so minute as to be beyond detection by any other method, is distinguishable from the action brought about by ethereal vibrations, such as those of beta or gamma rays, by the fact that it is completely inhibited by interposing a thin screen of mica between the wood and the film. Russell showed, further, that wood which had been exposed to strong sunlight before being placed in contact with the photographic film produces a far stronger effect than wood which has been kept in the dark.

Under these circumstances it became necessary to determine as far as possible with what constituent or constituents of the animal tissues their skotographic power was bound up, and subsequently whether that power conformed more to the type of a chemical substance, and particularly to hydrogen peroxide, or more to that exerted by ethereal vibrations.

It must be stated at the outset that a complete answer cannot be given to the above questions. It can rather be said what the skotographic power is *not* bound up with than what it *is*. Similarly it will be found that it is impossible to include it definitely in the chemical or the physical group of phenomena, since in certain respects it appears to resemble hydrogen peroxide and in others to resemble an ethereal vibration.

Attempts at Isolation of the Skotographic Substance.

The skotographic substance, or substances—for there is reason to believe that, in the liver at least, two substances exist which differ from one another in certain reactions but agree in affecting a photographic plate in the dark—is not protein, or fat, or carbohydrate, or colouring matter or mineral salts. Each of these constituents of the tissues has been definitely excluded. It is unnecessary to enter into a detailed account of the method in which this has been done, for it has been recorded elsewhere.⁴ The following short account will suffice.

That it is not *protein* is indicated by the fact that it is yielded by liver, for example, and not by spleen, while the same fact shows that it is not dependent upon the "cellularity" of the tissue as such. But the non-protein character can be shown by obtaining a completely protein-free extract of the tissue, and proving that this extract still possesses skotographic power. Fig. 7 shows that this is the case. It was produced in the following way: Liver of sheep was minced and allowed to stand under distilled water for eighteen hours. The watery extract was then acidified, boiled, and filtered. To the filtrate three times the volume of basic lead acetate in solution and suspension was added, and the resulting precipitate was filtered off. Then sulphuretted hydrogen was passed through the filtrate to precipitate the lead, and after the filtrate from this operation had been concentrated to small bulk upon a water bath it was added to twenty times its volume of absolute alcohol and allowed to stand for three weeks. The merest trace of precipitate was formed, and was filtered off, and the alcohol was removed by evaporation. The residual fluid was then used to write certain words on a plain glass plate with a camel-hair pencil, and this plate was exposed to the photographic film under skotographic conditions and separated from contact with the film by strands of cotton. It is seen that this watery extract of liver, in which none of the laboratory tests for protein shows the slightest trace of that material, possesses intense skotographic power. The process for

purification from protein has been checked at every stage, as well as the reagents used, and it is certain: (1) That the skotographic power is not introduced during the process, and (2) that the various precipitates formed are devoid of skotographic power, except in so far as they are contaminated with the corresponding filtrate.

That the skotographic substance is not *fat* as such is shown by the fact that a material which has been rigidly extracted with ether still possesses skotographic power. Thus, all the powdered tissues that were used to arrive at the conclusions given in the first lecture were extracted with pure ether in a Soxhlet apparatus for a minimum of six hours before being examined skotographically. This method extracts the phosphorized as well as the non-phosphorized fats.

Just as the watery extract of a liver, for example, possesses skotographic powers, so it is possessed by an ethereal extract. Into the question of the actual constituent of the ethereal extract which possesses the power it is impossible for me to enter. The chemical analysis I have not attempted, owing to its difficulty.

That the skotographic substance is not *carbohydrate* as such is proved by the facts that some of the extracts by which it is manifested contain carbohydrate while others are devoid of it, that it is manifested by ethereal extracts, and, as will be shown later, that it survives charring.

The fact that the skotographic power is independent of the water-soluble and the ether-soluble pigments of the tissue is shown by the persistence of effect if one of these extracts be decolorized by finely divided animal charcoal.

And, finally, that it is independent of the *mineral constituents* of the tissue—at least those which survive a temperature of 300° C. (see below)—is shown by the fact that the ash of a tissue which possesses marked skotographic power is entirely without effect upon a photographic plate in the dark.

The skotographic power of any tissue is completely removed if it be first extracted with water and subsequently with ether. This appears from the accompanying figure (8), in which the upper fourth shows the effect of the original substance, the second fourth shows the effect of a watery extract, the third that of an ethereal extract made subsequently to the watery; the bottom fourth, over which there is an absence of skotographic action, was exposed to the solid residue after the watery and ethereal extractions. A similar absence of skotographic power characterizes a tissue which has been extracted with acetone, as might be expected, since it dehydrates and also extracts fat.

Just as the skotographic power is contained in the watery and ethereal extracts so it is contained in one made by acetone, and by evaporating with care the constituents of the original skotographic material which are soluble in water and those which are soluble only in strong acetone may be divided. They are apparently the same as those which are contained in the watery and ethereal extracts when these are made separately.

But though it is clear that the skotographic power is not a property of protein as such, there is no doubt that it is a property of certain organic substances, as distinguished from inorganic, that are probably derived from protein. As will be said later the skotographic power is not destroyed even though the substance manifesting it be heated to a temperature of 300° C. Such a temperature chars the substance, and no doubt breaks up much of

the protein, but from it a material, often of polymorphic crystalline character, but highly deliquescent, can be separated which exerts a skotographic effect, and which is still organic in nature, as shown by the fact that it chars on further heating. By repeated solutions in water and precipitation with absolute alcohol this crystalline material can be divided into a honeylike non-crystallizable and a colourless crystallizable portion. The skotographic power resides chiefly, if not entirely, in the honeylike, highly deliquescent material. This material is dialyzable with ease, and carries with it the actual skotographic substance. Further than this analysis has not gone.

CONDITIONS DETERMINING THE MANIFESTATION OF SKOTOGRAPHIC POWER.

It has been shown above that I have not succeeded in isolating the skotographic substance, though it has been stripped of certain materials with which it is usually associated. It has therefore been necessary to investigate the skotographic property upon crude material, and for this purpose the ether-extracted, dried, and powdered original material and watery extracts that have been freed from protein in the way already described, have chiefly been used.

Relation of Substance to the Photographic Film: Contact: Proximity: Screens.

All the photographic effects to which reference was made in the first lecture were obtained by allowing the powdered substances to lie in actual contact with the photographic film, but there is no doubt that they can act at a little distance. This is better seen in considering the watery extracts, for when they were under investigation contact with the film was necessarily excluded. Attempts were made to determine the maximum distance at which recognizable photographic effect is produced by exposing a glass plate bearing the watery extract to the photographic plate at an angle. In this way, and using photographic plates specially sensitized for α rays, it was found that the watery extract used for producing Fig. 7 is still effective when it is separated from the film by a distance of 15 mm.

The question of screens between the material and the photographic film is one of fundamental importance in trying to arrive at a conclusion as to the nature of skotographic action, and must, therefore, be dealt with in some detail.

It may be stated at the outset that interposition of a screen of the order of thickness of that used in examining the effects of recognized radio-active materials such as uranium or thorium between the animal substance under investigation and the photographic film completely eliminates skotographic action. This is true whether the screen be metallic (for example, aluminium) or non-metallic (for example, mica), however intense the skotographic action may be in the absence of a screen, or however long (up to six weeks) the exposure may have been, and even though it had been carried out at 55° C.

On the other hand, the matter is not simple, as the following experiments show:

If a flat section of a betel nut or of liver be coated with a thin film of celloidin and the whole be exposed to a photographic plate in the ordinary way, it will be found that the greatest deposition of silver occurs on that part of the plate which was separated from the substance by the celloidin. That this effect is not due to the celloidin itself is shown by the fact that an old film of celloidin is entirely without skotographic effect, and in the betel nut experi-

DESCRIPTION OF PLATE.

Fig. 1.—Skotograph of dried, powdered, ether-extracted, non-malignant liver.

Fig. 2.—Skotograph of pure cholesterol gall stone. Action occurs well beyond the limits of the calculus.

Fig. 3.—Skotograph of dried, powdered, ether-extracted primary spheroidal cell carcinoma of breast.

Fig. 4.—Skotograph of dried, powdered, ether-extracted pulmonary metastasis from a case of spheroidal cell carcinoma of the breast.

Fig. 5.—Skotograph of a mixed phosphatic and uratic calculus.

Fig. 6.—Skotograph of a section of a betel nut. The dark crescent was at the seat of a film of celloidin two years old.

Fig. 7.—Skotograph produced by a protein-free watery extract of sheep's liver. Material prepared and skotograph taken March 24th, 1908. Compare with Fig. 10.

Fig. 8.—Skotograph showing that the power of affecting a photographic plate in the dark manifested by dried, powdered, non-malignant liver (top quarter) is entirely removed (bottom quarter) by extraction with water and subsequent extraction with ether. The extracts possess strong skotographic power.

Fig. 9.—Skotograph showing that the effective agent in cedarwood oil can be transported in a current of air. The air impinged on the photographic plate at the apex of the triangle of silver deposition.

Fig. 10.—Skotograph taken with protein-free watery extract of sheep's liver used to produce Fig. 7 over a year after its original preparation.

Fig. 11.—Skotograph of a phosphatic calculus removed by Percival Pott (died 1788).

Fig. 12.—Skotographs on Ilford α -ray and on Imperial special rapid plates produced by the same sample of dried, powdered, ether-extracted carcinoma material under identical conditions. Compare with Fig. 13.

Fig. 13.—Skotograph on Ilford α -ray and on Imperial special rapid plates produced by the same sample of sawdust under identical conditions. Compare with Fig. 12.

Fig. 17.—Skotographs of dried, powdered, and ether-extracted testis and ovary of herring. The testis produces the greater deposition of silver. (This specimen will be referred to in Lecture IV.)

Fig. 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

Fig. 7.

Fig. 8.

Fig. 6.

Fig. 10.

Fig. 9.

Fig. 12.

Fig. 11.

Fig. 13.

Fig. 17.

ment the celloidin had been painted on the nut two years before it was exposed to the photographic plate (Fig. 6).

In the second place, if an albuminate of thorium be made by allowing a solution of the nitrate to fall into egg-white, the resulting precipitate after drying and powdering fails to affect a photographic plate through a film so thin as that produced by brushing a minute quantity of egg-white over tissue paper, though a fair deposition of silver occurs when the albuminate is separated from the photographic film by tissue paper uncoated with egg-white. Such an albuminate of thorium contains about 10 per cent. of the radio-active element by weight. Even in this case it is possible that such direct skotographic effect as was produced was due to uncombined radio-active salt, since the precipitate was not washed for fear of hydrolysis. In several instances, where the precipitate with thorium was washed thoroughly with hot water before drying, it failed to produce an effect upon the photographic plate even though it were actually in contact with it during exposure; nevertheless, such samples of albuminate showed the presence of considerable amounts of thorium on analysis.

Hence under special conditions a vegetable or animal tissue may produce skotographic action through a thin film of celloidin and a recognized radio-active substance may fail to affect a plate through a film of egg-white of probably even a greater tenuity.

Effect of Temperature.

Within the limits of -3°C . and 100°C . it may be said that the skotographic action of the tissues is independent of the temperature provided that the exposure be longer at the lower temperatures. Thus a degree of silver deposition which can be attained by exposing a cholesterol gall stone for eighteen hours at 55°C . to the photographic plate can only be obtained after an exposure lasting for weeks at the room temperature, and probably for several months in the ice chamber at -3°C . Exposure of the substance to a plate at a dry heat of 100°C . is an unsatisfactory procedure, but if the exposure be shortened to about half an hour, silver deposition is not general, but is confined to the part with which the substance under investigation was in contact. The general temperature at which the skotographs in this research were taken was 55°C ., and the length of exposure was eighteen hours. Except in certain cases, Imperial special rapid plates were used, and development was by 1 in 20 Rodinal.

I have dealt above with the temperatures at which the substance has been exposed to the photographic plate, but the substance itself may be exposed to a much higher temperature without undergoing complete loss of skotographic power. I have not been able to determine with exactness the highest temperature to which a skotographic substance may be raised without undergoing complete loss of its power; but it is certain that a skotographic watery extract painted in a thin layer upon a No. 1 coverslip and exposed to dry air at about 280°C . for five minutes will probably afford evidence of skotographic power, while a similar coverslip exposed to about 300°C . will not. In any case it is certain that the skotographic power is not destroyed by a temperature (about 200°C .) which chars the substance itself and therefore in large measure disintegrates it.

Light and Darkness.

Leaving on one side such points as the fact that the change in the photographic plate is not due to the hygroscopic character of the partially purified skotographic substance, the gradual disappearance of the power on continued exposure to air, the curious way in which occasionally there occurs a recrudescence of skotographic power in a watery or acetone extract, the fact that the power is independent of the acidity, basicity, or neutrality of a watery extract—points which have been made the subject of many experiments and are of great theoretical interest—I pass to certain experiments instituted in order to compare the skotographic power of animal tissues with that manifested by woods and held by Russell to depend upon the presence of hydrogen peroxide.

Russell found that the skotographic power of a wood is profoundly influenced according as it has been exposed to light, and especially direct sunlight, or has been kept in darkness before exposing it to the photographic plate. If exposed to light, the skotographic power is always intensified; if kept in darkness, it is always diminished. In the case of animal tissues and of calculi of different kinds

this point has been carefully investigated by myself and by Colwell respectively. We agree in finding that our substances do not show the slightest difference in skotographic power, even though they may be exposed to direct sunlight or be preserved in total darkness for a period of six months before placing them in contact with the photographic film.

Volatility.

Inasmuch as the skotographic power of an animal substance disappears after about a fortnight if it be exposed freely to air, it was thought that the effect might be due to some volatile product. Apart from the fact that it is difficult to imagine a volatile substance which can withstand a temperature of 300°C ., below which the skotographic power is not destroyed, the question has been approached directly. By causing a current of air which had passed over cedar-wood oil, or clove oil, or oil of cajuput, or oil of origanum, to impinge upon a photographic film, it was found that volatility, as such, is not the effective cause of a skotographic effect since a skotographic effect was produced in the case of air charged with the volatile products of oil of cedarwood alone. Further, it has never been found possible to carry over the skotographic power of any of the substances used in the present research although they frequently give off volatile products with powerful odours that can be detected in the stream of air impinging upon the photographic film by the sense of smell. Fig. 9 shows the skotographic effect of a current of air charged with the volatile products of cedarwood oil.

Is Skotographic Action essentially due to Hydrogen Peroxide?

The experiments made bearing on this question were of two kinds. In one set Colwell took a cholesterol gall stone, which yielded a profound skotographic effect, and prepared from it a sample of purified cholesterol. Examined skotographically, this purified cholesterol was absolutely without effect. He now exposed a thin film (made by dissolving cholesterol in chloroform, pouring it on a glass plate, and allowing the chloroform to evaporate) to an atmosphere fully charged with hydrogen peroxide vapour for twenty-four hours at a temperature of 53°C . Similarly he exposed to hydrogen peroxide vapour a vesical calculus normally possessing no skotographic power. He found that now both cholesterol and vesical calculus affected the plate profoundly, but that within twenty-three hours of their removal from the atmosphere of hydrogen peroxide they had completely regained their original freedom from skotographic power. Probably the last traces of hydrogen peroxide had disappeared long before twenty-three hours, but of this there is no proof. This experiment bears upon the length of time during which skotographic power is held by a substance—a subject which will claim our attention shortly.

The second set of experiments concerns the so-called peroxydase effect shown by certain tissues. In its best-known form the peroxydase reaction is represented by the guaiacum test for blood. It depends upon an oxidation of the traces of guaiaconic acid present in the freshly-prepared tincture of guaiacum by oxygen that has been set free from hydrogen peroxide by a substance (peroxydase) present in the substance undergoing the test.

In examining a large number of dried and powdered tissues from this point of view, as well as from that of their skotographic power, I found, speaking generally, that the degree of peroxydase reaction manifested by them and measured against a standard graduated series of blue solutions, varies inversely with the skotographic power.

Table showing Results in Two Series of Ten Consecutive Specimens.

No.	Tissue.	Photographic Value.	Peroxydase Value.	No.	Tissue.	Photographic Value.	Peroxydase Value.
220	Kidney	6	0	320	Liver	6	2
221	Lung	4	6+	321	Kidney	3	4
222	Liver	3	5	322	Liver	5	3
223	Kidney	4	0	323	Kidney	3	5
224	Lung	2	4	324	Liver	5	0
225	Spleen	3	6+	325	Kidney	2	6
226	Liver	4	0	326	Liver	4	1
227	Kidney	2	0	327	Kidney	2	4
228	Spleen	2	4	328	Liver	3	5
229	Lung	0	2	329	Kidney	2	6

This fact would seem to indicate that a tissue possessing a large amount of a substance which splits up hydrogen peroxide has but little skotographic power, and conversely that a substance exerting a great skotographic power contains but little of the hydrogen peroxide splitting property. From this it is but a step to the conclusion (with Russell) that the skotographic property is in reality nothing more than a manifestation of the action of hydrogen peroxide upon a photographic plate.

But that this conclusion is not justified is shown by two sets of observations.

First, I have found that skotographic effect may be shown by watery extracts which from beginning to end of their exposure to the photographic plate contain peroxydase and are therefore in a condition to split up any hydrogen peroxide that may be formed immediately on its production. And secondly, the inverse relations of peroxydase and skotographic power do not obtain for dried and powdered carcinomatous material, for such material, whether it produce a great skotographic effect, or a slight one or no effect at all, is devoid of peroxydase. Amongst seventeen samples of dried and powdered carcinomatous material examined for the purpose only four contradictions were found. One of these is explicable by the fact that the growth was a pulmonary metastasis, and probably contained some pulmonary tissue which has, normally, a high peroxydase coefficient, and the remaining three, all of which were hepatic metastases, gave the least recognizable amount of peroxydase.

Duration of Skotographic Power.

Strictly speaking, the length of time during which a substance retains skotographic power can only be determined in the case of such materials as have actually been prepared. Thus, the material used in the production of Fig. 7 was prepared on March 24th, 1908, and inasmuch as a skotograph taken with the same substance a year later (Fig. 10) also shows effect, it is clear that the substance in question retained the power for that length of time. Numerous skotographs taken at intermediate times showed that the power was present throughout.

From the time of its preparation the substance was kept in a clear stoppered bottle in the laboratory without any special precautions. This experiment renders it probable that a substance which at any time shows skotographic power also possessed it at the time of its original formation, and this assumption becomes of importance in connexion with the skotographic power of calculi. Colwell has shown that cholesterin calculi will often produce intense skotographic action directly after their removal from the body, but he has also shown that intense skotographic power may be shown by a cholesterin gall stone that had been preserved in the museum of the hospital for over twenty-five years. In the case of vesical calculi he goes much further; for a calculus removed by Percival Pott, who died in 1788, shows well-marked skotographic power (Fig. 11), and faint but definite skotographic power is shown by a calculus removed from the body of a pre-dynastic Egyptian mummy, and therefore at least 7,000 years old.*

Use of Photographic Plates Specially Sensitized for X-ray Work.

Recently plates have been put upon the market which are specially sensitized for *x*-ray work. It was thought that a series of comparative tests of the same substances, when exposed to these and to ordinary plates, might indicate whether the skotographic power of the substance under examination was more akin to light or to *x* rays. Dried, powdered, and ether-extracted carcinomatous material from a metastasis in the liver and ordinary deal sawdust were examined in this way. It was found that the first appearance of action on the *x*-ray plate occurred at the end of the sixth hour of exposure, and on the Imperial special rapid plate not till the end of the seventh hour (the powdered growth being separated from the photographic films by thin layers of filter paper), and that the ultimate density of silver deposition was

greater also on the *x*-ray plate. When the material was not separated from the photographic film by filter paper, the first appearance of action was observable on both plates at the end of the first hour of exposure; but whereas it was quite marked on the *x*-ray plate, it was only just distinguishable on the other. In the case of sawdust these differences did not show themselves, the deposition of silver on the ordinary plate being very nearly, though not quite, as intense as that on the *x*-ray plate (Figs. 12 and 13).

Skotographic Action of Bacteria.

MacCormac⁵ has published the results of an investigation into the question whether skotographic action is exerted by bacteria. He examined in this respect 27 different varieties of micro-organisms, and from his table they may be grouped as below:

GROUP I. NO SKOTOGRAPHIC ACTION.

B. Rabinowitch, *B. Cohn I*, *B. Gärtner*, *B. phosphor. albiensis*, *B. intestinalis sporogenes*, *B. Danysz*, *B. mycoides*, *B. coli*, *V. cholerae*.

GROUP II. SKOTOGRAPHIC ACTION FICKLE.

Sarc. flava, *B. Flexner*, *V. Metchnikovi*, *B. Hoffmanni*, *B. megaterium*, *M. citreus agilis*, *B. pyocyaneus*, *B. phlei II*, *B. prodigiosus*, *B. typhosus*.

GROUP III. SKOTOGRAPHIC ACTION USUAL AND CONSIDERABLE.

Staph. pyogenes aureus, *Staph. pyogenes albus*, *B. tuberculosis*, *B. tuberculosis bovinus*, *B. diphtheriae*, "pathogenic throat yeast."

The importance of these researches will be indicated later.

SUMMARY.

In this and the preceding lecture I have shown that certain of the tissues of the human body show skotographic action (liver, kidney), while certain do not (spleen, lung); that a larger amount of this property resides in female tissues that possess it than in male of the same kind; that the amount present in the liver increases from infancy to the age of 55 years, with an unexplained drop in both sexes during the age-period 35-45, and that after 55, while it increases still further in the case of males, in females it undergoes a sharp diminution. I have shown that carcinomatous material possesses skotographic power, whether it be primary or secondary, and that female carcinomatous tissue has a higher skotographic value than male; and that the existence of a mass of carcinoma in a tissue modifies its normal skotographic value. I have found that certain substances commonly supposed to be causally related to carcinoma possess the skotographic power to a high degree (cholesterin gall stone), while others are devoid of it (clay pipe, paraffin). I have endeavoured to throw some light upon the nature of the skotographic action manifested by animal tissues and certain other substances, comparing their behaviour with that of woods in which the action has been regarded as dependent upon the formation of hydrogen peroxide, and with that of recognized radio-active substances. On this point I have adduced evidence showing that the animal tissues and other substances under examination cannot, so far as their action upon a photographic plate in the dark is concerned, be grouped conclusively with the recognized radio-active substances or segregated into a class such as that formed by the woods, and believed by Russell to affect a photographic plate in the dark by means of a purely chemical action. They occupy an intermediate position and have affinities with the woods on the one hand, and with the recognized radio-active substances on the other. Lastly, MacCormac, working in my laboratory, has shown that bacteria may be divided into groups according to their skotographic power, and that the only group which acts in this way with constancy and to a marked degree is a small one, which includes the pyogenetic staphylococci and the bacilli of tuberculosis and diphtheria.

The bearing of these observations upon the question of carcinoma must be left for consideration in the last lecture.

REFERENCES.

¹ Lazarus-Barlow, On the Effect Produced by Certain Animal Tissues on a Photographic Plate in the Dark, *Archives of the Middlesex Hospital*, vol. xiii, 1908 (Seventh Cancer Report), p. 111; Colwell, The Effects of Urinary and Biliary Calculi upon Photographic Plates in the Dark, *ibid.*, p. 98, and *Proc. Roy. Soc. Med.*, vol. 1, 1908 (Pathological Section), p. 127. ² Loc. cit. ³ *Phil. Trans. Roy. Soc.*, Series B, vol. cxvii (1904), p. 281, especially; other papers in same *Transactions* both before and after. ⁴ Lazarus-Barlow, *Archives of the Middlesex Hospital*, 1908, vol. xiii, p. 130. ⁵ *Archives of the Middlesex Hospital*, 1909, vol. xv (Eighth Cancer Report), p. 177.

*This calculus is preserved in the Museum of the Royal College of Surgeons, and was kindly lent by Mr. Shattock. Owing to its unique character, it was impossible to deal with it as with other specimens. Hence a flat surface could not be placed in contact with the photographic film. The original skotograph was shown during the lecture, but the markings, though definite, are too faint for reproduction in a plate without retouching, which is, of course, inadmissible.