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Faerber, Ernest Ecusiel

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INVESTIGATIONS INTO A NEW METHOD OF EXPERIMENTAL TAR CARCINOGENESIS IN ALBINO RATS.

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by

ERNEST ECUSIEL FAERBER,

Department of Physiology, University of the Witwatersrand.

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A. - INTRODUCTION.

Although the ultimate factors in the causation of cancer are not yet known, our knowledge of the properties of the cancer cell is vast; this is doubtless largely due to experimental cancer research which has assumed tremendous proportions since the beginning of the present century and especially within the last two decades. For the study of the cancer cell, cancerous tissue had to be made available, and it is mainly with this aspect of research that the significance of tar in experimental carcinogenesis was in the first instance concerned. The sensational advances in experimental tar carcinogenesis since its initiation by Yamagiwa and Itchikawa in 1914, such as the identification of the potent phenanthrene factors, and the demonstration of the similarity in chemical constitution between them and the sex hormones, bile acids, vitamin D, and the "organisers" of the embryo, can only stagger the casual observer who is able to correlate the regulation of normal and abnormal growth, reproduction and the propagation of the species. It is doubtful whether the first successful initiators of experimental tar carcinogenesis ever anticipated that the science would develop into its present extensive ramifications, and reveal to our ignorant minds the phenomenal planning and chemical organisation in nature, which had already been gleaned in the realms of inorganic chemistry.

B. - GENERAL REVIEW OF EXPERIMENTAL TAR CARCINOGENESIS.

The subject of experimental tar carcinogenesis has been very thoroughly reviewed by William H. Woglom (146), and by Seelig and Cooper (122). In the brief review to be presented hereunder, the salient features have been selected from these reviews, in addition to several features from other sources of information.

HISTORICAL:

The history of experimental tar carcinogenesis began in 1889 when Hanau (49) painted rats for months with gas tar or similar materials, but elicited only a chronic dermatitis. He was followed by Cazin (22), who in 1894 applied tar to dogs for 5 months, but did not produce carcinomata. We now know that these species are most resistant to the induction of skin cancer by the application of tar. A more suitable animal, i.e. the rabbit, was selected by Wacker and Schminke (142a) in 1911, and by Haga (46) in 1913, but the irritants were not applied for long enough to induce malignancy.

Bayon (8) in 1912 elicited abundant epithelial proliferation from the injection of gas works tar into the ear of a rabbit, but again the irritant was not applied for a sufficiently long period to effect malignancy.

The first successful attempt at experimental carcinogenesis was made in 1914 by two Japanese workers, Yamagiwa and Itchikawa (149) who produced papillomata on the ear of the rabbit by repeated applications of tar. In the following year they recorded the development of three cancroids (malignant growths) in the tarred animals.

This discovery initiated an era of intense activity in the realm of experimental tar carcinogenesis, some idea of the degree of which may be gauged from the fact that from 1927 to 1931, over 500 papers were published on this aspect of cancer research.

THE SUSCEPTIBILITY OF VARIOUS SPECIES TO TAR PAINTING:

The most susceptible animals to experimental tar cancer are the rabbit and the mouse (146). Yamagiwa (148) stated that tar painting will not produce carcinoma in rats or fowls, and the literature contains copious references to unsuccessful attempts to attain this object. The following order of

susceptibility to tar tumours was suggested by Twort and Twort (137): mouse, rabbit, rat, guinea-pig and fowl; these authors (136), however, estimate that in a group of 100 animals, 5% will be very susceptible, 5% very resistant and 90% will show an average reaction to tar.

Yamagiwa's (148) statement regarding the resistance of rats and fowls to the experimental induction of tumours by tarring is borne out by the following examples:-

Paszkiewicz (107, 108) painted white rats every two or three days, or gave them subcutaneous injections every eight to ten months, but none developed a tumour.

Leitch (75, 76, 78) failed to produce a tumour in guinea-pigs after 2½ years of tarring, and in rats not even a papilloma was produced after one year of tarring.

Polletini (110) applied tar to rats for 6 months with no result, and Mertens (95) was similarly unsuccessful with guinea-pigs.

Itchikawa (54) regarded rats as insusceptible and guinea-pigs were found to be insusceptible by Dentici (36). Halberstaedter (47), Teutschlaender (130, 131), Borrel, Bouz and de Coulon (16), and Bloch (14).

According to Borrel, Bouz and de Coulon (16), fowls do not react to tarring, and according to Teutschlaender (130) neither do pigeons.

Choldin (24) produced only papillomata in two chickens by painting with tar, but later (25) obtained an infiltrating cancroid following repeated applications of tar to the skin of the neck of a fowl, and a sarcoma after twelve subcutaneous injections of an ethereal extract of tar into a chicken.

Buschke and Lange (18), Borrel, Bouz and de Coulon (16), Halberstaedter (47), Deelman (33), Teutschlaender (130) and P. Möller (96) have all been unsuccessful with rats. Dentici (36) observed a simple papilloma in one rat out of 30 after

176 days' painting.

Calvanico (19) painted and injected rats with tar for over a year, and observed merely evidence of hyperplasia and atypical growth, while Cholewa (27) obtained negative results in ten rats treated over seven months.

The first successful attempt recorded of the production of a carcinoma in the skin of a rat was that of Herly (50). Further successful attempts as a result of tar painting were those of Guilera (45), who obtained one cancer in a batch of 40 rats after three months, and of Watson (143) who obtained cancers in four rats out of a litter of eleven by painting the skin with tar after a preliminary application of petroleum ether extract of rat tissues. The object of this preliminary application was to make the tar more absorbable, it being maintained that the reason for the resistance of the rats' skin to carcinogenesis was its great thickness and impenetrability

Yamaguchi, Nishiyama, and Suzuki (154) found white mice more susceptible to tar than black, and Sciesinski (120), Suzuki (127) and Grynfeltt and Harant (43) obtained similar results with rats.

Babes (3) actually secured a cancer of a rabbit's ear within about two weeks after the application of tar - surely a record of its kind.

Fukudi (40) found that tar cancer can be transplanted more successfully in white than in black mice.

Leroux (80), however, found black rabbits more susceptible to tar cancer than white ones.

THE SUSCEPTIBILITY OF TISSUES OTHER THAN THE SKIN:

Although rats, guinea-pigs and fowls are resistant to experimental carcinogenesis by tar painting, tissues other than the skin of these animals are more susceptible.

Maisin and Picard (86, 87) inserted small blocks containg paraffin, tar and scharlach R in the urinary bladder, pleural

cavity and peritoneum of rats. One of these had in addition an injection of a drop of tar into the bladder 34 days after insertion of the block. The animal died 115 days after the commencement of the experiment, and a papillary carcinoma was present on the posterior wall of the bladder.

Puccinelli (111) was unable to produce malignant growths in the bladders of rats by similar means.

Menetrier (92) produced a papillary carcinoma of the stomach of a rat by injecting tar into its perigastric, perihepatic and perirenal regions.

Teutschlaender (132, 133) obtained a carcinoma of the uterus in one of seven rats in which tar had been introduced per vaginam. The tumour developed 242 days after the first injection and 173 days after the last.

Russell (115, 116) obtained sarcomata in two rats out of four survivors which had received a subcutaneous injection of 10-15 mgms. tar weekly for one year. The first sarcoma appeared 11½ months after the commencement of the experiment, and the second six months after the cessation of the experiment.

Kazama (55, 56) observed an adenocarcinoma of the gall-bladder following the injection of tar into its cavity, and Leitch (78) revealed that the gall-bladder of the guinea-pig is somewhat susceptible to the induction of cancer by the introduction of tar, a fact confirmed by Polettini (110) who observed an adenoma with invasive potentialities from injections of tar into its cavity.

Although no carcinomata have been described in the fowl, sarcomata have been produced by Carrel (20) and by Murphy and Landsteiner (98). It is interesting to note that Carrel described his tumour as the most malignant of all known tumours, as it might kill its host in 4 or 5 days. The present author (38) has described a spontaneous, transplantable sarcoma in a

mouse, which might also kill its host in 4 or 5 days, but when one considers the respective lengths of life of the mouse and the fowl, one must give priority to Carrel's tumour as regards this claim. Murphy and Landsteiner (98) obtained their tumour by injecting 0.5 c.c. of a mixture of equal parts of minced chicken embryo and tar extract into adult hens; every two weeks thereafter 0.2 c.c. of tar extract was introduced into the resulting mass in the breast. Two of three survivors after 5 months had spindle-cell sarcomata. One of the tumours has been successfully transplanted, but Sturm and Murphy (126a) have demonstrated that these sarcomata cannot be transmitted by filtrate or desiccate.

Laser (69) subjected a fowl to four intravenous and two subcutaneous injections of tar at weekly intervals. Cultures of embryo spleen grown in vitro on the plasma of the fowl's blood (which it liquified immediately) were then implanted in a normal fowl after four passages. A sarcoma appeared after two weeks, which metastasised extensively.

Leitch (79) injected tar into the region of previously created embryomata, and in 1 - 18 months observed malignant tumours in three out of four hens thus injected.

In man, two epitheliomata have been recorded as a result of tar painting. De Jong, Meyer and Martineau (34) observed the development of a squamous cell carcinoma in a man 85 years old after eight years' constant painting of an eczematous area with coal-tar ("goudroline"). The patient applied this to allay the itching against the advice of his physician.

Veiel (140) reported the case of a 68-year old man who had suffered from recurrent eczema of the scrotum for 23 years. He had frequently applied a 33.1/3% alcoholic solution of pine tar, thus producing an inflammatory lesion with papules; one of these became malignant.

Vaughn (139) has suggested that kangri cancer may be a

tar instead of a heat variety as it and the skin surrounding it are begrimed with a material resembling tar or soot.

IRRITATION AS A FACTOR IN TAR CANCER:

Soboleva (124) showed in 1926 that oil of mustard applied to the skin tends to inhibit tar tumour growth. This was confirmed by Berenblum (10, 12) in respect to tumours caused by both tar and dibenzanthracene. Berenblum concluded that mustard gas acts on the cell rather than on the cancer-inducing agent, but Deloof (35) has shown that the inhibitory action of mustard gas depends on its age.

Berenblum (11) demonstrated that repeated freezing led to the development of cancer in mice, but did not hasten the development of tar tumours.

Heat as an irritant was found to hasten the development of tar tumours in mice by Choldin (26), and in rats by Cholewa (27) but this aspect of carcinogenesis has been the subject of much controversy.

THE RESULTS OF TAR INJECTIONS:

While earlier attempts at carcinogenesis were confined to painting of the skin, the injection of tar was found to produce interesting results, and its effects were rapidly investigated.

Yamagiwa and Itchikawa (150, 151) produced carcinoma by injecting a mixture of tar and hydrous wool fat into the breast of the rabbit, and Yamagiwa, Suzuki and Murayama (154) obtained a fibromyxosarcoma by similar means.

Yamagiwa and Murayama (152) obtained cancroids or adenocancroids in 23 out of 188 rabbits (12%) treated as above. "As the glandular epithelium of the breast does not respond to tar, only cancroids from the ducts, hair follicles, or surface epithelium were obtained, or adenocancroids from the ducts."

Although successful in the hands of the Japanese workers,

this technique was found not to be efficacious by Seedorf (121) in 39 rabbits.

Kennaway and Sampson (60), however, produced a duct papilloma in the breast of a rabbit by introducing tar into the main ducts.

Peracchia (109) injected tar subcutaneously into 120 rats and 50 mice, with no production of a sarcoma.

Tedeschi (128) obtained negative results in rabbits from intravenous injection of tar, while Beck (9) reported an increased susceptibility to carcinogenesis through a similar procedure.

Ishibashi and Ohtani (53) obtained papillary adenomata in the stomach of rabbits by submucosal injection of coal-tar. Only a slight hyperplasia resulted in the intestines of the same animals similarly treated.

Leitch (77) obtained papillomata by rectal injection of tar in rabbits.

Kimura (61) found adenocarcinomata in several guinea-pigs 140 days after introducing coal-tar into their bronchi.

Lacassagne and Monod (66) obtained a sarcoma of the testis in a rabbit after two intratesticular injections of tar at a fortnightly interval, and Russell (115) obtained a transplantable spindle-cell sarcoma from weekly subcutaneous injections of tar in 50 male mice.

Martin (90) made intravenous injections for several months and then inserted small pieces of tar subcutaneously in 38 animals. 22 developed malignant growths as against none of a control series which had not received intravenous inoculations.

Löwenthal (81) obtained three sarcomata in 42 mice from intraperitoneal injection of tar in oil.

Melczer (91) obtained alveolar sarcomata in 12 rats of 30 in which tar had been rubbed in the gluteal region; six out of 35 developed similar tumours after a similar procedure in the mammary region.

Appelmans (2) reported the development of 18 carcinosarcomata after tarring, and Shimoda (123) one carcinosarcoma, the metastases of which were composed entirely of sarcomatous elements.

Lungs. It appears that the lungs are next to the skin in order of frequency, as the commonest site of tar tumours.

Schabad (117) concluded that the mouse's hereditary predisposition to primary lung cancer could be increased by the application of tar. This was confirmed by Koose and Cordes (64) who suggested that the tarring facilitated the entrance into the circulation of a bacteriophage-like carcinogenic organism.

Mercier and Gosselin (94) and Mercier (93) described lung tumours of the nature of lymphadenomata consequent upon the intraperitoneal injection of tar.

Garschin and Pigalew (41) described hyperplasia in the lung tissue of rabbits following injection of tar therein, while Uno (138) obtained two carcinomata from injection of tar into the pleural cavities of 30 mice.

Gastro-intestinal tract. Bonne (15) found papillomata in the stomachs of 17 mice out of a group of 300 whose skins had been tarred (presumably due to ingestion from licking), and Voronoff and Alexandrescu (142) obtained a metastasising gastric carcinoma in one rat out of ten to which a mixture of tar, lanolin, aniline oil and toluylenediamine had been administered.

Macchiarulo and Bungeler (82) injected tar into the parotid glands of rats in the hope of producing "mixed parotid tumours" as they occur in man. These, however, were not observed.

Schabad (118) failed to produce tumours in mice by repeated introductions of tar into the rectum.

Badile (4) failed to produce tumours in the appendices of 32 rats, which were ligated and into whose lumina tar was injected.

Genkin and Dmitruk (42) obtained papillomata and adenomatous overgrowths by injecting tar into permanent appendicular fistulae in rabbits.

Biliary system. Clements (31) failed to produce tumours by introducing a mixture of tar and cement into the gall-bladder.

Urinary system. Latteri (70, 71) obtained papillomata and carcinomata in the renal pelves of guinea-pigs and rabbits by introducing therein, by means of nephrotomies, a mixture of tar and cement.

Genital system. Cioli (29) injected tar into the uteri of white mice per vaginam. He obtained carcinomata only when this procedure was preceded by oophorectomy.

Momigliano (97) obtained carcinomata in seven of 45 rats whose uteri were injected with tar directly through a laparotomy wound or fistula.

Badile and Maurizio (5, 6) injected tar by a direct laparotomy approach into the uteri of 34 mice, and observed three carcinomata.

Orru (101) obtained two carcinomata in rats by a similar procedure.

Spirito (126) failed to produce tumours in rabbits by injecting tar into the uteri through utero-abdominal fistulae.

Casabona (21) obtained negative results after injecting tar into the body of the uterus of 60 rats, and questions the validity of positive results.

Fels (39) obtained three carcinomata in mice in whose vaginae tar had been repeatedly deposited.

Badile (4a) injected tar into the seminal vesicles of ten rats between two ligatures to eliminate the possibility of escape. A basal-cell epithelioma developed in one rat after 16 months. In three rats, only epithelial metaplasia was observed.

Eye: Anardi (1) painted with tar the corneae of 60 mice. He obtained only cancers of the lids.

THE INFLUENCE OF VARIOUS FACTORS ON THE DEVELOPMENT OF TAR CANCER.

a. Pregnancy.

Maisin and De Smedt (84) mentioned the rarity of pregnant mice in a tarred group, but Lecloux (73) and Parodi (102) observed no interference with reproduction in tarred mice, and Krotkina (65) no interference in the case of rabbits.

Ciechanowski and Sciesinski (28) concluded that pregnancy increases the susceptibility of the rabbit to the development of tar tumours.

Tedeschi (129) and Lazzarini (72), however, state that pregnancy has no effect.

b. Castration.

According to Maisin, Desmedt and Jacqmin, (85), castration of male mice did not prevent the development of tar tumours, although it appeared that papillomata in castrates became cancerous at an earlier period.

Parodi (103) found that castration of both sexes had no influence on the growth of tar carcinoma in mice, and he (105) also states that it does not affect the development of tar cancer.

c. Diet.

Passey (106) conducted experiments in which one group of 50 white mice was fed on a diet very rich in vitamin A, and another on a diet in which that factor had been eliminated as completely as possible. Both were painted with a basic ether-soluble fraction obtained from chimney soot. "There was no important difference in the number of malignant tumours produced" (42% in the vitamin rich, 47% in the vitamin-poor group.)

Similar results were obtained by Roussy, Leroux and Peyre (114).

Watson and Mellanby (145) came to the conclusion that diet exercised no appreciable influence on the development of tar tumours.

Twort and Twort (136), however, reached a different conclusion, viz. that the effect of diet depends on the essential tumour susceptibility of the mice. They state that in a susceptible strain, good nutrition allows for early hyperplasia of the epithelium with an enhanced tendency to malignant transformation.

This view-point was experimentally confirmed by Rochlin (112, 113).

Imura and Toshiro (52) found that feeding rats on a vitamin A-free diet during the period of tar painting does not influence the production of pathological changes in the skin.

According to Oike (100) a diet rich in brain, albumen and vitamin B protects rabbits against the development of tar cancer, and a diet rich in fats, regardless of its vitamin content, leads to rapid death of the animal from tar cancer.

The latter conclusion is supported by Guerin (44), Wolkoff (147) and Vles, de Coulon and Ugo (141).

Maisin and Pourbaix (88) showed that a liver diet accelerated tar carcinogenesis, as well as pancreas and intestinal mucosa, while brain, thymus, marrow, dried gastric mucosa, and dried lymph nodes retarded it. Brain and other tissues are said to contain two factors - a growth-promoting substance, which is water-soluble, and a growth-inhibiting substance soluble in ether. They argue further and conclude that in the interaction of these two factors "a cell growth regulatory mechanism" is established in the organism.

THE EFFECT OF TAR ON PLANTS.

Komuro (62, 63) treated the root-tips of young plants with coal-tar, and subsequently found vacuolisation of the cytoplasm and nucleus, the presence of hypo- and hyperchromatic nuclei, fragmentation of nuclei, and giant cell formation. Finally, knots of tissue are formed, which the author compares to tar carcinomata.

THE CHEMISTRY OF TAR IN RELATION TO THE CARCINOGENIC PROCESS.

The presence of several hundred substances has been recognised in coal tar, though not more than a hundred have been definitely isolated (125).

Bloch and Dreifuss (13, 37) stated that the carcinogenic agent in coal tar is contained in a fraction with a high boiling point (370 - 440°C.) which is soluble in benzol. Such fractions caused extremely malignant tumours in 100% of mice in four months.

Maisin, Romme, and Jacqmin (89) stated that tar distilled at 450°C. was non-carcinogenic, as well as the constituents coming over below 200°C. at 200 - 300°. and at 300°C. These authors failed to produce cancer with chemically pure coal tar constituents, such as anthracene, phenanthrene, carbozole and acridine.

Deelman (33) found that the carcinogenic agent is present in a toluol, benzol, or acetone extract of pitch, and that the remaining pitch is then inactive.

Maisin (83) showed that a benzene extract of pitch obtained by evaporating at 350°C. a sample of coal tar from a horizontal retort was particularly active. It was generally agreed that the carcinogenic principle could be removed with benzene.

Murray (99) obtained a very potent sample by extracting coal tar successively with water, alcohol and ether.

Bloch (14) stated that the carcinogenic activity of tars varies with their source, as the agent is entirely lacking in some tars.

Schreuss and Zurhelle (119) found wood tar as efficacious as coal tar.

The relationship between the chemistry of tar and its carcinogenic potency was established by Kennaway (57) who stated that lignite tar, gasworks tar, producer-gas tar, and

coke-oven tar were known to be carcinogenic, while blast-furnace tar was not. He drew attention to the industrial and experimental evidence that the carcinogenic factor may distil over at from about 250o to above 500°C. He examined the known compounds of high boiling point in tars, and suggested that the carcinogenic principle might be among the unknown compounds which might be present in minute quanities only. Acridine, which had often been suggested as the cancer-producing factor of tar because of its irritating effect, was applied to 200 mice, a large proportion of which lived for 9 months without developing cancer. "Kennaway therefore concludes that irritation alone does not produce cancer, and recalls the observation of Legge (74), who found no carcinoma among 175 cases of chrome ulceration in dyers, tanners, and bichromate manufacturers."

Kennaway (58, 59) furthermore showed that when isoprene, CH_2: CMeCH: CH_2, and acetylene, HC ≡ CH, are passed through a tube and heated to 820°C. and 700°C. respectively, a mixture of compounds is formed which is more carcinogenic than many samples of tar. This suggested that the active principle was simple in constitution, and that it contained only carbon and hydrogen, i.e. was a hydro-carbon.

Tar produced at 920°C. from yeast or from human skin also caused carcinoma in mice.

From further studies Kennaway concluded that the temperature at which the active principles are formed bears no relationship to their carcinogenic activity.

Elaborating on Kennaway's work, Twort and Fulton (135) found that a tar made from pinene was carcinogenic, and Twort and Ing (134) and Watson (144) produced a potent carcinogenic tar from turpentine.

The sensational sequence of events, which led to the discovery of the great carcinogenic potency of dibenzanthracene was initiated by Hieger (51) who had observed a resemblance

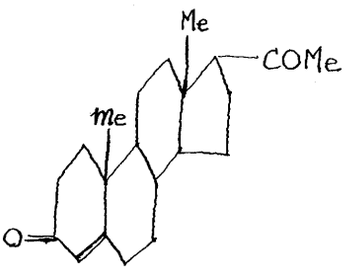
between the fluorescence spectrum of benzanthracene and a carcinogenic coal tar. Benzanthracene, however, was found to be only feebly carcinogenic. In 1929, Clar (30) a German chemist, published a method for synthesis of 1:2:5:6 - dibenzanthracene, and Kennaway, who had been trying for a number of years to identify the carcinogenic factor in tar, and his group of workers immediately undertook the study of its biological effects. It was found to be highly carcinogenic, and with its appearance, a new era in experimental cancer research commenced, for through its agency, cancer could be produced in the laboratory at the will of the experimenter.

C. - RATIONALE OF THE PRESENT INVESTIGATION

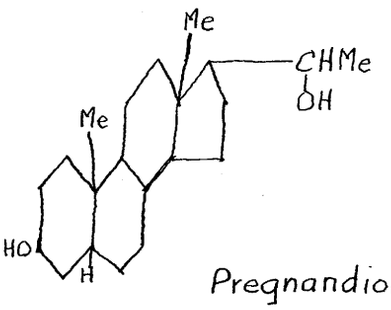
The experiments to be described hereunder were designed by the author in order to produce cancerous tissue within the shortest time possible for purposes quite remote from a purely statistical investigation. The technique used was considered to be a very drastic one, although at the time of commencement of these investigations, the author was not acquainted with the previous attempts at experimental tar carcinogenesis with reference to the genital system.

The experimental animals at the author's disposal were albino rats which are known to be very resistant to the induction of skin cancers by the painting of tar. In the search for a new method it was reasoned that if carcinogenic tar could be introduced into the lumen of the uterus and retained there, such as by occlusion of the lumen distally it might theoretically give rise to cancer within a fairly short time, because of the possible reactivity of the uterine epithehum to the carcinogenic phenanthrene substances in the tar, the latter was assumed on the basis of the structural relationships between the carcinogenic phenanthrene substances and oestrone.

Sterol substances with one double bond in the phenanthrene nucleus, such as progesterone and

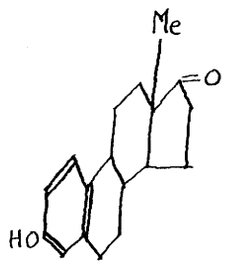


Progesterone

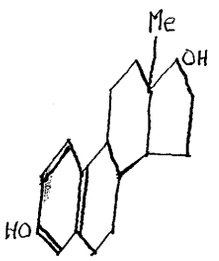


Pregnandiol

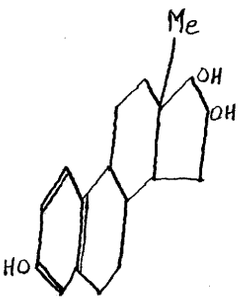
corticosterone, have no proliferative effect on the cells of the uterine epithehum. On the other hand, the sterol substances that have such an effect, have all, at least, three double bonds in the nucleus (147a).



Oestrone
(Ketohydroxyoestrin)

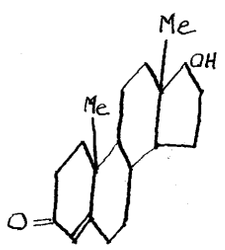


Dihydro-oestrone

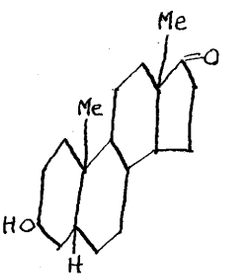


Oestriol

Furthermore, testosterone with one double bond



Testosterone



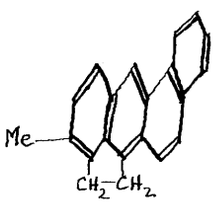
Androsterone.

in the nucleus, is ten times as active as androsterone which is fully hydrogenated '147a). Likewise, progesterone, with one double bond is active whereas pregnandiol, which is fully hydrogenated, is inactive, it is therefore tempting to associate the cellular proliferative activity with the degree of unsaturation of the phenanthrene nucleus, if the various side-chains are temporarily ignored (38a).

In addition to structural relationships, it is known that there are also functional relationships between these substances and the body sterols. For example, Havas and Gal (49a)

showed that administration of methylcholanthrene, (although not present in tar), to rats is accompanied by a great deposition of fat on the internal organs, and they suggested some functional relationship between this substance and the sex hormones.

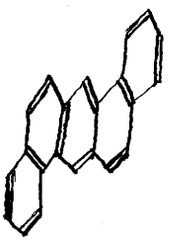
Cook, Dodds, Hewett and Lawson (32) furthermore showed that 3:4 benzpyrene and 5:6 cyclo-penteno 1:2 - benzanthracene were oestrogenic as well as



Methylcholanthrene carcinogenic.



3:4-benzpyrene



1:2:5:6:dibenzanthracene.

A further demonstration of functional relationship was that of Chalmers and Peacock (23) who showed by an ingenious method of direct observation, employing the fluorescent properties of dibenzanthracene and benzpyrene, that when these substances in colloidal solution are injected intravenously, they leave the blood after a few minutes to appear in the body fat. From this, they again slowly disappear during the ensuing two hours, and are concentrated and eliminated in an altered and water-soluble form in the bile.

This indicates that the metabolism of these carcinogenic substances in the body bears definite relationships to the metabolism of the bile acids, which contain the sterol nucleus.

Although the mechanism of carcinogenesis through the unsaturated hydrocarbons is at present unknown, it has been suggested that it might have to do with their complete degree of unsaturation (38a). A possible mechanism supported by experimental facts has been suggested and discussed elsewhere (38a). Oestrone causes an increase in the respiration of the uterus (60a, 90a), and various female sex hormonal extracts greatly increase the oxidase reaction in epithelial cells of the uterine

mucosa and glands (61a). Owing to the similarity in chemical constitution between oestrone and the carcinogenic phenanthrene substances, it is suggested that the latter may act through the same cellular respiratory system as oestrone, possibly the oxidase-cytochrome system. In precancerous conditions produced by tar (113a) and carcinogenic agents (36a) the respiratory processes are enhanced appreciably. Boyland (16a) has indeed observed that 1:2:5:6 dibenzanthracene increased the indophenol reaction of a yeast oxidase preparation. The suggestion has therefore been made (38a) that the ultimate cause of cancer might lie in an affection of the oxidase-cytochrome system.

The advantages of inserting tar in solid form into an occluded portion of the uterine cavity are

(1) The possibility of prolonged action of the hydrocarbons present in it, and

(2) A better opportunity for localised action or the confinement of its action to fewer epithelial cells in whose respiratory enzymes it might bring about the alterations leading to cancer, (on the above basis).

There is no justification for the assumption that the action of tar from within the lumen is comparable to the action of an agent present in the blood-vascular system such as oestrone. It is, however, interesting to note that Lacassagne (67,68) has pointed out that mammary cysts contain folliculin, and has suggested that the accumulation of ovarian hormones in the breast and their consequently intensified action on the mammary epithehum is the direct stimulus to malignant proliferation.

On the basis of this, an investigation into the effect of tar from within the lumen of the uterus should prove interesting.

D. EXPERIMENTAL

Crude coal-tar (obtained from the City Engineer's Department, Municipality of Johannesburg) was used. Before each operation, it was slightly warmed until it was sufficiently fluid to be capable of injection through a syringe. The syringe used was an all-metal dental syringe specially altered as to accommodate a larger nozzle. Immediately before pouring the warm tar into the barrel, the syringe was well rinsed with ether, benzol or acetone, as this was found to prevent adherence of the piston to the walls of the barrel.

Three series of injections were done. In the first, the vagina was ligatured with catgut at the site of its bifurcation, and tar was injected into the lumina of both uterine horns. In the second series, one horn of the uterus was ligatured and tar was injected into the lumen of the proximal end. In the third series, both ovaries were excised, one horn of the uterus ligatured, and tar injected into the lumen of the proximal end.

Different operative procedures were employed with each of these series, but before describing them, it would be well to record some observations regarding the anaesthesia. It was found that rats were more amenable to operative procedure if they had been deprived of food several hours previously. Care was also exercised in conveying the animal from its cage to the anaesthetic jar, as a pre-operative excitement was found to be predisposing to a respiratory embarrassment and often death during the surgical procedure. The latter feature was so consistent that if an animal was unduly excited, it was discarded. The anaesthetic used was ether. Swabs soaked in

this were placed in the jar, and when the vapour had had time to diffuse through the interior, the rat was quickly and carefully introduced inside. Anaesthesia was maintained during the operation by placing cotton wool swabs soaked in ether over the animal's snout, due care being taken to prevent contact between the swab and the tissues of the face. Criteria of recovery from the anaesthetic were an increase in the respiratory rate, and an accentuation of the reflexes, such as the conjunctival and sneezing reflexes. An over-dose of ether was invariably associated with gasping and accordingly the anaesthetic was withdrawn at manifestations of this phenomenon. Whether an animal that had ceased to respire was destined to recover or to expire could be ascertained from the condition of the eyes. A deep pink or purple colour in the eyes invariably denoted recovery, while a pale, glassy, anaemic appearance signified very little chance of recovery even if cardiac stimulants like adrenaline were injected and continual artificial respiration was attempted.

OPERATIVE PROCEDURES:

The author found it good practice to ascertain the anatomical relationships of the regions in a preserved animal before attempting the operation.

For the first series of experiments the animal was placed on its back, and its head averted to one side, if possible, to avoid inhalation of salivary secretions. The hair over the whole ventral abdominal region was removed by means of scissors, in which process care was taken not to injure the mammae which may be concealed by the hair. It was found best to remove the hair in a different place from that where the operation is to be performed, as the hairs otherwise tended to adhere to the instruments and to gain entrance to the wound. The region was then moistened with saline or rectified spirits. A straight incision into the skin was made in the median line about 2 cm. long, and $\frac{1}{2}$ cm. proximal to the urethral orifice. The

incision was continued through the subcutaneous tissue, fascia, and muscle, until the contents of the abdominal cavity had been exposed. The coils of the intestine were displaced until the vagina was made visible. The vagina was then secured at its bifurcation and slightly raised. A thread of catgut was passed under it through its membranous attachments, and the structure firmly ligatured immediately below its site of bifurcation. Care was taken to pass the catgut between the vaginal wall and the two nutrient arteries situated laterally.

The syringe nozzle was now inserted into the lumen of either horn of the uterus or at the bifurcation of the vagina, or into the lumina of both uterine horns, and 0.1 - 0.2 c.c. of tar injected therein. The tar could easily be seen entering the lumen. If a little tar escaped at the withdrawal of the syringe, it was cleansed with a swab, although this is quite unnecessary as far as the possibility of a developing peritonitis is concerned. The incised muscle, together with the peritoneum was then sutured with a continuous catgut suture, and the skin likewise. Acriflavin was applied to the surface of the skin.

In the second series of experiments, the hair was removed from the left dorsal lumbar region. A vertical incision, about 1.5 cm. in length, was made parallel to, and about 1.5 cm. lateral to the spinal column, between the os coxae and the last rib, but nearer the latter. Similar incisions were made through the subcutaneous tissue, fascia and muscle. This usually exposed the left horn of the uterus, or else it could be easily found. It is easily distinguishable from the gut by a pinkish-purplish tinge, its smaller size, firmer consistency, and the peculiar arrangement of its vessels. The horn was secured and a thread of catgut passed around it, through the membranous attachment and between the horn and the left uterine artery, thus ensuring retention of the blood supply. It was firmly ligatured about 1.0 - 1.5 cm. from the ovary. The syringe nozzle was then inserted into the lumen of the horn proximal to the ligature, and 0.1 - 0.2 c.c. tar injected. Any tar oozing out at the site

of injection was cleaned off with a swab. Catgut sutures were then applied as above to the muscle and to the skin, and acriflavin applied superficially.

In the third series of experiments, the hair was removed from the median dorsal lumbar region. An incision of about 2 cm. was made in the skin in the dorsal median line. The skin was then bluntly dissected from the muscle, and an incision of about 1.5 cm. made in the muscle about 1 cm. away from , to the left of, and parallel to, the vertebral column. The left ovary was then secured and pulled to the exterior of the wound. A thread of catgut was then passed around the ovary, and that structure firmly ligatured. The adjoining 0.5 cm. or so of the uterine horn was included in the ligation of the ovary, and the organ was well retracted exteriorly together with its enveloping adipose tissue, if present, in order to ensure complete removal of the whole of its tissue and, if possible, any accessory tissue present. The ligatured tissue was then excised as near to the ligature as possible. The remainder of the horn was then replaced, and the muscle sutured; the right ovary was removed in a similar manner, and in addition, a further ligature was applied to the right uterine horn about 1.2 cm. distal to the first ligature. The second ligature was passed between the horn and the artery, so as to preserve the blood supply. 0.1 - 0.2 c.c. tar was then injected into the portion of horn between the two ligatures as above. The muscle on the right side was then sutured and the skin likewise.

A local anaesthetic, Codrenine (cocaine + adrenaline) was occasionally applied to the subcutaneous tissue. The adrenaline has a useful function besides constricting the vessels, and preventing a too rapid absorption of the anaesthetic. It prevents bleeding, and after the operation, when the sutures have been applied , and the cut ends approximated, the vessels again relax, and the small amount of bleeding that takes place facilitates organisation and healing of the tissues.

TABLE 1.

First series - Vagina ligatured at its bifurcation. Tar injected into both horns of uteri.

Rat No.	No. of days alive after date of injection	Cause of death
11	434	Multiple lung abscesses with pyometra.
32	409	Multiple lung abscesses - gangrene of small gut.
33	462	CANCER OF UTERUS WITH LIVER METASTASES.
40	292	Pyometra.
41	293	Multiple lung abscesses.
42	315	do.
46	88	Laparotomy - Multiple abscesses in peritoneal cavity.
47	107	Multiple lung abscesses.
45	39	Accidental overheating of rat chamber, (discredited).

Second series - Left horn of uterus ligatured. Tar injected into proximal portion of horn.

Rat No.	No. of days alive after date of injection.	Cause of death
30	205	Broncho-pneumonia.
8	231	Broncho-pneumonia.
36	251	Multiple lung abscesses.
37	183	Broncho-pneumonia.
38	266	Multiple lung abscesses.
39	167	Broncho-pneumonia.
3	149	Broncho-pneumonia. (DISCREDITED).
9	83	Accidental over-heating of rat chamber.
35	58	do. do.
43	40	do. do.
2a	35	do. do.
34	22	Laparotomy.
44	7	Strangulated hernia.
1	48	Pyaemia.

TABLE I - Contd.

Third Series - Bilateral ovariectomy. One horn ligatured inferiorly and tar injected proximal to ligature.

Rat No.	No. of days alive after date of injection	Cause of death
OA	548	Killed - no tumour present
OB	544	Killed - do.
OC	270	Multiple lung abscesses.
OD	252	do. do.
OE	209	do. do.
OF	320	do. do.
OG	229	do. do.

A D D E N D A

Bilateral ovariectomy without injection of tar.

O	272 (after date of operation).	Multiple lung abscesses.

0.1 c.c. tar injected under peritoneal layer of ileum.

21	217	Multiple lung abscesses.

In the above operations, asepsis was observed throughout, all instruments being sterilised before use. This is actually quite unnecessary, as rats very seldom, if ever, develop post-operative sepsis. The author has had only one death from sepsis in his experience. This has probably been due to the animal's natural resistance, as occasionally when the catgut sutures have given way, andthe gut has projected exteriorly out of the wound subsequent to an operation, the animal has lived quite normally after resuturing of the wound.

E. RESULTS.

Eight rats were injected according to the first operation described, i.e., after ligation of the vagina at its bifurcation, and 15 were injected in one horn of the uterus. Shortly after the completion of this the rat chamber accidentally became overheated, and five rats were sacrificed - one of the first series, and four of the second. Post-mortem examinations conducted on the rats revealed that the horns of the uteri proximal to the site of ligature were intensely dilated and filled with a slightly turbid blood-stained fluid, although the tar originally injected was still present. Seven rats were then bilaterally ovariectomised, and tar injected as described above. The object of this was to enhance the irritative effect by withholding the uterine secretions, and simultaneously, to observe the effect of a probable devitalisation of the uterine epithelium on its susceptibility to malignant transformation.

The majority of the rats used in this series were young, probably under one year of age. That the ovarian tissue had indeed been removed was judged by the subsequent increase in weight of the animals.

The results of this experiment are indicated in the accompanying tables. It will be seen that out of 22 animals (excluding the 8 that died from influences irrelevant to the object of the experiment), only one developed a tumour.

Complete post-mortem examinations were conducted on all the rats, and the tar could in every case be discerned in the uteri. It will be seen that the majority of the rats died of multiple abscesses in the lungs. That these were abscesses and not malignant growths was unquestionable because of (1) the capsule enclosing the abscess (v. Fig. 7), (2) the abundant pus (bacteriological examination showed the organism to be a streptococcus), (3), the absence of a primary malignant focus, especially in the uteri, (4), the frequent absence of emaciation of the animal, and (5) the frequent occurrence of abscesses in animals that had not been injected with tar.

The uterine horns were dilated and filled with fluid proximal to the sites of ligation, in several rats. In the majority of animals, however, the uteri were not dilated at all; yet the tar was present in the lumen. The significance of this is unknown, but two explanations suggest themselves; (1) the ligature might have eventually yielded under the pressure, and the secretions been able to escape without e scape of the tar, or (2) the fluidmight have appeared only at certain periods of the menstrual cycle, and been reabsorbed at other periods.

Unfortunately, if no gross evidence of tumour was present in the uteri at post-mortem examination, the animal in toto was discarded. As the original object of this experiment was to obtain tumour tissue for purposes quite remote from those of a statistical investigation, no attention was paid at the time to the microscopic appearance of the uterus in those cases that were apparently normal and unassociated with tumour development.

Gangrene of the tail was seen in two rats, Nos 41 and OF. In the case of rat OG, there was commencing gangrene of the tail. In the case of rat No. OF (Fig. 2)

the gangrene was typical of that produced by ergot in the experiments of Suzman, Freed and Prag (126b). The significance of this lies in the suggested etiological relationship of the ovarian secretion to gangrene (thrombo-angeitis obliterans v. Suzman, Freed and Prag, 126b) and the fact that both rats Nos. OF and OG were bilaterally ovariectomised.

THE TUMOUR :

In the case of rat No. 33, the left uterine horn was somewhat dilated and filled with inspissated white material presumably pus. This material was unfortunately not examined microscopically. The reasons for presuming that it was pus are :

(1) The identity in its consistency with the pus of the frequent and undoubted lung abscesses from which the majority of the animals died. Although the pus from the lung abscesses was also inspissated microscopical examination revealed only pus cells and debris.

(2) The wall of the uterus of rat No. 33 was extremely necrotic, and the superficial cells especially were undergoing necrosis. The extent of this necrosis made an adequate histological photograph of the epithelial downgrowths impossible. The necrotic effect was believed to be due to the proteolytic ferments of the pus cells.

About ½ cm. proximal to the bifurcation, the wall of the left horn was considerably thickened, irregular, and white in appearance for about 1 cm. in length (Fig. 1). The lumen in this part was very narrow and occupied by the same inspissated material. In addition, the liver showed three or four superfical white areas, the largest of which was 4 mm. wide. On further investigation, these were found to extend into the liver substance. The lungs contained numerous white masseswhich appeared to be mainly abscesses. No signs of malignancy were subsequently observed on microscopical examination of the lungs.

Histological examination of the uterine horn showed numerous epithelial downgrowths and cell-nests - typical of a squamous epithelioma - which infiltrated the muscular coats of the uterus (Fig. 3). The metastases of the liver, on examination, showed similar cell-nests in its substance (Fig. 4).

MISCELLANEOUS OBSERVATIONS AND PROCEDURES DURING THE COURSE OF THE EXPERIMENTATION.

Brief reference has been made above to the great resistance of rats to infection. Some practical instances of this, which occurred during the period of experimentation may be mentioned.

Rat No. 9 was injected with tar per operation on 16.3.37 On the following morning it was found that the rat had eaten up the catgut sutures in the skin, and that the wound was open and gaping. The skin was sutured again, and the wound healed up completely without complicating sepsis.

Rat OG was the subject of a bilateral ovariectomy and tar injection on 30.6.37. When examined the following morning, the wound was found to be open and gaping. It was restitched, whereafter the animal recovered without complicating sepsis.

Rat No. 45 was injected with tar per operation on 30.4.37. When examined the following morning, a large portion of the vascular adipose tissue that is common in the pelvis in association with the urogenital system, was seen extruding through the incision in the skin. The catgut sutures were still intact. The tissue was haemorrhagic and gangrenous. Under ether anaesthesia, the stiches in the skin were opened, the projecting tissue was secured as far down in the abdomen as was possible (where it was not gangrenous) and it was there ligatured with catgut. The gangrenous portion was cut off, and the rest replaced in the abdominal cavity. An extra stitch was applied to the rectus abdominis muscle, and the skin sutured. The animal made an uneventful recovery.

It was decided in the initial stages of the experiment to ascertain how the animals were responding to the treatment with tar. Accordingly, rat No. 30, which had had tar injected into the lumen of its left uterine horn on 13. 3. 37 was made

the subject of a laparotomy on 12. 4. 36, i. e. 30 days after the date of injection. An incision about 1-inch in length was made to the immediate left of, and parallel to, the original incision. The underlying fasciae and transversus abdominis muscle were similarly incised. In the loose connective tissue, particles of solid tar were observed, ensheathed by dense connective tissue. A streak of white fibrous tissue in the transversus abdominis muscle denoted the site of the original incision. The left uterine horn was found with some difficulty, owing to apparent adhesions of the gut and viscera to the parietal peritoneum. When found, it contained a fairly large lump of solid tar, and the catgut ligature appeared to have caused a definite blockage in the lumen. The uterus in the region of the original ligature showed a change suggestive of necrosis, while dark purple strands, presumably of uterine muscle were regenerating from the part distal to the ligature in a proximal direction, encircling the apparently necrosed proximal part. The ovary showed numerous, distinct, thick, white strands, The wound was sutured and the animal recovered.

Although the idea of regenerating uterine muscle may not be compatible with human pathology, it should be borne in mind that the potentialities of growth of tissues of the lower animals may far exceed those of the tissues of man. The albino rat is especially a case in point. Since it is extremely resistant to infection, as the examples quoted by the author point out, its tissues would not be so prone to the action of toxins, as for example, the tissues of the mouse. The author has difficulty in applying to the dark purple strands mentioned any designation other than that of regenerating uterine muscle. In man the powers of regeneration of skeletal muscle are probably no greater than those of uterine muscle. Yet the author has mentioned (p. 25, p. 27) several instances of

extreme power of regeneration in the skeletal muscle of rats. It is therefore probably permissble to assume a similar potentiality for its uterine muscle.

The particular period of the oestrous cycle at which the operative procedures were undertaken was disregarded, but doubtless these varied. For instance, although the uterine horns were usually small in size at the operations, the uterus of rat No.35 was exceptionally large and hypertrophied, indicating possibly the oestrous stage. A vaginal smear of rat No.41 indicated that it was in oestrus at the time of operation.

At the laparotomy of rat No.34 during which it died (22 days after the date of injection), the uterus immediately proximal to what appeared to be the original site of ligation was seen to be intensely dilated, not unlike a balloon, transparent, vascular, and filled with a fairly clear fluid. Bits of tar were scattered at wide intervals on the walls of the distended part and in the adipose connective tissue in relation to it. The ovary was small.

At the post-mortem examination of rat No.44, which died seven days after an injection of tar into the proximal end of the ligatured left uterine horn, about 1-inch of ileum was found to be herniating through the original incision in the muscle. This was gangrenous, but the rest of the gut healthy. The edge of the muscle surrounding the herniated gut was rounded off and smooth, but the pressure appeared to have been sufficient to cause strangulation and death by obstruction. The round, smooth edge of the surrounding muscle in this case is a manifestation of the great proliferative and adaptive potentialities of the rat's tissues.

A variation in the anaesthetic technique was attempted in rat No.3, which was injected with about 0.7 c.c. of an ether + olive oil (85 : 15) solution. As this was insufficient to produce a general narcosis, the anaesthesia was supplemented by the open, soaked swab method of application, and the operation completed.

Rat No.11, which had been operated on on 16.2.37 from a ventral, median, abdominal approach, was made the subject of a laparotomy on 14.5.37, i.e. 87 days after the date of injection, to ascertain the cause of a swelling of varying consistency, which had appeared at irregular intervals for some time previously. When the skin had been incised numerous engorged blood vessels were seen in the subcutaneous tissue underneath. A few drops of Codrenine were applied, whereupon the vessels immediately contracted and practically disappeared from view. The incision through the subcutaneous tissue was continued, and this revealed complete absence of the usual muscle layers. Instead, a portion of the caecum projected forward. A search showed that the muscle was completely divided in the median line and the edges were lined by adipose connective tissue with occasional adhesions to the ventral abdominal wall. The edges were far apart, leaving a large space between. Hence the lump previously felt was merely protruding caecum, and the different degrees of firmness in consistency were due to varying states of the caecal contents. The left uterine horn was then secured. This appeared normal, and contained numerous black nodules of tar for about $\frac{1}{2}$-inch of its length. The separated edges of the muscle were then sutured with catgut, and the skin likewise. The hernia was thus repaired.

The only case of death due to sepsis in the author's experience was that of rat No.2R, on which a bilateral oophorectomy had been performed on 14.5.37. Three days later, i.e. on 17.5.37, the animal died in coma, and a subsequent post-mortem examination revealed accumulations of pus around the catgut sutures in the muscle.

In the case of rat No.46, a large, firm mass, 1-inch in diameter, was palpated in the abdominal cavity 81 days after the date of operation. The rat was furthermore emaciated and restless. Seven days thereafter, a laparotomy was performed, during which the animal died. As the original object of this

experiment was to obtain tumour tissue for other purposes, the rat, barring a portion of the tumour, was unfortunately not preserved. Fig.5 represents a sketch of the abdominal cavity and its contents as it was found post-mortem. The hard mass that had been palpated previously proved to be a large diverticular sac from the right uterine horn with greatly thickened walls and containing a thick greenish material. Two or three similar diverticula arose from the rest of the length of the right horn, which was itself greatly dilated and had greatly thickened walls. The left horn was likewise greatly dilated and thickened. Attached to the parietal layer of the peritoneum, were three, white spherical nodules, of smaller width than the uterine diverticula. These were incised and found to be of a cystic nature, containing a thick, greenish material like the uterus and having greatly thickened walls. One of these metastatic nodules was preserved, and the microscopic appearance of its wall is indicated in Fig.6. It is seen to consist only of fibrous tissue with no indications whatsoever of muscular tissue.

The greenish material was examined microscopically, and was found to consist entirely of polynuclear leucocytes. An emulsion was made from some of it, and 1/4 c.c. of this injected into a young male rat, and some solid pus transplanted to the right axillary region of a young female rat. These subsequently showed no ill-effects.

It is difficult to interpret the nature of these nodules, but they are regarded by the author as a further manifestation of the great proliferative capacity of the rat's tissues. They may be to a certain extent analogised to human fibroids which have become detached from the uterus and affixed to the parietal peritoneum.

SELECTED POST-MORTEM EXAMINATIONS:

Rat No.1 (injected 25.5.37, found dead 22.6.37), Pelvic lymph glands enlarged, soft, and purulent. Large abcesses in the right lung.

Rat No.37 (injected 17.4.37, found dead 17.10.37). Portion of uterine horn proximal to site of ligation was dilated and filled with fluid.

Rat No.8 (injected 17.3.37, found dead 3.11.37). Uterus was unaffected, although it contained small particles of tar. Lungs showed large, soft, whitish masses, which on microscopical examination showed no signs of malignancy.

Rat No.21 (injected 31.3.37, with tar under serous coat of ileum, found dead 3.11.37). Animal emaciated. Portion of ileum under whose serous coat tar was injected was intensely dilated, and its walls were hypertrophied. Large, firm areas were also present in lung, but showed no signs of malignancy on microscopical examination.

Rat No.40 (injected 21.4.37, found dead 7.2.38). Abdomen was found to be distended, and some coils of the gut and several ill-defined masses of firmer consistency were palpable 14 days before death. Three days before death, a firm, median mass was palpable in the pelvic region.

Post-mortem examination: The rat was not emaciated. The uterine horns just above their junction were intensely dilated and filled with pus. One pouch of the left horn was enlarged to form a spherical mass, the diameter being about that of a shilling-piece. This was of bluish-black appearance and vascular. On section, it contained a grumous whitish material, suggestive of pus. Specks of tar were visible in the pus.

Rat OG (ovariectomised and injected 30.6.37, found dead 14.2.38). No apparent change in uterus. The animal was somewhat adipose and there was commencing gangrene of the tail. Cause of death: suppurative broncho-pneumonia.

Rat No.42 (injected 26.4.37, found dead 7.3.38). The uterus was not dilated and was filled with a blackish, tarry material.

Rat OC (ovariectomised and injected 14.6.37, found dead 11.3.38). Uterine horns were apparently atrophied and contained

tar. Animal somewhat adipose. Cause of death : suppurative broncho-pneumonia.

Rat No. 11 (injected 16.2.37, laparotomy 14.5.37, found dead 26.4.38). White patches along uteri: Large, white encapsulated abscesses in lungs. No sign of malignancy on microscopical examination.

Rat OF (ovariectomised and injected 19.6.37, found dead 5.5.38). Gangrene of tail. Multiple abscesses in lungs. No apparent malignancy in uterine horns which were atrophic.

F. DISCUSSION

An examination of the review above will reveal that previous attempts at carcinogenesis in the genital system of rats by the application of tar have yielded conflicting results. Thus, while Momigliano (97) claimed to have produced carcinomata in seven of 45 rats by direct injection of tar through a laparotomy wound or fistula, Orru (101) two carcinomata by a similar procedure and Teutschlaender (132,133) one carcinoma of the uterus in one of seven rats in which tar had been introduced per vaginam, Casabona (21) obtained negative results with 60 rats, and questions the validity of positive results.

Owing to the variations in the method used in these investigations, it is not possible to compare the results with those of the above methods.

THE STATISTICAL VIEWPOINT

One positive result out of a series of eight compared to negative results in two series of seven each does not enable one to draw any definite conclusions from the statistical viewpoint. Such a solitary result might as well be due to chance as to variations in the experimental technique. What is deemed to be of greater significance is the number of negative results. If the drasticity of the

procedure be considered, one is justified in concluding that the results of these investigations, i. e., one malignant neoplasm out of 22 animals indicate an extreme degree of resistance of the uterine epithelium of the rat to malignant transformation. Esp̂ecially is this so, if cognisance is taken of the factors set out in the rationale of this investigation, viz., the similarity in chemical constitution of the agents in the tar to oestrone, to which the epithelium of the uterus is so sensitive.

The number of animals employed, viz. 22, is not considered too small to make the above deduction for the following reasons :-

(1) The great degree of resistance of rats generally to experimental tar carcinogenesis. This is accepted by most laboratory workers; it is amply supported by the review in this dissertation, and further restatement of it recurs time and again in the literature.

(2) The tables hereunder indicate all the authors quoted in this dissertation in whose experiments the number of animals used is stated. The first table indicates the authors whose experimental animals - irrespective of species - exceeded in number those employed by the present author, viz., 22. The second table indicates those authors whose experimental animals were below 22 in number. It will be seen that there are 16 in the first table and 8 in the second. Thus 33-1/3% of the authors quote figures below 22. In addition to this factor, it should be borne in mind that the experimental technique employed by the author was more exacting than many employed by the first group of authors, which often involved merely painting with or subcutaneous injection of tar.

TABLE I

Experimental animals exceed 22 in number

Author	No. of animals	Positive results
Anardi (1)	60	?
Badile (4)	32	0
Badile & Maurizio (5,6)	34	3
Bonne (15)	300	17
Casabona (21)	60	0
Dentici (36)	30	1
Guilera (45)	40	1
Lowenthal (81)	42	3
Martin (90)	38	22
Melczer (91)	30 35	12 6
Momigliano (97)	45	7
Passey (106)	50	21
Peracchia (109)	120 50	0 0
Russell (115)	50	1
Uno (138)	30	2
Yamagiwa & Murayama	188	23

TABLE II

Experimental animals below 22 in number

Author	No. of animals	Positive results
Badile (4a)	10	1
Cholewa (27)	10	0
Leitch (79)	4	3
Murphy & Landsteiner (98)	3	2
Russell (115,116)	4	2
Teutschlaender (132,133)	7	1
Voronoff & Alexandrescu (142)	10	1
Watson (143)	11	4

SUSCEPTIBILITY AND RESISTANCE TO CANCER

Why the tissues of the rat should be so resistant to experimental tar carcinogenesis is not known. The factors which determine susceptibility and resistance to carcinogenesis generally are ill-understood at the present time.

There is firstly particular tissue susceptibility which differs in different species. For example, the statement is frequently made in the literature, and supported by the review in this dissertation that the skin of the rat is exceedingly resistant to carcinogenesis by the painting of tar, while its connective tissue is much more susceptible to sarcomatous development from the subcutaneous injection of tar. On the other hand the skin of mice is very susceptible to experimental cancer from the painting of tar, while its connective tissue is more resistant to the development of sarcoma. Watson (143) believed that the resistance of the skin of the rat was due to its thickness; he confirmed this experimentally by application of a petroleum ether extract of rat tissues prior to painting with tar, his object being to make the tar thereby more absorbable. His viewpoint, unfortunately, is not universally accepted; in fact the results of the present author's experiments and those of others suggest that the resistance applies to other epithelial surfaces as well. This indicates how complicated the subject is.

The genetic factor is evidently also of great importance. If mice are interbred for a number of generations, they develop a special propensity for the development of mammary cancer. Such cancer might occur spontaneously or as a result of what may normally be a trivial derangement in the equilibrium of the breast tissue, e. g. injury. The male mice of such a strain readily develop cancer of the breast from the prolonged

injection of oestrin (Lacassagne). This propensity to cancer is manifest only in the breast. It has been repeatedly shown that tar painting of the skin meets with no greater response in such animals than in animals of a non-susceptible strain. This genetic derangement or alteration affects therefore only the epithelial cells of the gland and not the skin.

Furthermore, if the newly born of the mice susceptible to breast cancer suckle at the breasts of those not susceptible, their susceptibility disappears. Hence the milk contains an ill-understood factor which is necessary for the transmission of the neoplastic proclivity to the young.

With our present state of ignorance of what may be called "the chemistry of genes", it is not possible to explain these phenomena of susceptibility. It has often been stated that in man, the likelihood of inter-breeding, ([redacted]) as a factor determining susceptibility is very remote, although we know of families with a special proclivity for cancer, such as carcinoma of the breast (Sampson Handley). Why the incarcerated epithelial cell in a gastric ulcer should become malignant in 8 or 9 cases out of a hundred, and remain orderly in the remainder, while perhaps they are subject to the same metabolic stimuli, is a mysterious issue. The genetic factor is probably also at play. For example, twins may develop retinoblastomata independently and simultaneously. Then we have the familial incidence of polyposis coli.

The problem extends to diseases other than cancer. In man, for example, the apparently strong in physique may succumb to acute infectious diseases, like pneumonia, while the lean and scraggy survive. Again, some viruses affect healthy people as rapidly as unhealthy people, e. g. the virus of infantile paralysis. The explanation of susceptibility and resistance to cancer lies in the future of scientific research.

THE ORIGIN OF THE TUMOUR

The surface from which the tumour developed consisted of squamous epithelium. The epithelial downgrowths displayed the typical structure of keratinising epithelium. This will explain the presence of cell-nests in the tumour metastases. The significance of this feature is difficult to interpret. It is recognised that in man cell-nests may be present in carcinoma of the cervix (97a). This may be regarded as a latent potentiality of the cervical epithelial cells. Such a potentiality would tend to manifest itself more frequently in animals of a lower order, such as the rat. Just as the rat is more lowly in the evolutionary order, so are its cells more embryonic in behaviour than those of the higher orders. Evidence for this has been presented above, with respect to the proliferative potentialities of its skeletal and smooth muscle. We also know from numerous cases cited in the literature that malignant cells of the same tissue of a rat or mouse may assume the most diverse forms with growth. A neoplasm commencing as a definite carcinoma may display the typical appearance of a sarcoma with continued transplantation and vice versa. Hence the expression of a latent potentiality in the cell of a rat may perhaps be considered as a peculiarity of the species.

The metaplasia in the case of the author's tumour from a columnar epithelium to a squamous type under the influence of tar would best be regarded as such an expression. It is well to remember that the chemical "organisers of the embryo are closely related chemically to the carcinogenic phenanthrene substances present in tar. This is all the more reason why a latent primitive propensity in the uterine epithelial cell should show itself under the influence of a carcinogen.

THE SIGNIFICANCE OF THE NEGATIVE RESULTS

Although no importance from a statistical viewpoint may be attached to the solitary tumour obtained, the fact that no tumours arose in the ovariectomised series is considered of greater significance. It appears from these experiments that removal of the ovaries in young animals - which would possibly have a devitalising effect on the uterine epithelium - did not make the epithelium more prone to malignant change. The fact that mostly young animals were ovariectomised distinguishes this devitalisation of the uterine epithelium from the physiological devitalisation that occurs at the menopause, and therefore eliminates the consideration of cancers ordinarily occurring at the "cancer age". Epithelial cells of the uterus thus deprived of oestrin atrophy. Although a directly opposite conclusion to the above was reached in the case of mice by Cioli (29) who obtained carcinomata from the injection of tar into the uterus only after oophorectomy, the experimental evidence pointing to the fact that devitalisation is of no importance in carcinogenesis, is abundant. Why this is so, it is not possible to discuss here in detail. A brief summary may be appended.

It appears that unless the metabolism of a tissue is characterised by a high glycolysis normally, its respiratory enzymes have to undergo a certain number of oxidations and reductions before malignancy can develop. The evidence for this is abundant (38a). We know that oestrone enhances the respiration of the uterus (60a, 90a), and that the indophenol reaction of the uterine epithelial cells is enhanced by various female sex hormone extracts (61a). If deprived of oestrone through ovariectomy, the epithelial cells of the uterus are subject to less respiratory stimuli. Consequently the respiratory substances cannot undergo the initial number of

of oxidations and reductions which are necessary prior to malignant transformation within the same time as if oestrin were present.

This may account for the resistance of the ovariectomised series in the above experiments to the development of cancer.

THE DEVELOPMENT OF TAR CANCER IN VITAMIN-DEFICIENT TISSUE

Mention has been made above of the conclusions of Passey (106) and of Roussy, Leroux and Peyre (114) that elimination of Vitamin A from the diet made no important difference in carcinogenesis in mice, and of the conclusion of Imura and Toshiro (52) that the same obtained in rats.

Passey has shown in recent years that omission of Vitamins A and B from the diet of rats did not favour the development of gastric carcinoma subsequent to feeding of the parasite, Gongylonema neoplasticum .

The reason why deficiency of Vitamins A and B although associated with epithelial hyperplasia, does not enhance the chances of cancer development is probably similar to the above. It has been mentioned that tissues, unless characterised by a high glycolysis, apparently have to undergo an initial number of oxidations and reductions before malignancy can develop. For example, in the case of cancer of the skin of animals caused by the carcinogenic hydrocarbons, we know that these substances enhance the respiratory processes and increase the oxidase reaction.

The epithelial hyperplasia caused by Vitamin A deficiency is probably due to the following mechanism : In plants the precursor of Vitamin A, phytol, forms an integral part of the chlorophyll complex. There is also a definite quantitative relationship between the carotenoids and chlorophyll in green leaves. Plants can synthesise phytol, $C_{20}H_{39}OH$, and Vitamin A, $C_{20}H_{29}OH$ from CO_2, and water through

ultra violet rays. Metazoa cannot synthesise them, hence are dependent on plants for their source. In plants the function of Vitamin A has to do with oxygen carriage or the catalysis of oxidation. In metazoa the function is probably similar, and it is very probable indeed that Vitamin A functions in conjunction with tetrapyrrolic porphyrin respiratory substances, such as cytochrome, peroxidase, and catalase, just as in plants, it functions with chlorophyll, a tetrapyrrolic porphyrin substance.

With deficiency of Vitamin A, respiration may be impaired, and the glycolysis of affected cells thereby increased. We know that with growth and differentiation, respiration gradually controls glycolysis, and that with interference with respiration under certain conditions, glycolysis may gain sway, and abnormal cellular proliferation ensue. Since the respiration of cells in Vitamin A deficiency is thereby diminished, even though epithelial hyperplasia, (due to glycolysis) is present, the probable explanation for the resistance of these cells to experimental tar cancer is the fact that the respiratory enzymes of the cells cannot undergo the oxidations and reductions necessary prior to malignant transformation.

It is impossible to discuss this subject in its entirety here. The author intends to do so elsewhere in the immediate future. Nevertheless, the evidence is sufficiently suggestive to indicate why omission of Vitamin A from the diet does not hasten the appearance of tar cancer or cancer due to irritant parasites.

It appears that malnourishment or devitalisation does not expedite the development of cancer. If anything, it retards it. For example, the natives of South Africa, with respect to whose health nutritional surveys have been carried out, are said to be malnourished. This is tantamount to some degree of devitalisation of their tissues, especially in view of their subnormal vitamin intake. Yet in Johannesburg

the incidence of cancer in the Bantu population is five times less than that in Europeans (12a). The same applies to the incidence of cancer in the poorer classes and slums as compared with that of the well-fed classes. Here the incidence is said not to vary appreciably, yet it has to be considered whether the tissues of the former are not in a malnourished state, especially as regards vitamins, and whether they may not be considered to be somewhat devitalised.

G. SUMMARY

1. Experimental tar carcinogenesis is briefly reviewed in most of its aspects.

2. Investigations into a new method of experimental carcinogenesis in albino rats are described, and the rationale for these discussed.

3. They consist of : (a) ligaturing the vagina and injecting tar into the uterine lumina: (b) ligaturing a uterine horn and injecting tar into the lumen of the proximal end; and (c) as in (b) with removal of both ovaries in addition.

4. Only one neoplasm was obtained out of 22 animals thus treated - a squamous epithelioma of the left uterine horn, with metastases in the liver. This tumour arose from the series of injected animals (eight in number) whose vaginae had been ligatured.

5. Several phenomena observed during the period of experimentation are described and briefly discussed. These include the occurrence of gangrene (thrombo-angiitis obliterans?) in an ovariectomised animal and its possible significance, instances of the extreme degree of resistance of rats to infection, and the great proliferative potentialities of the rat's tissues.

6. Devitalisation of the uterine epithelium in young animals - regarded as following on removal of the ovarian tissue - did not make it more probe to malignant transformation in these experiments.

This is discussed in relation to wider applications.

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