UNIVERSITY OF CINCINNATI

May 10 1949

I hereby recommend that the thesis prepared under my supervision by Louis Soffer

entitled Radiosulfonamides for Cancer Research

be accepted as fulfilling this part of the requirements for the degree of Doctor of Philosophy

Approved by:

Francis C. Ray

Hoke S. Greene

Ian R. MacGregor

Form 668—G.S. and Ed.—500—3-42

RADIOSULFONAMIDES FOR CANCER RESEARCH

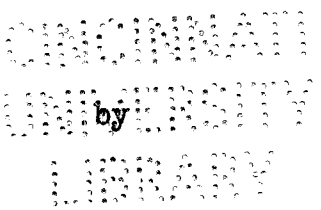
A dissertation submitted to the

Graduate School of Arts and Sciences
of the University of Cincinnati

in partial fulfillment of the
requirements for the degree of

DOCTOR OF PHILOSOPHY

1949



Louis Soffer

B.S. Washington University 1942
M.S. University of Cincinnati 1948

ACKNOWLEDGEMENT

For the stimulating criticism and skillful guidance of Dr. Francis Earl Ray we wish to express our sincere appreciation.

We also wish to thank Dr. Charles D. Stevens for many helpful discussions and his support of this work.

TABLE OF CONTENTS

1) O N C O L O G Y

1.1) Introduction

Cancer research, or oncology, is not a science in itself, but the simultaneous and frequently coordinated activity of many independent scientific disciplines, such as clinical medicine, surgery, pathology, radiation physics, genetics, immunology, endocrinology and biochemistry. The common aim of these approaches has been the elucidation and control of neoplastic transformation (1).

Claiming one life of every twenty persons born (2), cancer has been called "the most challenging disease which confronts biological science". (3). No region of the body is exempt from malignant tumors. There are no early symptoms in cancer of the esophagus, stomach, rectum, intestines, pancreas, kidney, lung and other internal organs which call attention to the disease at a stage where it is curable in any large proportion of cases (2).

Cancer involves the most basic phenomenon in biology, namely, growth. According to Ivy, the disease is characterized by abnormal, unlimited, unregulated growth of tissue which destroys the host which nourishes it. It is unique among diseases because the host is consumed by its own flesh. The thought of this fact, the frequently associated pain, and the insidiousness and treachery of its attack place cancer first among the most dreaded diseases.

1.2) Historical

1.21) Cancer as a Descriptive Science

Cancer as a disease was recognized in antiquity and, indeed, its very name (from the Latin "crab") is derived from the ancient description of its frequent clinical course. Neoplastic diseases or tumors are mentioned in the oldest remnants of Indian and Persian literature, and in the Papyrus Ebers (1500 B.C.) (4). They were then treated by excision and with escharotics, among the latter being an arsenical ointment used in Egypt.

By the time of Hippocrates (about 400 B.C.) a multitude of descriptive facts concerning malignant growth in tissues and organs had accumulated. Hippocrates was the first to differentiate between indolent ulcer ("carcinoid") and progressive malignant tumor ("carcinoma"). These terms have carried over into modern concepts of cancer, although the word "carcinoma" is today restricted to tumor of glandular epithelium in a typical arrangement.

Galen (150 A.D.) described two types of tumors, one benign and the other malignant: the latter was called "cancer".

In the period that followed until the time of the Renaissance little of value was learned about cancer. Methods of treatment were subject to the fashions of the time. Some surgical procedures, however, appear to have been remarkably

successful.

With the general awakening that was the Renaissance, oncology received a great stimulus. Astruc (1684-1776) was the first to employ chemical methods in cancer research. He ashed cancer and muscle tissues and reported that he found them to contain "the same salts". Le Dran (1685-1770) studied neoplasms (malignant growth), and conceived the role of the lymph in the process in which cancer cells are transported to distant tissues, a phenomenon known today as metastasis. Peyrilhe tried to demonstrate a virus principle originating from tumors, a theory still of interest today. Hunter (1728-1793) affirmed that tumor tissues were nourished by the body like normal tissues and develop following the same biological laws. Potts, in 1775, accurately described the cancer of chimney sweeps and traced its etiology. Some 150 years later this observation led to the work on carcinogenic hydrocarbons.

In 1820 Mannoir announced that each tumor results through morbid changes of the tissues from which it arises. Six years later microscopic studies by Raspail showed that growth of tissues results from the multiplication of cells. In 1838 J. Mueller published his classical studies on microscopic findings in malignant tissues. During the perio[illegible] of Virchow careful histologic studies were further extended.

By 1860 the main types of tumors had been described

accurately and classified mainly on the basis of microscopic structure. The existence of benign tumors, various types of carcinoma (tumor of the glandular epithelium) and the distinctness of sarcomas (tumors of connective tissues) and epitheliomas (tumors of epithelial cells) were accepted facts.

In the middle of the 19th century, when it had become generally agreed that a cancer consisted of cells, two hypotheses regarding the nature of cancer received recognition. One maintained that normal cells can be transformed into cancer cells by irritation or infection of some type; the other, that the cells which form cancer were cancerous from embryonal life as the result of an hereditary blemish or an embryonal rest of cells excluded from the line of normal development.

By 1900 it had been shown that neoplasia could be of local origin and that metastasis arose from transported cells.

These were the principal contributions of the descriptive scientific approach to the problem of the cause of cancer. The descriptive science of cancer added nothing to the treatment of the disease, since therapeutics is entirely an experimental science. And, it is generally agreed that the cause of a disease remains a theory until established by controlled experimentation.

1.22) Cancer as an Experimental Science

The study of cancer as an experimental science began just prior to the turn of the century, when cancer was first transplanted in laboratory animals by Hanau (1889) and Moreau (1891) - a fact which was soon confirmed by Leo Loeb (1901) and Jensen (1902).

Workers of the Schneeberg mines in Germany for years had been known to have a strangely prevalent fatal disease of the lungs. It was later identified as cancer of the lung caused by inhalation of radioactive particles from the ore of the mines (5).

Shortly before World War One, Fibiger (6) found that feeding of cockroaches which were infested with certain parasites to rats resulted in the production of carcinoma of the stomach.

Cancer had been known for a long time to be an occupational hazard for chimney sweeps. In 1918 Yamagiwa and Ichikawa reported the production of carcinomas in rabbits by application of coal tar (7). Some 15 years later Kennaway andCook (8) discovered the carcinogenicity of certain polycyclic hydrocarbons. Methylcholanthrene was by and large found to be the most potent compound of this group. The mechanism by which the carcinogenic hydrocarbons exert their biologic action is not yet understood. Hartwell (8) published in 1941 a list of some 700 chemical compounds which had been

tested for carcinogenic activity up to 1939, and reported many of them active.

Clinicians have pointed out the possibilities of hereditary factors in neoplasia ever since Warren's report on a "cancer family" in 1837 (9). In 1914 Little began to breed mice with the aim of developing cancer-resistant and cancer-susceptible strains. Several strains of animals with naturally high and naturally low incidence of various types of malignancies are today available for research (10). At first sight this seemed to prove the influence of heredity.

By the use of pure strain mice, Bittner (1936), however, could show that an extrachromosomal factor plays an important role in rendering mice susceptible to neoplasia. This factor is present in the milk of mice and was therefore called the "milk factor" or "milk influence". When mice of a high mammary tumor strain are suckled by foster mothers from a low mammary tumor strain, the nursed animals show a low tumor incidence. The complimentary procedure, feeding high tumor strain milk to low tumor strain infant mice produced high tumor strain animals (11).

The first attempt at chemical therapy of cancer that was at least partially successful was made by Huggins (12) in 1940. His demonstration that estrogens cause epithelial atrophy and consequent shrinkage of the total bulk of the hypertrophied prostate gland in dogs laid the basis for clinically

successful androgen deprivation of cancerous human prostates, either by orchiectomy or with estrogens. That vitiation of androgen is the key to the suppression of neoplastic activity in the human prostate may be demonstrated by the fall of the serum level of acid phosphatase, which is always in excess in cases of prostatic carcinoma and particularly in the presence of metastasis.

In further work Huggins reported that remissions of varying length of time occurred in 18 patients among 20 with disseminated prostatic cancer treated with orchiectomy, and that 4 of the patients showed no clinical (laboratory) evidence of cancer at this time. He concluded that the anti-androgenic therapy of cancer of the prostate demonstrates that a clinical change in the internal environment of the host "had brought about a long continuing regression of a malignant neoplastic process" (13).

Somewhat along these lines, a study of the influence of synthetic estrogens upon advanced malignant neoplasia was announced by Haddow, Watkinson et al., in 1944 (14). This group reported that 10 of 22 cases of late malignant disease of the breast treated with triphenylchlorethylene showed significant, although temporary, retardation or even partial regression. However, no evidence was obtained suggesting the drug will prevent metastasis. The results obtained with cancers other than of the breast were much less satisfactory.

In 1943, Nettleship and Henshaw noted that urethane hastened the appearance and raised the incidence of lung tumors in certain strains of mice (16).

A relatively new point of attack, dietary control of cancer, was begun in recent years. Tannenbaum (15) reported in 1942, that a diet restricted in caloric content per se definitely inhibits the formation of induced epithelial tumor, induced sarcoma and spontaneous breast tumor in mice. Some three years later, Huseby, Ball and Visscher reported the striking information that the final incidence of mammary carcinoma in virgin C3H mice maintained from weaning on a diet supplying the same amounts of protein, vitamins and salts as an ad libitum fed control group received, but restricting the caloric intake by one-third, was 0% as compared to 72% for the control animals. (17). The authors postulated that the mechanism by which caloric restriction per se reduced the incidence of mammary carcinoma was probably pituitary insufficiency producing

(a) a lowered level of ovarian secretion

(b) a relative refractoriness of the mammary gland to estrogenic substances.

1.3) Effects of Radiations on Living Cells

1.31) Types of Radiations

Because radiation therapy is an accepted treatment of cancer it is well to discuss the more important types of radiations.

The activity or strength of a radioactive material is the direct result of its rate of decay. A source which decays such that $3.7^{x}10^{7}$ atoms change per second is of 1-millicurie strength (18).

X-radiation is electromagnetic radiation, emitted in quanta, of wave length 0.05-10 A. On account of their greater penetrating power it is more convenient to measure the energy absorbed in a given volume than the total energy incident on a surface. Therefore use is made of the fact that when absorption takes place in air the latter becomes conducting and the saturation current through a given volume of air is a measure of the rate of energy absorption in that volume of air. X- as well as gamma radiation absorption is measured in units called "roentgens". One roentgen is that quantity of X- or gamma radiation such that the associated corpuscular emission per 0.001293 g. of air produces, in air, ions carrying 1 electrostatic unit of quantity of electricity of either sign. The roentgen is also the unit of dose employed and corresponds to the liberation of $2.082^{x}10^{9}$ ion-pairs per cm^{3}

of air at standard conditions of temperature and pressure, involving an energy dissipation of 0.1083 ergs / cm^3 of air. In terms of dosage to animal tissue one roentgen is equivalent to the absorption of 83 ergs / g. of tissue.

Gamma rays are natural X-rays of short wave length whose absorption in tissue is entirely by Compton recoil thus producing electrons of all energies from zero up to 2.0 Mev. (million electron volts). As has been mentioned gamma rays are measured in terms of roentgens.

Beta rays are fast electrons emerging from nuclei in radioactive transformations. Since the "roentgen" by definition is used to designate only X or gamma absorption, the "roentgen equivalent physical" is employed for the equivalent rate of absorption produced by beta and other types of radiation. According to Evans, "one equivalent roentgen of beta radiation per day is delivered to tissue containing 57/E microcuries per kilogramof pure beta ray emitter whose maximum beta ray energy in mev is E" (19).

Alpha rays are nuclei of helium atoms emitted spontaneously by radioactive materials and also obtained artificially with cyclotron. Their range in tissue is very short; therefore, they are only usable with materials obtainable in thin films. Alpha rays, with their larger size, produce many more ionizations per micron path in tissue than do electrons; the comparison of the relative

efficiencies, per ionization, of electrons and alpha rays is valuable for testing theories of the biological action of radiation.

A radiation dose of 0.1 roentgen per day was the tolerance level for X- and gamma radiation permitted on the atomic energy projects and is considered roughly to produce no biologic effects. Thus the maximum safe concentration of an isotope which emits beta rays only would be 5.7/E microcuries per kg., where E is the maximum energy.

Since the effect on tissue of equivalent energy absorption of different types of radiation varies for gamma beta and other radiations, the unit "roentgen equivalent man" (r.e.m.) is used to designate the amount of radiation which produces the same biologic effects in man as one roentgen of X- or gamma radiation. On this basis the maximum permissible exposure value has been set at 0.1 r.e.m./ 24 hr. day.

1.32) Mechanism of the Action of Ionizing Radiation

Lea (20) classifies alpha, beta, gamma radiations, X-rays, protons and neutrons as ionizing radiations. The principal means of energy dissipation by an ionizing radiation in its passage through matter is ejection of electrons from the atoms through which it passes. An atom so ionized is left positively charged, and is referred to as an ion. It is possible that some actions of radiation of biological significance aredue to this separation of electrical charge, but

in most cases it seems more plausible to attribute the action to chemical change resulting from ionization. For when an atom is ionized the molecule of which it is a part almost certainly undergoes chemical change.

A second method by which radiation dissipates energy in tissue is by excitation. This means the raising of an electron in an atom or molecule to a state of higher energy and is a less drastic process than the complete ejection of an electron.

Practically all the energy dissipated by radiation in tissue ultimately becomes degraded to heat energy. Thus, a dose of 10^5 roentgens is sufficient to raise the temperature about $0.25^{\circ}C$. This small temperature rise for a large dose of radiation means that temperature change is quite inadequate to explain the biological effects of ionizing radiations in the way in which temperature rise accounts for most of the biological effects of, for example, short-wave wireless waves. On the other hand, the highly localized nature of the energy dissipation means that the energy, which eventually causes a rise of temp of $0.25^{\circ}C$. in the tissue as a whole, is initially confined to a small proportion of the atoms and might be considered to produce a large rise in temperature of these atoms. This is the basis of the "point-heat" theory of Dessauer (1923) (21). The concept of an ionization as a "hot spot" is considered less satisfactory than the concept of an atom ionized leading to chemical changes in its molecule.

Ionizing radiations constitute a useful therapeutic and experimental tool but, at the same time, a dangerous one. It is increasingly important that clinical effects of radiation be understood and methods of therapy be explored and made available.

Researches in clinical physiology and biochemistry have been concerned with (1) ascertaining whether different kinds of ionizing radiation have similar effects upon the mammalian body, (2) measuring sensitive reactions as a function of dose in the hope of setting permissible exposure limits, (3) searching for sensitive biological indication of exposure, (4) describing the course of radiation damage sufficiently to suggest profitable directions for research into the mechanism of such damage that might eventually lead to effective therapy.

Recently the results of investigations on the action of radiation on animal tissue, carried out in connection with the Plutonium Project, have been published. Conclusions were drawn, from findings on experimental animals, with regard to the problem of human protection against penetrating radiation.

According to Prosser (22) every kind of ionizing radiation is similar in its clinical action whether it be penetrating external radiation or internal radiation from deposited materials.

The most sensitive organ systems are the blood-forming organs, the gastro-intestinal tract and the gonads. The peripheral circulation and the heart are also effected. Terminally, there may be damage to the kidney and even to

the central nervous system. Some of these effects are probably direct and some indirect as a result of toxic agents, or of hypoxia, infections, or other secondary factors.

No single clinical reaction is peculiarly specific for irradiation damage. There are striking similarities to anaphylactic shock and many of the delayed effects of irradiation can be duplicated by various toxic chemical, e.g., anemia in benzene and phenylhydrazine poisoning, and tumor induction by coal tar derivatives.

Experiments studying the effects of X-rays on mice, rabbits and guinea pigs by Lorenz, Heston, et al., showed that long-continued absorption of penetrating radiation is associated with damage even in doses of 0.11 r. given in 8 hrs/day. These authors define a permissible dose for chronic irritation as one in which the damage cannot be expressed in pathological changes. Experiments indicated that the blood picture is of little value in determining radiation damage, except in cases of obvious over- exposure. Carcinogenic action and sterility effects dominate the picture of radiation injury. Mice and guinea pigs exposed to 1.1 r. showed an essentially normal blood picture for total accumulated doses of over 1000 r.; yet an appreciable decrease in life span and an increase in tumor incidence occured. It is known, at least in the case of the ovarian tumors, that the tumor inducing dose is cumulative and independent of dose level. Hence it is of paramount importance that the present

permissible maximum dose of 0.1r./day be not exceeded. Human ovaries are somewhat less radiosensitive than mice ovaries (24). Approximately 700 r. in divided doses is necessary for permanent sterilization while a single whole body exposure of 300 r. to LAF_1 mice produces sterility. As ovarian tumors in mice are similar in type to those observed in human beings, we may have to assume, unless shown otherwise, that in the latter similar degenerative processes may be induced by chronic irradiation and lead to tumor formation. It follows that the permissible dose of 0.1r./day should be reduced for women to perhaps 0.02r./ day or that the time of exposure should be reduced to a few years (21).

As far as genetic changes are concerned, the present permissible dose gives a sufficiently wide margin of safety so that a detectable increase in the rate of gene mutations and translocations will not occur.

In certain types of work, e. g., handling of radioactive substances, a larger permissible dose to the hands may be considered safe if the rest of the body does not receive more than the permissible dose of 0.1 r. Since their data showed no radiation injury to the skin of experimental animal exposed to 8.8 r. given in 8 hrs./day up to a total dose of 10,000 r., Lorenze concluded that it seemed possible to increase the permissible dose to the hands to 1 r./day.

It has long been assumed by some that beta rays would produce in biological systems effects identical with X- and gamma rays since the latter radiations expend their

energy by the ejection of high speed electrons from the atoms of the biological materials in which they are absorbed. This assumption has proven valid by experimental work by Raper (25) and others at Clinton Laboratories on two biological materials which were small compared to the range of beta particles; fern spores and eggs of the fruit fly, Drosophila melanogaster. With larger biological objects, however, the distribution of energy absorption differs markedly between beta rays and the more penetrating electromagnetic radiations and consequent differences in effect are to be expected.

In the irradiation of laboratory animals with beta rays from external thick phosphorous sources all the energy is absorbed in a superficial layer of tissue which in the mouse comprises about 1/3 the animal's volume, in the rat about 1/6, and in the rabbit about 1/20. Thus, in effect, total surface irradiation with beta rays furnishes a means of studying the effects of high doses of an ionizing radiation delivered almost exclusively in larger animals to a single organ, the skin (25).

1.3) Radiosensitivity of Tissue

Radiation therapy, in addition to surgery, is so far the closest approach to an effective weapon in the fight against cancer. Therapeutic X-ray apparatus and radium are standard equipment on every tumor service. However, both possess certain innate shortcomings. In many cases it is

impossible to subject only tumor tissue to radiation with the result that healthy tissue may be adversely affected. When treating an internal neoplasm skin tolerance is often reached before a sufficient radiation to check tumor growth has been administered. The use of radium which might obviate the latter difficulty, is limited to tumors which can be reached by radium applicators.

No simple scheme permits one to decide whether or not a tumor is radiosensitive. It is felt by some as information accumulates that the "behavior of tumors to radiation " is a very individual problem and that the responses of particular types is only expressible in terms of averages (26).

Radiosensitivity may mean rapid tumor regression, slower progressive regression over a period of from days to weeks, or slow chronic atrophy requiring months or perhaps a year for completion. Of course, the same mechanisms are not responsible for these various responses nor do they occur in the same types of tumors. Radiosensitivity does not mean the certainty of cure by irradiation nor does radioresistance imply that a given tumor is not curable by irradiation. When one states that a carcinoma of the breast, for example, is radiosensitive, it should be implied that the particular neoplas is sensitive according to the accustomed scale of behavior of tumors of the breast and not according to the scale of sensitivity applied to some other tumor, such as lymphosarcoma.

Radiosensitivity, in general, increases with increasing embryonal quality of the tumor cells, and with increasing degree of anaplasia (that is, with increasing degree of reversion of cells to a more primitive and undifferentiated form). However, not all anaplastic tumors are radiosensitive, or equally sensitive.

The effect of radiation is always complex. It involves not only the tumor cells but the tissues of the host, and possibly, general reactions on the part of the host. The ultimate effect of the radiation must always result from a nice balance between tumor effect and response of the host tissue. The relative importance of the two mechanisms varies in different tumors. In some instances the known sensitivity is such that the radiologist may endeavor to destroy the tumor cells. In others he can but hope for restraint of growth by moderately affecting the tumor cell and trusting that the response of the host tissue will play a decided role. Under such circumstances, tumor cells may remain in an indolent state in the midst of fibrous tissue for many years without giving rise to further damage (26).

1.4) Frontiers in Cancer

1.41) Some Provocative Biological Problems.

The advances in the basic knowledge of cancer have not yet answered with certainty some of the fundamental biological questions involved. (3).

1. What is the difference between benign and malignant tumors?

Fundamentally it is best to view benign and malignant tumors as manifestations of the same neoplastic principle, the benign tumor or cell representing an arrested or inhibited stage of a potentially malignant process.

2. Does the cancer cell represent a transformation of a normal cell or a cell abnormal from embryonic life?

The evidence seems to indicate that malignant cells may arise either from embryonal residues or from normal cells. But it is really immaterial whether a normal cell or an "embryonal residue" is changed. The important question is:

3. What is the nature of the process induced in the normal cells, or if one prefers, the "embryonal residue" when they become cancerous?

It would appear that the rational approach to a cure or control of cancer would be to ascertain either the factors which are responsible for causing unregulated, invasive, destructive growth or those which are responsible for limiting and regulating normal growth.

4. How autonomous is a neoplasm?

"Autonomous" implies growth unregulated and independent of the host except for nourishment. If a malignant growth is entirely autonomous, nothing manufactured in the body of the host except food of some type could influence it. This is not true of some cases of cancer of the prostate with metastasis to the bone in man, since Huggins and his colleagues (13) have observed the disappearance of metastasis in bone and the primary tumor after castration or the administration of a large dose of stilbesterol to the patient. The cells of tumors of the prostate and breast which are markedly benefited by modifying the sex hormone supply do seem to be sufficiently transformed to be not entirely autonomous.

5. What factors are concerned in the induction of tumors?

The intriguing studies of the natural occurrence of mammary cancer in inbred strains of mice have indicated that at least 3 factors are involved (1) a genetic constitution, or pattern, or inherited susceptibility, (2) the female sex hormone, and (3) the "virus-like" agent transmitted in the milk of mice (11)(15)(17)(27).

Many other examples exist showing the complexity of the endegenous and exogenous factors concerned in the genesis of cancer in mice. To render the problem still more complex, the species differences are marked, and the influence of factors which predispose to cancer in one species may re-

tard in another species. For example, though reproduction and nursing unequivocally predispose to cancer of the breast in the strains of female mice studied, they have the opposite effect on women.

6. What is the effect of diet on the evidence of cancer?

Diet has demonstrated its importance in inhibition of tumor induction and has been shown to exert a significant effect in rat hepatoma (25). The possible effects of diet on human tumors will be discussed more thoroughly in Section 2.

1.42) The Virus Theory (28)

Evidence (29) has existed for more than forty years indicating that ordinary cells are able to behave as if cancerous in response to extraneous influences, thus justifying the inference that activating cause for tumors need only work on capabilities which cells innately possess. This view opposes the conception of cancer as a "secret bound up with life itself". There is no need to suppose that tumor cells are the outcome of some inherent fundamental cell change, a favorite supposition.

As presented by Rous there are two clearly defined classes of carcinogens, (1) "provocative" carcinogens, and (2) the "actuating" carcinogens.

The "provocative" chemical carcinogens have been mentioned previously. As yet all attempts at obtaining a virus of some sort from cancer has failed. But this does

not mean such agents do not exist. Chicken tumors, for example, which have yielded viruses regularly for a long time may cease for considerable periods to yield any and then, as unaccountably, do so again (30)(31).

Certain obstacles to supposing that viruses causes the generality of tumors have forced those interested in the concept to make several assumptions:

(1) To account for world-wide distribution of tumors and for the occurrence of growths of peculiar type at widely separated intervals of space or time, it has been necessary to suppose that the body carries resident viruses just as it does resident bacteria. These are indigenous viruses, ordinarily harmless, but now and again becoming so changed in response to conditions as to work on the cells around them, with the creation of tumors. Such viruses might reach young creatures in early life by transfer from parent to offspring, either in utero, or after birth. They would remain inactive until a provocative carcinogen happened to work on the cells with which such a virus was associated. It might then undergo alternation and take a hand in cell affairs for the first time, giving rise to a tumor. The new pathogenic variant would not be transmitted to other animals under natural circumstances for reasons as yet obscure. It would be a dead-end virus, not concerned in the production of other neoplasms, though the harmless source virus liable to

the same or other variations would be passed on.

The discovery of the "milk factor" provided an instance which embodies this conception almost point for point (32)(33)(34)(11)(35)(36). Furthermore, it has been shown that estrogens (37) when provided in excess, enhance the natural liabilities to "milk factor" tumors; also, one of the potent carcinogenic hydrocarbons, methylcholanthrene, a substance devoid of estrogenic effect, causes tumors to appear very soon, and in great numbers, when applied to mice of a strain in which the milk factor is present. (38).

In summary, we may say that as experimental satistics reveal, cancer "may only be prevented by preventing human beings". Nevertheless, there are many who are reasonably optimistic of great discoveries in the next ten or twenty years.

2) GASTRIC CANCER IN MAN

2.1) Introduction

Gastric cancer is the most frequent of all malignant growths. "There are more deaths from cancer of the stomach than from all malignant tumors of the lip, tongue, cheek, tonsil, pharynx, larynx, salivary glands, thyroid, male and female breast, ovary, uterine cervix, and corpus uteri combined". (39).

Of 150,000 annual deaths from cancer in the U.S., approximately 40,000 persons die of gastric cancer. Moreover as the proportion of aging people in the population increases it is obvious that the total number of deaths from cancer, as well as gastric cancer will increase.

Cancer of the stomach is "silent" or produces no symptoms in 25 - 30% of autopsied persons dying of the disease (3). In fact the evidence indicates that the disease is overlooked in 50% of the cases which are attended at our best general hospitals, for the incidence of gastric cancer, according to mortality statistics, is 16% of all cancers, whereas the incidence according to the records of general hospitals is only from 6 - 8 % of all cancers. Not only does a large percentage of silent cases exist, but at least 20% of the cases died of a surgically resectable cancer or one without metastasis beyond the adjacent lympth nodes (39).

The surgical mortality from gastric resection has

been reduced to such an extent that, in the opinion of Ivy, it may be now stated that if there were some simple method for the diagnosis of early gastric cancer, the mortality from this disease could be reduced from 30 - 50%, and perhaps even more. The uncovering of such a diagnostic tool is one object of this investigation.

2.2) Occurrence and Etiology

The disease is predominantly found in males in the ratio of about 2 to 1. The peak age incidence is between 50 and 70, but about 8% of cases are patients under 40 years of age (40).

Present statistics indicate that cancer of the stomach occurs twice as frequently in the Dutch as in the British. Although the evidence is equivocal the cause has been ascribed to the greater consumption of irritants in the food and drink of the Dutch (41). English statistics have shown that cancer of the mouth, tongue, throat, larynx, skin and stomach are much more frequent in poorer sections of society - areas which are exposed to irritation by environmental influences in the shape of food and drink (42). Such observations strongly indicate that extrinsic factors are concerned in the genesis of cancer of the stomach. This should warrant and stimulate a thorough search among food and drink for carcinogens. An agent in heated fats and something produced by heating cholesterol will cause sarcoma occasionally when injected subcutaneously in mice or rats. Nevertheless, it has not been established that the feeding of heated fat, cholesterol, or irradiated cholesterol will cause cancer of the stomach in the experimental animal. However, the fact that Peacock (44) has demonstrated existence of carcinogenic substances in heated fats should render them suspect when taken daily over years.

Little of an undisputed nature can be said on the direct etiology of gastric cancer. According to Ewing, the existing knowledge of early stages of the disease points to chronic nutritional and functional disorders, connected with dietary habits, as essential steps in its development. Stout (44) lists several factors which may be regarded as chronic irritants - gastric ulcers, benign epithelial neoplasms and hyperplasia. Just how frequently gastric ulcers develop into gastric cancer is a hotly debated question - a certain number of cases do develop. Hurst (45) believes that chronic gastritis precedes gastric cancer in 75% of the cases, chronic gastric ulcer in 20%, simple adenoma in 4.5%, and generalized polyposis in 0.5% but no unanimity of opinion exists in regards to such a percentage distribution.

Comfort and Vanzant have observed that the gastric secretory function is subnormal in patients with carcinoma of the stomach (46) and for a large number of cases studied, the mean secretory activity had been subnormal for an average interval of 11 years before the diagnosis of cancer was made (47).

Rhoads (48) advances the suggestion that certain forms of gastritis are in fact precancerous alterations, that they are the result of deficiency and that they may be cured by adequate dietary supplement. However, it is emphasized that in no sense is it maintained that gastric cancer, or any large

part of it, is the result of dietary deficiency, nor is there any suggestion that the malignant change once established, can be reversed by any means.

2.3) Treatment

Surgery and radiation therapy are the two available treatments for gastric cancer.

Most authorities agree that the only chance for a complete cure of carcinoma of the stomach is surgical resection of the lesion (39)(40). The resection rate for carcinoma of the stomach has greatly increased in most clinics and the operative mortality has decreased. But, unless the resection is carried out at a very early stage in the growth of the tumor, the patient's life is being prolonged, at the very best, for a few years. In 1943, Pack summarized the results of 303 total gastrectomies. The mortality was 37% and there were only 16 patients (5%) who were known to have lived more than three years. It seems logical that this operation should be reserved for smaller rather than larger lesions, for with well-advanced cases the operative mortality is almost prohibitive, and the number surviving 5 years is too small.

A gastric ulcer should preferably be treated radically rather than by local excision for in some instances the surgeon, no matter how capable, cannot tell at operation whether the lesion is benign or malignant (40).

Considering the very small proportion of patients who eventually benefit by surgical treatment of carcinoma of the stomach, the contributions no matter how small of any other form of treatment would be welcome. In 1896, Despeignes (49) applied X-rays to the treatment of gastric cancer. He

and many others who have succeeded him in hoping for a curative effect of radiation have been disappointed. Werner in 1915 published the results of the treatment of over 100 patients with cancer of the stomach by X-rays with some temporary results but no permanent cures. Evans and Leucutia (51) Govet and Regaud (52) had similar experiences. Radiotherapy, however, may contribute unexpected relief and remissions. (53).

In spite of some radiosensitivity shown by these tumors radiotherapy usually fails because of the pathological character of gastrointestinal carcinomas in general and because of the widespread visceral extension and metastasis particularly present in carcinoma of the stomach.

Livingston and Pack (39) give a detailed discussion of the radiosensitivity of gastric tumors and the obstacles, restrictions and dangers attendant on radiation therapy.

2.4) Avenues of Approach to the Problem

Today the available possible approaches to the gastric cancer problem are many. As seen by Barrett (54), little is known of the role of heredity in gastric cancer, although the available evidence indicates genetic factors play a part.

There is as yet no conclusive proof of the causal relationships between gastric cancer and diet, and more experiments are desirable. There is no direct evidence of hormonal influence on gastric cancer, but more clinical and animal experimentation is indicated. The experimental production of atrophic gastritis appears highly desirable. Its relation to gastric cancer is unknown, but the majority of gastric cancers are associated with atrophic gastritis. Pernicious anemia and achlorhydria appears to have some unknown relationship to gastric cancer. The proof of the exact relationship of gastric carcinoma to gastric ulcer is lacking. Polyps, though their number is small in relation to the total of gastric cancers, play a definite role.

Barrett feels that one of the most pressing needs at present is for new and improved methods of diagnosing early gastric cancer. It is our purpose to throw some light on this problem by preparing radioactive compounds which may localize selectively in or near a gastric tumor. Such materials should prove extremely useful in the diagnosis and possibly treatment of the disease. This matter is discussed in more detail in the next section.

3) RADIOACTIVE SUBSTANCES IN DIAGNOSIS AND TREATMENT OF CANCER OF THE STOMACH

3.1) Selective Localization of Chemical Substances in the Body.

There are a considerable number of known instances where elements and compounds localize in certain organs and tissues with a high degree of selectivity. Outstanding examples are the deposition of calcium and phosphorus in bone and the uptake of iodine by the thyroid. Radiophosphorus, administered as a phosphate, hasbeen used in the treatment of leukemia and lymphosarcoma. Unfortunately, bone takes up larger amounts of the element than do the tumors, and the rather intense radiation to which the bone marrow is exposed limits the use of radiophosphorus in the treatment of these diseases (55).

Radioiodine has been used extensively in the investigation of thyroid physiology. Hamilton and Soley (56) have measured the uptake of radioiodine by the thyroid in patients by placing a Geiger-Mueller counting tube against the neck and directly over the isthmus of the gland. Iodide is also concentrated in the gastric contents and by the salivary glands (57).

Among the organic compounds known to localize are the contrast media used in X-ray diagnosis for the visualization of the gall bladder or urinary tract, such as tetra-

iodophenolphthalein, 2-oxo-5-iodopyridine-N-acetate (Iopax), monoiodomethane sulfonate (Skiodan), and many others.

As a result of Warburg's studies on tumor glycolysis a search was made for compounds which would suppress tumor metabolism, and thereby tumor growth, without being toxic to the organism of the host. A number of dyes and other organic substances were tested by Karczag (58) for their ability to inhibit the glycolytic activity of zymase and whole yeast, under the assumption that those compounds which inhibited the zymase, but not the yeast, were specific glycolysis inhibitors without being protoplasma poisons. Such compounds were to be used in cancer therapy. However, the results on experimental animals were disappointing. As a result of his studies, Karczag emphasized the importance of considering the non-specific properties of compounds to be used as chemotherapeutic agents, such as their solubility, dispersion, diffusibility, action on the reticulo-endothelial system, etc.

In connection with tumor glycolysis, it is known that lactic acid is produced in tumors within the living animal. Lactic acid is a relatively strong acid, its ionization constant being 1.37×10^{-4} (59). Its formation in the tumor produces a lowering of pH. Greenstein (60) records certain measurements made on a rat hepatoma 31 in which the pH of the tumor changed from 7.0 to 6.4 within 30 minutes after an intraperitoneal glucose solution injection. A control non-

tumor liver showed practically no change in pH.

This effect is of considerable interest as it might lead to a means of depositing a sulfonamide-type compound in or near a tumor. An injection of glucose a short time prior to the ingestion of a sulfonamide might very conceivably, through lowering the pH in the tumor, suppress the ionization of an acidic sulfonamide, thus reducing its solubility and forcing its deposition in the cancer tissue.

A desirable compound would require a considerable solubility differential, that is, its solubility at a pH of 6.4 should be perhaps one half the solubility at the pH of blood serus, 7.4.(We are assuming that the drop in pH in human tumor is comparable to that shown by the rat). It can be seen from the work of Schmitt and colleqgues that such solubility behaviour is entirely possible. In their experiments with six sulfonamides the average solubility at a pH of 6.4 was less than one half the solubility at 7.4 (6).

For acids ionizing as follows

$$SH_2 \rightleftharpoons H^+ + SH^-$$

$$SH^- \rightleftharpoons H^+ + S^=$$

Michaelis (62) has derived the following relationship between solubility and pH.

$$\Lambda = \lambda\left(1 + \frac{K_I}{(H^+)} + \frac{K_I K_{II}}{(H^+)^2}\right) \qquad \text{where}$$

Λ = total solubility = concentration of all the material present regardless of the form in which it is pre-

sent, whether as the undissociated molecule or ion.

λ = partial solubility = concentration of the undissociated molecule in the saturated solution.

K_I = primary ionization constant.

K_{II} = seconday ionization constant.

(H^+) = concentration of hydrogen ion.

The partial solubility, λ, could be determined by measuring solubility in the presence of added H^+, such as in dilute hydrochloric aicd solution (63), The (H^+) from the hydrochlorid acid would serve to inhibit the ionization of the sulfonamide so that the value of the solubility obtained would be, effectively, the partial solubility.

We may now set up two equations for the solubility-pH relationship at the two pH values in question, 7.4 and 6.4.

$$\Lambda_{7.4} = \lambda\left(1 + \frac{K_I}{(H^+)_{7.4}} + \frac{K_I K_{II}}{(H^+)^2_{7.4}}\right) \qquad \Lambda_{6.8} = \lambda\left(1 + \frac{K_I}{(H^+)_{6.4}} + \frac{K_I K_{II}}{(H^+)^2_{6.4}}\right)$$

We may neglect the $\frac{K_I K_{II}}{(H^+)^2}$ term since it will be of no consequence. Eliminating λ from the two equations and setting $\Lambda_{7.4} = 2\,\Lambda_{6.4}$ we obtain

$$2\left(1 + \frac{K_I}{(H^+)_{6.4}}\right) = 1 + \frac{K_I}{(H^+)_{7.4}}$$

Solving for K_I:

$$K_I = \frac{(H^+)_{7.4}\,(H^+)_{6.4}}{(H^+)_{6.4} - 2(H^+)_{7.4}} = \frac{(3.98\times10^{-8})(3.98\times10^{-7})}{(3.98\times10^{-7}) - 2(3.98\times10^{-8})} = 4.98\times10^{-8}$$

For the assumptions we have made, the above is the value of the ionization constant, that is, the degree of acidity, for a promising sulfonamide of the general structure R - SO_2NH .....HN_2OS -R where R is a neutral grouping. This information would be of value in consideration of materials to synthesize.

In relating the in vitro activity of N^1 - substituted sulfanilamides to their acidity, Bell and Roblin (64) have shown that as pK_a of the sulfonamide increases the bacteriostatic activity passes through a maximum and then decreases. This maximum pK_a is approximately 6.5 (65). A sulfonamide of $K_I = 5.0 \times 10^{-8}$ (calculated above to be desirable) would have a pK_a of 7.3, a value close to Bell and Roblin's value for maximum bacteriostatic activity.

In 1909 Goldman (66) observed that certain dyes seem to be concentrated by tumors. These findings have been confirmed and advanced by other investigators (67).

Duran-Reynolds reported in 1939 that T-1824 (Evans Blue) and other poorly diffusible dyes injected intravenously localize selectively in spontaneous and transplantable tumors growing in mice, rabbits and chickens (68). Brunschwig and colleagues extended this work, reporting that selective localization of the dye occurred in a majority of a series of human subjects presenting a variety of malignant neoplasms. Further, benign neoplasms did not show localization of the dye (69).

The first synthesis of a radioactive dye was re-

ported by Tobin and Moore (70). They prepared radioactive dibrom-Trypan Blue and radioactive dibrom-Evens Blue by brominating O-tolidine with Br^{82}(half life 34 hours) and coupling the radioactive dibrom-o-tolidine with H-acid or Chicago acid, respectively. Moore's group investigated the distribution of these radiobrom dyes in tumor mice and reported considerably more of the radioactivity in the tumor than in the skin. It was concluded that the "radioactive colloid permeates into and...irradiates tumor tissue wherever the tissue may be and no matter how widespread the metastasis...". The fact that the liver and other organs took up a large amount of radioactivity showed that the degree of selectivity in these particular compounds was much less than to be desired.

The selective uptake of dyes by malignant neoplasms can not be attributed to any specific characteristic of the tumors. It may be due to a difference in the pH of the cells or to increased capillary permeability. The latter is a phenomenon which occurs in certain pathological conditions other than cancer; for example, in abscesses or burns (71).

The dyes which stain tumors have also been found to localize in abscesses. Further, substances other than dyes were found to localize in tumors. Duran-Reynolds (loc. cit.) observed the accumulation of foreign sera in a number of animal neoplasms, as demonstrated by precipitin reactions. Dyes such as Evans Blue and Trypan Blue are known to combine

with plasma protein, particularly albumin, in quantitative proportions. (71)(72). This would seem to indicate that the accumulation of these dyes in the tumors is due to the presence of ceftain proteins.

Although no localization of a chemical material in malignant tissue is known to be involved the following experiments are noteworthy. Boyland (73) administered large and repeated doses of certain aromatic amines containing sulfur, such as 4,4'-diminodiphenyl sulfoxide and sulfanilyl sulphanilic acid, and reported the inhibition of tumors in mice. With further investigations complete inhibition of growth of spontaneous tumors in 4 of 4 mice was obtained by methylene blue, 4,4'-diminodiphenyl sulfoxide and 4,4'-diaminodiphenyl ether, the latter being generally most effective (74). In general the inhibition of tumor growth continued only for the period of dosing. When the dosing was stopped normal growth was restored.

It is our belief that derivative of these and similar compounds with S^{35} incorporated in the molecule, merit our study.

3.2) S^{35}

Of all the elements sulfur is second only to nitrogen in the frequency of its use as an index to protein metabolism. This is so because most, if not all, proteins contain one or more of the sulfur-containing animo acids, such as cystine, methionine or djenkolic acid, and because

sulfur plays an important role in numerous oxidation-reduction reactions in the body.

Of the three radioactive isotopes of sulfur, S^{35}, with a half-life of 87.1 days (75), is particularly well adapted for studies dealing with sulfur metabolism.

The nuclear reactions that would possibly yield S^{35} are as follows:

(a) S^{34} (d, p) S^{35}

(b) S^{34} (n, γ) S^{35}

(c) Cl^{37} (d, α) S^{35}

(d) Cl^{35} (n, p) S^{35}

Reaction (a) is not favored because (1) the small quantity of S^{34} in normal sulfur (2) the specific acticity obtainable is low because of dilution with normal sulfur and (3) targets containing a high percentage of sulfur cannot be prepared to withstand intense deuteron bombardment.

Reaction (b) is not indicated for reasons (1) and (2) given for reaction (a).

Reaction (c) is advantageous, particularly if carried out using a metallic chloride, the cation of which also yields a valuable radioactivity, such as RbCl. However, the apparent average cross-section for reaction (c) is 5-10 times lower at 14 Mev than that for reaction (a).

Reaction (d) represents the best method for S^{35} production with the uranium pile providing the source of

neutrons. Despite the rather high potential barrier to proton escape (about 4 Mev) the reaction is found to occur with thermal neutrons. The upper energy limit for the beta rays from S^{35} is about 0.17 Mev, with no appreciable gamma radiation observed. (76).

The assay of S^{35} is accomplished with the Geiger-Mueller counter, electrometer ionization chambers or the electroscope. A modified Lauritsen electroscope of high sensitivity for S^{35} assay has been developed recently (77).

3.3) Statement of the Problem.

The aim of this research was to work out suitable methods for the preparations of a series of compounds which could be made radioactive by the incorporation of S^{35} in the molecule, and which held promise of

(1) localizing in or near a gastric tumor

(2) exerting a possible therapeutic effect at the point of deposition either through their chemical nature or their radioactivity or both

(3) detection by means described below, thus being of potential diagnostic value.

If a compound containing the short-range beta emitter S^{35} localized in a gastric tumor the radioactivity should preferably be determined from within the stomach. This can be accomplished by fastening a piece of photographic film or a small Geiger-Mueller tube in place of the bucket of a Rehfuss tube or the lamp of a gastroscope.

Shapiro, Bloch and Schiff (78) have investigated gastric excretion of sulfadiazine, sulfapyridine and sulfathiazole in man, in a search for a substance applicable to the detection of gastric malignancies. None of these compounds were found satisfactory.

Work recently carried out in this laboratory has indicated that tumor mice show a greatly different uptake picture of radioactive iodosulfapyridine than normal mice (79). This is significant, because if it can be extended to human beings, it would be a possible means of diagnosis

for the presence of malignancy, and possibly the extent of malignancy. Further, were a radioactive material to localize in the tumor itself, the opportunity for therapy, as well as diagnosis, would present itself.

We have selected the sulfonamide linkage as a basic structure in which to incorporate S^{35} because (1) the function is apparently not attacked by enzymes (2) sulfonamides have a well known therapeutic value and (3) sulfonamides have been reported to localize in certain tissues (80).

Consideration of factors which might likely affect the concentration of a substance in neoplastic tissue suggests:

(1) the chemical nature of the compound

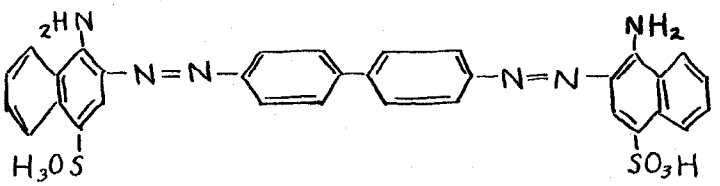
(2) The pK of the substance

(3) the molecular weight

(4) the solubility in water and body fluids.

A suitable compound should have active functions such as acid or basic groups so that combination might be effected readily with the tissue. Also, sulfonamides have been shown to reduce the effective vitamin intake of animals by suppressing the intestinal flora, and the reduction of essentail vitamins in the diet of tumor bearing mice is known to inhibit the growth of tumors (74).

Most of the dyes that visibly concentrate in tumor tissue can be related to the Congo reds, a type that is fast to cotton without a mordant.



Congo red

There may be no relation between such localization and the essential Congo red structure, but it would seem advisable to preserve some semblance to this structure.

With the above considerations in mind, together with Boyland's success with 4,4'-diminodiphenyl ether, the following variations in structure have suggested themselves:

(1) $RSO_2NH-C_6H_4-C_6H_4-HN_2OSR$

(2) $NH_2C_6H_4-C_6H_4-HN_2OSR$

(3) $CH_3CONH-C_6H_4-C_6H_4-HN_2OSR$

(4) $RSO_2NH-C_6H_4-S-C_6H_4HN_2OSR$

(5) $NH_2C_6H_4-S-C_6H_4-HN_2OSR$

(6) $RSO_2NH-C_6H_4-CH_2-C_6H_4-HN_2OSR$

(7) $CH_3CONH-C_6H_4-CH_2-C_6H_4-HN_2OSR$

(8) $NH_2C_6H_4-CH_2-C_6H_4-HN_2OSR$

(9) $RSO_2NH-C_6H_4-O-C_6H_4-HN_2OSR$

(10) $NH_2C_6H_4-O-C_6H_4-HN_2OSR$

The molecular weights as well as solubility of the compounds may be varied by changing R. As this substituent may be either aliphatic or aromatic and the latter either simple or substituted a large number of possibilities exist.

The solubilities may be increased by sulfonation or salt formation and decreased by alkylation.

These materials will all contain S^{35} in the sulfonamide function.

4) DISCUSSION AND PROCEDURE

4.1) Radioactive sodium p-toluenesulfonate and p-toluenesulfonyl chloride.

It is proposed that radioactive sodium p-toluenesulfonate be prepared by the sulfonation of toluene with concentrated sulfuric acid containing a small amount of radioactive sulfuric acid.

The method of preparation described in the literature (81) was designed to obtain as good ayield as possible of the sulfonate in a short time, considering both the toluene and the sulfuric acid as being non-critical. It was our purpose, on the other hand, to obtain a maximum yield of the sulfonate on the basis of the amount of sulfuric acid used. Therefore it was essential to modify the given method. This was accomplished by carrying out the reaction with stirring in a water-removing apparatus, using excess toluene. The temperature was permitted to rise to 195°C. These modifications led to an average yield of 60%, whereas that reported in the literature was 38%.

Para-toluenesulfonyl chloride was obtained in maximum yield and in purest state by heating sodium p-toluenesulfonate with a mixture of phosphorus pentachloride and phosphorus oxychloride.

4.2) The Benzidine Series

4.21) N,N'-di-p-Tosylbenzidine (82)

This substance was prepared by reacting benzidine and p-toluenesulfonyl chloride in pyridine. Pyridine was chosen for its excellent solvent properties, its possible catalytic effect, and the likelihood of its furthering the reaction by removal of hydrogen chloride. After recrystallization from acetone a pure sample was found to melt at 248°, five degrees higher that the melting point given by Willstätter.

4.22) N-Acetylbenzidine

This material was prepared by the method of Cain (85). Dilute (75%) alcohol was used as the solvent. Recrystallization from 50% alcohol yielded a colorless material melting at 199-200°C. The yield was 18.6% if one assumed only the monoacetyl product was produced. Cain reports a melting point of 199°C.

4.23) N-Acetyl-N'-p-tosylbenzidine

This material was obtained by reacting N-acetylbenzidine (p.59) with p-toluenesulfonyl chloride in pyridine solution in the usual way.

4.24) N-p-Tosylbenzidine

This substance was obtained by the hydrolysis of an alcoholic solution of N-acetyl-N'-p-tosylbenzidine with

20% hydrochloric acid. The material as prepared was quite sensitive to oxidation both in solution and in the solid state. Its solutions would color even when well-stoppered and charcoal was inadequate for securing a colorless material.

4.3) The Diphenyl Sulfide Series

4.31) N,N'-di-p-Tosyl-di-p-aminodiphenyl sulfide

This material was prepared from bis(p-aminophenyl)-sulfide (p. 62), p-toluenesulfonyl chloride and pyridine by refluxing on the steam bath. The product was also obtained when the unheated mixture was permitted to stand at room temperature for several hours with occasional shaking.

4.32) N-p-Tosyl-di-p-aminodiphenyl sulfide

This compound was prepared by reducing N-p-tosyl-p-amino-p'-nitrodiphenyl sulfide in glacial acetic acid, stannous chloride and concentrated hydrochloric acid.

4.4) The Diphenylmethane Series

4.41) N,N'-di-p-Tosyl-di-p-aminodiphenylmethane

This substance was prepared without difficulty in the usual way from p,p'-diaminodiphenylmethane, p-toluenesulfonyl chloride and pyridine.

4.42) N-Acetyl-di-p-aminodiphenylmethane

Unless special means are employed attempted monoacetylation of 4,4'-diaminodiphenylmethane will yield, for the most part, the diacetyl product. This is explained as the inevitable result of the complete blocking of resonance between the amino functions by the methylene linkage.

To obtain the monoacetyl compound acetic anhydride was added very slowly and with vigorous stirring to a large excess of the diamine in benzene solution. As the reaction proceeds the probability of getting the diacetyl derivative increases. Because of the insolubility of this material it was immediately evident when this substance began to form, and the reaction could be stopped.

4.43) N-Tosyl-N'-acetyl-di-p-aminodiphenylmethane

This substance resulted from the tosylation of N-acetyl-di-p-aminodiphenylmethane (p. 67) with p-toluenesulfonyl chloride and pyridine. On two occasions, however, a material was obtained, which, instead of melting at 169° showed a constant melting point of 136-137°. A mixed melting point determination showed no depression, but instead,

a sharply defined transformation at 137° with no further visible change until 169°, at which point there was complete melting. It was quite possible that these were polymorphic forms; however, there was apocryphal evidence of quantitative as well as qualitative nature that the lower melting form was a pyridine adduct - that is, two molecules of pyridine had combined with the normal product. It is regrettable a lack of material prevented a complete elucidation of this matter.

N-tosyl-N'-acetyl-di-p-aminodiphenylmethane was also prepared using an acetic acid-sodium acetate mixture in place of pyridine. This reaction was quite as satisfactory as with pyridine, and the yield was comparable. The product was identical with that of the pyridine reaction and melted at 168-169°.

4.44) N-Tosyl-di-p-aminodiphenylmethane

This substance was obtained by hydrolyzing an alcoholic solution of N-tosyl-N'-acetyl-di-p-aminodiphenylmethane with 20% hydrochloric acid.

4.5) The Diphenyl Ether Series

4.51) N,N'-di-p-Tosyl-di-p-aminodiphenyl ether

This material was obtained in the customary way from bis(p-aminophenyl)ether and p-toluenesulfonyl chloride in pyridine solution.

4.52) N-Acetyl-p-amino-p'-nitrodiphenyl ether

This material was prepared by condensing p-hydroxyacetanilide (p/ 72), p-bromnitrobenzene and sodium hydride with a Cu catalyst in a concentrated methyl carbitol solution at 150-155°. The recorded yield was strictly dependent on close adherence to very limited ranges of temperature and concentration and duration of heating.

$$CH_3CONH\text{-}C_6H_4\text{-}ONa + Br\text{-}C_6H_4\text{-}NO_2 \longrightarrow$$

$$CH_3CONH\text{-}C_6H_4\text{-}O\text{-}C_6H_4\text{-}NO_2 + NaBr$$

It was observed in some cases, where the reaction had been less successful, that the unreacted p-bromnitrobenzene would precipitate first, and in a fairly pure state, when the crude reaction product was recrystallized from aqueous alcohol. This occurred because this substance is only slightly soluble in alcohol and much less so in aqueous alcohol. The filtrate from the p-bromnitrobenzene separation

made turbid with water and permitted to stand for some hours, would then yield a fairly pure condensation product.

4.53) p-Nitro-p'-aminodiphenyl ether

This material was obtained by hydrolysis of N-acetyl-p-amino-p'-nitrodiphenyl ether in alcoholic solution with 20% hydrochloric acid. The amine hydrochloride separated readily from the solution.

4.54) N-Tosyl-p-amino-p'-nitrodiphenyl ether

This compound was obtained in the usual way from p-nitro-p'-aminodiphenyl ether, p-toluenesulfonyl chloride and pyridine, as well as by the acetic acid-sodium acetate procedure used with N-acetyl-di-p-aminodiphenylmethane.

Analysis for sulfur by the micro Parr method gave results that were low and that varied among themselves. Apparently the material was being only partially oxidized. Qualitative tests for sulfur as sulfide ion were positive. Analysis for nitrogen by micro Kjeldahl confirmed the structure.

4.55) N-Tosyl-di-p-aminodiphenyl ether

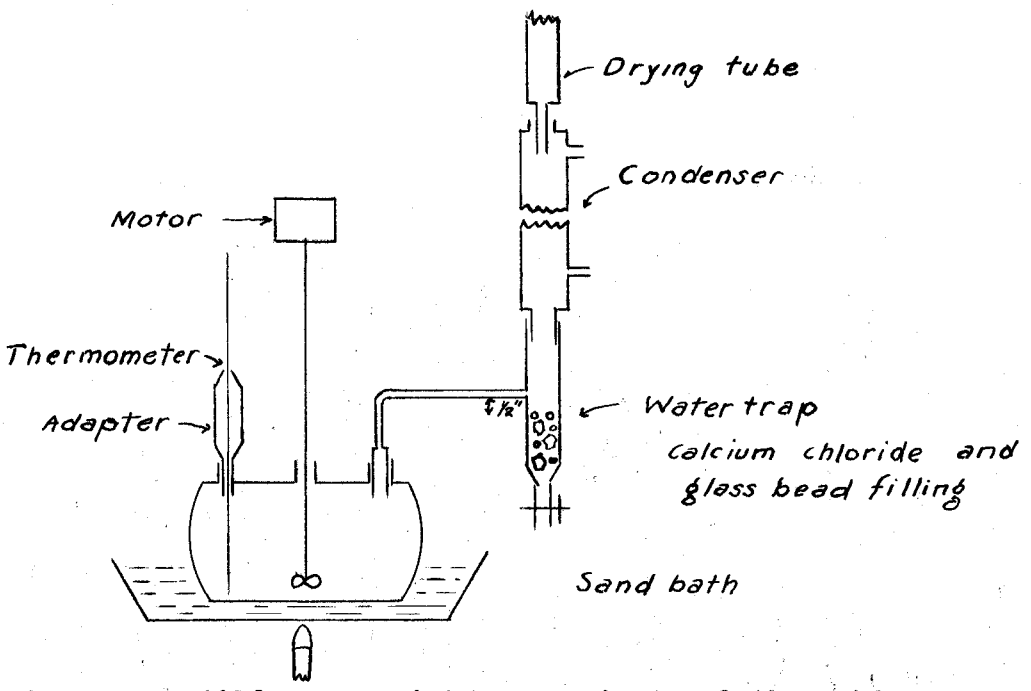
This substance was prepared by reducing an acetic acid solution of N-tosyl-p-amino-p'-nitrodiphenyl ether with stannous chloride and hydrochloric acid, followed by neutralization with ammonia and extraction with benzene. Analysis for nitrogen by micro Kjeldahl confirmed the structure.

The material was also obtained, but in poor yield, by reacting one equivalent of p-toluenesulfonyl chloride with one equivalent of p,p'-diaminodiphenyl ether. The resulting mixture, after acidification, contained the ditosylated material, the desired monotosylated substance as the hydrochloride, and the unreacted diamine as the dihydrochloride. The first named substance was insoluble in dilute hydrochloric acid solution; the latter soluble materials were separated through fractional crystallization. The reaction seemed of value only for structure confirmation.

5) EXPERIMENTAL

5.1) Preparation of Sodium p-Toluene Sulfonate

Toluene was purified as recommended in the literature (83). A 500 ml., 3-neck, round bottom, standard taper flask was equipped as shown below with a tapered thermometer, a mercury-seal stirrer, and a side arm water trap to which was attached a condenser stoppered with a calcium chloride drying tube.



The water trap was filled to within one inch of the side arm with purified toluene. To the flask was added 10 ml. (0.18 mole) concentrated sulfuric acid and 20 ml. (0.188 mole) of purified toluene. The mixture was heated with stirring such that the temperature rose to 195° during one hour.

During this time droplets of water could be seen in the trap before being taken up by the drier. The flame was removed, the mixture cooled to 100° and 3-4 ml. toluene added with thorough stirring. Heat was again applied and a cloudiness soon reappeared in the trap. When the temperature rose to 195°, the flame was again removed and the mixture cooled as before. Another portion of toluene was added. This process was repeated through four or five additions, with simultaneous removal of wet toluene from the bottom of the trap, until no more water (cloudiness) was in evidence on heating. The reaction was then deemed complete. The side arm was emptied partly and the flask heated to 150° to remove excess toluene, the latter being visible as condensate in the side arm. The total time required was five hours.

When the toluene had been removed the hot reaction mixture was poured into 100 ml. of water and flask rinsed with a few more ml. from a wash bottle. The acid solution was partly neutralized by carefully adding 11 g. of sodium bicarbonate; 30 g. of sodium chloride were then added and the mixture heated to boiling, adding more water, if necessary, to bring the salt into solution. A small amount (2-3g.) of a yellow, waxy solid was filtered off at this point and from its melting point, after recrystallization from alcohol, identified as di-p-tolyl sulfone, $CH_3C_6H_4SO_2C_6H_4CH_3$. The saturated solution was cooled thoroughly in an ice bath and the crude sodium p-toluene sulfonate collected on a Büchner funnel.

Crystals in the beaker were rinsed into the funnel with filtered mother liquor, the cake pressed dry, and washed into 25 ml. saturated sodium chloride solution.

Recrystallization was accomplished by dissolving the cake in 50 ml. of water, adding 10 g. of sodium chloride, a further 20-25 ml. of water being required to bring the latter into solution. The solution was stirred fot a few minutes with 0.5 g. of charcoal and filtered hot by suction through a fine filter paper. Because water had been added to the various washings, the solution was concentrated from the present volume of 100 ml. to the initial 70-75 ml. Crystallization was then permitted to take place without disturbance in an ice bath and the crystals collected and washed as before. The yield of colorless crystals was 19.7-22 g. or 56.6-63.2% of theoretical. Fieser (81) reports a yield of 37.8%. Further small amounts of the sulfonate could be recovered by concentrating the filtrate but these were contaminated with increas- amounts of sodium chloride. The sulfonate was identified and its purity tested by preparation of the p-toluidine salt (M.pt. 197°).

5.2) Preparation of p-Toluenesulfonyl Chloride

To 10 g. (0.051 moles) of sodium p-toluenesulfonate, dried at 140°, in a 200 ml. round bottom flask equipped with a condenser sealed with a calcium chloride drying tube was added 5 g. (0.024 moles) of phosphorous pentachloride and 10 ml. (0.109 moles) of phosphorous oxychloride. The mixture was refluxed with frequent vigorous shaking on an oil bath at 160° for 1.5 hours. Excess phosphorous oxychloride was removed at the pump and the mass washed into 400 ml. of ice water. After stirring vigorously the sulfonyl chloride was permitted to settle and was filtered off. The nearly white solid was washed with a little cold water and dried in a vacuum desiccator over phosphorous pentoxide. The material weighed 8.6 g. (89.6% of theoretical) and melted at 69°C.

5.3) The Benzidine Series

5.31) Preparation of N,N'-di-p-Tosylbenzidine

Because the use of technical grade benzidine led to difficulties in purification of the product materials the crude substance was treated as recommended in the literature (84).

In a 500 ml. round bottom flask equipped with a reflux condenser was placed 40.0 g. (0.25 moles) of purified benzidine, 95.3 g. (0.50 moles) of p-toluenesulfonyl chloride and 150 ml. (1.86 moles) of dry C. P. pyridine. The dark solution was refluxed gently for one hour on an oil bath, cooled, and poured into 400 ml. of cold water. Concentrated hydrochloric acid (35 ml.) was added with vigorous stirring until the mixture was definitely acidic. The lumps of solid were broken up until quite well dispersed. The dark gray solid was permitted to settle, filtered on an Büchner and washed with several small portions of dilute hydrochloric acid. The dried material melted at 236-239° and weighed 62 g.

Purification was effected through recrystallization, after charcoal treatment from aqueous acetone. Successive recrystallizations until a colorless product was obtained displayed melting points of 244-246°, 248° with no further change. The yield of large, colorless crystalline plates was 50 g. or 40.6% of theoretical. The melting point given in the literature was 243°. (82).

5.32) Preparation of N-Acetylbenzidine (85)

In a 1 liter, round bottom, 2-neck standard taper flask, equipped with a mercury-seal wire stirrer, and a small dropping funnel, was placed 18.4 g. (0.1 mole) of benzidine dissolved in 100 ml. hot 75% alcohol. To the solution was added dropwise, with stirring, 9.6 ml. (0.10 moles) of acetic anhydride and the mixture was stirred for a further 30 minutes after the addition was complete. The mixture was neutralized with concentrated ammonia and filtered hot to remove diacetylbenzidine and unreacted amine. Water was added to the filtrate until a turbidity was produced, the solution then allowed to stand in the cold. The gray solid was filtered, pressed dry, and washed with small amounts of cold water. The crude material weight 9.5 g. and melted at 187-195°.

Recrystallization from 50% alcohol, using charcoal, afforded a colorless material melting at 199-200° and weighing 4.2 g. (18.6% of theoretical if one assumed only the monoacetyl product was produced.)

5.33) Preparation of N-Acetyl-N'-p-tosylbenzidine

In a 100 ml. round bottom flask equipped with a mechanical stirrer was placed 2.26 g. (0.01 mole) of N-Acetylbenzidine, 1.91 g. (0.01 mole) p-toluenesulfonyl chloride and 10 ml. of pyridine. The solution was warmed with stirring on the water bath for one hour, the color changing from

purple-red to orange in a few minutes. The solution was added slowly and with stirring to 125 ml. of ice water. The cold solution was made acid with concentrated hydrochloric acid and was left for several hours in the ice bath. The lightly colored solid when filtered off weighed 3.1 g. and melted at 218-221°. The material was recrystallized from aqueous alcohol and charcoal and dried at 120-130°. The yield was 2.8 g. (73.8% theoretical) of colorless microcrystalline needles melting at 227.5°C.

Analysis: Subst., 0.008133, 0.004907; $BaSO_4$, 0.004960, 0.002948.

Calc. for $C_{21}H_{20}N_2O_3S$; S, 8.43.

Found: S, 8.37 , 8.25.

5.34) Preparation of N-p-Tosylbenzidine

In a 100 ml. round bottom flask equipped with a condenser was placed 1.0 g. (0.0026 moles) of N-acetyl-N'-p-tosylbenzidine dissolved in 20-25 ml. alcohol. To the hot solution was added 25 ml. of 20% hydrochloric acid. The mixture was heated to reflux temperatur , solution being complete within ten minutes, and refluxed vigorously for one half hour longer. The solution was concentrated at the pump with warming to remove alcohol, cooled, and neutralized with concentrated ammonia with stirring. The solid was permitted to settle, filtered, and washed with a few ml. cold water containing a few drops of alcohol. This procedure yielded 0.9 g.

of lightly-colored material melting at 158-164°. Recrystallization from aqueous alcohol and charcoal resulted in 0.8 g. (91% of theoretical) of near-colorless crystalline material melting at 164-165°. Further purification neither removed the color nor raised the melting point. It was observed that exposure to air tends to rapidly darken the aqueous alcoholic solution of this substance.

Analysis: Subst., 0.004336, 0.005055

$BaSO_4$, 0.002841, 0.003294

Calc. for $C_{19}H_{18}N_2O_2S$:S, 9.47

Found: S, 9.0, 8.95.

5.4) The Diphenyl Sulfide Series

5.41) Preparation of bis(p-nitrophenyl)sulfide

The most satisfactory method was that of Fuson and Melamed (86).

To a solution of 10 g. (0.063 moles) of p-nitrochlorbenzene in 25 ml. absolute alcohol in a 500 ml. round bottom flask equipped with a reflux condenser was added a solution of 7.5 g. (0.032 moles) of hydrated sodium sulfide, $Na_2S.9\ H_2O$. The mixture rapidly darkened on refluxing for 3 hours on the steam bath. After a time the color changed from dark red to orange and crystals began to appear. The mixture was cooled, the mother liquor dedanted and the crystals filtered and washed with a few ml. of cold absolute alcohol. Recrystallization from a mixture of 10 ml. absolute ethanol and 12 ml. glacial acetic acid afforded beautiful, long, flat yellow needles melting at 160°. The yield was 4.0 g. or 45.7% of theoretical.

5.42) Preparation of bis(p-aminophenyl)sulfide

The method used was essentially that of Fuson and Melamed (86).

In a 500 ml. round bottom flask equipped with a mechanical stirrer and a dropping funnel was placed 3.4 g. of metallic tin and 2 g. (0.00725 moles) of bis(p-nitrophenyl)-sulfide dissolved in 32 ml. glacial acetic acid. An equal volume of 20% hydrochloric acid was added dropwise at such

a rate that the solution remained clear. The mixture was maintained in the water bath at 75-80° for two hours, at the end of which time the solution was colorless. After cooling and filtering to remove particles of tin, concentrated sodium hydroxide was added with stirring until the initial precipitate of hydrous tin oxides had completely redissolved. The free amine was filtered off, recrystallized from benzene-petroleum ether mixture and allowed to stand in the cold. After filtration and drying 1.4 g. (89.5% of theoretical) of a white solid was obtained which melted sharply at 107°.

At the end of the reduction period an alternate path of operation may be taken as follows: The solution was permitted to stand overnight permitting the crystallization of the tin chloride complex of the diamine in colorless, glistening needles. These were filtered off, particles of tin removed, and then the needles were taken up in water. The free diamine was liberated in the usual way with excess alkali. Recrystallization from benzene-petroleum ether yielded 1.3 g. (83.2% of theoretical) of white crystals melting at 107-108°C.

5.43) Preparation of N,N'-p-Tosyl-di-p-aminodiphenyl sulfide

In a 100 ml. round bottom flask equipped with a reflux condenser was placed 2.16 g. (0.01 moles) of bis(p-aminophenyl)sulfide, 3.82 g. (0.02 mole) of p-toluenesulfonyl chloride and 15 ml. of dry pyridine. The dark solution was heated for one hour on a water bath, cooled, and poured into 200 ml. cold water. Concentrated hydrochloric acid was added with

stirring until the mixture was definitely acidic. The white floc was permitted to settle. It filtered readily after standing a few hours and was dried in a desiccator over phosphorus pentoxide. The crude almost white material melted at 184-189°. After several recrystallizations from absolute alcohol and charcoal 4.3 g. (82% of theoretical) of a colorless crystalline material was obtained which melted sharply at 195°.

Analysis: Subst., 0.005831; $BaSO_4$, 0.007897.

Calc. for: $C_{26}H_{24}N_2O_4S_3$: S, 18.33

Found: S, 18.40.

5.44) Preparation of p,p'-Nitroaminodiphenyl sulfide (87)

To a 500 ml. round bottom flask equipped with a wire stirrer, a dropping funnel and a reflux condenser was added a solution of 2.76 g. (0.01 mole) of bis(p-nitrophenyl)sulfide dissolved in 90 ml. of a mixture of 2 parts of absolute alcohol to one of benzene. To this solution at 50°C was added dropwise with stirring 250 ml. of ammonium sulfide solution prepared by absorbing hydrogen sulfide gas in cold concentrated ammonia. The mixture was kept at this temperature for 30 minutes after the addition was complete; the temperature was raised and a mild reflux carried out for five hours. The solution was taken to dryness on the water bath and the residue extracted 3 times with 5% hydrochloric acid. After removing any small particles of solid material by filtering through a wad of glass wool, the filtrate was neutralized

with concentrated ammonia. The yellow floc was permitted to settle undisturbed in the cold, filtered off, washed with water, and dried at 100-110°. The crude yellow solid melted at 137-140° and weighed 1.5 g. Recrystallization from a benzene-alcohol solvent pair provided 1.1 g. (44.7% of theoretical) of tiny yellow-orange needles melting sharply at 143°.

5.45) Preparation of N-p-Tosyl-p-amino-p'-nitrodiphenyl sulfide.

In a 100 ml. round bottom flask equipped with a reflux condenser was placed 2.46 g. (0.01 mole) of p,p'-nitroaminodiphenyl sulfide, 1.90 g. (0.01 mole) of p-toluenesulfonyl chloride and 10 ml. of pyridine. The yellow solution was warmed for one hour on the water bath. The solution was added slowly with stirring to 125 ml. of ice water. The cold solution was made acid with concentrated hydrochloric acid and the yellow-white precipate permitted to stand overnight. The white solid was filtered, washed with dilute hydrochloric acid, and with water. The material, after several recrystallizations from benzene-petroleum ether mixture, melted at 157.5-158.5°c. and weighed 3.2 g. (80% of theoretical).

Analysis: Subst., 0.003456, 0.012477

$BaSO_4$, 0.004003, 0.014418

Calc. for: $C_{19}H_{16}N_2O_4S_2$: S, 16.01

Found: S, 15.9 , 15.85

5.46) Preparation of N-p-Tosyl-di-p-aminodiphenyl sulfide

To a hot solution of 2.0 g. (0.005 mole) of N-p-tosyl-p-amino-p'-nitrodiphenyl sulfide in 20 ml. glacial acetic acid was added, during one minute, 4.5 g. (0.02 mole) of tin salt ($SnCl_2 \cdot 2H_2O$) dissolved in 8 ml. hot concentrated hydrochloric acid. After warming at 60°C. for 30 minutes the solution was cooled and transferred to a separatory funnel. An equal volume of benzene was added and the solution was then neutralized by carefully adding concentrated ammonia with vigorous shaking. The benzene layer was removed, fresh benzene added, and the extraction repeated. The extract was dried over anhydrous calcium sulfate, filtered through glass wool, and the benzene removed at the pump.

The light yellow solid was dissolved in 30% alcohol and permitted to stand overnight. Upon seeding crystallization began immediately. After standing a few hours the crystals were separated and washed with water containing a few drops of alcohol. The yield was 1.5 g. of crystals melting at 140-143°C. Recrystallization from 30% alcohol and charcoal yielded 1.3 g. (70.4% of theoretical) of colorless glistening microcrystals melting at 142-143°.

Analysis: Subst:, 0.008199 ; $BaSO_4$, 0.010223.

Calc. for: $C_{19}H_{18}N_2O_2S_2$: S, 17.30

Found: S, 17.12.

5.5) The Diphenylmethane Series

5.51) Preparation of N,N'-di-p-Tosyl-di-p-aminodiphenylmethane

In a 500 ml. round bottom flask equipped with a reflux condenser was placed 3.96 g. (0.02 mole) of p,p'-diaminodiphenyl methane, 7.64 g. (0.04 mole) of p-toluenesulfonyl chloride and 30 ml. of pyridine. The clear, dark red solution was heated on the water bath for on e hour and poured with stirring into 300 ml. cold water. Concentrated hydrochloric acid was added with vigorous stirring until the mixture was definitely acidic. The pink brittle solid that formed was allowed to stand for one hour in the cold. The material was transferred to a Büchner, washed with several portions of dilute hydrochloric, and then with cold water. Two recrystallizations with aqueous alcohol and charcoal, followed by drying at 120°, yielded 6.2 g. (61% of theoretical) of colorless crystals melting at 186-187.5°C.

Analysis: Subst.: 0.011442 : $BaSO_4$, 0.010250

Calc for: $C_{27}H_{26}N_2O_4S_2$: S, 12.65.

Found: S, 12.31

5.52) Preparation of N-Acetyl-di-p-aminodiphenylmethane

To a 1 l. round bottom flask equipped with a wire stirrer and a small capillary-tipped dropping funnel was added

a solution of 5.94 g. (0.03 mole) of p,p'-diminodiphenylmethane in 200 ml. of dry benzene. The stirrer was started and a solution of 0.944 ml. (0.01 mole) of acetic anhydride in 10 ml. of benzene was added slowly through the capillary dropping funnel at room temperature. This addition normally required 2-2½ hours. The lightly colored solution remained clear until approximately 7/8 of the reagent solution had been added. The appearance of a turbidity indicated the formation of N, N'-diacetyl-dipaminophenylmethane and denoted the end of the desired reaction. The small amount of white floc was permitted to settle and removed by filtration. The filtrate was neutralized with concentrated ammonia and was allowed to stand overnight, or, for shorter periods, in an ice bath. The white, crystalline material was filtered off and the filtrate retained. On standing overnight, or for several hours in the cold, another crop of crystals were secured. The combined material was washed thoroughly on the filter with petroleum ether and dried in a desiccator over phosphorus pentoxide. The crude material weighed 1.2 g. and melted in the range 123-126°. Pure Material was obtained by recrystallization from a minimum amount of boiling water containing a small quantity of charcoal. The latter effectively removed some unreacted diamine which could be seen as oily droplets in the boiling aqueous solution. The yield was 1.0 g. of large pearly platelets melting at 133-134°C.

Analysis: Subst., 0.004625, 0.004681; 0.00988N HCl, 3,80 ml., 3.92 ml.

Calc for: $C_{15}H_{16}N_2O$: N, 11.66

Found: N, 11.38, 11.59

5.53) Preparation of N-Tosyl-N'-acetyl-di-p-aminodiphenylmethane

In a 100ml. round bottom flask equipped with a reflux condenser was placed 2.40 g. (0.01 mole) of N-acetyl-di-p-aminodiphenylmethane, 1.90 g. (0.01 mole) of p-toluenesulfonyl chloride and 10 ml. of pyridine. The orange solution was warmed for one hour on the water bath and the solvent removed at the pump. The reddish oil remaining was taken up in aqueous alcohol and permitted to stand overnight. The translucent pink platelets which separated were transferred to a Buchner funnel, washed with water containing a few drops of alcohol, then with water. A sample of the dried material melted at 167-168° (see p. 49). Recrystallization from aqueous alcohol and charcoal yielded 3.25 g. of micro needles melting at 167.9-169.2°. Yield was 82.5%.

Analysis: Subst., 0.005720; 0.00988N HCl, 5.83ml.
Calc. for: $C_{22}H_{22}N_2O_3S$; N, 7.10

Found: N, 7.15

5.54) Preparation of N-Tosyl-di-p-aminodiphenylmethane

In a 100 ml. flask equipped with a reflux condenser was placed 1.97 g. (0.005 mole) of N-tosyl-N'-acetyl-di-p-aminodiphenylmethane in 20 ml. of alcohol. To the hot solution was added 25 ml. of 20% hydrochloric acid and the mixture refluxed for 30 minutes. The solution was concentrated

at the pump to remove alcohol, and concentrated ammonia was added dropwise with stirring until the mixture was alkaline. After standing overnight, the white precipitate was filtered, washed with water, and dried. The crude material melted at 137-140°. Recrystallization from aqueous alcohol and charcoal afforded 1.4 g. (79% of theoretical) of an almost white crystalline material melting at 156-158°C.

Analysis: Subst., 0.007974, 0.004354; $BaSO_4$, 0.005215, 0.002880.

Calc. for: $C_{20}H_{20}N_2O_2S$; S, 9.11

Found: S, 9.0, 9.1.

5.6) The Diphenyl Ether Series

5.61) Preparation of N,N'-p-Tosyl-di-p-aminodiphenyl ether

Because the use of technical grade p,p'-diaminodiphenyl ether led to untoward difficulties in purification of the product materials, and seriously lowered the yields, the commercial material was treated as recommended in the literature (84).

In a 100 ml. round bottom flask equipped with a reflux condenser was placed 2.0 g. (0.01 mole) of p,p'-diaminodiphenyl ether, 3,82 g. (0.02 mole) of p-toluenesulfonyl chloride and 15 ml. dry pyridine. The dark solution was heated for one hour on the water bath, cooled, and poured into 400 ml. cold water. Concentrated hydrochloric acid was added with stirring until the mixture was definitely acidic; the pink solid that separated being permitted to stand overnight. After filtration, washing with dilute hydrochloric acid, and with water, there was obrained a light-pink colored material melting at 170-175°. Several recrystallizations from aqueous alcohol and charcoal afforded a white crystalline substance weighing 3.6 g. and melting at 179-180°. The yield was 71% of theoretical.

Analysis: Subst., 0.016603, 0.008586; $BaSO_4$, 0.014730, 0.007729.

Calc. for $C_{26}H_{24}N_2S_2O_5$; S, 12.61

Found: S, 12.22, 12.41

5.62) Preparation of p-Hydroxyacetanilide (88)

In a 1 l. round bottom flask equipped with a reflux condenser was placed an intimately ground mixture of 22 g. (0.15 mole) of p-aminophenol hydrochloride and 12.5 g. (0.15 mole) of anhydrous sodium acetate. A mixture of 21.5 ml. (0.37 mole) of glacial acetic and 16.6 ml. (0.17 mole) of acetic anhydride was added and the flask heated on the oil bath with frequent shaking at 135-140° for 1½ hours. The pink mixture was cooled, the solid filtered off, washed with a few ml. of cold water, and pressed dry. Sodium carbonate was added carefully to the filtrate until it was slightly alkaline. The mixture was cooled, the solid filtered off, washed as before, and pressed dry. The combined products were recrystallized from water and charcoal. The yield of almost white crystalline prisms was 14.4 g. (63.2% of theoretical) melting at 167-168°.

5.63) Preparation of N-Acetyl-p-amino-p'-nitrodiphenyl ether

In a 500 ml. 3 neck, standard taper conical bulb flask equipped with a mercury-seal stirrer and a reflux condenser connected to a mercury trap, the purpose of which is to exclude air from the reaction mixture, were placed 3.0 g. (0.02 mole) of p-hydroxyacetanilide, 4.0 g. (0.02 mole) of p-bromnitrobenzene, 0.48 g.

(0.02 mole) of sodium hydride, and a pinch of Cu powder (89). The materials were stirred thoroughly for a few minutes. To the homogeneous mixture was added with caution 5 ml. of methyl carbitol which had been distilled from calcium hydride over the range 190-194°. The flask was heated in a metal bath at 150-155° for 2½ hours. The brown, somewhat viscous liquid was poured into cold water with vigorous stirring, a few ml. of hot water sufficing to wash out the flask. There was usually a small amount of tar-like material at this stage. After standing overnight, the yellow-brown crystalline solid was separated on a Büchner funnel and washed with a little water. Recrystallization from aqueous alcohol and charcoal resulted in 1.5-1.7 g. (28.32% of theoretical) of bronze-colored, microcrystalline needles melting at 152.5-153.5°.

Analysis: Subst., 0.003478, 0.004482;
0.00988N HCl, 2.52 ml. 3.34 ml.
Calc. for: $C_{14}H_{12}N_2O_4$: N, 10.3
Found : N, 10.0, 10.3.

5.64) Preparation of p-nitro-p'-aminodiphenyl ether

In a 100 ml. flask equipped with a reflux condenser was placed 1.36 g. (0.005 mole) of N-acetyl-p-amino-p'-nitrodiphenyl ether in 15 ml. of alcohol. To the hot solution was added 25 ml. of 20% hydrochloric acid and reflux was maintained for 45 minutes. On standing, the amine hydrochloride separated in light-yellow, feathery needles. These were filtered off, washed with a small amount of cold water and dried at 120°.

The salt melted at 215-217° and weighed 0.75 g.

Concentrated ammonia was added slowly with stirring to the filtrate and the yellow precipitate permitted to settle. After the usual filtering, washing and drying, the free base weighed 0.28 g. and melted at 128-129°. The total crude yield on the basis of the free amine was 87%. Recrystallization of the free base from aqueous alcohol and charcoal yielded a yellow, finely crystalline material with a constant melting point of 134-135°.

Analysis: Subst. 0.003067, 0.002485;
0.00988N HCl, 2.69 ml., 2.18 ml.
Calc for : $C_{12}H_{10}N_2O_3$: N, 12.18.
Found: N, 12.18, 12.12.

5.65) Preparation of N-Tosyl-p-amino-p'-nitrodiphenyl ether.

In a 100 ml. round bottom flask equipped with a reflux condenser was placed 1.33 g. (0.005 mole) of p-nitro-p'-aminodiphenyl ether hydrochloride, 0.95 g. (0.005 mole) of p-toluenesulfonyl chloride and 5 ml. of pyridine. The clear, light-brown solution was heated for one hour on the water bath and poured into 200 ml. of cold water. Concentrated hydrochloric acid was added with stirring until the mixture was definitely acidic. On standing a crystalline, nearly colorless material separated. This was filtered and washed with a few ml. of water. Recrystallization of the gray-yellow solid from

alcohol and charcoal yielded 1.45 g. (75.5% of theoretical) of fine crystals melting at 154-155°C.

Analysis: Subst., 0.002834; 0.00988N HCl, 1.50 ml.

Calc. for: $C_{19}H_{16}N_2O_5S$: N, 7.29

Found: N, 7.32.

5.66) Preparation of N-Tosyl-di-p-aminodiphenyl ether

To a hot solution of 1.92 g. (0.005 mole) of N-tosyl-p-amino-p'-nitrodiphenyl ether in 20 ml. glacial acetic acid was added, during 1 minute, 4.5 g. (0.02 mole) of stannous chloride dihydrate dissolved in 8 ml. hot concentrated hydrochloric acid. After warming at 60° for 30 minutes the solution was cooled and transferred to a separatory funnel. An equal volume of benzene was added and the solution neutralized by carefully adding concentrated ammonia with vigorous shaking. The benzene layer was removed, fresh benzene added, and the extraction repeated. After drying over anhydrous calcium sulfate, the extract was filtered through glass wool and the benzene removed at the pump. Recrystallization of the red solid from aqueous alcohol and charcoal afforded 1.1 g. of white microcrystalline needles melting at 141-142°. The yield was 77.5%.

Analysis: Subst., 0.003611, 0.001852

0.00988N HCl, 2.07 ml., 1.05 ml.

Calc. for: $C_{19}H_{18}N_2O_3S$: N, 7.91.

Found: N, 7.91. 7.84.

An alternate preparation of this material, but in very poor yield, was as follows:

Commercial bis(p-aminophenyl)ether was purified to some extent through conversion to the dihydrochloride (90). A mixture of 2.73 g. (0.01 mole) of bis(p-aminophenyl)ether dihydrochloride, 1.91 g. (0.01 mole) of p-toluenesulfonyl chloride and 5 ml. of pyridine was warmed on the water bath for one hour. The dark solution was taken to near dryness to remove excess pyridine. One hundred and fifty ml. of water and sufficient concentrated hydrochloric acid to make the mixture acidic were added. The solution was refluxed with charcoal for 30 minutes, filtered while hot to remove the N, N'-di-tosyl-di-p-aminodiphenyl ether, and the filtrate concentrated to ½ its volume. On standing overnight a small amount of tarry material separated from the purple solution. Several recrystallizations from aqueous alcohol and charcoal yielded 0.15 g. of almost white microcrystalline needles melting at 140-141°. A mixed-melting point determination with the substance obtained from the reduction of N-tosyl-p-amino-p'-nitrodiphenyl ether (see 5.66) confirmed the identity of the two materials.

(6) SUMMARY

It is commonly believed that the mortality from gastric cancer could be greatly reduced, perhaps by 50% or more, if there were some method of diagnosis or therapy.

Evidence has been presented indicating that it would be of value to prepare certain sulfonamides for possible localization in cancer tissue. These materials, made radioactive, would have value as possible diagnostic and therapeutic agents. The substances listed below have been prepared with this purpose in mind. All, except the first, are new materials.

1. N,N'-di-p-tosylbenzidine
2. N-acetyl-N'-p-tosylbenzidine
3. N-p-tosylbenzidine
4. N,N'-di-p-tosyl-di-aminodiphenyl sulfide
5. N-p-tosyl-p-amino-p'-nitrodiphenyl sulfide
6. N-p-tosyl-di-p-aminodiphenyl sulfide
7. N,N'-di-p-tosyl-di-p-aminodiphenylmethane
8. N-tosyl-N'-acetyl-di-p-aminodiphenylmethane
9. N-tosyl-di-p-aminodiphenylmethane
10. N-tosyl-p-amino-p'-nitrodiphenyl ether
11. N-tosyl-di-p-aminodiphenyl ether
12. N,N'-di-p-tosyl-di-p-aminodiphenyl ether

In addition, the following intermediate materials are prepared for the first time:

13. N-acetyl-p-amino-p'-nitrodiphenyl ether

14. p-nitro-p'-aminodiphenyl ether

15. N-tosyl-p-amino-p'-nitrodiphenyl ether.

7) BIBLIOGRAPHY

(1) Greenstein, J. P., Biochemistry of Cancer, Academic Press, Inc., New York (1947) p. 1.

(2) Pack, G. T. and Livingston, E. M., Treatment of Cancer and Allied diseases, Paul B. Hoeber, Inc. (1940).

(3) Ivy, A. C., Biology of Cancer, Science, 106, No. 2759 (November 14, 1947).

(4) Ewing, J., Neoplastic Diseases, W. B. Saunders Co., Philadelphia (1940).

(5) Pircham, A. and Sickl, H., Am. J. Cancer 16, 681 (1932).

(6) Fibiger, J. Ztschr. f. Krebsforschung 13, 217 (1913). 14, 295 (1914).

(7) Yamagiwa, K. and Ichikawa, K. J. Cancer Research, 3, 1 (1918).

(8) Hartwell, J. L., Survey of Compounds which have been tested for Carcinogenic Activity, U. S. Public Health Service (1941).

(9) Ewing, J. loc cit., p. 95.

(10) Little, C. C., Am. J. Cancer 15, 780 (1931), J. Nat'l. Cancer Inst. 1, 727 (1941).

(11) Bittner, J. J., A.A.A.S. Research Conference on Cancer (1945) p. 63.

( ) Shimkin, M. B. and Andervont, H. B., ibid., p. 97.

Andervont, H. B., A Symposium on Mammary Tumors in Mice, p. 133 (1945).

(12) Huggins, C., Science 97, 541 (1943).

Peck, R. I., J. Amer. Med. Ass'n. 127, 17 (1945).

(13) Huggins, C., J. Amer. Med. Ass'n. 131, 576 (1946).

(14) Haddow, A., Watkinson, J. M., et al. Brit, Med. J., 2, 393 (1944).

(15) Tannenbaum, A. , Cancer Research, 2, 460 (1942).

(16) Nettleship, A. and Henshaw, P. S., J. Nat'l. Cancer Inst., 4, 309 (1943).

(17) Huseby, R. A., Ball, Z. B., Visscher, M. B., Cancer Research, 5, 40 (1945).

(18) Pollard, E. and Davidson, W. L., Applied Nuclear Physics, J. Wiley and Sons, Inc., New York (1942).

(19) Glasser, O., Medical Physics, Year Book Publishers, Inc. Chicago, Illinois (1944).

(20) Lea, O. E. Actions of Radiations on Living Cells, The MacMillan Co., N. Y. (1947).

(21) Dessauer, F., Z. Phys. 20, 288 (1923).

(Jordan, P., Naturewissenchaften 26, 693 (1938).

(22) Prosser, C. L., et. al., Radiology 49, (3), 299, Sept., 1947

(23) Lorenz, E., Heston, W. E. et al., Radiology 49 (3), 269-365, Sept. 1947.

(24) Peck, W. S., McGreen, J. T., et al., Radiology 34, 176-183 Feb. 1940.

(25) Raper, J., Radiology 49, 314, Sept. 1947.

(26) Steward, F. W. and Farrow, J. H. in "Treatment of Cancer and Allied Diseases", Pack, G. T. and Livingston, E. M., P. B. Hoeber, Inc. (1940).

(27) White, F. R., J. Nat'l. Cancer Inst. 5, 41 (1944).

(28) Rous, P., Am. J. Cancer 28, 233 (1936).

(29) Fischer, B., Munchen med. Wchnschr. 53, 2041 (1906).

(30) Gye, W. E. and Andrews, C. H., Brit. J. Exper. Path. 7, 81 (1926).

(31) Claude, A., Science 85, 294 (1937).

(32) Little, C. C., Science 78, 465 (1933).

(33) Bittner, J. J., Science 84, 162 (1936).

(34) Bittner, J. J., Tr. College Physicians, Philadelphia, 9, 129, (1941).

(35) Fekete, E. and Little, C.C., Cancer Research 2, 525, (1942).

(36) Bittner J. J., Ann. N. Y. Acad. Sci. 49, 69-73 (1947).

(37) Gardner, W. W., Cancer Research 1, 345 (1941).

(38) Engelbreth-Holm, J. J., Cancer Research 1, 109 (1941).

(39) Livingston, E. M. and Pack, G. T., End-Results in the Treatment of Gastric Cancer, P. B. Hoeber, Inc., New York (1939).

(40) Ackerman, L. V., and del Regato, J. A., Cancer, Diagnosis Treatment and Prognosis, C. V. Mosby Co., St. Louis (1947) pp 515-552.

(41) Ivy, A. C., J. Nat'l. Cancer Inst. 5, 313 (1945).

(42) British Registrar General's Report: Abstr. Jour. Am. Med. Assn. 90, 704 (1928).

(43) Peacock, P. R., J. Nat'l. Cancer Inst. 7, 407, 1947.

(44) Stout, A. P., Human Cancer, Lea & Fibiger, Philadelphia, (1932) p. 89.

(45) Hurst, A. F., Lancet 2, 1023 (1929).

(46) Comfort, M. W., Vanzant, F. R., Tr. Am. Gastroenterol. Assoc. 264-268 (1933).

(47) Ibid., Am. J. Surg. 26, 447 (1934).

(48) Rhoads, C. P., J. Nat'l. Cancer Inst. 1, 551 (1941).

(49) Despeignes, V., Lyon med. 82, 428, 503 (1896).

(50) Werner, R., Strahlentherapie, 601-626 (1914-1915).

(51) Evans, W. A., and Leucutia, T., Am. J. Roentgenol. 793-801 (1923).

(52) Grosset, A. and Regaut, C., Bull. Acad. de med. Paris 110, 379 (1933).

(53) Merrit, E. A., Am. J. Roentgenol. 36, 324-331 (1936).

(54) Barrett, M. K., J. Nat'l Cancer Inst. 7, 127 (1946).

(55) Kenney, J. M., Cancer Research 2, 130 (1942).

(56) Low-Beer, B. V. A., et al. Radiology 39, 573 (1942).

Lawrence, J. H., Scott, K. G., et al., International Clinics 3, 33 (1939).

(56) Hamilton, J. G. and Soley, M. H., Am. J. Phsiology, 131, 135, (1940).

(57) Bloch, H. S., Doctoral Dissertaion, University of Cincinnati 112 (1946).

(58) Karczag, L., Biochem. Ztschr. 230, 411 (1930).

(59) Glasstone, S., Elements of Physical Chemistry, D. Van Nostrand Co., N. Y. (1947) p. 504.

(60) Greenstein, loc. cit., 273-277. See Ref. (1).

(61) Schmitt, L. H. et al., The Toxicity of sulfamerazines and sulfamethazines J. Pharm. & Exptl. Therap. 81, 17-42 (1944).

(62) Michaelis, L., Hydrogen Ion Concentration, The Williams and ~~Wi~~ Wilkins Co., Baltimore, (1926) p. 77.

(63) Paul, Zeitschr. f. physiol. chemie, 31, 1, 64 (1900).

(64) Bell, P. H. and Roblin, R. O., J. Am. Chem. Soc., 64, 2907-8 (1942).

(65) Northey, E. H., The Sulfonamides and Allied Compounds, Reinhold Publishing Corp., New York (1948) p. 7.

(66) Goldman, E. E., Beitr. Klin. Chir., 64, 192 (1909).

(67) Hess, M., J. Path and Bact. 51, 309 (1940).

Kolman, K., Schweiz. med. Wochenschr. 65, 533, (1935).

Ludford, R. J., Proc. Roy Soc., S. B. 104, 493 (1929).

(68) Duran-Reymolds, F. Am. J. Cancer 35, 98 (1939).

(69) Brunschwig, A., Schmitz, R. L., Clarke, T. H., Arch. Pathology 30, 902 (1940).

(70) Tobin, L. H. and Moore, F. D., J. Clin Invest. 22, 155 (1943).

Moore, F. D., Tobin L. H., Aub., J. C., J. Clin. Invest. 22, 161, 1943).

(71) Moore, F. D. and Tobin, L. H., J. Clin. Invest. 21, 471 (1942).

(72) Chapman, L. M., Greenberg, D. M., Schmidt, C. L., J. Biol. Chem. 72, 707 (1927).

Rawson, R., Am. J. Physiol. 138, 708 (1943).

(73) Boyland, E., Biochem. J. 32, 1207 (1938).

(74) Ibid., Biochem. J. 40, 55 (1946).

(75) Andersen, E. B., Z. Physik Chem., 32B, 237 (1936).

(76) Kamen, M. D. Phys. Rev. 60, 537 (1941).

(77) Henriques, F. C. et al., Ind. Eng. Chem. Anal. Edit. 18, 349, (1946).

(78) Shapiro, N., Bloch, H. S. and Schiff, L., Gastroenterology 3, 39 (1944).

(Bloch, H. S. et al., Gastroenterology 4, 421 (1945).

(79) Stevens, C. D., Private communication.

(80) Helander, Acta Physiologia Scandinavia 10, 103 (1945).

(81) Fieser, L. F., Experiments in Organic Chemistry. E. C. Health and Co., Boston, (1941), p. 139.

(82) Willstätter, R. Kalb, L., Ber. 37, 3772 (1904).

(83) Fieser, L. F., loc. cit., p. 364.

(84) Weygand, C., Organic Preparations, Interscience Publishers, Inc., New York, 238, (1945).

(85) Cain, J. C., J. Chem. Soc. 95, 717 (1909).

(86) Fuson, R. C. and Melamed, S., J. Org. Chem. 13, (5) 691 (1948).

(87) Kehrmann, F. and Bauer, E., Ber. 29, 2365 (1896).

(88) Woroshtzow, N. N., Jr. Pr. (2) 84, 530 (1911).

(89) Blatt, A. H., Organic Syntheses, Coll. Vol. II, 446 (1947).

(90) Haeussermann, C., and Teichmann, H., Ber. 29, 1449 (1896).